الجمهورية الجزائرية الديمقراطية الشعبية

People's Democratic Republic of Algeria

Ministry of Higher Education and Scientific Research

جـامعــة الاخـوة منتــوري

كليــة العـلوم الطبيعــة والحيــاة

Faculty of Natural and life Sciences

قســم الكيميــاء الحيويــة

Biochemistry - Molecular and cellular biology Department

و البيولوجية الجزيئية و الخلوية

Thesis presented by

Malika BOULMELTOUT

To obtain

Doctorate Degree in Sciences

Speciality: Biochemistry

Option: Toxicology

Topic :

Modulation of the Apoptotic Effects of Terpenes by Acting on the Mitochondrial Enzymatic System In Hepatic Normal and Cancer Cells

Publicly presented in front of the jury composed of :

President: Youcef NECIB (Professeur, University Frères Mentouri, Constantine)

Supervisor: Mesbah LAHOUEL (Professeur, University MSB, Jijel)

Examiner : Souad AMEDDAH (Professeur, University Frères Mentouri, Constantine)

Examiner: Amar ZELLAGUI (Professeur, UniversityOum El Bouaghi)

Examiner : Mohamed SIFOUR (Professeur, University MSB Jijel)

2018/2019

ACKNOWLEDGEMENTS

First of all I am grateful to Allah for giving me health, courage and patience to accomplish my thesis. Thanks to the greatest Allah.

Very deep gratitude goes out to my research director Prof Lahouel Mesbah, Professor at Mohamed seddik Benyahia University, faculty of natural sciences and life, head and researcher in molecular toxicology laboratory; for giving me the opportunity to work with him in his laboratory preparing this thesis. The success and final outcome of this work required a lot of guidance and assistance. I am extremely privileged to have got this all along the completion of my thesis. All that I have done is only due to such supervision and assistance and would not forget to express my deep gratitude.Many thanks for giving me this chance since I have learned so much about pharmacological and toxicological studies of natural substances and drugs through my research; for teaching me excellent reflexes of being good researcher and scientist, his kindness and excellent manners dealing with beginners who know that they are away from this field ; for being so patient, his precious advises that led me to accomplish this thesis; his scientific and human qualities as well as his extraordinary strength doing research has made of him an example of hard-working that I truly hope I will potentially be able to keep on profiting from that for so long. Similar gratitude goes out to Prof Zellagui A. without his contribution, this could not have been accomplished, thank you for your kindness, help and best of all for providing those precious molecules that we have explored and made use of them in this work. Many thanks to Mme Ameddah S and Mr Menad A for their help and back up.Before; passing to anyone else; I would thank so much all my colleagues, friends and collaborators at the molecular toxicology laboratory, University of Mohamed seddik Benyahia in particular both lab- engineers Mrs Bousegera Yahia and Ziad, my deep gratitude for your help and accessibility.

I would like to address my sincere thanks to the members of the jury for honoring me and accepting to judge my work .My deep gratitude for all

professors Prof Necib Y. for being the president of that jury, for his support, Prof Ameddah S. as a member of the jury many thanks, for being accessible to evaluate my thesis; both of them from Frères Mentouri University, I would not forget to express my thanks towards Prof Sifour from Mohamed Seddik Benyahia again for honoring me and being accessible to evaluate my thesis. Many thanks go out to all my teachers and colleagues at Frères Mentouri University.

I would not forget to address my sincere thanks and gratitude to Prof Gillian Barratt at university Paris-Saclay ex-Paris-Sud (France) for having me in her laboratory completing my work, her accessibility, precious advises and support. Without forgetting to thank Valérie Nicolas from MIPSIT Platform of the UMS IPSIT University Paris-Saclay, France for their amazing helps with confocal imaging microscopy and their kind guidance.

I address my sincere thanks towards my friends Mme Kebssa W, Mme Benftaima H, Prof Hamra-karoua, Prof Chellat Smain, Prof Belhouane R, Prof Mekkiou R for her accessibility, her endless support. Many thanks; for my collegues in chemistry‘s department. Prof Boukhalfa C. for her precious advises; Mme Kerouache Z, Mme Chellat A for her endless support, great patience, help and hardworking with me to fulfill my work and all of her family in particular Soundous; Mmes Haouam Z, Mahbouba A, Nacira for their help accessibility and support.

I would not terminate without thanking my family that has a great place in my heart and has been the source of my energy, courage and joy as well as strength. For the love, support and prayers of all members of my family all along my studies.

Finally, I would like to express my sincere thanks to everyone who has helped me in a very close or far away position for fulfilling my thesis.

DEDICATES

To the memory of my parents who have always been supporting me to achieve my goels, may Allah forgive them and cover them with his mercy inchaallah.

To my dearest brothers MahmoudRiadh, Abdelbaki and Zoheir.

To my dearest sisters Chahrazed, Abla and Houria.

To my dearest nephews and nieces whose births have brought meaning hope and joy to my life

To my dearest friends Chellat A. Kerouache Z. , Haouam Z., Dekdouk N., Mahbouba, Nacira and everyone who has known me along my work throughout these's years, and everyone whom I forgot to cite.

Malika

Table of contents

List of tables

List of figures

Abbreviations list

AA	Amino Acid
Acetyl-COA	Acetyl Co enzyme A
ACO	Aconitase; Prx3red) reducedperoxiredoxin; Prx3ox) oxidized peroxiredoxin; Q) coenzyme Q;
AD	Alzheimer's disease
ADP	Adenosinediphosphate
AIF	Apoptosis Inducing Factor
AlphaGDH	Alpha-glycerophosphate dehydrogenase
ALS	Amyotrophic lateral sclerosis
ANT	Adenyl nucleotide transporter
APAF-1	Apoptosis activating factor -1
ATP	Adenisinetriphosphate
B(a)P	Benzo [a] pyrene
B16F1	Melanoma cell line
B-cl2	B-cell lymphocytes-2
BSA	Bovine Serum Albumin
CAT	Catalase
CDNB	Cyanidedinitrobenzene
C-I	Complex I
CoA	Coenzyme-A
COQ	Coenzyme Q
CP	Cyclophosphamide
Cyp D	Cyclophilin D a protein of the matrix
CYP1A1	Cytochrome1A1
Cyt. b5	Reductase cytochrome b5 reductase
DD	Death Domain
DED	Death Effector Domain
DHOH	Dihydroorotate dehydrogenase
DIABLO	Direct IAP Binding Protein with Low pI)
DNA	Deoxyribonucleotide Acid
EPI	Epirubicine
FADD	Fas-associated death domain
FADH2	Reduced Flavinribonucleotide
FAO	Hepatocytes cell line
FEC	Fluorouracil-Epirubicin-Cyclophosphamide
FMN	Flavin Mono Nucleotide
Gpx	Glutathione Peroxidase
GR	Glutathione reductase
Grx2ox	Glutaredoxin-2 oxidized
Grx2red:	Glutaredoxin-2 reduced
GSH	Reduced Glutathione
GSSG	Oxidized Glutathione
GST	Glutathione-s-Transferase
HD	Huntington's disease
IDH1	Isocitrate dehydrogenase

Abbreviations list(Over))

IM	Inner mitochondrial membrane
KDa	Kelodalton
KGDHC	Alpha-ketoglutarate dehydrogenase complex
LK1	Ferulenol
LK3	Coladin
LPO	Lipoperoxidation
MAOs	Monoamine oxidases A and B
MCF-7	HumanBreastcancer
MDA	Malonedialdehyde
MDH	Malate dehydrogenase
MMPs	Membrane Metaloproteins
MPTP	Mitochondrial permeability Transition Pore
NAD^+	Oxidized Nicotinamide Adenine
NADH	Reduced Nicotinamide Adenine
NOS	Nitrogen Oxygen Species
OM	Outer mitochondrial membrane
PDHC	Pyruvate dehydrogenase complex
PGPx	Phospholipid hydroperoxide-glutathione peroxidase
PTP	Permeabitlity Transition Pore
RNA	Ribonucleotide diphosphate
ROS	Reactive Oxygen Species
SDH	Succinate dehydrogenase
SH	Sulfhydryl group
SOD	Superoxide Dismutase
T	Control
TBARS	Thiobarbuturates
TH	Transhydrogenase
TNF	Tumor Necrosis Factor
TNF-R1	Tumor Necrosis Factor Receptor or TRAIL
Trx2ox	Thioredoxin-2 oxido
Trx2red	Reduced thioredoxin-2
TrxR2	Thioredoxin-2 reductase
VDAC	Voltage-dependent anionic channels
WST-1	Westernsouternblotingtechnique-1
XIAP	X-linked inhibitor-of-apoptosis protein

Introduction

Introduction

Cancer has been considered to be the worse disease threathning the life's quality of human beings. This disease is a major health problem in both developed and developing countries. Being the second leading cause of death worldwide, high death rate associated with it, serious side-effects of chemotherapy, many cancer patients seek alternative and/or complementary treatments of disease E/RAZEK et al; **2003**; Savithramma et al; **2014.**The latter indicated that cancer is the degenerative disease brought about by several factors such as accumulation of toxins through carcinogenic food like fast food,colas,habits like smoking,drinking,stressful life style, toxic medicine and environmental pollution and all of them lower immunity causing cancer E/RAZEK et al; **2003;** Savithramma et al; **2014.** These autors also reported that the number of cancer cases is increasing and that the most common causes of cancer death are lung cancer (1.18 million deaths), stomach cancer (700.000 deaths),liver cancer (598.000 deaths). Other studies have demonstrated that exposure to polycyclic hydrocarbons aromatic, notably benzo (a) pyrane could increase cancer appearance- Benzo procarcinogenic, the metabolic achiration of B (a) P, principally via cytochrome P450 generates 7.8 – did-9.10-epoxy – benzo- (a) pyrane that is considered as the ultimate carcinogenic metabolite of B (a) P. during the metabolic process, the latter could directly or indirectly be metabolized to free radicals.

Liver cancer could be treated in different ways including chemotherapy. The use of anticancer drugs which destroy the dividing cancer cells to inhibit their proliferation, has been proved to be so limited that it cannot be reliable as far as efficacy and safety are concerned. Since the anticancer drugs destroy both normal and cancer cells, researchers have been oriented towards biologically active products in order to search for and seek cheaper, safer and more effective anticancer treatments. Moreover, Savithrama and his coworkers added that the control of cancer as the leading cause of death resides in the potential use of alternative therapies among which plant based natural products are being beneficial to combat cancer. These natural substances can potentially provide therapeutic products attacking different targets in cancer cells; such as umbellifera from ferula assafoetida seeds which significantly reduced cytochrome $\mathbf{p_{450}}$ and $\mathbf{b_5}$, enhanced the activities of glutathione-s-transferase, superoxide dismutase and catalase E/Razek et al; **2003;** Savithramma et al; **2014.**

Phytochemicals, including those obtained from fruits, vegetables, nuts and spices, have drawn a considerable amount of attention due to their ability to selectively kill tumor cells and

suppress carcinogenesis in preclinical animal models Aggarwal et al; **2006;** Russo. **2007;** Naithani et al; **2008;** Kaefer and, Milner. **2008;** Bishayee and Darvesh. **2010.**

A large number of these plant-derived substances have been shown to significantly prevent or delay cancer development in several high risk populations Kris-Etherton et al; **2002;** Riboli and Norat; **2003**.

With all what has been mentioned; we have been interested in sesquiterpene coumarins from Ferula roots methanol extract constituents to be tested on liver cancer and potentially explored as they have been reported in protecting from, and even treating liver cancer. Ferula species roots are the main source of sesquiterpene coumarins and globally terpenoids; the latter have been singled out to be explored in liver cancer prevention and treatment. Anticancer properties of terpens related to various mechanisms like concentrating oxidative stress, potentiating of antioxidants, improving detoxification potential, disrupting cell survival pathway and inducing apoptosis. However structurally some terpenoids are themselves inversely related to the risk of chronic diseases like cancer according to research published in current drug targets.

All theses properities make them valuable in combating cancer and be phenomenal for human beings health. Beyond that, many medicinal plants extracts are so rich in terpenoids such as monoterpenes found in the essential oils of citrus and other plants which makes them the best source of these naturally bioactive compounds Crowell.**1999;** Brunet . **1999**.

The cell has also disposed enzymatic systems capable of regulating its functions and making it adapted to different physiological situations. The mitochondrial enzymes play a key role in maintaining cellular homeostasis. Detoxifying enzymes (glutathion –s- transferase and superoxide dismutase) coupled with the mitochondrial enzymes called enzymes of xenobiotic metabolism (EXM) assure xenobiotic elimination outside the cell this function is completed through the line with the mitochondrial enzymatic system (Electron Transport chain and translate) assuring the regulation of mitochondrial respiration.

The majority of anticancer drugs (xendoliotics) could get chemically transformed and are often activated into a more toxic metabolite via microsomal cytochrome P450 the oxidative stress could occur when the production of reactive oxygen species attack the tissular enzymatic defense capacity.

After a hyper detection of reactive metabolites (following the activation of enzymes such as superoxide dismutase, oxidases) or upon detoxifying effect, the latter could be secondary of oxygenated reactive metabolites action of DNA, which can bring about the dysfunction or inactivation of enzymes of detoxification (glutathione -s- transferases, peroxidases, catalases).

Our objectives, hence are :

1- Evaluation of the cytotoxic effect of terpenic substances (Ferulenol, coladin and lapiferin) alone or associated with anticancer drugs over rat liver cancer *in vivo* and over liver cancer cell lines (FAO hepatocytes and B16F melanoma Lang cells) *in vitro.*

2- Evaluation of oxidative stress induced by ferulenol alone or associated with anticancer drugs (doxorubicine) by MDA determination and superoxide anion.

3- Search and seek for alternative natural anticancer therapeutic agents.

Literature Review

BIBLIOGRAPHIC SYNTHESIS

I. Generality on mitochondria

Mitochondria are what is commonly called the "cell's power station" where cellular respiration reactions occur, allow the synthesis of ATP, an energy molecule directly usable by cellular reactions Stohs and Bagchi. **1995**. The existence of a primitive eukaryote without mitochondria is currently in doubt, and various studies have recently shown that a mitochondrial organisms are in fact due to secondary mitochondrial loss Morin et al; **2004**; Andreyev et al; **2005.** In addition, some of these organisms have hydrogenosomes that are thought to be derived from mitochondria Wathley et al; **1996**; Hauptmann et al; **1996**; Simonson et al; **1993**.

I.1. History of discovery and definition

The discovery of mitochondria is much more diffuse. In 1890, Altman discovered in the cytoplasm, granulations and filaments which denominated them bioblast (of Greek bios: life and blastos: germ). In 1897, Banda developed a very complicated technique that makes it possible to differentiate these formations in a precise manner in the protoplasm of animal and plant cells. It is he who gave these germs of life, the name of mitochondria (Greek mitos: filaments and chondria: granule). The organization of the mitochondria as we know it at present has been demonstrated by electron microscopy by Plade (1952) and Sjoystrand (1953). In 1959, Chevremont discovered the presence of a DNA molecule whose structure is fundamentally different and independent of that of nuclear DNA Kunduzova et al; **2002**.

Mitochondria are cellular compartments, which generally have the appearance of cylinders of 0.5 to 1 µm in diameter, up to a maximum length of 7µm. The number, shape, size and location of mitochondria vary depending on the activity of the cell; they also vary by organ and tissue. The cells contain many: estimated number from 1000 to 2000 in the rat hepatocyte Lofler et al; **1996**; Koza et al; **1996**.

Mitochondria are divided by bipartition or budding. The sequential microcinematography of living cells has shown that mitochondria are extremely mobile and malleable.They constantly change shape, merge and separate, and that they are often associated with microtubules during their displacement Zhang et al; **1998**; Mclennan et al; **2000**.

I.2. Origin

Mitochondria result from a phenomenon of primary endosymbiosis. This origin postulates that a proteobacterium (symbiont) was phagocytized by an anaerobic archeobacteria (host).It is at least a billion and a half years ago to form a primitive eukaryote from which all current eukaryotes derive. The bacterium at the origin of the mitochondria was, therefore, in a vacuole of endocytosis within the cytoplasm of the host cell, there was no digestion of the bacterium but made use of its potentialities that host was not Gardner. **2002**; Vasquez-Vivar et al; **2000**. The processes of transformation of the bacterium into a mitochondrion consist of morphological and physiological phenomena leading to the interdependence of the two actors. After the loss of the bacterial wall, the two membranes surrounding the organelle are most probably that of phagocytosis, for the outer membrane and that derived from the plasmalemma of the bacterium, for the inner membrane Tretter et al; **2004**.

I.3. General Organization and ultrastructure of mitochondria

The mitochondrial ulustructure is characterized by the existence of two limiting membranes, representing approximately 40% of cell membranes in a hepatocyte. The outer membrane and the inner membrane. These two membranes are separated by a space of 10 to 20 nm, intermembrane space or external chamber. The inner membrane delimits the mitochondrial matrix space Starkov et al; **2004**.

I.3.1. the outer membrane

It has a trilamellar structure consisting of 60% protein and 40% lipid. It is semi-permeable due to the presence of porins allowing molecules up to 5Kd to pass. These porins contain voltage-dependent anionic channels VDAC (Voltage-Dependent Anion Channel) that have a metabolic and regulatory action. The outer membrane also contains import receptors; translocase of the outer membrane TOM (translocase of the outer membrane), import complexes of cholesterol, and proteins of the family Bcl2 Kushnareva et al; **2002**.

I.3.2. The inner membrane

The molecular organization of the inner membrane differs completely from that of the outer membrane. It emits within the organelle tight folds called ridges whose number and shape vary according to the physiological state of the body. The inner membrane contains 80% of the proteins and 20% of lipid. It includes the complexes of the respiratory chain and ATP

synthesis which will be described below. (Carriers of ADP and ATP, specific cotransporters ensure the passage of elements such as pyruvate, fatty acids, Ca^{++}, enzymes of B-oxidation. Cardiolipin and electron transporters embracing cytochrome C and ubiquinol Murphy. **2004**; Shen et al; **2004**; Korshunov et al, **1997**.

I.3.3. The intermembrane space

The composition of this chamber is close to that of the cytosol. I is particularly rich in protons. It also contains key components involved in apoptosis which are caspases 2, 3 and 9, AIF (Apoptosis Inducing Factor) and cytochrome C. These molecules are released under the action of pro-apoptotic factors Hansford et al;**1997**.

I.3.4. The mitochondrial matrix

This slightly dense inner chamber in electron microscopy contains a highly concentrated mixture of many enzymes: involved in the replication, transcription and translation of DNA, as well as those needed for the various matrix metabolic pathways. It also contains granules of 50 nm diameter, cation accumulators (Ca^{++}, Mg^{++}), mitoribosomes, RNA, and mitochondrial DNA molecules Coogh et al; **1990**.

I.3.5. The mitochondrial genome

Due to their endosymbiotic origin, mitochondria have a distinct genome, distinct from nuclear genetic material. The genetic memory of a mitochondria, mitochondrial DNA (mit DNA), which accounts for less than 1% of the total cellular genome, is a 16569 bp small double-stranded circular molecule in humans. Each mitochondrion has two to ten molecules of mitochondrial DNA and each human cell contains from 103 to 104 molecules Singal et al; **1998**; Lambert et al; **2004**. DNA codes for 13 essential proteins between 83 involved in the respiratory chain (7 subunits of complex I, one of complex III, three of complex IV and two of complex V), 2 ribosomal RNA and 22 transfer RNA required for the synthesis of mitochondrial proteins Herrero et al; **2000**; Junemann et al;. **1998**. The mitochondrial genome is free of introns and flanking structures except for a loop of about 1000 bp. It is also characterized by its exclusively maternal origin and the absence of histones Turmpower. **1990**.

The absence of a DNA repair system and exposure to free radicals generated by oxidative phosphorylation expose the DNA to an increased mutability 10 to 20 times greater than that of nuclear DNA, leading to heteroplasmy Starkov et al; **2000**; Starkov et al; **2001**.

I.4. Structure and function of the mitochondrial respiratory chain

The four complexes that constitute the mitochondrial chain of electron transfer are the result of the association of about fifty polypeptides. Each complex is independent and contains prosthetic groups involved in a series of oxidation-reduction Papa et al; **1997b.**

I.4.1. Complex I: NADH ubiquinone oxidoreductase

It consists of 42 or 43 polypeptide subunits for an overall mass of 750 KDa. It has 7 redox couples: one FMN and 6 Fe / S centers, the initial transfer of electrons requires the NADH cofactor. The latter is oxidized on the matrix face of the membrane by NADH dehydrogenase. Complex I catalyzes the transfer of two electrons from NADH to ubiquinone, coupled with the expulsion of protons from the matrix to the intermembrane space, the electron transfer takes place through the FMN (Flavin Mono Nucleotide) and Fe / S centers. Complex I can be inhibited by rotenone Vidal-Puig et al; **2000**; Neger- Salvayre et al; **1997**.

I.4.2. Complex II: Ubiquinone oxidoreductase succinate

It is fully encoded by nuclear DNA. It participates in the Krebs cycle by catalyzing the re-oxidation of succinate to fumarate, which allows the transfer of two electrons to complex III through the oxidation of FADH2 and a pool of ubiquinone. This electron transfer is not coupled to the efflux of protons. The use of malonate inhibits the activity of this complex Arsenijevic et al; **2000**.

I.4.3. Complex III: Ubiquinol Cytochrome C Reductase

Ubiquinones are electron free carriers of complex I and II to complex III. The latter is a dimer (550 KDa), containing 10 polypeptides, among which cytochromes b, c1 and Fe-S-centered proteins. Complex III allows the transfer of electrons from ubiquinol to cytochrome C, a mobile transporter located in the intermembrane space. This transfer is associated with an efflux of protons. Antimycin A inhibits complex III Pre J. **1991**; Wilson et al;**1997**.

I.4.4. Complex IV: Cytochrome C oxidase

It is a dimer (320 KDa), containing 9 polypeptides, among which the 2 cytochromes a1 and a3, as well as copper proteins. Class A cytochromes are the only ones that are auto-oxidizable, ie capable of spontaneously oxidizing upon contact with O_2. This complex catalyzes the last redox reaction that reduces O_2 to H_2O by 4 electrons. This electron transfer is coupled to an efflux of protons towards the intermembrane space. Cyanide is an inhibitor of cytochrome c oxidase Bagchi et al; **1994**; Echtay et al; **2003**.

I.4.5. ATP synthase: FOF1-ATP synthase

ATP synthase couples the broadcast easy facilitated protons to the synthesis of ATP from ADP and Pi and thus makes it possible to transform the electrochemical potential difference generated by the complexes I, III and IV into chemical energy (FIG. 1) Bertrand. **2003**; Friguet et al; **1997**.

This complex is composed of two supercomplexes, the super hydrophobic transmembrane Fo complex allowing access of the protons to the second hydrophilic supercomplex F1, located at the matrix face of the inner membrane, F1 alone can only catalyze the hydrolysis of ATP. Hence its lack of ATPase, and it is the association of the two supercomplexes that allows the synthesis of ATP Smolka et al; **2000**; Cadet et al; **2002**.

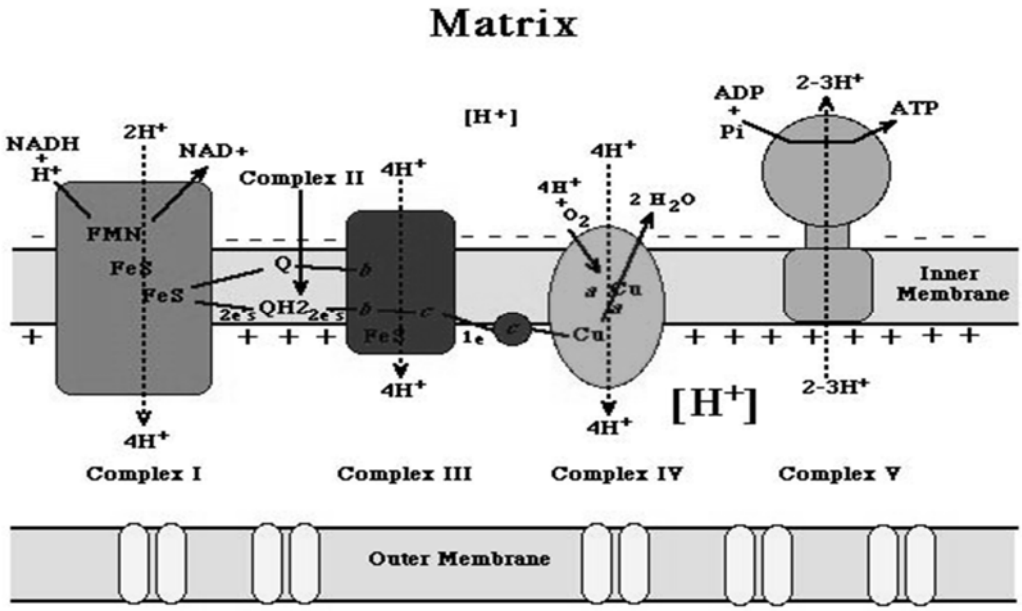


Figure 1: General structure and function of the mitochondrial respiratory chain Liu et al; **2002**; Cadet et al; **1999**.

I.5. The main functions of mitochondria

Mitochondria are the main source of energy produced in the form of ATP at the level of the respiratory chain; they are also involved in several functions: synthesis of steroid hormones, catabolism of fatty acids by B-oxidation, regulation of intra cytoplasmic Ca^{2+} participation in programmed cell death and ROS production Bohr et al; **2002b**.

I.5.1. ATP synthesis

The energy contained in the molecular bonds of metabolites from ingested foods is converted to ATP by mitochondrial oxidative phosphorylation. Mitochondria use several substrates, pyruvate, mainly derived from glycolysis of carbohydrates, is the major mitochondrial substrate. In the mitochondrial matrix, pyruvate is converted to acetyl CoA by the action of pyruvate dehydregenase.

During the tricarboxylic ring, the oxidation of the latter (Acetyl COA) produces NADH substrate molecules of complex I and FADH2, which is produced at complex II during the oxidation of succinate to fumarate. These two complexes transfer the electrons resulting from the oxidation of the substrates to the ubiquinone pool. The ubiquinol-reduced ubiquinone transports electrons to complex III which transfers them to cytochrome c. Then, at the IV complex, the electrons from cytochrome c oxidation are used to reduce molecular oxygen (O_2). This electron transfer pathway is also called the cytochrome pathway. These electron transfers are accompanied at the level of the complexes I, III and IV by an expulsion of protons in the intermembrane space. An electrochemical gradient of about 150 mV is thus generated and therefore represents a force pushing the protons back into the mitochondrial matrix. When these protons return by ATP synthase, the energy then released allows the synthesis of ATP from ADP and Pi Bohr, **2002a**, Comhair et al; **2002**.

I.5.2. Mitochondria and apoptosis

Mitochondria play an integral role in programmed cell death pathways: in the majority of cases, it is a necessary step, but sometimes only a place of potentialization of apoptosis. Mitochondria have a wide range of proteins involved in the regulation of this process Scandalias. **1993**, Gardner et al; **1995**; Meloo et al; **1999**; Ho et al;. **2004**. Indeed, the effector phase of apoptosis involves the opening of permeability transition pore "PTP" of mitochondria, and the release of apoptogenic molecules such as cytochrome c and AIF (Anti-

apoptotic Inducing Factor), capable of triggering apoptosis via caspase activation. This release phase is under the control of members of the B-cl2 family Antunes F et al; **2002**.

I.5.3. Mitochondria source of ROS

The mitochondrial respiratory chain (MRC) is the main source of ROS production within the cell Wathley et al; **1996**; Hauptmann et al; **1996**. Under the conditions of optimal functioning between 5 to 15% of the electrons fall of the respiratory chain prematurely. They are directly captured by oxygen molecules that are transformed not into water but into toxic superoxide radicals. These ROS are produced at the complexes of the respiratory chain mainly complex I and III Kelner et al; **2000**; Aurousseau et al; **2004**. This production occurs during the operation of the mitochondrial respiratory chain, a production that can be compared to the inevitable waste from industrial power plants. Thus, 1 to 4% of the oxygen consumed under basal conditions is assigned to the production of the superoxide radical. Although this production of superoxide radicals is usually low and concerns only a small percentage of the oxygen used, it can increase when breathing becomes intense or when inflammatory or nutritional disorders occur Achon et al; **2000**.

I.5.4. Mitochondria and calcium homeostasis

Intracellular Ca^{2+} is regulated by several processes. Its cytosolic concentration is adjusted by binding to non-membrane proteins, the endoplasmic reticulum and mainly mitochondria. Mitochondria are capable of taking large amounts of cytosolic Ca^{2+}. As a result, they act as safety devices against potentially toxic increases in cytosolic Ca^{2+}. It captures and releases Ca^{++} through different modes of transport. Thus the Ca^{2+} enters through the Ca^{2+} uniportors, and it is released essentially by an antiport transport (Na^{+} / Ca^{++}) / (Na^{+} / H^{+}) at the internal mitochondrial membrane Martin. **2003**. The matrix concentration of free Ca^{++} varies between 0.2 and 10 µM, a concentration necessary for the proper functioning of mitochondrial activities such as the regulation of the activity of key enzymes of energy metabolism (pyruvate dehydrogenase, isocitrate dehydrogenesis,...), the regulation of protein synthesis and that of nucleic acids. Variations in this concentration lead to mitochondrial dysfunction such as PTP openning and induction of apoptosis Liu, et al; **1995**; Pré. J. **1992**.

II. Oxidative stress

Oxygen, an essential element of life, could under certain conditions become a danger for the organism via the formation of reactive oxygen species highly reactive. Thus the normal

cellular metabolism of this element continuously produces small amounts of these reactive oxygen derivatives which are particularly toxic for cellular integrity Krinsky. **1992**; Drog. **2002**.

II.1. Definition of oxidative stress

To define the oxidative stress, it is necessary above all to define, what the free radicals are. A free radical is a chemical species containing a single electron, unpaired at its peripheral electronic orbit. This state confers on the molecule a great instability and reactivity towards other molecules. This imbalance is only transitory and is filled either by the acceptance of another electron or by the transfer of the free electron to another molecule. The probability of these two possibilities depends essentially on the instability of the free radical Pelletier et al; **2001**; Olson et al; **2001**. Free oxygen radicals (ROL) are also called "reactive oxygen species" (ROS) or, for the Anglo-Saxons, "reactive oxygen species" (ROS), "reactive oxygen intermediates" (ROI) or "reactive oxygen metabolites "(ROM) Hengarther,**2000;** Hengartner. **2000**. The term "free oxygen transport" is not restrictive. It includes the free radicals of oxygen itself, but also certain non-free radical reactive oxygen species whose toxicity is important Kroemer et al; **2000**.

Under normal conditions, there is a balance between the production and the removal of the ROS.The oxidative stress is classically defined as an imbalance in favor of the ROS. This imbalance results either from an increased production of free radicals compared with intact antioxidant capacities, or from a deficient state of the latter, or from these two cumulative situations Starkov et al; **2000**; Vaux et al; **1999**.

II.2. The reactive species of oxygen

Among all the radical species that may form in the cells, it is necessary to distinguish a restricted set of radical compounds which play a particular role in physiology and which we will call primary radicals. The other free radicals, called secondary radicals, are formed by the reaction of these primary radicals on the biochemical compounds of the cell, these primary radicals are derived from oxygen by one-electron reduction such as the superoxide anion (O^{2-}°) and the hydroxyl radical (°OH), or nitrogen such as nitric oxide (NO°) Murphy. **2004**. Other species derived from oxygen called active oxygen species such as singlet oxygen 10, hydrogen peroxide (H_2O_2) or nitroperoxide (ONOOH°) are not free radicals but are also reactive and can be precursors of radicals. All free radicals and their precursors are often

called reactive species of oxygen, whose reactivity is very variable depending on the nature of the radical (Table 1) Green et al;**1998**.

A first origin of the radical phenomena is the initial formation of the superoxide anion, the most common free oxygen radical. This superoxide anion can,then, disproportionately spay, or enzymatically be catalyzed to give oxygenated water (H_2O_2), which can in turn turn into hydroxyl radical (°OH), the most reactive ROS. The latter is formed by degradation of hydrogen peroxide in the presence of transition metals in their reduced form. Thus hydrogen peroxide associated with ferrous iron leads to the fenton reaction:

$$H_2O_2 + Fe^{2+} \longrightarrow {}^{\circ}OH + {}^{-}OH + Fe^{3+}$$

The hydrogen peroxide can also react with the superoxide radical resulting, again in the production of the hydroxyl radical. This reaction mechanism is called the Habber-Weiss reaction.

$$H_2O_2 + O^{-\circ}{}_2 \longrightarrow {}^{\circ}OH + {}^{-}OH +$$

Nitric oxide reacts with a superoxide radical ($O_2^{-\circ}$) to form peroxynitrite (ONOO°), a powerful oxidant. The alkoxyl (RO°) and peroxyl (ROO°) radicals can be generated by the action of $O^{-}{}_2$, OH and singlet oxygen on the unsaturated fatty acids Kluch et al; **1997**.

Table 1. Main ROS and comparison of oxidative powers Wallace. **1995**.

Superoxide anion	$O^{-\circ}{}_2$
Hydroxyle radical	°OH
Hydroperoxide radical	HOO°
Peroxile radical	ROO°
Alkoxyle radical	RO°
hydrogene peroxide	H_2O_2
Nitric oxide radical	NO°
Peroxynitrite	ONOO°
Hypochlorite	ClO
Oxidant power :	°OH > RO°> HOO°> ROO°> NO°

II.3. Sources and formation of reactive oxygen derivatives

Our cells are continually attacked by free radicals, essentially reactive oxygen species, whose formation is linked to both exogenous and endogenous factors.

II.3.1. Exogenous sources of ROS

The environments in which we live but also our way of life are at the origin of an increase in the production of the ROS in our organization Mivabella et al;**2000**.

II.3.1.1. Medications and their metabolism

Drugs, foreign substances, are capable of inducing a state of severe oxidative stress. At hepatic levels, drugs are subject to detoxification reactions that rapidly diminish their reactivity. Two types of biochemical reactions are used: the first phase of detoxification is provided by the enzymes of the P450 cytochrome oxidase family and involves, in particular, oxidation reactions, the second phase consists of the conjugation of the oxidized substrates with molecules such as gluconic acid or sulfate groups Vaux et l; **1999**. Drug metabolism by cytochrome P450 may be harmful, producing reactive metabolites that attack lipids, proteins, and DNA and may induce cancer events, cytotoxicity, and necrotic and / or apoptotic Kluch et al; **1997**.

- ***Doxorubicin***

Doxorubicin is an antitumour drug that is widely used in the treatment of a broad spectrum of cancers Brossy-Wetzel et al; **1998**. This drug belongs to the family of anthracyclines whose chemistry leads to the generation of free radicals.

Several enzymes including cytochrome P450 reductase, NADH dehydrogenase associated with mitochondrial complex I, NO synhtase catalyzes the metabolism of doxorubicin Alnemri et al; **1996**; Gupta et al; **2003**.

The quinone structure of doxorubicin allows them to behave as an acceptor of electrons in the presence of redox enzymes such as cytochrome P450. Indeed, the addition of a free electron transforms quinone into a semi-quinonic free radical with a release of superoxide anions, which would then have deleterious effects directly on DNA and macromolecules. In the presence of oxygen, superoxide radicals, hydroxyl radicals and hydrogen peroxide are formed which will also be toxic, leading to a state of oxidative stress Thorberry. **1998**. The

metabolism of doxorubicin is shown in Figure 2. *In vitro* and *in vivo* studies have shown that doxorubicin stimulates disturbances in cellular and mitochondrial Ca^{2+} homeostasis. Doxorubicin is able to induce both the mitochondrial pathway of apoptosis and the extrinsic pathway Thorberry. **1998**; Fesik et al; **2001**.

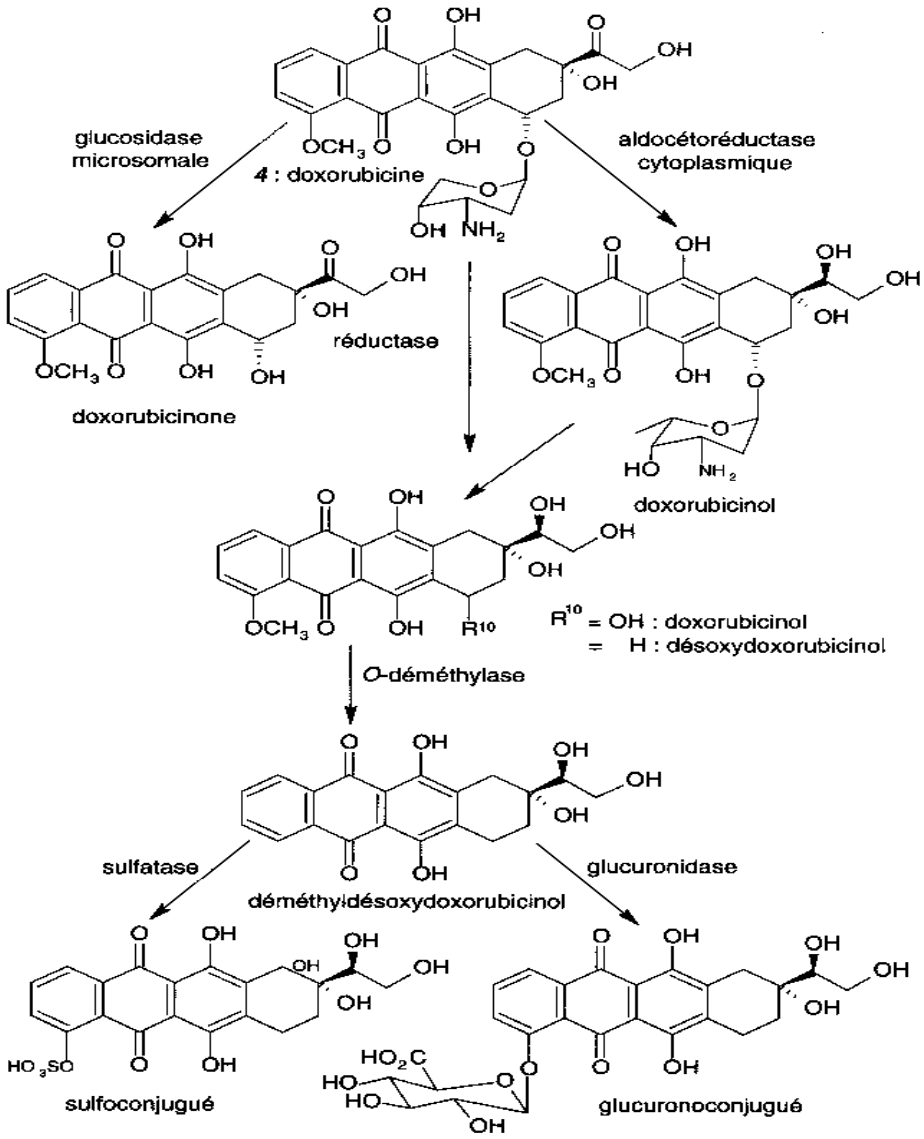


Figure 2. Liver metabolism of doxorubicin Lofler et al; **1996**.

II.3.1.2. Other exogenous sources

Electromagnetic radiation, some transition metals, combustion fumes (from cigarettes, wood, building materials), chemical products (antiseptics, drugs, pesticides, toxic products such as CCl_4 and solvents, asbestos dust and silica) are all factors contributing to the genesis of free radicals. Inhaled particles of silica and asbestos are sources of free radicals because they exacerbate phagocytosis, and because their surface is lined with iron salt. The radiation is

capable of producing free radicals, either by splitting the water molecule when it comes to X, γ ionizing rays, or by activating photosensitizing molecules when it comes to the ultraviolet rays that go through it. Mechanisms that produce superoxide anions and singlet oxygen. It should also be noted that intellectual or thermal stress, infectious agents and poorly managed physical exercise can generate oxidative stress Nakagawa et al; **2000**; Krajewski et al; **1993;** Gross et al; **1999**.

II.3.2. Endogenous sources of ROS

At the cellular level, there are several potential sources of free radicals, either at the level of mitochondria, membranes, endoplasmic reticulum or just the cytosol. In general, any biochemical reaction involving molecular oxygen is likely to be at the origin of a production of oxygen free radicals. The cellular sources of ROS are enzymatic and not enzymatic Muchmore et al; **1996**; Schendel et al; **1998**.

II.3.2.1. Xanthine oxidase

It is a soluble enzyme that generates ROS by reducing hypoxanthine to xanthine and xanthine to uric acid. This enzyme is present in the blood, the endothelial cells of the capillaries and very importantly in the liver and intestines. Under basal conditions, though the production of ROS by xanthine oxidase is low, it plays an important role in ischemia - reperfusion Antonsson et al; **2000**.

II.3.2.2. NADPH oxidase

It is an oxidase bound to the plasma membrane. It was initially described in phagocytic cells where it plays a fundamental role in the immune response in the fight against microorganisms. It is specialized in the production of the superoxide radical according to the following reaction.

$$2O_2 + NADPH \Longrightarrow 2O^{-\circ}{}_2 + NADP^+ + H^+$$

This normally dormant enzyme is activated when the phagocytic cell is stimulated. The resulting high consumption of oxygen is called "respiratory burst" in the international literature. This production of superoxide is at the origin of the synthesis of molecules such as H_2O_2 and hypochlorite (ClO^-), essential for destruction of the phagocyte material. This

production pathway is particularly stimulated during infectious processes Shimazu et al; **1998;** Vander. **1999**; Pollack et al; **2001**.

II.3.2.3. Nitric Oxide (NO)synthase

Many of the cells are capable of producing nitric oxide (NO) from argenine and oxygen in a NO synthase catalyzed reaction (NOS), as the following reaction may demonstrate:

$$\text{L-argenine} + O_2 + \text{NADPH} + H^+ \xrightarrow{\text{OUR}} \text{L- citruline} + \text{NO}^\circ + \text{NADP}^+$$

Indeed, studies carried out using purified enzymes have shown that NOS is capable of generating superoxide anions in situations of deficiency of its substrate, L- arginine, or of these activation cofactors Corbiere. **2003**; Ravagnan et al; **2002**; Yang et al; **1997**.

II.3.2.4. Metallic ions

Metallic ions such as iron and copper are remarkable promising radical processes: they transform hydrogen peroxide (H_2O_2) into hydroxyl radicals even more toxic, according to the fenton reaction described above, thus, accelerate lipid peroxidation. Under physiological conditions, the free concentration of iron or copper is particularly low, these metals being sequestered by specialized proteins so that this reaction does not take place. On the other hand, cellular destruction (hemolysis, hepatic cytolysis) leads to a release of these metals that can lead to oxidative stress Borner, **2003**; Xiaodong. **2001**; Lenaz.**1998**.

II.3.2.5. Peroxisomes

The peroxisome is an important source in the cellular production of ROS, because this organelle contains many enzymes generating hydrogen peroxide. All times, the latter is used as substrate by the peroxisomal catalase to carry out the detoxification processes present in the liver, and the kidney. It seems, however, that only a small amount of H_2O_2 could escape catalase Wei et al; **2002**.

II.3.2.6. Mitochondria

The predominant endogenous source of RL is, however, linked to mitochondrial activity. This organelle is considered as the main source of ROS in the cell by the functioning of the

mitochondrial respiratory chain, essentially through the leakage of electrons during their transfer to the mitochondrial respiratory chain (Figure 3) Morin, Thierry, Spedding, Tillement. **2001**; Karmasyn. **1991**.

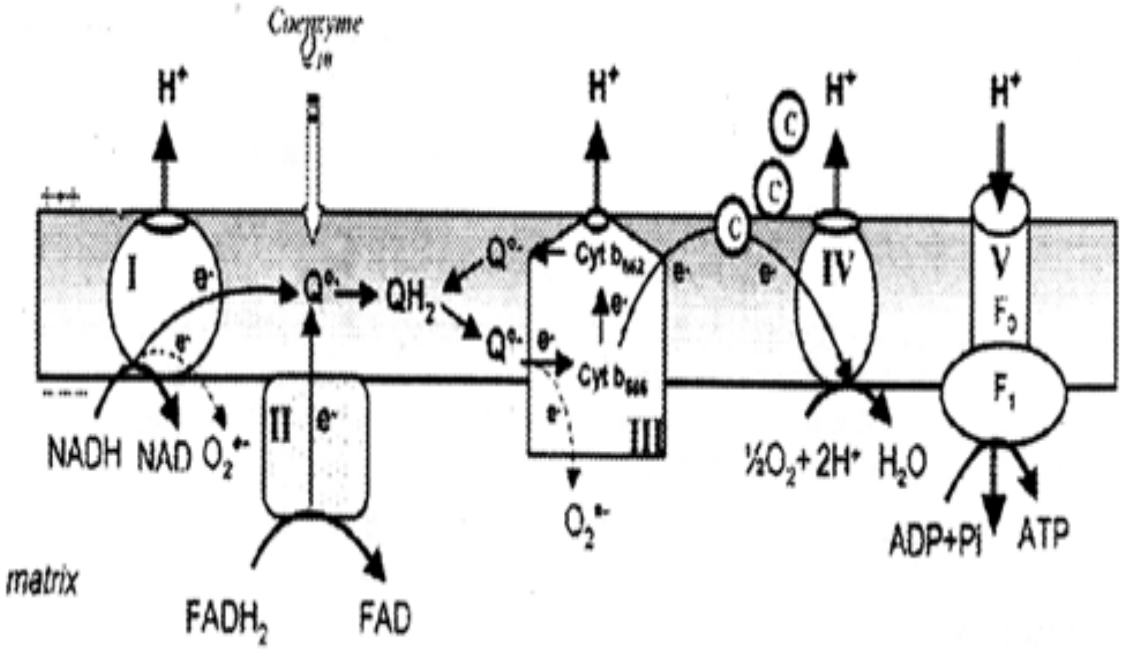


Figure 3. ROS production at the mitochondrial respiratory chain Starkov et al; **2001**.

II.4. Involvement of mitochondria in oxidative stress

In addition to ATP synthesis, the mitochondrial respiratory chain is a powerful source of ROS and is, therefore, involved in the genesis of most human and animal pathologies. It is estimated that under normal conditions, 1-4% of oxygen consumed is incompletely reduced to superoxide. In the mitochondria, there are nine sites responsible for this production. They are ubiquitous in mammalian mitochondria, but the expression and the production capacity differ according to the tissues. These sites have been shown, however, as ROS producers in appreciable quantity. The evolution of the concepts has confirmed the importance of complexes I and III in the production of ROSCadet et al; **2000;** Mytitinedu et al; **1994;** Reichmann et al;**1993**.

II.4.1. Cytochrome b5 reductase

Located in the outer membrane of the mitochondria, this protein is found in large quantities in mammals. It oxidizes cytoplasmic NADPH and reduces cytochrome b5 in the outer membrane, further, it participates in the regeneration of ascorbate by reducing the free radical

ascorbyl. The mitochondrial cychrome b5 reductase produces the superoxide anion with a very high rate that exceeds 300 nmol / min / mg protein Chadge et al; **1997**; Lee et al; **1999**.

II.4.2. Monoamine oxidase (MAO)

Also located at the level of the external limb, this enzyme catalyzes the oxidation of biogenic amines which is accompanied by the release of hydrogen peroxide (H_2O_2).

The monoamines oxidase of cerebral mitochondria plays a central role in the renewal of neurotransmitters. The oxidation of tyramine by the cerebral mitochondria of the rat produces H_2O_2 at a rate 50 times higher than that of the III complex inhibited by Antimycin. MAOs are the main source of H_2O_2 in tissues during ischemia Gu et al; **1996**; Wallace. **1995**; Mivabella et al; **2000**.

II.4.3. Dihydro-orotate dehydrogenase (DHOH)

This enzyme is located on the outer surface of the inner membrane. It catalyzes the conversion of dihydroorotate, a step in the synthesis of pyridine nucleotides. In the absence of its natural electron acceptor (coenzyme Q), reduced DHOH can produce H_2O_2*in vitro*. The production of the superoxide anion by DHOH is also suggested Cheng et al; **2000**;Ligeret et al; **2004**.

II.4.4. Alpha-glycerophosphate dehydrogenase

Also located on the outer surface of the inner membrane, it exists in large quantities in the brown adipose tissue, muscles and brain where it has a great activity.This FAD-enzyme, catalyzes the oxidation of glycerol-3-phosphate into dihydroxy-acetone phosphate and uses coenzyme Q as electron acceptor.This reaction is accompanied by the production of hydrogen peroxide Chin et al; **2003**; Morrissay et al; **2003**.

II.4.5. succinate dehydrogenase (SDH) or complex II

It is a flavoprotein located on the inner side of the inner mitochondrial membrane. It oxidizes succinate to fumarate. By using submitochondrial oxidizing succinate particles, the production of ROS can be inhibited by carboxin, an inhibitor of succinate dehydrogenase, but it is clear whether this intact respiratory chain complex is capable of generating ROS Nestel . **2003**; Raj Narayana et al; **2001**.

II.4.6. Aconitase

It is an enzyme of the mitochondrial matrix which participates in the tricarboxylic cycle catalyzing the conversion of citrate to isocitrateThis enzyme is inactivated by the superoxide anion which oxidizes its Fe-S center. Its activation leads to the production of the hydroxyl radical. Such a production is mediated by Fe^{2+} ions Bruneton . **1993**; Bruneton . **1999**.

II.4.7. Alpha-ketoglutarate complex

It is a matrix enzyme tightly bound to the inner mitochondrial membrane. It catalyzes the oxidation of alpha cetoglutrate to succinyl-COA. Two recent studies on isolated purified enzymes, cardiac mitochondria in cattle, and cerebral mitochondria in rats have shown that this enzyme complex is capable of generating superoxide anion and hydrogen peroxide Gerhard. **1993**; Marfak. **2003.**

II.4.8. Production of ROS by complex I

Complex I is one of two major inputs to the mitochondrial respiratory chain for the reduction of reduced equivalents derived from the Krebs cycle. Several studies have been done to explore the mechanism of ROS production at this complex. Unfortunately, these studies give no idea about the exact site of this production. Some have suggested that the latter is located between the flavin moiety and the rotenone binding site while others have suggested that it is the flavin moiety itself Nestel. **2003**; Raj Narayana et al; **2001**; Bruneton. **1993**; Bruneton. **1999**; Gerhard. **1993**, -------, Deprey et al; **2000**.

Overall data in this topic support the view that ROS are most likely produced by one of the Fe-S centers and that (N-1a) Fe-S is the potent candidate. According to these complex studies I produce superoxide anion from NADH when inhibited by rotenone, by an independent mechanism of membrane potential Corbiere. **2003** and, from ubiquinol by a potential dependent mechanism called "reverse transfer". "TRE" electrons Harada et al; **1999**; Muchmore et al;**1996**. This inverse electron flow is a series of reactions taking place at the level of the respiratory chain leading to the transfer of electrons against the gradient of the redox potential of the electron carriers, from ubiquinol to NAD^+ and not oxygen Schendel et al; **1998**; Antonsson et al; **2000**. If the succinate is used as a substrate in the absence of rotenone, the production of superoxide is very important, which has revealed the importance of the inverse electron flow of complex II to complex I. This reverse flow is due to the fact

that the succinate-derived electrons can go back to complex I and reduce NAD^+ to NADH releasing electrons leading to the formation of superoxide Shimazu et al; **1998**.

This production is regulated by the amplitude of the membrane potential.Thus a decrease of 10% of this potential causes an inhibition of the production of ROS by 10%. It is also inhibited by the acidification of the medium, by any energy-using process such as ATP synthesis, Ca^{++} transport and decouplers Vander. **1999**; Pollack et al; **2001**; Corbiere. **2003**. TRE is also inhibited by rotenone (Figure 4) Ravagnan et al;**2002.** Ravagnan et al; **2002**.

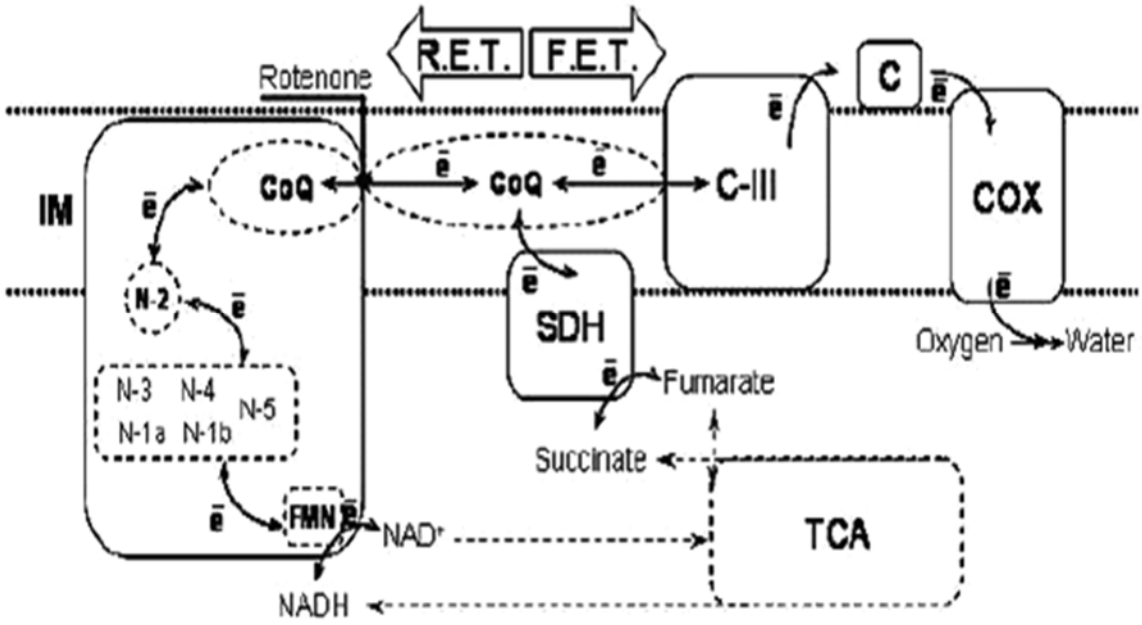


Figure 4: ROS Production by complex III and Q cycle. Krinsky.**1992;** Drog**. 2002.**

II.4.9. ROS production by complex III and Q cycle

The production of ROS by Complex III represents a remarkable example of deductive research in the biochemical sciences. It is capable of robust production of $O_2^{-\circ}$. This production is the result of the leakage of the electrons at the level of the cycle Q. In fact these electrons can react directly with the dissolved oxygen and form the free radical superoxide anions (figure. 5).

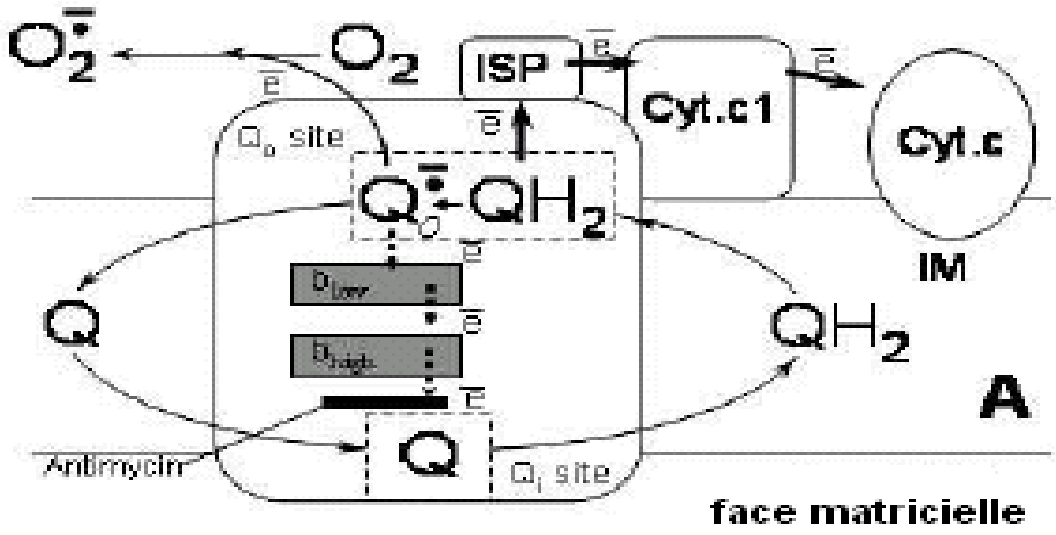


Figure 5: Mechanism of superoxide production at complex III of the mitochondrial respiratory chain. Pelletier et al; **2001**.

To consider this cycle Q, it is necessary to follow the path followed by 2 molecules of ubiquinol (QH2) penetrating simultaneously in the cycle (Figure 6). The two QH2 molecules each deliver an electron to cytochrome C1 (via Fe-S) which results in the formation of an ubisemiquinone anion.

(Q- °) step (i). The protons released during this reaction pass into the itermembaranaire space. Q - is then oxidized to ubiquinone by cytochrome b566 - step (ii). A molecule of ubiquinone (Q) produced by this pathway is then reduced in ubisemiquinone by an electron of cytochrome b562 (which itself was reduced by cytochrome b566 - step (iii).) A second electron of b562 then reduces Q - ° in QH2 The other molecule of Q formed in step (ii) is reduced in QH2 by complex I or II or by another flavoprotein ubiquinone -reductase and the cycle can then continue (Figure 6) Lenaz.**1998**, Wei et al; **2002**.

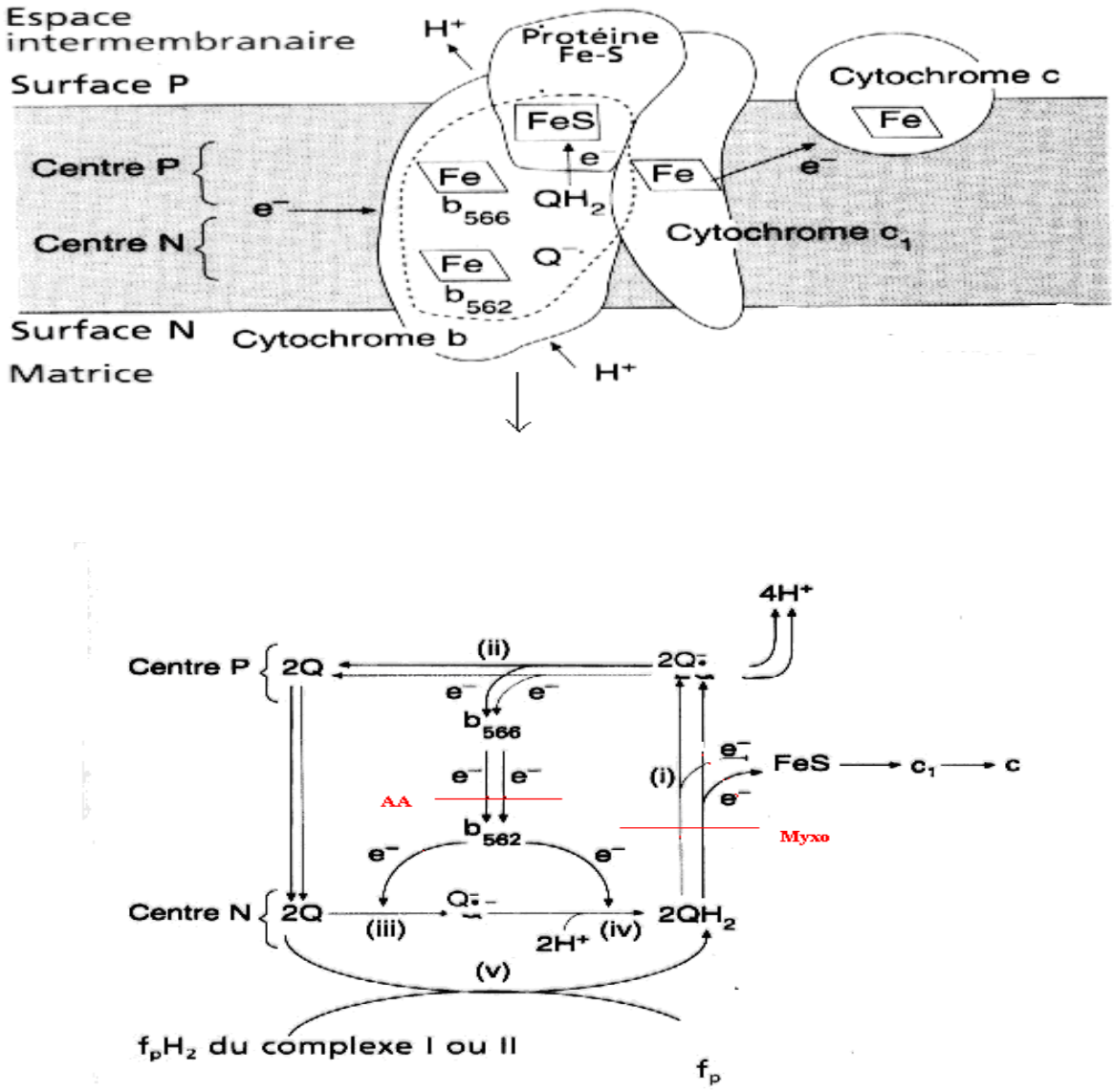


Figure 6: Cycle Q Mechanism of action and inhibitors of complex III (adapted from Peter et al; **2002.** AA: Antimycin A, Myxo: Myxothiazol.

The effects of complex III specific inhibitors played the most important role in deriving the site and source of ROS production at this complex. Myxothiazol (Myxo) prevents the flow of electrons from ubiquinol to the Fe-S center, thereby inhibiting the formation of ubisemiquinone. Antimycin A (AA) disrupts the transfer of electrons from cytochrome b562 to ubisemiquinone in the N-center, thereby causing the accumulation of the unstable radical form that transfers an electron to molecular oxygen to form the anion superoxide Morin et al; **2001**; Karmasyn. **1991**; Cadet et al;. **2000**; Mytitinedu et al; **1994**; Reichmann et al; **1993**; Chadge et al; **1997**. Myxothiazol prevents the effect of AA thereby inhibiting superoxide production in mammalian mitochondria Karmasyn.**1991**; Cadet et al; **2000**. In the absence of AA, the production of superoxide by the complex III is minimal Chin et al; **2003** and it is

therefore probable that *in vivo*, the complex I is the major source of ROS during the normal transfer of the electrons and especially through the transfer resumes.

Several authors have shown that complex I produces superoxide towards the matrix face of the inner membrane while complex III generates it towards both sides; matrix and cytosolic Lee et al; **1999**; Gu et al; **1996**.

II.5. Modulation of mitochondrial production of ROS:

It is important to note that mitochondrial production varies according to the tissues studied. Mitochondria in post-mitotic tissues have the highest ROS production Mytitinedu et al;**1994**. Several authors have shown that the mitochondrial production of ROS is only detectable after the use of specific electron transfer inhibitors: rotenone for NADH-linked substrates and antimycin A for FADH2-related substrates. This production is also regulated by constituents of the internal mitochondrial membrane known as decoupling proteins (uncoupling protein, UCP) which allow the dissipation of energy related to the electrochemical potential Reichmann et al; **1993**; Chadge et al; **1997**. In overexpressing macrophages UCP2, the production of $H_2O_2$is reduced. In addition, macrophages of mice whose UCP2 gene has been invalidated produce more ROS than wild-type mice. The rate of production of ROS is also a function of the flow of electrons in the respiratory chain Wallace. **1995**; Mivabella et al; **2000**.

II.6. The oxidative damage of ROS

When the defenses are overwhelmed, the prooxidant / antioxidant equilibrium is disturbed, the excess ROS will, therefore, damage the biological macromolecules (lipids, proteins, carbohydrates and nucleic acids) thus causing irreversible modifications, but also secondary lesions due to cytotoxic and mutagenic character of metabolites released especially during the oxidation of lipids. The body can react against these compounds by producing antibodies, which can unfortunately also be auto antibodies creating a third wave of chemical attack Cheng et al; **2000**;Ligeret et al; **2004**.

II.6.1. Lipid peroxidation

Lipids and mainly their polyunsaturated fatty acids are the preferred target for attack by free radicals and in particular the hydroxyl radical Chin et al; **2003**.

II.6.1.1. Mechanism of lipid peroxidation and products formed

Mammalian membranes are rich in polyinstered fatty acids and are, therefore, very sensitive to oxidative stress. Whatever the triggering event, the sequence by which lipoperoxidation occurs can be summed up in three distinct steps: the initiation phase, the propagation phase and the termination phase (Figure 7) Morrissay et al; **2003**; Nestel; **2003**.

Lipid peroxidation is a chain reaction, initiated by the attack of a radical R such as the hydroxyl radical on an unsaturated fatty acid (LH) by abstraction of a hydrogen atom. In an aerobic environment, oxygen adds to the lipid radical to give the peroxyl radical (LOO°). Once initiated, the peroxyl radical can propagate the peroxidative chain reaction by abstraction of a hydrogen atom from another unsaturated fatty acid. The hydroperoxides formed (LOOH) can undergo several modes of evolution: be reduced and neutralized by glutathione peroxidase or continue to oxidize and fragment into acid aldehydes and alkanes (ethane, ethylene, pentane ...) which from their volatility is eliminated by the pulmonary route. The cleavage of the LOOH molecule can release various toxic aldehydes including malondialdehyde MDA and 4-hydroxynonenal (HNE). These latter have been extensively studied and are widely used as markers of lipid peroxidation Raj Narayana et al; **2001**; Bruneton. **1993**.

Ultimately, the propagation system must be stopped. This termination step is attained when two free radicals meet and covalently share their electrons or when antioxidant systems come into play. To this end, the strategy commonly used by the cell is to form a electronically stable compound. This new electronic structure has a low reactive power, which must be regenerated in its antioxidant form or eliminated by the body. This is particularly the case for tocopherol, ascorbate and the glutathione system Beecher. **2003**; Bruneton. **1999**.

- **Chain reactions of lipid peroxidation :**

Initiation

R

↓

LH L °

Propagation (n times)

$$L^{\circ} + O_2 \longrightarrow LOO^{\circ} \text{ (peroxyl radical).}$$

$$LOO^{\circ} + LH \rightarrow LOOH + L^{\circ} \text{ (hydroperoxide)}$$

Termination

$$L^{\circ} + L^{\circ} \longrightarrow L\text{-}L$$

$$LOO^{\circ} + L^{\circ} \longrightarrow LOOL + O_2$$

Figure 7: Chain of lipid peroxidation. Aurousseau. **2002.**

R: free radical, (LH): unsaturated fatty acid, (LOO °): peroxyl radical, LOOH: hydroperoxide, L-L and LOOL: elctronically stable compound

II.6.1.2. Metabolism of MDA

In the liver, MDA can be converted into CO_2 and H_2O by successive metabolisations. MDA is converted to malonic semi-aldehyde acid by mitochondrial aldehyde dehydrogenase.The malonic semi-aldehyde decarboxylates spontaneously to acetaldehyde which is oxidized by acetaldehyde dehydrogenase to acetate which is then metabolized. Some of the MDA can be converted to malonate which is converted to COA malonyl and decarboxylated to acetyl COA. In the urine, different metabolites of MDA are found, derived from the degradation of conjugates (MDA-proteins, and MDA-deoxyguanosine). The metabolism of MDA is responsible for the appearance of methyl ethyl ketone in the urine. MDA is, therefore, rapidly metabolized, while disrupting the metabolism and excretion of other lipid metabolites Gerhard. **1993**; Marfak. **2003.** (pp 175,176).

II.6.1.3. Mitochondrial and cellular consequences of lipid peroxidation

Lipid peroxidation destabilizes the cell membranes and in particular the inner membrane of the mitochondria, at the level of which electron transport is organized, the destruction of the phospholipidic bilayer of the mitochondrial ridges increases the premature fall of these electrons. This process leads to overproduction of ROS. A vicious circle can thus be initiated. It will result in the destruction of the cell or, worse, the genesis of cells whose DNA is mutated. The membranes become rigid, lose their selective permeability, and under extreme

conditions, may lose their integrity. However, Brand's group recently observed that 4-HNE can directly activate mitochondrial decoupling by direct action on UCPs and could thus reduce the mitochondrial production of ROS. This mechanism could be a means of regulating the production of ROS by mitochondria during lipid peroxidation in order to limit mitochondrial damage Deprey et al; **2000**; Harada et al; **1999.** (pp 177.178).

II.6.2. Oxidation of proteins

Proteins are also targets for free radicals. All amino acids and especially those containing a sulfhydryl (SH) group are potential targets of ROS attack, particularly by the hydroxyl radical Pincemail. **1998**. (p 179).

II.6.2.1. Mechanism and products formed

The oxidative modifications of the proteins by the ROS cause the introduction of a carbonyl group in the protein. These oxidation reactions are classified into two categories: on the one hand, those which break the peptide bonds and, on the other hand, the modifications of the peptides by addition of products resulting from lipid peroxidation, such as 4-HNE. Such modifications generally lead to a loss of the catalytic or structural function of the affected proteins and become much more sensitive to the action of proteases and in particular of the proteasome. The oxidized proteins also become very hydrophobic, either by suppression of the ionizable amine group or by externalization of central hydrophobic zones. The two main biological markers of protein oxidation are the formation of protein canbonyls and nitrotyrosine groups Pincemail . **1995**; Kawaltoxski et al; **2001**.

II.6.2.2. Oxidized protein repair systems

With the notable exception of the oxidation of cysteines and methionines for which specific systems of reversion of oxidation have been demonstrated, the oxidation of other amino acids appears as an irreversible phenomenon Reichmann et al; **1993**. At the cellular level, the degradation of oxidized proteins is mainly provided by the proteasome. However, the most damaged proteins can become resistant to this degradation. Thus, it has been shown that 4-HNE-modified proteins not only become resistant to degradation by the proteasome, but can also act as a proteasome inhibitor for the degradation of other oxidized proteins Chadge et al; **1997**; Lee et al; **1999**.

The oxidized proteins will also be supported by specific proteins called stress proteins or heat shock proteins (HSP), which allow the cell, respond to stress quickly. Among these proteins, are members of the family of HSP70 and HSP72 Gu et al; **1996**.

II.6.3. DNA Oxidation: Trained Products and Repair Systems

Although DNA is the memory of all the biochemical composition of living beings, it is a molecule very sensitive to attack by radicals of oxygen. There are two types of DNA in the cell; nuclear DNA and mitochondrial DNA. The latter is the preferred target of oxidation by ROS. Thus, the level of oxidized bases would be 2 to 3 times higher in DNA mit compared with nuclear DNA Wallace. **1995**.

Five main classes of OH-mediated oxidative damage can be generated. Among them, oxidized bases, abasic sites, intra-catenary adducts, strand breaks and DNA-protein bypass Mivabella et al; **2000**; Matsriski et al; **1996**. These various damages are strongly implicated in the phenomena of cancerization and aging, so mutations of the mtDNA could be involved in cell death phenomena. The most widely used biological marker of DNA oxidation is the detection of modified bases Alan , Miller. **1996**; Clifford. **1999**.

Elimination of oxidative damage to DNA is an important mechanism to limit their mutagenic and cytotoxic effects. For this, many DNA repair systems are used by the cell. Oxidized bases are removed essentially by two mechanisms: Basic excision repair (BER) or nucleotide excision (NER, Excision Nucleotides) Hanazaki et al; **1994;** Mira et al; **2002**.

II.6.4. Oxidation of carbohydrates

Although the chemistry of the radical attack of carbohydrades has been much less studied than that of other macromolecules, the fact remains that ROS attack mucopolysaccharides and especially cartilage proteoglycans. The OH radical reacts with the carbohydrates by removing a proton from one of the carbon atoms thereby forming a centered carbon radical. Moreover, glucose can be oxidize under physiological conditions, in the presence of metal traces, releasing ketoaldehydes, H_2O_2 and OH°, which will lead to protein cuts or their glycation by attachment of ketoaldehyde (leads in diabetics to fragility of their vascular walls and their retina) Williams et al; **2004**.

II.7. Antioxidants

Free radicals occur spontaneously and in a continuous way within our organism, the types of these radicals produced as well as their places of production and propagation are varied, the rapid antiradical effect is fortunately polymorphous at the same time preventive and curative. An antioxidant can be defined as any substance that is capable, at relatively low concentrations, of competing with other oxidizable substances and thereby slowing or inhibiting their oxidation. This definition applies to a large number of substances comprising enzymes with specific catalytic properties but also small lipo- or water-soluble molecules. Cellular antioxidants are therefore enzymatic and not enzymatic Cheng et al; **2000**; Ligeret et al; **2004**.

II.7.1. ROS defense systems

The main antioxidant enzymes are superoxide dismutase, glutathione peroxidase and reductase, as well as catalase. More recently, other enzymes with antioxidant properties have been revealed; thioredoxin peroxidases Chin et al; **2003**.

II.7.1.1. Superoxide dismutase (SOD)

As its name suggests, SOD is an enzyme that catalyzes the dismutation of the superoxide anion into hydrogen peroxide and oxygen. There are three isoforms of SOD: a cytosolic, nuclear and extracellular form, associated with copper and zinc ions, (Cu / Zn-SOD), a mitochondrial form associated with manganese (Mn-SOD) and another form associated with iron, (Fe-SOD), predominant in bacteria and plants Morrissay et al; **2003**; Nestel . **2003**.

The mitochondrial SOD, located exclusively in the matrix, allows the quasi-instantaneous disproportionation of the O_2^{-0} produced at the level of the chain of the electron carriers thus protecting this organelle against the attack of the ROS Raj Narayana et al; **2001**. Mice lacking the so-called "Mn-SOD knauckaut" enzyme survive only a few days after birth while those with only 50% activity of this enzyme are viable and fertile. Although Mn-SOD seems essential for life, this is not the case for the cytosolic form, even though it plays an important role in the elimination of ROS Bruneton **1993**; Beecher **2003**. The activity of this enzyme is unevenly distributed in the tissues. In the mouse, the activity in the liver and kidneys is highest, followed by that of the brain and heart, muscles and spleen, and eventually the lungs with the lowest activity; 20 times lower than in the liver Bruneton. **1999**.

II.7.1.2. Catalase

It is a heme enzyme that acts synergistically with SOD since its role is to accelerate the disproportionation of hydrogen peroxide into water and molecular oxygen. In tissues, catalase activity is highest in the liver followed by kidneys, lungs, heart and brain Chanyallon et al; **1994**. This enzyme is contained in peroxisomes, cytoplasm and mitochondria. In the heart, some heights think that it is present in the mitochondria only where it constitutes 0.025% of the total proteins. The contribution of this enzyme is insignificant compared to that of glutathione peroxidase, another enzyme that detoxifies hydrogen peroxide Schendel et al; **1998**.

II.7.1.3. Glutathione peroxidase and reductase (Gpx) and (GR)

Glutathione peroxidase (Gpx) is the most important H_2O_2 detoxifying enzyme in mammals. It is a selenium enzyme found in the cytosol, mitochondria, endoplasmic reticulum and nucleus. The role of this enzyme is to reduce,on the one hand,hydrogen peroxide in water molecule,and on the other hand,hydroperoxides organic (ROOH) in alcohol. During this reaction which requires the intervention of two molecules of glutathione (GSH), it is transformed into glutathione disulfide (GSSG) Cook et al; **1996**; Bankova. 2**005**; Bankova et al; **2002**.

$$H_2O + 2GSH \xrightarrow{\text{Gpx}} 2H_2O + GSSG$$

$$ROOH + 2GSH \xrightarrow{\text{GPx}} ROH + H_2O + GSSH$$

Glutathione reductase (GR), also localized in the cytosol and in the mitochondria, is responsible for regenerating GSH from GSSG. During this reaction, glutathione reductase uses a cofactor, NADPH Melina et al; **2000**; Harborne et al; **2000**.

$$GSSG + NADPH + H^+ \xrightarrow{\text{GR}} 2GSH + NADP^+$$

This reaction produces $NADP^+$ which will be regenerated into NADPH for later use by another enzyme, G6PD (glucose 6-P dehydrogenase):

$$NADP + G6PD \longrightarrow NADPH + H^+$$

At the level of the mitochondria, NADPH is renewed by an enzyme called $NADP^+$ dependent isocitrate dehydrogenase Kuresh et al; **2002**. The mitochondrial GPx is localized at the level of the matrix and in the intermembrane space. The enzymatic activity of this enzyme is highest in the liver, kidney, and heart, and lowers slightly in mitochondria of the brain and skeletal muscles Julies et al; **2002**.

II.7.1.4. Thioredoxin peroxidases (Prx)

Thioredoxin peroxidases (Prx) have recently been discovered and are the subjects of much work concerning their antioxidant functions Gerhard . **1993**. They are six in mammals and are located in the cytosol, mitochondria, peroxisomes, associated with the nucleus and membranes. These proteins exert their antioxidant role through a peroxidase activity, where H_2O_2, peroxynitrite and many hydroperoxides are the substrates. They play a very important role in detoxification due to their large quantity (0.1 to 0.8% of soluble cellular proteins) and their wide distribution in the cell Elicoh-Middleton et al; **2000**.

Two isoforms of thioredoxin peroxidase (Prx 3 and Prx 5) have been found in mammalian mitochondria. Prx 3 is ubiquitous in various rat tissues, it is present in large quantities in the heart and adrenal gland followed by liver and brain. Prx 5, the newest member of this family, is ubiquitous in cattle tissues, with the highest level in the testes Remesy et al; **1996**.

II.7.2. Non-enzymatic antioxidants

II.7.2.1. Cytochrome C

The mitochondrial intermembrane space contains about 0.7 mM of cytochrome C capable of removing the superoxide anion. Cytochrome C can be alternatively reduced by the superoxide anion and, then, regenerated by its normal electron acceptor cytochrome-C oxidase Hollman et al; **1999**. This fascinating ability of this defense system, to produce energy while detoxifying the potentially harmful superoxide anion has earned it the title of "ideal antioxidant". The antioxidant properties of cyt-C have been confirmed *in vitro*, but the

physiological role and *in vivo* efficacy of this superoxide scavenger system remains to be investigated Hollman et al; **1997**.

II.7.2.2. NADPH

Some heights assume that NADPH can serve as a direct non-enzymatic antioxidant. Mammalian mitochondria contain high concentrations of NAD (P) H (approximately 3-5 mM each), which by capturing centric oxygen radicals, prevent damage to proteins and mitochondrial DNA Manach et al; **2004**.

II.7.2.3. Ubiquinone or CoQ

Ubiquinone is known for its vital role in energy production.It transfers electrons between complex I and complex III of the mitochondria. It has also interesting antioxidant properties by effectivelly protecting the proteins and lipids of the mitochondrial and cellular membranes against peroxidation.

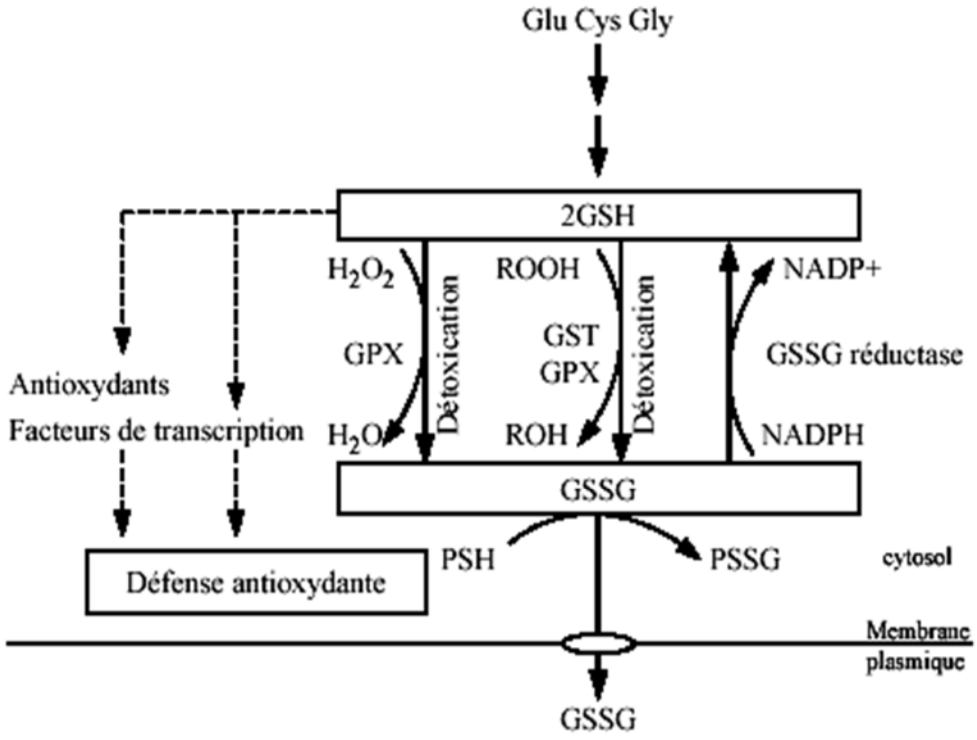


Figure 8. Metabolism and antioxidant functions of GSH in the cell Day et al; **1998**.

GSH (as shown in Fig 8) is consumed either by a cytosolic GPx, or by GST and GPx to reduce hydrogen peroxide or organic peroxides generated by aerobic metabolism, respectively. To maintain the cellular redox balance, GSSG is reduced to GSH by GSSG reductase at the expense of NADPH, thus forming a redox cycle. Under severe oxidative stress conditions, the ability of the cell to reduce GSSG to GSH may be exceeded, inducing its accumulation in the cytosol. To maintain cellular redox balance, GSSG is actively exported or conjugated to a protein thiol (PSH) to form a mixed disulfide conjugate (PSSG).

II.7.2.4. Thioredoxins

Thioredoxins (Txn) are small proteins (12 KDa) endowed with an intrinsic oxidation-reduction activity, like all the proteins with thiol groups (-SH). They are able to reduce ROS. Three variants of thioredoxin have been cloned and characterized: Txn1 cytosolic, Txn2 mitochondrial localization and Txn3 specific expression in spermatozoa. Once oxidized Txn is reduced by thioredoxin reductase Manach et al; **1997**.

II.7.2.5. The metalothioneins

Metalothioneins are small proteins made up of about thirty amino acids, one-third of which are cysteines. The first function described for these proteins is the detoxification of heavy metals. Their role as an antioxidant is demonstrated in different cases of oxidative stresses such as stress induced by doxorubicie. These proteins were able to remove the hydroxyl radical, chelate iron and copper ions and thus limit the Fenton reaction Deprey et al **2000**; Wall et al; **1999**.

II.7.2.6. Vitamin E

Vitamin E is the family of tocopherols (Alpha, Beta, Gamma and Delta), it is the alpha and gamma that have the most interesting antioxidant properties. Vitamin E, like ubiquinone, is concentrated in membranes where it is particularly effective in limiting lipid peroxidation. It can, therefore, intervene directly at the level of the membranes where it traps free radicals before they reach their targets or by stopping the chain of lipid peroxidation. The vitamin E then becomes a radical and can be supported by another antioxidant molecule such as ubiquinone or vitamin C Spencer et al; **1999**; Rice-Evans et al; **2001;** Crespy et al; **2004**.

II.7.2.7. Other non-enzymatic antioxidants

In the water-soluble medium, several molecules seem to have antioxidant power. This is particularly the case of vitamin C or ascorbic acid; excellent ROS scavenger and protects the cell substrates from oxidation, but this vitamin could also have pro-oxidative properties Spencer et al; **1999**.

Since iron and copper in free form are particularly promising for radical damage, these metals are physiologically sequestered and transported by so-called ion chelating proteins, such as transferrin, ferritin, lactoferrin, cerruloplasmin and albumin, which act as primary antioxidants Vanessa et al; **1999**.

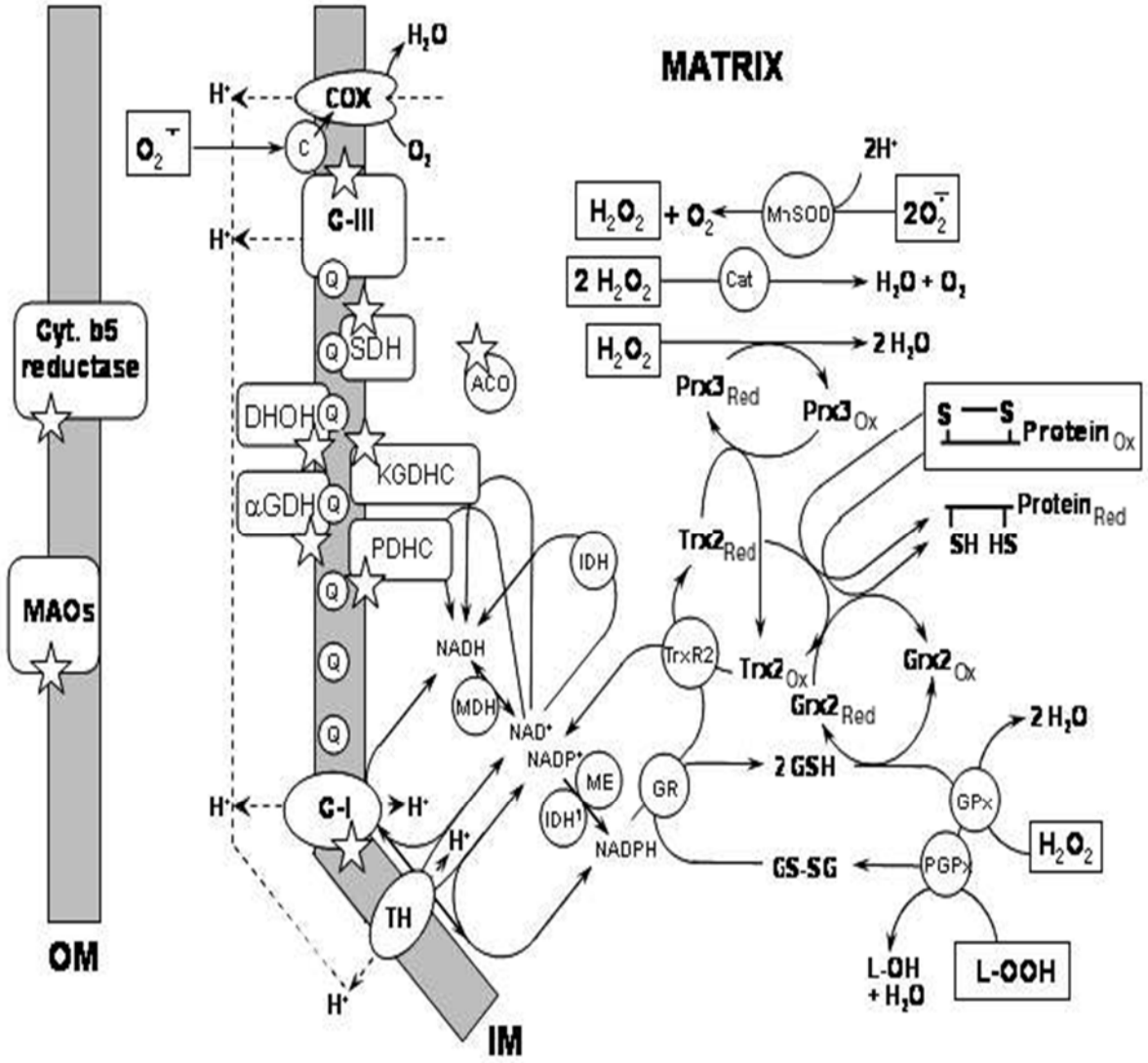


Figure 9: Main sources and system of detoxification of ROS in the mitochondria Gee et al; **2000**.

(ACO) aconitase; Prx3red) peroxiredoxin; Prx3ox) oxidized peroxiredoxin; Q) coenzyme Q; DHOH) dihydroorotate dehydrogenase; KGDHC) alpha-ketoglutarate dehydrogenase complex; alphaGDH) alpha-glycerophosphate dehydrogenase; PDHC) pyruvate dehydrogenase complex; IDH) isocitric dehydrogenase; Trx2red) reduced thioredoxin-2; Trx2ox) thioredoxin-2 oxido; Grx2red) glutaredoxin-2 reduced; Grx2ox) glutaredoxin-2 oxidized; TrxR2) thioredoxin-2 reductase; MDH) malate dehydrogenase; IDH1) isocitrate dehydrogenase; GR) glutathione reductase; GSH) reduced glutathione; GS-SG) oxidized glutathione; GPx) glutathione peroxidase; PGPx) phospholipid hydroperoxide-glutathione peroxidase; C-I) Complex I; TH) transhydrogenase; Cyt. b5 reductase) cytochrome b5 reductase; MAOs) monoamine oxidases A and B; OM) outer mitochondrial membrane; IM) inner mitochondrial membrane. ROS species which is detoxified of descriptive compartments indicating the sources of ROS.

The single acid, major terminal product of purine metabolism, has a major antioxidant activity.It can interact, at physiological concentration, with ROS and in particular by trapping $O_2^{-\circ}$ and OH, it appears to be the most potent plasma antioxidant in terms of reactivity with ROS.However, it is not active against lipid radicals Felgines et al; **2000**.

The trace elements that have a nutritional origin (zinc, copper, selenium, magnesium ...) participate in the defense process against ROS as cofactor of antioxidant enzymes. The deficiency in one or more of these trace elements generally results in a greater sensitivity to oxidative stress and the development of free radical diseases.

These antioxidants are supplemented by dietary intake of other substances such as carotenoids and flavonoids essentially. As a result, with these protective agents, there is a certain tolerance of the body vis-à-vis oxidative stress Grasfe et al; **2001**.

II.8. ROS intervention during physiological processes

The biological consequences of oxidative stress will be extremely variable depending on the dose and cell type. Light stress will increase cell proliferation and adhesion protein expression. Moderate stress will facilitate apoptosis, while strong stress will cause necrosis and violent stresses will disrupt membranes, resulting in immediate lysis. The paradox of free radicals in biology constitute, therefore, extremely dangerous species, capable of generating a considerable number of diseases, while being indispensable species to life. They fulfill many

useful functions which, apart from phagocytosis, have recently been discovered Manach et al; **1995**.

Free radicals contribute to the functioning of certain enzymes, the transduction of cellular signals, the immune defense against pathogens, the destruction by apoptosis of tumor cells, the cell cycle, cell differentiation, the regulation of dilation capillary. Besides it contributes to the functioning of certain neurons and especially those of memory, the fertilization of the ovum, the regulation of genes; phenomenon called redox control of genes Rechner et al; **2000**; Harada et al; **1999**.

The phagocytosis of bacteria and parasites by macrophages or polynuclear is accompanied by a production of ROS so brutal and intense that it is referred to as"Oxidative burst" ie respiratory explosion. Within the phagosome, the activation of NADPH oxidase and the action of SOD and NO synthase, lead to the formation of a highly corrosive mixture of O_2^- H_2O_2, and ONOOH, which destroys by oxidation the set of bacterial components Chanvallon et al; **1994**.

Free radicals constitute also a system of signal transmission, this system is present in unicellular organisms, and it has been preserved in evolved beings, including mammals which have systems of free radical production and signal detection and transduction systems. ROS can, therefore, be considered as intra- and extracellular messengers. They make it possible to induce the cellular response to numerous stresses, thermal, ultraviolet, xenobiotic, allowing the expression of defense genes and the modulation of the expression of genes coding for the antioxidant enzymes. In man, the antioxidant genes most inducible by oxidative stress are those of manganese SOD, catalase, thioredoxins and HSP70 whereas those of Cu-Zn SOD and glutathione peroxidase are not very inducible; Nimesulide, an anti-inflammatory and analgesic drug, is reported to cause severe hepatotoxicity. Nimesulide reduced mitochondrial activity, depolarized mitochondria and caused membrane permeability transition (MPT) followed by release of apoptotic proteins (AIF; apoptosis inducing factor, EndoG; endonuclease G, and Cyto *c*; cytochrome c).Antonio et al; **1998**; Hirano et al; **2000** ; Brijesh Kumar Singh et al ; **2012**.

Needing a certain amount of reactive oxygen species, the body does not seek to destroy them but to control their level to avoid oxidative stress. This explains the very fine regulation of the anti-oxidant genome and these mechanisms of adaptation. Antioxidant therapy should not

forget these useful activities of free radicals on pain of failure; see paradoxical toxic effects Boyle et al; **2000**.

III. Mitochondria and apoptosis

III.1. Definition of apoptosis

Apoptosis or programmed cell death is a physiological process by which supernumerary or dysfunctional cells are removed from the body. It is one of the areas of recent research that can better understand why there are so few cancers, given the incredible number of mitoses in the body and the precise mechanisms that control them. Apoptosis is, therefore, necessary for the development and maintenance of the proper functioning of any living organism. It plays an important role in embryogenesis, in morphological changes, in cellular homeostasis in tissue repair and tumor regression. Unfortunately, the disruption of this process either by excessive activation or lack of intervention is involved in the pathophysiology of many diseases (cancers, neurodegenerative diseases, AIDS ...) Anderson et al; **2000**; Russo et al; **2000**; Ishige et al; **2001**; Halliwell **1994**.

The word "apoptosis" was introduced in 1972 by Kerr et al., and refers to the programmed fall of the leaves in autumn (from the Greek apo for removeness, ptosis for fall) Heijnen et al; **2001**.Apoptosis, a type of active cell death, refers to a sequence of characteristic morphological changes: Condensation of the cytoplasm and chromatin, fragmentation of the cell and nucleus into apoptotic bodies which are then released, and will be phagocytized without any inflammatory reaction, as opposed to necrosis, which is an uncontrolled, violent and rapid death, often manifests itself as an immediate burst of the cell causing the collapse of internal homeostasis and is associated with inflammatory reactions Chun et al; **2003**; Ritta **2000**.

The apoptotic process can be subdivided into three distinct phases: The initiation phase depends on the nature of the stimulation and the cell type targeted by the latter, the regulation phase depends on the activity of the proteins of the Bcl-2 family, and the execution phase which depends on the activity of cysteine proteases or caspases. These two last phases are closely related to the activity of the mitochondria. During this process the mitochondrial membrane is permeabilized in one way or another, causing the release of caspase activating substances such as cytochrome c or AIF regulated by Bcl-2 family proteins Dugas et al; **2000**; Moris et al; **1995**; Brown et al; **1998**.

III.2. Major Molecular Actors of Apoptosis

III.2.1. The caspases

Caspases are important mediators of cell death. These are cysteine proteases specific for aspartic acid. The term caspase has been proposed by Alnemri et al.**1996;** Clark et al; **2016**; Yen et al; **1997.** "C" represents active site cysteine and "aspase" defines the strict specificity of cleavage of these proteases after an aspartic acid Kessler et al; **2002**. 14 different caspases have been highlighted so far. These caspases can be subdivided into initiating caspases (caspases 2, 8, 9 and 10), responsible for the triggering of caspase activation cascades, thereby allowing the amplification and integration of proapoptotic signals, and in caspases effector (caspase 3, 6, 7 and 14) that degrade specific cell substrates Kujumgier et al; **1999**; Pham et al; **1993**. All caspases have a conserved structure and are synthesized as inactive or zymogenic precursors. Activation of caspases involves proteolytic cleavage of the zymogenic form. However, it should be noted that very few data are available on the functions of several caspases and that their roles are still controversial.

The pharmacological activation of caspases using small molecules is an effective approach in the degradation of cancer cells or at least to remove resistance to anticancer drugs. Blocking the expression of the genes that encode caspase inhibitors is another powerful strategy in cancer therapy Terschuk et al; **1997**; Pham et al; **1993**; Sato et al; **1996;** Ghazal et al; **1992**.

III.2.2. Members of the Bcl-2 family

Members of the Bcl-2 (B-cell leukemia / lymphoma 2-like proteins) family are key players in mitochondrial permeabilization. This family, containing about 15 members, can be divided into 2 groups depending on their activities. Proteins with antiapoptotic activity (Bcl-2, Bcl-XL, Bcl-w ...) are partly located at the level of the outer membrane mitochondrial thereby preventing the dissipation of membrane potential and the release of cytochrome c and AIF, and proteins having a proapoptotic activity (Bax, Bid, Bad ...) which can directly induce these modifications Raj Narayana et al; **2001**; Yang et al; **1998**; Huang et al; **1997**; Gupta et al; **2001**.

All the proteins of the Bcl-2 family contain a hydrophobic carboxy-terminal domain of 20 amino acids allowing their anchoring in the intracellular membranes, at the level of the mitochondria but also at the level of the endoplasmic reticulum and the nucleus. The main function of these regulators is to control the release of proapoptotic factors, such as

cytochrome c, from the mitochondrial intermembrane space to the cytosol Yamagishi et al; **2002**; Ong et al; **2000**; Erica weinstin et al; **2005**.

III.3. Mechanisms of permeabilization of mitochondrial membranes during apoptosis

Several models can explain the release of solutes from the mitochondria to the cytosol, a step corresponding to the effector phase of apoptosis.

III.3.1. MPTP mitochondrial permeability transition pores

This MPTP is a nonselective channel that can be formed by the apposition of transmembrane proteins resident at the level of the inner membrane and at the level of the outer membrane of the mitochondria. The various studies carried out show that this pore is mainly formed by the combination of ANT (adenyl nucleotide transporter), VDAC (voltage-dependent anionic channels) and cyclophilin D (Cyp D), a protein of the matrix (Figure 10) Bankova et al; **2000**; English et al; **1992**.

The opening of the pore can be induced by various physiological effectors such as calcium, the decrease in the concentration of adenine nucleotide or inorganic phosphate, the production of oxygen free radicals or the change in pH. In addition this (PT) pore has been previously implicated in clinically relevant massive cell death induced by toxines, anoxia, (ROS), and calcium over load. The PT pore complex could be regulated by caspases and members of the Bcl-2 family : Bcl-2 can prevent this opening whereas Bax causes a fall in the mitochondrial membrane potential and promotes its opening Marzo et al; **1998;** Vander Heiden et al; **1997;** Cheng et al; **1997**; Kalkavan et al; **2018.**

The opening of the pore increases the permeability of the inner mitochondrial membrane to proteins of molecular weight less than 1.5 kda. This leads to a dissipation of the mitochondrial membrane potential, a chemical imbalance between the cytoplasm and the mitochondrial matrix, and a decoupling of oxidative phosphorylation, causing osmotic swelling that can lead to rupture of the outer membrane Alexandre. **1999**.

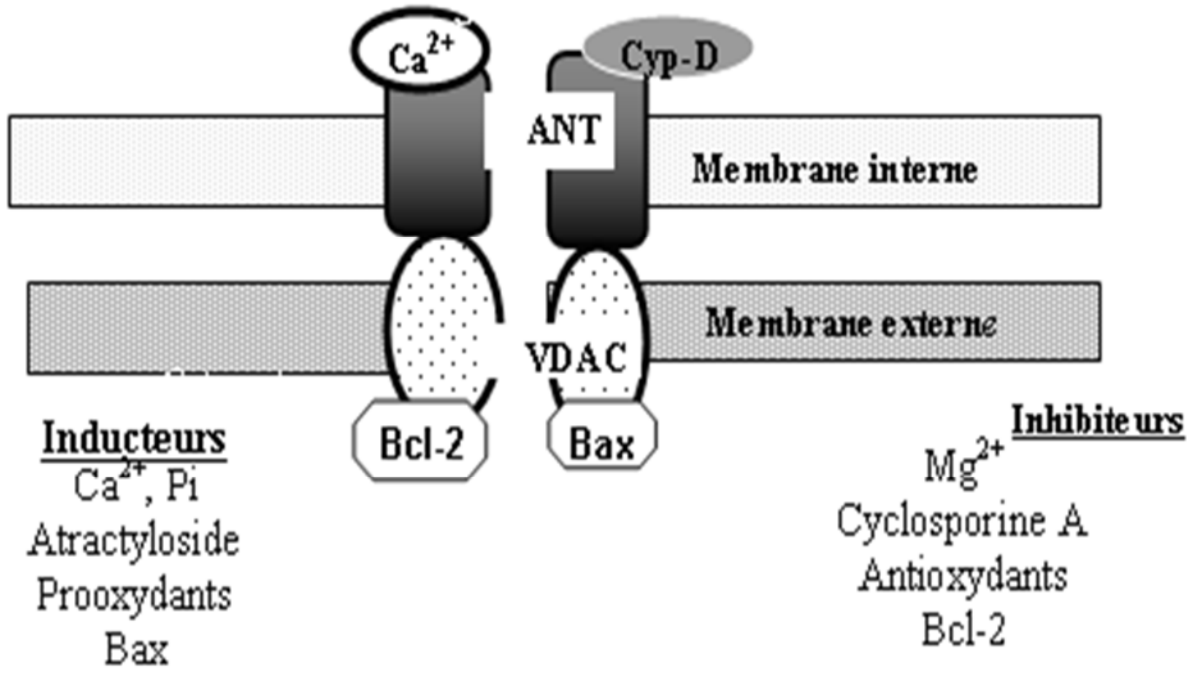


Figure 10: Principal components of the PTP mitochondrial permeability transition poreEvangelist-Rodrigus et al; **2001**.

III.3.2. Rupture of the mitochondrial outer membrane

This model involves the hyperpolarization of the internal membrane preceding the release of cytochrome C in some systems. This hyperpolarization results from the inability of the exchange between cytosolic ADP and mitochondrial ATP. This exchange is normally carried out by voltage-gated anion channels or VDAC located at the outer membrane and by the adenyl nucleotide transporter or ANT located at the inner membrane. This lack of exchange seems to inhibit F1F0-ATPase activity, which prevents the return of H^+ ions to the matrix and therefore contributes to hyperpolarization. Such an increase in mitochondrial membrane potential may cause osmotic swelling of the matrix leading to mitochondrial outer membrane rupture and triggering of the mitochondrial pathway of apoptosis Kokaleci et al; **2003**.

III.3.3. The pore formed by members of the Bcl-2 family

The hypothesis of a channel capable of passing apoptogenic proteins from the mitochondrial intermembrane space is studied. This channel could be formed by certain members of the Bcl-2 family given the strong homology of Bcl-XL with the subunit of diphtheria toxin, capable of forming a membrane pore. It has been suggested that Bcl-2 family proteins such as Bax may insert at the mitochondrial outer membrane and oligomerize to form a channel, but that such a

channel may be large enough to pass from small proteins remains to be demonstrated Amoros et al; **1992**.

III.4. Molecular mechanisms and different pathways of apoptosis

There are now several major signaling pathways leading to apoptotic cell death. The first, called the intrinsic pathway, involves mitochondria, which play a central role in the mechanisms of apoptosis. Another pathway is initiated on the surface of the cell by membrane receptors; it is the extrinsic pathway or death receptor pathway Christov et al;**1998**.

III.4.1. The mitochondrial pathway or intrinsic pathway

Many stimuli, such as therapeutic agents, UV radiation, stress molecules, lack of growth factors, seem to induce apoptosis by the mitochondrial pathway. The intermembrane space contains cytochrome c, certain procaspases (2, 3 and 9), Smac / Diablo, AIF and endonuclease G proteins. The permeabilization of the outer and inner membranes causes the dissipation of the membrane potential and the release of all these proteins, proapoptotic in the cytoplasm. The mitochondria can then develop two apoptotic pathways: one dependent on caspases, the other is independent Wollenweber et al; **1997**.

III.4.1.1. The mitochondrial pathway dependent on caspases

The release of cytochrome c is the major step in the induction of apoptosis by this route.

- **Cytochrome c**

Cytochrome c is encoded by a nuclear gene and is synthesized as a precursor that is unable to participate in the induction of apoptosis. This precursor is imported into the mitochondria where it matures. Cytochrome c is sequestered at the mitochondrial intermembrane space where it exerts its physiological function as an electron transporter between the III and IV complexes of the respiratory chain Harada et al; **1999**. In 1996, Liu et al. showed that cytochrome c was necessary for the activation of caspase-3. It is now established that cytochrome c released into the cytosol is responsible for the formation of apoptosome. Recently, the invalidation of the gene coding for cytochrome c confirmed the crucial importance of this protein in apoptosis. These studies show that no other cellular protein can replace cytochrome c for the oligomerization of Apaf-1 and for the activation of caspase-3,

induced by cellular stress or by a mitochondrial targeting agent Melina et al; **2000**; Park et al; **2002**.

- **Apoptotic process**

The increase of the permeability of the inner membrane of the mitochondria results in its swelling by massive entry of solute into the matrix and an alteration of the outer membrane sufficient to release the products of the intermembrane space, inter alia the cytochrome c. The oligomerization of cytochrome c, and APAF-1 in the presence of ATP allows the recruitment of procapase 9 to form a very large complex (700 to 1400 KDa) called apoptosome (Figure 11), within which the procaspase 9 activates and initiates a caspase cascade by cleaving caspases 3 and 7, major effector proteases of apoptosis responsible for nuclear DNA fragmentation and cell self-destruction Park et al; **2004**

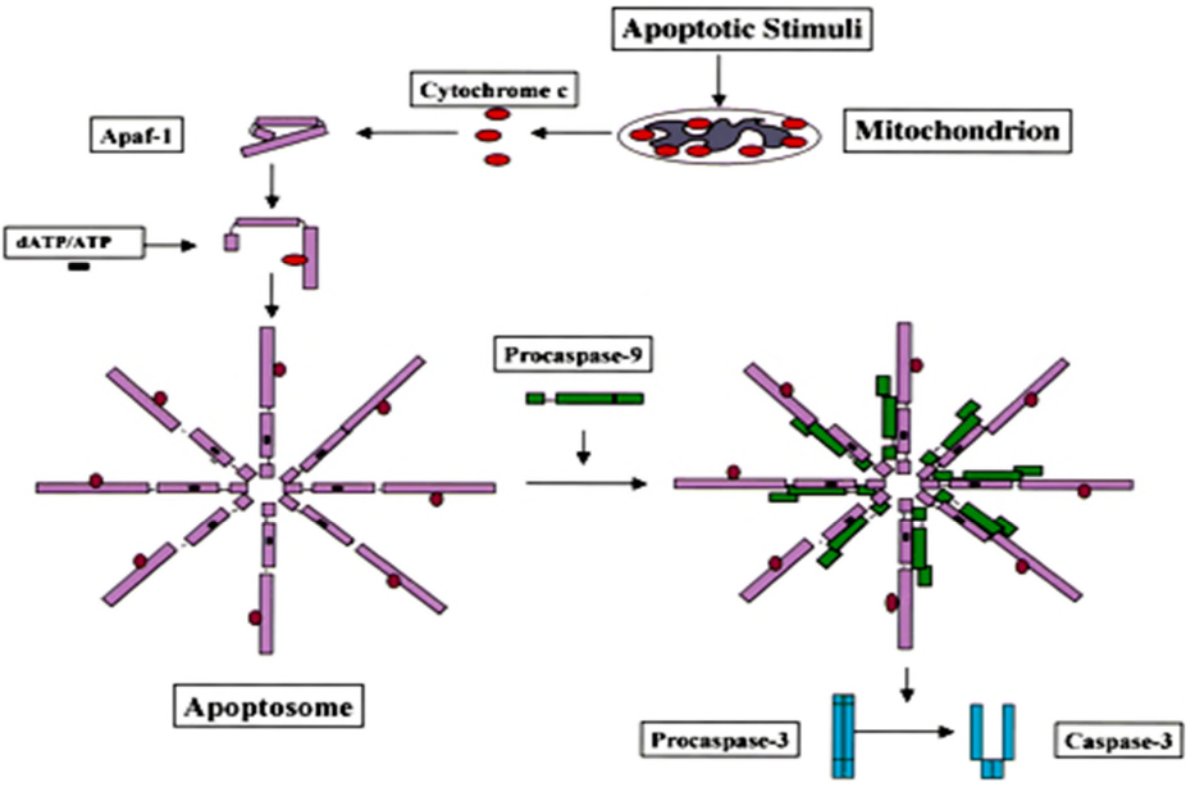


Figure 11: Formation of apoptosome, activation of caspases and induction of the apoptotic process Kumazawa et al; **2003**.

III.4.1.2. The mitochondrial pathway independent of caspases

Several proteins contained in the intermembrane space can induce apoptosis directly without activation of caspases. This is the case of the apoptotic induction factor AIF and endonuclease

G which, once released from the mitochondria, are translocated into the nucleus causing condensation of the chromatin and cleavage of the DNA into large fragments (Figure 12) Murat et al; **2002**.

- **AIF protein**

It is a bifunctional protein with probably oxidoreductase activity and a proapoptotic role. After exposure of the cell to a proapoptotic stimulus, the AIF translates from the intermembrane space to the cytosol and then to the nucleus. The effects of AIF as an apoptogenic molecule have been studied both *in vitro* and *in vivo*. *In vitro*, AIF has been shown to generate peripheral condensation of chromatin by direct interaction with DNA without sequence specificity. In addition, AIF causes, in the presence of cytosolic extracts, permeabilization of the mitochondrial outer membrane and consequently the release of cytochrome c and procaspase 9. *In vivo*, the same observations (condensation, fragmentation, release of mitochondrial proteins) have been observed Maceijeuvicz et al; **2001**.

- **Endonuclease G**

Endonuclease G is a non-specific mitochondrial nuclease that is highly conserved in eukaryotes. It is encoded by a nuclear gene and is probably involved in the replication of the mitochondrial genome. During apoptosis, endonuclease G is released from the mitochondria and translocated into the nucleus. It digests the DNA in the absence of caspase activity and in the absence of caspase-dependent nuclease, thus, generating high molecular weight DNA fragments but also oligonucleosomal fragments Ghisalberti. **1997**.

III.4.2. The death receptor pathway or extrinsic pathway

The extrinsic path involves a reception of the signal at the level of the plasma membrane. Several known receptors belong to the family of TNF (Tumor Necrosis Factor) receptors such as Fas, TNF-R1 (Tumor Necrosis Factor Receptor) or TRAIL (TNF-related apoptosis-inducing ligand). Once the message is perceived, the transmission is carried out via the FADD (Fas-associated death domain) adapter protein. This protein has two interaction domains, one named DD (Death Domain) for the receiver and the other domain named DED (Death Effector Domain) that interacts with caspase-8. Subsequent activation of caspases-3, 6 and 7 by proteolytic cleavage of caspase-8 induces cell self-destruction by cleaving the essential components to maintain cell life Decastro, Higashi. **1995**; Shimazawa, Chikamatsu, Morimotov, Mi Shima. **2005**.

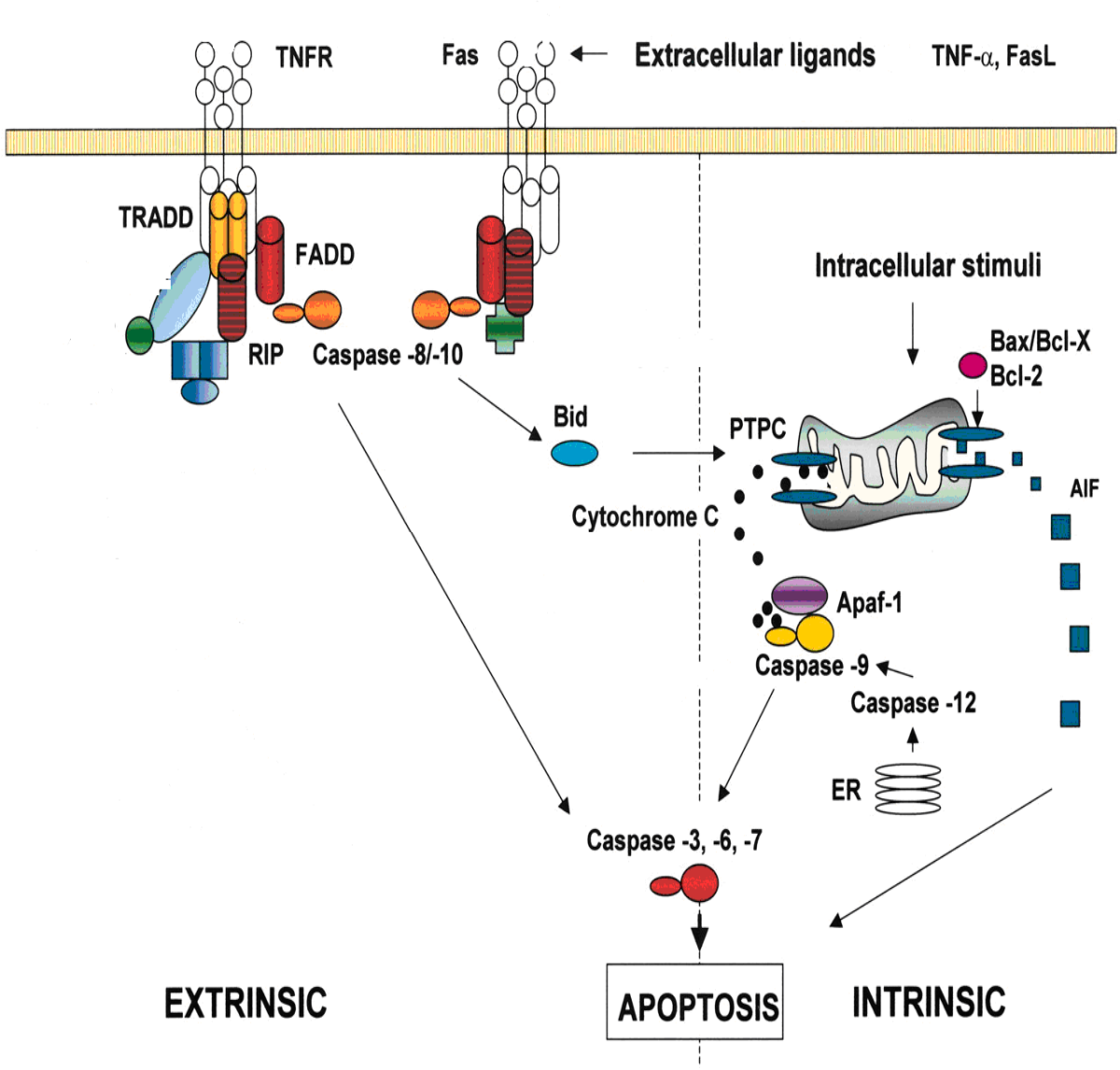


Figure 12: Intrinsic and extrinsic pathway of apoptosis and factors released from mitochondria during the apoptotic process Marfak. **2003.**

III.5. Regulation of the mitochondrial pathway of apoptosis

III.5.1. Apoptosis inhibiting proteins or IAPs

IAPs are proteins that inhibit cell death by preventing cleavage of caspases. XIAP (X-linked inhibitor-of-apoptosis protein) is one of the best-known molecules of this family; it can inhibit the activation of the initiating caspase 9 as well as that of the effector caspases 3 and 7. In fact, it can bind to the active caspase 9 and prevent it from acting on the procaspase 3 or sequestering the procaspase 3 at the apoptosome level by simple binding, which blocks the apoptotic pathway Isla et al; **2001**.

III.5.2. The inhibitory Smac / DIABLO protein of IAPs

Smac (Second Mitochondria-derived Activator of Caspase) and its homologous DIABLO (Direct IAP Binding Protein with Low pI) are recently identified proteins that block the antiapoptotic activity of IAPs and, consequently, the activation of caspases. Smac / DIABLO protein is strongly expressed in the heart, liver, kidneys, spleen and in several cancerous lines. Smac / DIABLO interacts with IAPs and prevents their binding to caspases 3, 7 and 9 Su et al; **2000**.

III.5.3. Other regulatory proteins

Members of the Bcl-2 family can also regulate the mitochondrial pathway by acting on the release of cytochrome c but also by binding to Apaf-1. The regulation of apoptosis by these proteins results from the level of expression between pro or anti-apoptotic proteins. While the cells expressing more proapoptotic proteins will be sensitive to death; the others will be resistant Morrissay et al; **2003**; Nestel. **2003**.

Thermal shock proteins or Hsp are also inhibitors of apoptosis. Various studies show that Hsp-70 and Hsp-90 can bind to the domain of Apaf-1, thus preventing it from oligomerizing and activating procaspase 9. Hsp-27 blocks the oligomerization of Apaf-1 by binding to cytochrome C Miyak et al; **1997**.

IV. Mitochondria, stress, apoptosis and human pathologies

Oxidative stress is involved in many diseases as a triggering factor or associated with complications of evolution. The primary cause of these diseases is mitochondria by the excessive production of ROS. Most of these diseases occur with age as aging decreases antioxidant defenses and increases mitochondrial production of ROS and consequently triggers apoptosis. Oxidative damage affects replication and transcription of mtDNA and results in a decline in mitochondrial function which in turn leads to enhanced ROS production. Cui et al; **2012.**The reduction of oxidative stress could be achieved in three levels: by lowering exposure to environmental pollutants with oxidizing properties, by increasing levels of endogenous and exogenous antioxidants, or by lowering the generation of oxidative stress by stabilizing mitochondrial energy production and efficiency. Poljsak .**2011.**Oxidative stress is a crucial factor for cancer progression and therapy. The later is a physiological state where high levels of reactive oxygen species (ROS) and free radicals are generated. Several

signaling pathways associated with carcinogenesis can additionally control ROS generation and regulate ROS downstream mechanisms. Subbroto Kumar Saha et al ;**2017.**

By showing abnormal biological molecules and suppressing certain genes, oxidative stress will be the main cause of several diseases: cancer, cataracts, amyotrophic lateral sclerosis, pulmonary edema, accelerated aging. Oxidative stress is also one of the factors that potentiate the development of multifactorial diseases such as diabetes, Alzheimer's disease, rheumatism and cardiovascular diseases. It also plays a role in the appearance of other atherogenic factors such as the increase of insulin resistance and activation of endothelial cells releasing prooxidant mediators. It is the combination of several factors; antioxidant deficiency, overload of prooxidant factors and genetic factors that will lead to pathogenesis;Oxidative stress is related to disease development, incidence of malignancies and autoimmune disorders.Murad et al; **2002**; Anu Rahalet al; **2014.**

Among the diseases related to stress, mitochondrial dysfunction and / or apoptosis consequences of oxidative stress include:

IV.1. Aging

Aging is characterized by increased mitochondrial production of ROS, modification of redox potential, GSH depletion and ATP. These changes facilitate lipid peroxidation and the opening of the PTP. Harman's radical theory of aging "free radical theory of aging" links senescence to the damage of superoxide radicals and other ROS generated during mitochondrial respiration Burdik G A. **1998**.ROS are associated with the pathophysiological parainflammation and autophagy process in the course of the age-related macular degeneration.Małgorzata et al; **2016**.This theory postulates that the random alterations of mtDNA are responsible for the energetic decline accompanying senescence Another theory, the mitochondrial theory of aging "mitochondrial theory of aging" proposes that the aging is the result of the accumulation of the mtDNA damaged by the ROS leading to the dysfunction of the complexes of the chain and eventually senescence.It is generally accepted that the abilities of antioxidant systems decrease with age, which results in the gradual loss of balance of antioxidant-prooxidant balance and accumulation of oxidative damage. Mitochondrial SOD activity has been shown to increase significantly in elderly patients with decreased levels of GPx, Cu-Zn / SOD and catalase Benskota et al; **2001**;Astridillo et al; **2000**.

IV.2. Ischemia-reperfusion

Ischemia is the interruption or reduction of blood flow to a tissue or organ. During the ischemia episode, the interruption of the blood circulation causes a decrease of the oxidative phosphorylation, and consequently a decrease of the concentration of ATP. This causes inhibition of Na^+ / K^+ -ATP-dependent pumps resulting in elevation of cytosolic Ca^{2+}. At first, the absence of oxygen does not seem deleterious since it inhibits the functioning of the respiratory chain and lowers the potential of the internal membrane which limits the entry of Ca^{2+} into the mitochondria. But when the tissue is Reperfused, the massive influx of oxygen causes a sudden resumption of the functioning of the respiratory chain, remaining the membrane potential of the mitochondria. It can, then, massively capture concentrated Ca^{2+} in the cyto in the cytosol, thereby again inhibiting ATP synthesis. In addition, the relative excess of oxygen causes the production of excess free radicals that induce oxidative stress. During ischemia followed by reperfusion, mitochondrial Ca^{2+} and free radicals are initially increased, favorable conditions for the opening of the giant pore. This second phase results in apoptosis of the cells. It is observed that brief ischemia leads to apoptosis whereas prolongation of ischemia leads to death by necrosis. There are several agents acting on mitochondria that inhibit apoptosis, such as caspase inhibitors and cyclosporine Paris et al; **1975**; Boharun et al; **1996**.

IV.3. Neurodegenerative Diseases

Mitochondrial dysfunction leads to oxidative damage detected in several neurodegenerative diseases. Parkinson's disease is characterized by the progressive loss of dopaminergic neurons from the Nigro-Striatal system. The electron microscopic study of the brains of parkinsonian patients reveals that these dopaminergic neurons die by apoptosis. This death is due to the increase in ROS synthesis during catecholamine metabolism Rustin et al; **1994**. It has been shown that mitochondrial complex I activity is decreased in parkinsonian fibroblasts. It has also been shown to decrease platelet mitochondrial I, II and III activity as well as lower total COQ levels compared to healthy subjects Lahouel. **2005**.

Alzheimer's disease (AD), which is characterized by a progressive loss of memory in the short term and then in the long term, by a decrease in intellectual capacities and, sometimes, by motor disorders is associated with a significant neuronal loss following the reduction of the activity of complexes II, III and IV of neuronal mitochondria Lowry et al; **1951**. Amyotrophic lateral sclerosis (ALS) is characterized by progressive loss of motoneurons leading to skeletal

muscle atrophy and paralysis. The pathophysiology of approximately 20% of familial ALS results from the mutation of the Cu / Zn / SOD gene Borner. **2003**; Xiaodong. **2001**. Huntington's disease (HD) is characterized by reduced activity of mitochondrial complexes II and III Ahmed et al; **2007**.

IV.4.Cancer

The relationships between oxidative stress and cancer are very close. ROS involved in the activation of procarcinogenic carcinogens, creating DNA lesions, amplifying proliferation signals and inhibiting antioncogenes like p53 (tumor suppressor gene) and proapoptotic members of the bcl2 family. P53 is able to induce cell cycle arrest, senescence and apoptosis. It is observed that the p53 protein can act on the PTP and the release of the cytochrome c and the AIF and that it undergoes mutations in the majority of the cancers in the human This pathology also results during a reduction of the apoptotic process Ahmed et al; **2007**; Lahouel et al; **2007**; Gamal Edeen et al. **2010**.

IV.5. Mitochondrial encephalomyopathy: EPM

EPMs are one of the diseases linked to mitochondrial DNA mutations. These mutations cause dysfunction of the mitochondrial respiratory chain and oxidative phosphorylation. A recent study has shown an induction of apoptosis in the muscles of patients with mitochondrial DNA mutations encoding genes in the respiratory chain compared to those with mutations in structural genes. Increased levels of malondialdehyde and carbonyl proteins were observed in subjects with mitochondrial encephalomyopathies Boussenane et al; **2009**; Chehma et al; **2005**.

IV.6. Ataxia of Friedrich

Friedrich's ataxia is characterized by a lack of movement coordination and hypertrophic cardiomyopathies. It is due to the decrease of frataxin which is a regulator of the mitochondrial ferric pool leading to a significant accumulation of iron associated with the oxidative damage observed in this pathology Ouglissi Dehhak et al; **2008**.

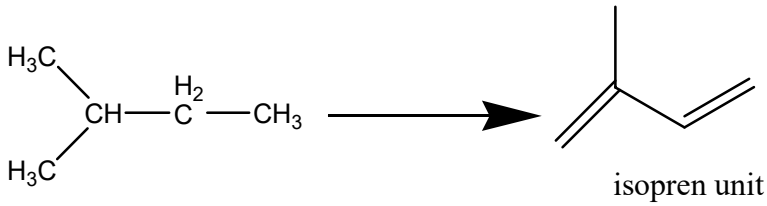
V. Terpenes and Terpenoids

V.1. Definition

Terpenes are reported to be the largest and most diverse group of plant secondary metabolites, produced by a variaty of plants.

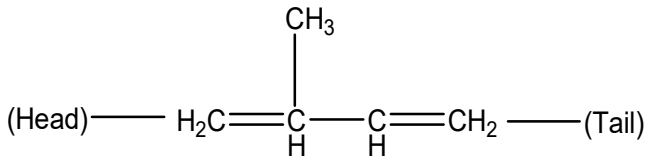
The diffrence between terpenes and terpenoids is that tepenes are hydrocarbons whereas terpenoids have additionnal functionnal groups. Most authors use this term terpenes to include terpenoids. The isoprene unit, a coummon 5- carbon unit, has been reported to be the basic building block of terpenes, and it has a molecular formula of C_5H_8. However terpenes bassic molecular formula are multiple of $(C_5H_8)_n$ where n is rhe number of isoprene units , which is called the biogenelic isoprene role or C_5 role Ružička Leopold. 1953.

According to the latter, the isoprene units would be builed either as chains or they could be arranged for rings.



isopren unit

With the exception of isoprene itself, isoprenoids or terpenoids are dimers, trinners, tetramers or polymers in which the isoprene units are joined in ahead to tail manner (shangan khan IWI..)



However, the original 5-C unit is difficult to be determined due to extension metabolic modifications.

V.2. Terpenes biosynthesis

Terpenoids represent the largest class of natural products with a diverse arry of structures and functions. Many terpenoids have reported therapeutic properties.

Terpenoids, with around 64000 known compounds, are considered the largest and most diverse class of natural products. Terpenoids are secondary metabolites mostly produced by plants and some by bacteria or yeast. They occur in various chemical structurs in a usual assortment of linear hydrocarbons or chiral carbocyclic skeletons with different chemical modifications such as hydroxyl, ketone, aldehyde and peroxide groups. Different terpenoidal molecule have been reported to have antimicrobial, antifungal, antiviral, antiparasitic, antihyperglycemic, antiallergenic, anti-inflammatory,antispasmodic, immunomodulatory and chemotherapeutic properties. They can also be used as natural insecticides and protective substances in storing agricukture products. This diverse array of tepenoid structures and functions has incited great interest in their medicinal use and commercial applications as flavors, fragrances and spices. Moreover, terpenoids recently emerge as strong players in the biofuel market. Among the terpenoids with established medical applicatios are the antimalarial artemisinin and the anticancer taxol. Wang et al; **2005**; Thoppil et al ; **2011**; Guan et al; **2015**; Ajikumar et al; **2008**.

The building process of terpenes does not involve the isoprene unit itself, but rather it makes use of isoprene activated forms, thus it embraces two parts:

A- Synthesis of activated 5-C Units

According to Shongen Khan, isoprene unit is synthesized from acetyl-COA through mevalonic acid pathway, by joining three molecules of acetyl-COA together in stepwise manner to give a rise to a six-carbon intermediate mevalonic acid. The latter is then pyrophosphorylated by utilising 2 ATP molecules to form mevalonic acid pyrophosphate (MVA-PP).

Followed by decarbokylation and dehydration, the activated 5-C unit called as isopentenyl pyrophosphate (IPP) results. The latter can be isomerized to another actiavted 5-C unit known as dimethylallyl pyro-phosphate (DPP). Both of these activated forms represent the terpenes building blocks (Shongan Khan IWI).

B- Condensation of activated 5-C units IPP and DPP to form terpenes

The ultimate condensation of activated 5-C units IPP and DPP is briefly described as follows:

- The gathering or unification of IPP and DPP to form 10-C geranyl pyrophosphate (GPP) which is the precursor of monoterpenes.

-GPP reacts with another molecule of IPP to give rise to 15-C farnesyl pyrophosphate (FPP) that is the precursor of sesquiterpenes.

- The reaction of FPP with another molecule IPP forms the 20-C compound geranyl-geranyl pyrophosphate (GGPP) which is the precursor of diterpenes.

- The dimerization of FPP give rise to 30-C compound which, after elimination of two pyrophate groups (2PP), gives rise to squalene. The latter is the precursor of tritepenes and steroids.

- The dimerization of GGPP form the 40-C compound which form phytoene by elimating two pyrophoshpate groups (2PP). Phytoene is the precursor of tetraterpenes.

- Polyterpenes are polymers containing large number of isoprenyl units.

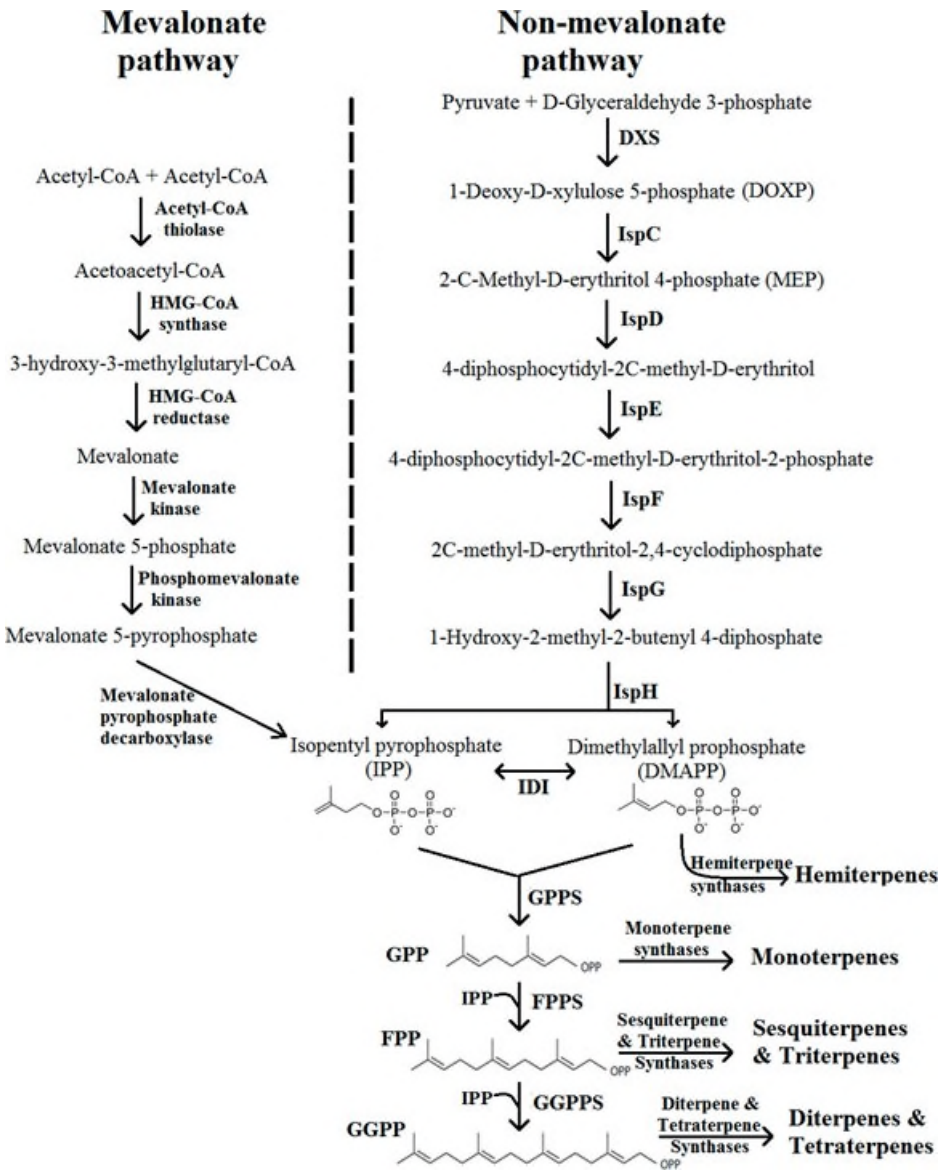


Figure 13 : Biosynthetic pathways for terpenoid production. Ingy et al; 2017

Sesquiterpenoids are widely distributed in nature and represent the most prevail-ing class of terpenoids. They are acyclic, monocyclic, bicyclic or tricyclic C_{15} compounds synthesized from the substrate FPP by sesquiterpene symhases.Ingy I. Abdallah et ;2017.

V.3. Terpenes classification

Based on the number of carbon atours and isoprene units present in their structure, terpenes are classified into:

- Hemiterpenes
- Monoterpenes, they consist of 10-C atoms or two isoprene units.
- Sesquiterpenes, these compounds contain 15-C atoms or three isoprenes.

- Diterpenes, these contain 20-C atoms or four isoprene units.
- Triterpenes, these cosist of 30-C atoms or six isoprene units.
- Tetraterpenes, these contain 40-C atoms or eight isoprene units.
- Polyterpenes, these consist of large number of isoprene units.

V.4. Properties of terpenes

Blue – green algae (cyanobacteria) have evolved as the most primitive, oxygenic plant – type photosynthetic organisms. They were the first which produced molecular oxygen as a byproduct of photosynthetic activitiy. Living in habitats with potentially damaging photooxidative conditions as well due to hight irradiation and oxygen concentrations; those blue – green algae cells must have evolved protective mechanisms to cope with reactive oxygen species to preventdamage of biologically important macro molecules.Regelsberger et al; **2002.** In addition hydrogen peroxide (H_2O_2) and organic peroxide can be removed by antioxidant enzymes via disproportionation and redaction to water or the correspnding alcohols. All the cyanobacteria possess peroxiredoxines witch use thioredoxin or other reduced thiols to get rid of hydrogenperoxide and lipid peroxide Regelsberger et al; **2002.** Due to high light intensities accompagnied by oxygen saturation without being able to escape of the light, under this condition plant have thus developed a strategy for defence composed of avoidance reactioncombined with a set of defence chemicalsGrassmann et al; **2002.**In order to take advantage of plant defence, investigators have tried to explore the underestimated group of chemicals of known as terpenoids that are effective pro and antioxidant in lipid peroxidationprocesses Grassmann et al; **2002.**

V.4.1. prooxidant properties of terpenes

Terpens could serve as mediators of the ROS scavenging system and have the potential to act as prooxidants or antioxidants depending on the biological environent of the cells. The proxidant effect could be cytotoxic such as Maytenus Procumbens on Hela cells or triggers the DNA damage witch leads to apoptosis upon ROS generation.Momtaz, et al; **2013.**

V.4.2. antioxidant properties of terpenes and mechanisms of action

The biotransformation of bioactive natural compounds could modify their Plants have a long history as therapeutic tools in the treatment of human diseases and have been used as a source of medicines for ages. In search of new biologically active natural products, many plants and herbs used in traditional medicine are screened for natural products with pharmacological

activity.Our focus has been on sesquiterpene coumarins as one of several categories of terpenoids.Despite many research reports about these natural compounds indicating their vital role in combating several life threathning diseases, thre still lacks on their mechanisms of actions and structure- activity relationship studies. Sesquiterpene coumarins are found in some plants of the families Apiaceae (Umbelliferae), asteraceae (Compositae) and rutaceae. the coumarin moietyis often umbelliferone (7-hydroxycoumarin) but scopo-letin (7-hydroxy-6-methoxycoumarin)and isofraxidin(7-hydroxy-6,8-dimethoxycoumarin) are also found.These coumarins are linked to a C_{15} terpene moietythrough an ether linkage. Another group of sesquiter-pene coumarins is the prenylated 4-hydroxycoumarins where the link between the coumarin and the C_{15} terpene moiety is a C–C-bond at carbon 3 of the coumarin moiety. Finally, the prenyl-furocoumarin-type sesquiterpenoids are a separate group of sesqui-terpene coumarins based on the suggested biosynthetic pathway.Gliszczynska et al; **2012.**

V.4.3. Ferula and cancer

Certain compounds have been demonstrated to have chemopreventive and / or cytotoxic activities towards many cancer cell lines. The roots of (ferula narthex) rich in terpenes coumarins, whith have been reported as chemopreventive agents against cutaneous carcinogenesis in mice Saleem et al. **2001;** Saleem et al; **2004**. Similarly, the chloroformic extract of the roots of F. persica inhibited the activation of matrix metalloproteinases (MMPs) whitch play a crucial role in tumor invasion and inflammation, in particular umbrelliprenine and persica sulphide B whitch appeare to be implicated in this type of activity Shahverdi et al**; 2006.**

V.4.4. Sesquiterpene derivatives

The following details concerning sequiterpenes derivatives have been taken from Alkhatib. **2010.**

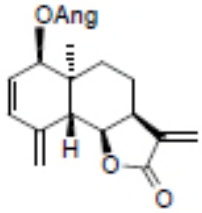
1. Sesquiterpene lactones:

Two types of sesquiterpene lactones can be distinguished: derivatives of eudesmane and those of guaiane. Here are examples of these compounds (Table A).

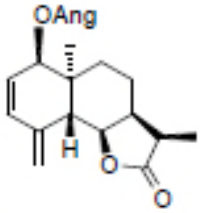
Table A. Source of some sesquiterpene lactones.

Composé	Source	Ref.
Déhydroopodine	*F. varia*	[Suzuki *et al.*, 2007]
	F. oopoda	[Serkerov, 1969]
Oopodine	*F. varia*	[Suzuki *et al.*, 2007]
	F. oopoda	[Serkerov, 1971]
Féropodine	*F. oopoda*	[Serkerov, 1971]
Sémopodine	*F. oopoda*	[Serkerov, 1976]
8α-angéloyloxy-10β-hydroxyguai-3-ène-6,12-olide	*F. varia*	[Suzuki *et al.*, 2007]
Diversolide A	*F. diversitata*	[Iranshahi *et al.*, 2008c]
Diversolide D	*F. diversitata*	[Iranshahi *et al.*, 2008c]
Diversolide G	*F. diversitata*	[Iranshahi *et al.*, 2008c]
Diversolide F	*F. diversitata*	[Iranshahi *et al.*, 2008c]

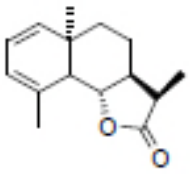
Derived from eudesmane

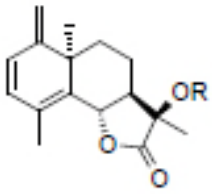


Déhydroopodine



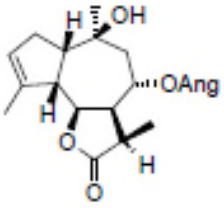
Oopodine



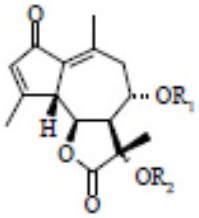


I **›podine**

Guaiana **derivatives**



8α-angéloyloxy-10β-hydroxyguai-3-ène-6,12-olide

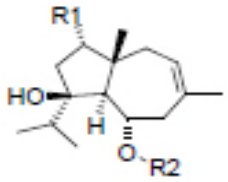


	R_1	R_2
Diversolide A	Ang	Ang
Diversolide D	Ver	Ac
Diversolide G	Bnz	Ac
Diversolide F	Ver	Sen

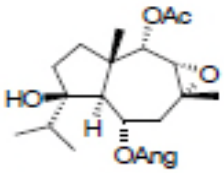
2. The daucan esters:

More than 150 daucan derivatives have been isolated from the genus Ferula, especially from the subgenus Peucedanoides. Esters of jaeschkenadiol (ferutinin) are very common in these species (Table B).

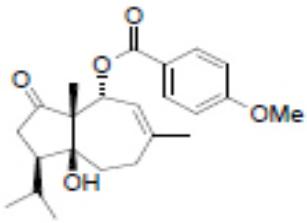
Table B. Source of some derivatives of daucan.



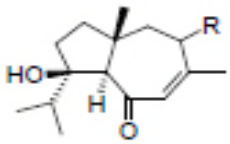
	R_1	R_2
Jaeschkeanadiol	H	H
Férutinine	H	*p*-OH-Bnz
Férutidine	H	Anis
2α-Hydroxyférutidine	OH	Anis
Téférine	H	Van



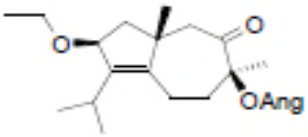
Lapiférine



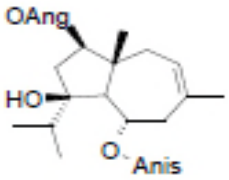
Anisate de siol



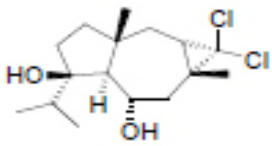
4β-hydroxy-5αH-dauca-7-ène-6-one R=H$_2$
4β-hydroxy-5αH-dauca-7-ène-6,9-dione R=O



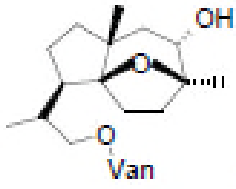
3α-éthoxy-8β-angeloyloxydauca-4-ène-9-one



Tunetanine A



Jaeschkenol



13-Vanilloloxydaucol

Coumarin derivatives

The coumarin derivatives are widespread in the genus Ferula, especially the ethers of coumarin-sesquiterpenes. Cyclic coumarin farnesyl and coumarins are also isolated from different Ferula species (Table C).

Table C. Source of some coumarin derivatives.

Composé	Source	Ref.
Umbelliprénine	*F. aitchisonii, F. arrigonii, F. assa-foetida, F. conocaula, F. eugenii, F. iliensis, F. linkii-TF, F. violaceae*	[Abd El-Razek *et al.*, 2003]
Persicaoside C	*F. persica*	[Iranshahi *et al.*, 2008a]
Persicaoside D	*F. persica*	[Iranshahi *et al.*, 2008a]
Farnésiferol B	*F. assa-foetida, F. kopetdagensis, F. szowitsiana*	[Abd El-Razek *et al.*, 2003]
Farnésiferol C	*F. assa-foetida, F. caspica, F. kopetdagensis*	[Abd El-Razek *et al.*, 2003]
Acide galbanique	*F. assa-foetida, F. kokanica, F. kopetdagensis, F. szowitsiana, F. violaceae*	[Abd El-Razek *et al.*, 2003]
Conférone	*F. conocaula, F. foetidissima, F. inciso-serrata, F. iliensis, F persica, F. sumbul, F. teterrima*	[Abd El-Razek *et al.*, 2003]
Conférol	*F. assa-foetida, F. conocaula, F. foetidissima, F. inciso-serrata, F. iliensis, F. korshinskyi, F. lipskyi, F. moschata, F. pallida, F. persica,F. sumbul, F. tuberifera*	[Abd El-Razek *et al.*, 2003]
Mogotlacine	*F. mogoltavica*	[Abd El-Razek *et al.*, 2003]
Fésélol	*F. assa-foetida, F. diversivittata, F. iliensis, F. korshinskyi, F. moschata, F. pallida, F. pseudooreoselinum, F. sumbul, F. tingitana F. vesceritensis*	[Abd El-Razek *et al.*, 2003] [Oughlissi-Dehak *et al.*, 2008]
13-Hydroxyfésélol	*F. tunetana*	[Jubran *et al.*, 2010]
Tunétacoumarine A	*F. tunetana*	[Jubran *et al.*, 2010]
Isosmarcandine	*F. caucasica, F. gummosa, F. sinica*	[Abd El-Razek *et al.*, 2003]

Farnésiferol A	*F. assa-foetida, F. badrakema, F. caspica, F. kokanica, F. linczevskii, F. mogoltavica, F. samarcandica* *F. vesceritensis*	[Abd El-Razek *et al.*, 2003] [Oughlissi-Dehak *et al.*, 2008]
Coladine	*F. arrigonii* *F. communis, F. linczevskii, F. linkii, F. sinica*	[Appendino *et al.*, 1997] [Abd El-Razek *et al.*, 2003]
Coladonine	*F. linczevskii, F. communis, F. tingitana*	[Abd El-Razek *et al.*, 2003]
Szowistsiacoumarine A	*F. szowitsiana*	[Iranshahi *et al.*, 2007]
Szowistsiacoumarine B	*F. szowitsiana*	[Iranshahi *et al.*, 2007]
Férulénol	*F. communis*	[Abd El-Razek *et al.*, 2003]
Fésumtuorine B	*F. sumbul*	[Zhou *et al.*, 2000]
Fésumtuorine C	*F. sumbul*	[Zhou *et al.*, 2000]
Fésumtuorine E	*F. sumbul*	[Zhou *et al.*, 2000]

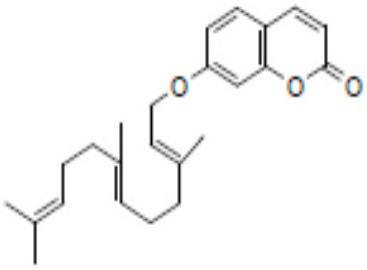
1. The coumarin-sesquiterpene ethers:

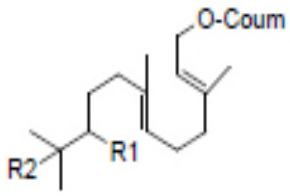
These are often coumarins substituted by linear sesquiterpenes, monocyclic or bicyclic.

- Coumarins substituted by linear sesquiterpenes

Umbelliprenine is common in this genus. It has been isolated from 10 species.

Recently, two glucosidic derivatives have been isolated from the methanolic extract of roots of F. persica, persicaoside C and D.





-

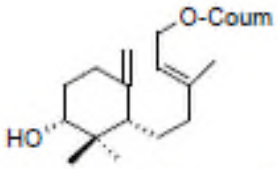
Umbelliprénine

Persicaoside C R_1=OH, R_2=O-βGlu-βGlu

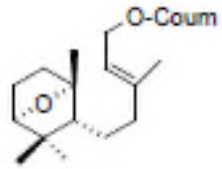
Persicaoside D R_1=O-βGlu-βGlu, R_2=O-βGlu-βGlu

Coumarins substituted by monocyclic sesquiterpenes

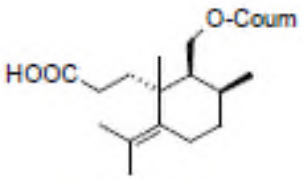
Galbanic acid is the major constituent of the resin of fetidase and galbanum.



Farnésiferol B



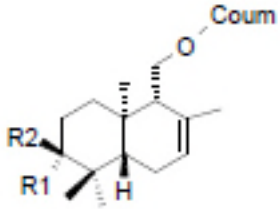
Farnésiferol C



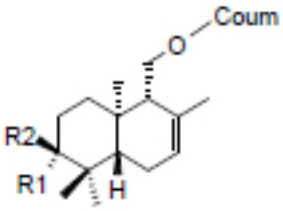
Acide galbanique

- Coumarins substituted by bicyclic sesquiterpenes

About 74 molecules were isolated from the subgenus Scorodosma, Merwia and Euferula. The sesquiterpene part of these compounds is often of the drimane type.

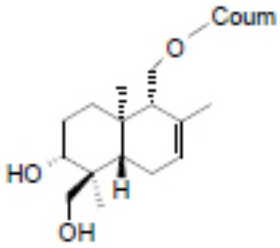


Conférone R_1, R_2= O Conférol R_1= H R_2= OH

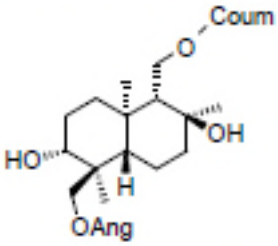


Mogotlacine R_1= H R_2= OH

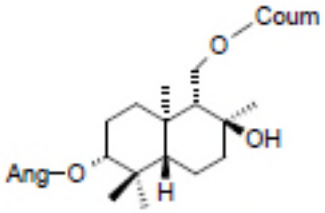
Fésélol R_1= OH R_2= H



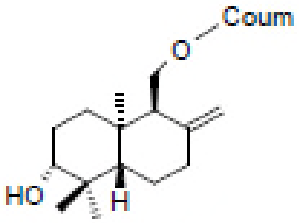
13-Hydroxyfésélol



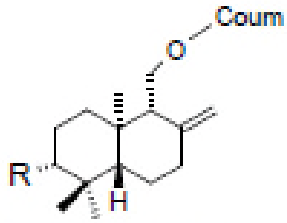
Tunétacoumarine A



Isosmarcandine

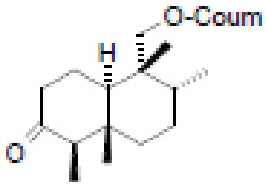


Farnésiferol A

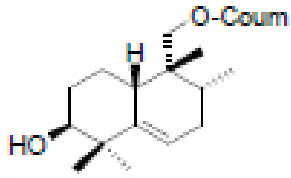


Coladine R= OAc

Coladonine R= OH



Szowistsiacoumarine A



Szowistsiacoumarine B

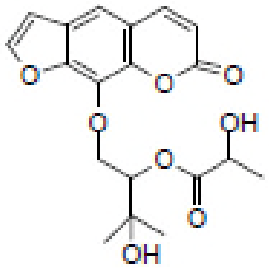
1. prenylated coumarins

Ferulenol and its derivatives or responsible for the toxicity of the species ferula.communis var breviedia which provoques the deadly hemoragic disease (ferolosis)

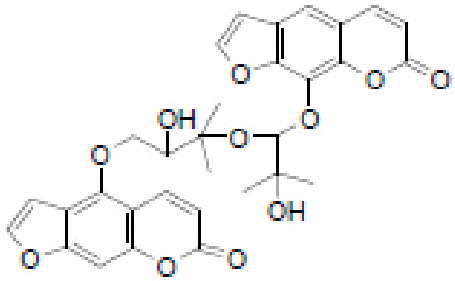
Férulénol

2. the furanocoumarins

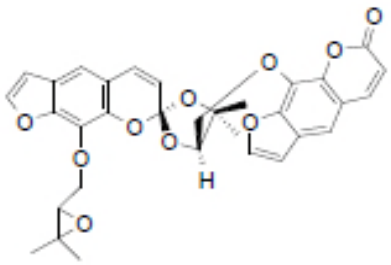
The presence of these compounds is limited to some species of ferula in particular F.sumbul which contains 21 furocoumarins



Fésumtuorine B



Fésumtuorine C



Fésumtuorine E

MATERIALS and METHODS

VI. MATERIALS and METHODS

The isolation and structure elucidation of two new sesquiterpene coumarins from a methylene chloride extract of *F. vesceritensis* have been reported Murray. **1989**; Rustin et al; **1994**. It is evident from the literature and previous investigations that the genus Ferula possesses a high biological activity Murphy. **2004** which prompts us to study the anti-cancer activity for the major isolated compound, lapiferin, from *F. vesceritensis* with different extraction methods.

On our continuation of investigation carried out on the chemical and pharmacological studies on the roots of *F. Vesceritensis* Murray. **1989**; Rustin et al; **1994**; Hofer et al; **1984**; Gamal Eldeen, Hegazy et al; **2010**. We report the specific anti-cancer activity of isolated compounds.A sesquiterpene coumarins; Ferulenol, coladin and carotene sesquiterpene designed lapiferin (10-acetoxy-6-angeloyloxy-8,9-epoxy-*trans*-caxotan-4-ol) for the first time from *F. vesceritensis* as a new natural source against human cancer cells.

1-1 Plant material

1-1-1 Classification

Branch: Spermatophytes (seed plants)
Under Spur: Angiosperms (Magnoliophyta:
flowering plants) Class: Dicotyledons
(Magnoliopsida)
Order: Caryophyllales
Family: Apiaceae
Genre: Ferula
Species: Ferula vesceritensis Coss. & Durieu ex Batt.

1-1-2 Description and tradional use

Ferula vesceritensis (Batt.), which has the synonym *F. tingitana L. var*, is an endemic plant to Algeria and Libya, where it is used extensively in traditional medicine to treat cancer and inflammatory diseases Lamnaouer et al; **1987;** Likhachev et al; **1992**. *F. vesceritensis* is indigenous to Algerian Sahara. According to ethno botanical investigation, fruit decoction is used in folk medicine to treat headaches, fever and throat infections, while the livestock avoids grazing it.

1-1-3 Collection of the plant material

F. vesceritensis roots were collected during the flowering stage in March 2011 near Biskra, approximately 300 miles southeast of Algiers, by Dr. Amar Zellagui, Department of Chemistry, Constantine University, where a voucher specimen was deposited in the herbarium of botanical department under the number (AM #112).

1-1-4 Extraction and isolation

Roots of *F. vesceritensis* (1.5Kg) were crushed and extracted with CH_2Cl_2–MeOH (1:1) at room temperature. The extract was concentrated in vacuum to yield 30 g of oily residue. The residue was fractionated on a silica gel column (6 ·120 cm) eluted with hexane, followed by a gradient of hexane–CH_2Cl_2 up to 100% CH_2Cl_2 and CH_2Cl_2–MeOH up to 15% MeOH.

After analyses on TLC using vanillin as revelator, similar fractions were gathered and subjected to further separation on Silica gel and Sephadex LH-20 columns. Main fraction 2, 3, and 4 [hexane–CH_2Cl_2 (3:1), (1:1), (1:3)] were considered owing to their terpenoids content. Main Fractions 2 and 3 were gathered and subjected to further fractionation on silica gel to afford 3 fractions.

Fraction 1:

This fraction was subjected to purification using Sephadex LH-20(2×40 cm) and eluted with n-hexane-CH_2Cl_2 (7:4) to afford 3 more sub-fractions which were in turn subjected to further purifications as follows:

Subfraction 1 was further purified through Sephadex LH-20 (1 x 30 cm) and eluting with *n*-hexane-CH_2Cl_2-MeOH (7:4:0.25) and was then purified by thin layer chromatography developed in *n*-hexane–diethylether (1:2) on a 0.2 mm aluminium sheets silica gel 60 F^{254}.To afford compound LK1(70 mg).

Subfraction 2 and 3 were purified through silica gel to yield other compounds.

Fraction 2:

This fraction was further purified by silica gel CC (2 · 40 cm) eluted with hexane–EtOAc (4:1), and then separated by TLC to afford LK8 (5 mg).

Fraction 3:

The fraction 3 was submitted to double purification through Sephadex LH-20 and eluting with *n*-hexane-CH_2Cl_2-MeOH (7:4:0.25) (1 x 30 cm) to afford compound **7**lk51 (55 mg).

1-2- Biological assays

For the *in vivo* study to evaluate the prooxidant effect of ferulenoland, an aqueous-alcoholic solution is prepared at a final dose of 50 mg / kg and 1% ethanol.

For the *in vitro* study on cultured cells, the Ferulenol is prepared in stock solution in ethanol which will be subsequently diluted in the coon's modified F12 culture medium with a final concentration of ethanol of 0.05 to 0.5 % maximum, in each well.

V1.2.1.Chemicals

The Benzo (a) pyrene used in our study was obtained from Sigma Aldrich (cat # B10080); it was solubilized in olive oil at a concentration of 50 mg / kg.

V1.2.2.Treatment of animals

V1.2.2.1. Animal species used and animal housing conditions

A total of 40 female Wistar Albino rats weighing between 200 and 250 g provided by the Pasteur Institute of Algiers (Algeria) are used in the study.

The accommodation of the animals is done in a pet shop maintained at room temperature (20 - 26° C), relative humidity: 55-60% and a day-night cycle 12h-12h. The animals are kept in opaque plastic cages at the rate of 8 rats / cage. The drink and the food composed of natural ingredients prepared commercially in the form of croquettes are served ad libitum. Rats are quarantined for 7 days prior to use.

V1.2.2.2. Induction of experimental pathology: Liver cancer

After the adaptation period of the animals, we started the treatment by chemical induction of an experimental model of liver cancer with benzopyrene. For this, a dose of 50 mg / kg conveyed in 1ml of olive oil of B (a) P was administered to the animals by intraperitoneal (IP) route Sinha **1972.**

V1.2.2.3. Treatment of animals with Ferulenol and anti-cancer drugs

For the study of the effect of ferula roots methanol extract, the animals were also divided into 5 groups (8 rats per group)

Group 1: Controls (C): served as a control by receiving a dose unique IP of 0.5 ml of olive oil

Group 2: Benzo [a] pyrene (B): animals receiving a single injection of a dose of 50 mg / kg B (a) P dissolved in 0.5 ml of olive oil IP.

Group 3: Ferulenol + Benzo [a] pyrene (BP) animals receiving ferulenol at a dose of 50 mg / kg orally for 5 days and the B (a) P 50 mg / kg given on the 6^{th} day IP channel.

The use of 50 mg /kg as a dose is based on the fact that this falls in the range of effectiveness since beyond that the dose could be toxic and bellow thet it is going to be ineffective. Our objective was to keep the animals alive and test the mechanisms of actions of the tested compounds.

Group 4: Benzo [a] pyrene + anticancer combination (BA): Animals receiving B (a) P at a dose of 50 mg / kg ip. After 16 weeks, a chemotherapy regimen consisting of a combination of 5-fluorouracil (5 FU) at 15 mg / kg + cyclophosphamide (cyclo) 15 mg / kg + epirubicin (epi) at 2 mg / kg was administered to the animals. .

Group 5: Benzo [a] pyrene + ferulenol + anticancer combination (BPA): Animals receiving B (a) P at a dose of 50 mg / kg ip, after 16 weeks (period required for liver tumor development in rats), the animals received 1 ml of ferulenol solution orally corresponding to the dose of 50 mg / kg for 5 days. The combination of 5 FU (15mg / kg) + cyclo (15mg / kg) + ear (2mg / kg) was administered on the 6th day.

The dose for ferulenol was chosen to be 50 mg/Kg to avoid the toxicity of experimental animals and inefficacity.

The drugs chosen for the study are those used clinically and at the same doses in the treatment of breast and liver cancer; it is the FEC protocol (Fluorouracil-Epirubicin-Cyclophosphamide)

5-Fluorouracil: 600 mg / m^2 corresponding to 15 mg / kg

Epirubicin: 80 mg / m^2 corresponding to 15 mg / kg

Cyclophosphamide: 600 mg / m^2 corresponding to 15 mg / kg.

VI.3. Collection of biological tissues and fluids

Before sacrifice of the animals, blood is collected in heparinized tubes by puncture of the retro-orbital sinus. After centrifugation at 3300 rpm for 10 min, the serum is collected and aliquoted and frozen at -20 °C for biochemical and enzymatic analyzes.

The animals were sacrificed after 20 weeks of the beginning of induction of the pathology by Benzo (a) pyrene (sufficient time for the development of bronchopulmonary cancer).

At the time of sacrifice, the liver of each rat were removed, washed in 9% NaCl solution, weighed, and examined macroscopically for signs of inflammation or cancer. Then each liver was divided into three fractions:

- A part was stored at -20 ° C for tissue assays of oxidative stress;
- A second part of the liver was immersed in the fixative fluid (10% formalin) for histopathological evaluation;
- The last part was used for protein extraction.

VI.3.1. In vitro study

V1.3.1.1. Measurement of stress on isolated mitochondria

Isolation of liver mitochondria

All stages of mitochondrial isolation are made on contact with ice.

The liver is surgically collected after sacrifice of the animal, 1 g is weighed and quickly put in a beaker then rinsed with homogenization buffer then cut into pieces and thoroughly rinsed again to remove a maximum of blood. The pieces of the liver are then finely ground in a DOUNCE potter (KONTES, Glass company an ISO-9001 streered firm, New Jersy USA) with 4 ml of the homogenization buffer. After homogenization, the mitochondria are isolated by a standard differential centrifugation technique with a Sigma 3-16 K centrifuge as follows: The recovered homogenate is centrifuged a first time at 2000 xg for 10 min at 4 ° C to remove unwanted cell debris. The supernatant is recovered and centrifuged at 17,500 xg for 10 min

and its pellet is resuspended in 1.5 ml of homogenization buffer and then centrifuged for 10 minutes at 600 xg for maximum mitochondria. The supernatant containing the mitochondria is centrifuged at 19,000 xg at 4 °C for 10 minutes.

The supernatant is then removed and the surface of the pellet is rinsed with the buffer (5 ml). After wards, this pellet is then taken up with 1 ml of the buffer and then centrifuged twice at 19,000 xg before obtaining the final mitochondrial suspension. The mitochondrial solution is immediately placed in ice at 4 ° C for the measurement of superoxide anion and mitochondrial swelling after Bradford's protein assay Chacon et al; **1991**.

V1.3.1.2. Protein assay by the method of Bradford (1976)

The total protein concentration of the samples is determined by the technique developed by Bradford, Bradford. **1976**. This method consists of a colorimetric assay using Coomassie blue which has the property of adsorbing on proteins in a non-specific manner and independently of their sequence. This adsorption is accompanied by a modification of the absorption spectrum of the molecule with a blue shift. The change in absorbance is proportional to the protein concentration in the sample.

100 ul of the suspension or the cytosolic fraction diluted$^{1/10}$ with distilled water (100 ul BSA for the standard range) are added to 4 ml of the Coomassie Brilliant Blue reagent (BBC). After stirring and incubation at room temperature for 5 min, the reading of the absorbance is carried out at 595 nm. The protein concentration expressed in grams is deduced from the calibration curve produced under the same conditions by BSA (Bovine Serum Albumin)

V1.3.1.3.Measurement of O_2^- productionby mitochondria

The superoxide anion is the main free radical generated by mitochondria, particularly by complex I and III of the respiratory chain. Its generation was evaluated by measuring the reduction of nitrobluetetrazolium (NBT^{+2}) by the superoxide anion to monoformazane, which absorbs at 560 nm.

Generation of the superoxide anion was evaluated according to the method of Ohkawa et al; **1979**. The mitochondrial suspension used (1 mg of protein / ml) in a final volume of 1.2 ml of reaction mixture (25 ° C) containing 1 µM CsA, 100 µM NBT in the incubation buffer (250 mM sucrose, 5 mM KH_2PO_4, pH = 7.4) supplemented with 1 µM of rotenone.

The reaction is triggered by the addition of (6 mM) succinate and the nitroblurtetrazolium reduction rate was measured at 560 nm.

V1.3.1.4.Demonstration of PTP opening: mitochondrial swelling: Mitochondrial

Swelling is an indicator of the permeability of the mitochondrial membrane, resulting from a change in osmotic equilibrium caused by the opening of the PTP which leads to entry of solutes into the mitochondria. The swelling is evaluated by means of a spectrophotometer by measuring at 540 nm the decrease of the absorption of the mitochondrial suspension

The demonstration of the mitochondrial swelling *in vitro* is carried out on suspended mitochondria according to the method Bridger. **1998;** Kroemer et al; **1998** .

The system contains 1 mg / ml of suspended mitochondria in a final volume of 1.8 ml of breathable buffer supplemented with rotenone at 1 µM (to prohibit the reverse electronic transfer of complex II to complex I) and 6 mM succinate. After one minute of incubation 2.25 µl of 25 µM calcium are added to induce swelling. The decrease in absorbance at 540 nm is recorded every minute for 5 minutes.

VI.3.1.5. Evaluation of antioxidant activities in vitro

V1.3.1.5.1.Determination of Superoxide Dismutase (SOD) Activity

This test was performed according to Beauchamp et al; **1971**

For the measurement of Superoxide Dimutase (SOD), knowing that SOD is a very important antioxidant enzyme that maintains a low concentration of the superoxide anion. It catalyzes the dismutation of superoxide anion of oxygen into hydrogen peroxide and oxyen according to the following reaction:

$$2O_2^{-\circ} + 2H^{+}\mathbf{SOD} \longrightarrow O_2 + H_2O_2$$

The enzymatic activity of SOD is determined according to the method of Friedrich et al; (1971) (274). This enzymatic activity depends on the ability of the enzyme to inhibit the reduction of nitro tetrazolium (NBT) by the superoxide anion upon the photoreaction of oxygen and riboflavin in the presence of an electron donner such as methionine according to the following reaction:

Riboflavin + O_2 ⟶ photoreaction Superoxide

Super oxide + NBT ⟶ Formazan

The reduction of NBT by the superoxide anion to formazan was monitored at 560 nm. A unit of SOD activity is defined as the quantity of the enzyme necessary for the inhibition of the reduction of NBT by 50%.

% inhibition = [(control abs - abs of the test) / control abs]× 100

SOD IU / mg protein = % inhibition × 6.35

The reaction mixture contains 2 ml of reaction medium (10^{-2} M sodium cyanide, NBT solution 1.76 x 10^{-4} M, EDTA 66 x 10^{-3} M and riboflavin 2 x 10^{-6} M), 5 µl of the enzymatic source (cytosol or mitochondrial matrix) and 5µl of extract to be tested at the different concentrations. The mixture was irradiated with a 15 watt lamp for 10 minutes.

The control is prepared under the same conditions but without an enzymatic source. The absorbance was measured at 560 nm and the values were expressed in IU / g protein.

V1.3.1.5.2.Determination of Catalase Activity (CAT)

It is an antioxidant enzyme that exists in high concentration in the liver and red blood cells and acts synergistically with SOD.

Removal of Nitrogen Peroxide to Water and Molecular Oxygen According to the Following Reaction: H_2O_2 **CAT** $H_2O + O_2$

Catalase activity was determined by adopting the (Clairbone 1985) method as described in (p275) Claiborne. **1985.** The reaction principle is based on the disappearance of H_2O_2 in the presence of an enzymatic source at 25 °C in brief the measuring cuve contains 1 ml of phosphate buffer (0.1 M KH_2PO_4, pH 7.2), 0.950 ml of hydrogen peroxide (0.019 M), 0.025 ml of enzymatic source and 0.025 ml of different concentrations of the test extract. The reading of the optical density (OD) is performed at 560 nm every minute for 2 minutes. The enzymatic activity (EA) is expressed in IU / g protein according to the following relationship:

IU / g of protein = (- 2.3033 / T. Log A_1 / A_2) / g of protein.

Knowing that:

A1: Absorbance at time 0 min.

A2: Absorbance after 1 min.

T: Time interval in mn. 3.

V1.3.1.5.3.Measurement of Cytosolic Glutathione S-Transferase (GST)

The activity of GST was determined according to the method described by Habig et al; **1974**. Whose principle is based on the formation of a complex between the GST artificial substrate, 1-chloro-2,4-dinitrobenzene (CDNB) and glutathione (GSH) as cofactor, in the presence of the enzyme. The absorbance of the colored complex is detected by spectrophotometer at 340 nm.

For the assay, we used a reaction mixture which contains 1700 µl of phosphate buffer (0.1M, pH 6.5) and 100 µl of CDNB (20 mM). After incubation of the reaction mixture at 37 °C for 10 min, the reaction is started by the addition of 100 µl of the homogenate and 100 µl of glutathione (20 mM).

The variation in the optical density due to the appearance of the GST-CDNB complex is read at 340 nm every minute for 5 min.

The activity of the GST is expressed per µmol of conjugated CDNB / min / mg protein, using the following calculation:

$$\text{IU / ml of enzyme} = (\Delta A_{340}/\text{ min Test} - \Delta A_{340}/\text{ min White}) / (Vt)\ (Fd)$$

(9,6) (Vez)

Vt: total volume (ml) of the test;

Df: dilution factor;

9.6: millimolar extinction coefficient of conjugated glutathione-1-chloro-2,4-dinitrobenzene at 340 nm;

IU/ ml of enzyme = µmol substrat/min.ml enzyme

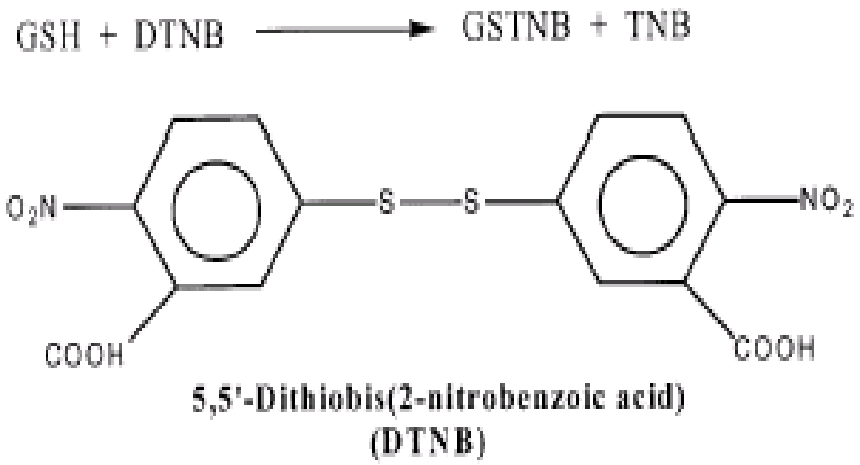
Vez: volume (ml) of the enzyme used

mg of protein/ml of enzyme

IU/ mg protein = µmol product/min.mg protein

VI.4. Non Enzymatic Antioxidant Capacity Measurement: Glutathione (GSH)

Glutathione is a tripeptide composed of three amino acids: glutamic acid, cysteine and glycine. It is present in all animal cells at concentrations ranging from 0.5 to 10 mM and in the order of µM in plasma. The mitochondria contain about 10-12% of cellular glutathione (67). For the GSH assay, we used the Ellman colorimetric method (p 276) Ellman. **1959**. with the DTNB reagent (5,5'-Dithiobis 2-nitrobenzoic acid). The principle of the reaction consists in the oxidation of GSH by DTNB, which releases the thionitrobenzoic acid (TNB) which has an absorption at 412 nm according to the following reaction:



5,5'-Dithiobis(2-nitrobenzoic acid)
(DTNB)

For this, 1 g of liver is cut and homogenized with 3 volumes of trichloroacetic acid TCA (5%) using a DOUNCE mill. Homogenized and centrifuged at 2000 rpm, 50 µl of supernatant are then diluted in 10 ml of phosphate buffer (0.1 M, pH = 8). 20 µl of the DTNB (0.01 M) are added to 3 ml of the dilution mixture. After 15 min of incubation of the optical density at 412 nm with a white prepared under the same conditions with TCA (5%). The GSH level is deduced from a standard range of glutathione under the same conditions as the assay and the concentrations are expressed in millimoles of glutathione per gram of liver.

VI.5. Cellular Oxidative Damage Measurement: MDA Assay

The method of Okhawa et al. 1979 (p 277), Ohkawa et al; **1979**. is used for this assay. The assay principle is as follows: The MDA reacts with two molecules of TBA (thiobarbituric acid) in an acidic medium (pH 2-3) and at 100 ° C hot to give an absorbent pink colored pigment at 530 nm and extractable by organic solvents such as n-butanol. For this assay we used 1g of liver supplemented with 3ml of KCL solution (1.15%) and then ground by a homogenizer of DOUNCE (KONTES .Verre management company ISO-9001 New Jersey USA). 0.5 ml of the homogenate, was added to 0.5 ml of 20% trichloroacetic acid and 1 ml of 0.67% thiobarbituric acid (TBA). The mixture is heated at 100 ° C. for 15 minutes, cooled and then 4 ml of n-butanol are added. After centrifugation for 15 minutes at 3000 rpm, the optical density was read over the supernatant at 530 nm. The level of MDA is deduced from a standard range under the same conditions using a solution of tetraetoxypropane (PET) which gives MDA after their hydrolysis.

The measurement of malondialdehyde (MDA) can be performed globally by reaction with thiobarbituric acid known as thiobarbituric acid reactive substances (TBARS). For that, the spectrophotometric method of Zini et al; 1999 is used, the principle of which is as follows: in acidic medium and at 95-100 ° C each molecule of malondialdehyde (MDA) resulting from lipoperoxidation reacts with two molecules of thiobarbituric acid (TBA) leading to the formation of a pink complex (MDA-TBA) whose absorbance will be measured at 530 nm.

0.5 ml of 20% TCA and 1 ml of TBA (0.67% in 50 mM NaOH) are added to 0.5 ml of homogenate obtained by grinding the liver tissue in KCl (150 mM) to volume (weight / volume) using a DOUNCE homogenizer. Subsequently the mixture is placed in the water bath for 15 minutes at 100 ° C, cooled with tap water to stop the reaction and added 2 ml of n-butanol. The whole is centrifuged for 15 minutes at 3000 rpm.

After centrifugation is complete, the supernatant is recovered for measuring the optical density at a wavelength of 530 nm against a blank made of 0.5 ml of distilled water treated in the same way. Absorbances were converted to MDA equivalents in nmol / mg protein using a TMP solution (its hydrolysis gives MDA).

VI. 6. Preparation of PEG liposomes Pegylated liposomes:

Composed with HEPC (Hydrogenated Egg Yolk Phosphatidylcholine), CHOL (Cholesterol) and DSPE-PEG2000 (Methoxypolyetheleneglycol (Mw 2000)-

distearylphosphatidylethanolamine) (185:1.00:015, molar ratio), were prepared as previously described by Zucker et al; **2009.** Briefly, the lipids were dissolved in chloroform, and after evaporation of the organic solvent with rotary evaporator (Bu¨chi Rotavapor R-215, Switzerland) under reduced pressure at room temperature. The resulting lipid film was hydrated at 70 C in 250 mM ammonium sulfate (pH5.4) to form multilamellar vesicles (MLV). The liposome size was reduced by stepwise extrusion in two steps, firstly through 0.2 lm and then through 0.1 lm pore-diameter polycarbonate filters (Whatman, Maidstone, Kent, UK). Each extrusion step was performed 5–10 times at 70 C using a high-pressure extruder (Northern Lipids, Inc. Burnaby, BC, Canada). The mean diameters of the resulting liposomes were determined by photoncorrelation spectroscopy (PCS) on a Malvern Zetasizer (Zetasizer Nano ZS Zen 3600, Malvern instruments, UK) at 25 C, DOX loading

DOX (Chemos GmbH, Germany) drug was loaded into preformed liposomes via a transmembrane pH gradient method and the free DOX was removed by gel filtration. Briefly, the liposomal suspension, prepared as described above, was passed through a Sephadex G25 gel-filtration column (PD-10 Desalting Columns, GE Healthcare Ltd, Sweden) pre-equilibrated by PBS pH7.4 to remove the ammonium sulfate solution. Then, a solution of DOX (10 mg/mL) was added at pH 7.4 to create a transmembrane PH gradient (drug to lipid ratio 1/20 w/w). This suspension was stirred for 1 h at 60 C. Subsequently, unloaded DOX was removed by Sephadex G25 column preequilibrated with PBS pH7.4. The amount of encapsulated DOX in PEG liposomes (DOXEnc) was determined by measuring absorption at 483 nm using UV–visible spectrophotometer (Uvikon 930, Kontron Instruments, Germany) after lysis of the liposomeswith 10% SDS (w/v) Mayer et al; **1990.**Drug encapsulation efficiency (EE%) was calculated as follows: EE% ¼ DOXEnc DOXTot 100

VI.7. DOX releas

The chemical stability of the entrapped drug was investigated by recording the UV–visible absorbance spectra of free and encapsulated DOX. (Alyene **2015).**

VII. Cell Cultures and cytotoxicity tests

VII.1. Cell cultures

Cell culture maintaining and cell culture medium preparation:

Coon’s FAO modified / F12 preparation incomplete.

Following sigma's instructions, 90% less of final required volume has been measured at room temperature, autoclaved, water was added to the mixiture.The powdered medium was gently stirred dissolved, then 2,68 g of sodium bicarbonate was added with gentle stirring to 1L of the final volume of the medium being prepared until dissolved (but not over mixed).

While stirring, the pH adjusted to 7.15 with pH meter using HCl (with the intention that the final pH will be 7,4, since it may rise during filtration and incubation. Next, additional autoclaved water was added to bring the solution to the final volume, gently stirred, and then immediately sterilized by filtration using membrane filters (Millipore express).

Pus and TRP 99500 0,22 μm

0,2 μm PES.

All the steps of medium's preparation have been done under the laminar flow hood using clean glass wave and autoclaved water. Then, the medium solution was stored in the lower part of filtration unit.

According to sigma instructions, the supplements FBS and PBS could be added prior to filtration or aseptically at the end; in preference they were added just before use (to avoid Pen-/stryp, Penicillin / streptomycin degradation!).

PBS already made in the laboratory tubes taken from the freezer.

100% PS (penicillin – stryptomycine)

10% FBS (Fetus Bovine Serum)

- Bringing up FAO cells from frozen state

FAO cells transported from sigma on ice. Immediately, the box was open to thaw cells - By

Putting the vial containing the cells at 37°C in water – bath without allowing the vials to immerse in water. The vials remained in water for a few minutes. Under the laminar flow hood a sterile plastic Flask T – 75 has been prepared for FAO cells transfer (as thawed) from sigma, by putting 15 ml of coon's ham modified / F12 pre – prepared and warmed up at 37°C for ~ 24 hrs.

T – 75 Flask containing FAO cells in 15 ml coo's ham modified / F12 medium, has been put in the incubator 5% CO_2, 37°C. After checking the cells under the inverted contrast phase microscopy, the cells looked good. Later, checking for cells attachment to the surface of the plastic T – 75 Flask.

Taking out the T – 75 Flask, gently Checking the FAO cells attachment to the surface of the Flask under the microscope – IF poorly attached – removal of the medium (the old medium) and added ~ 15 ml of Fresh coon's modified / F12 medium – transfer the cells into 50 ml conical centrifuge tube (all work under Laminar Flow Hood).

Centrifuge at 300 × g for 5 min the supernatant was carefully removed put into T - 25 Flask (to see whether there is any cells that may attach). Then the pellet resuspended in 15 ml Fresh Coon's / F12 medium, back to T – 75 Cm^2 Flask. After microscopic Check, the Flask has been put back into the incubator 5% CO_2, temperature 37°C till confluency.

Note : The centrifugation step and resuspenssion of the pellet – to remove the DMSO from the cell preparation and replace it with water since DMSO is toxic to cells but replaces water "during freezing", which expands when cells freeze and causes them to burst.

FAO cells counting + medium change counting cells after different times of incubation.

- A hemocytometer
- Hand counter
- Microscope inverted contrast phase
- Cell suspension (1plate) – 48 hrs incubation
- Plastic Pasteur pipettes
- Micropipettes p20, p200, p1000
- Trypan blue, PBS, Trypsin, EDTA

Procedure

- Medium removal from the wells (one well at the time)
- Addition of FBS – washing wells and loosing the action of trypsin 2 ml each
- Addition of trypsin (1 ml) per well

Covering the well surface – put the plate in the gallenic incubator for 3 min. Then taken out – removal of the plate.

Microscopic observation to see the cells are off the bottom of the well. The bottom of the well mixed with plastic Pasteur pipette; 20 ml taken to load the hemocytometer or both edges.

Observation under the inverted microscope and counting cells.

1 plate (3 wells) :

1) $4{,}25 \times 10^5$ cell / ml trypsil
2) $4{,}2 \times 10^5$ cell / ml trypsil
3) $5{,}75 \times 10^5$ cell / ml trypsin

Again, tried to complete cell – counting to establish the cell growth curve (Growth rate us time) and determine the subling time.

Again –

Refreezing of one ampoule of FAO cell, the latter put into 18 ml milieu "complete" + 2 ml serum $\Longrightarrow$ split into 2 ×25 cm^2

In Coon's modified F12 milieu "complete "composition – added 20 ml of PBS and one tube of penicillin / streptomycin to 400 ml milieu.

Refreeze cells, trypsinization, counting

$14{,}8 \times 10^5$ d /ml – 3ml

Split into 3 × 75 Cm^2 – 1ml cell + 14 ml milieu

After harvesting cells, trypsinization, get to counting cells.

Counting or preparing a suspenssion of $7{,}64 \times 10^4$ cell / ml

VII.2. Cytotoxic effects of Ferulenol, Coladin

Preparing two plates with 48 wells

(8 × 6) with 180 µl = 13,700 d

Addition of the products over the plates

Dilution of LK1 (Ferulenol)

Solution in ethanol at concentration of 3,984 M – dilution at 1M (LK8 Coladin) 251 µl + 749 µl ethanol

Then at 200 µM, 200 µl + 800 µl ethanol three dilutions × 10 in series in complete culture milieu to get 200 µM

The same schema of dilution for the crude product LK1 (Ferulenol absolute ethanol diluted 0,1% × 10 three times.

Doxorubicin

Solution taken from the refrigerator at 1,985 mg / ml with a concentration of 3,423 mM (PM DOX - HCl = 579, 98).

Dilution 200µl + 3212 µl complete milieu

To get 200 µM

After that filtration using 0,22µM

Addition of 10 µl of each final dilution completed is necessary with 10 µl milieu in order to get 10 µl or equivalent.

Plate :

Col 3 . B – G 20 µl milieu control

Col 4 . B – G 10 µl EtOH Ab + 20 µl milieu Control EtOH (CEtOH) +Exp. Prep. Exp –

preparation

Col 5. B – G 10 µl 0,1% EtOH + 20 µl milieu Control EtOH

Col 5. B – G 10 µl 0,1 % EtOH Ab + 20 µl milieu Control EtOH

Col 6. B – G 10 µl Doxo + 20 µl milieu Doxo

Col 7. B – G 10 µl LK_1 + 10 µl milieu LK_1

Col 8. B – G 10 µl K_3 + 10 µl milieu K_3

Col 9. B – G 10 µl Doxo + 10 µl LK_1 LK_1 + Doxo

Col 10. B – G 10 µl Doxo + 10 µl K_3 LK8 (K_3) + Doxo

Incubation of the plate for 24 hours and 48 hours

After incubation of 20 µl MTT at 5 mg / ml (PBS, filtrated using 0,22 µM) in each well of the plate. Incubation for 3 hours, removal of the milieu and addition of DMSO (volume variable due to defective pipette).

Reading with filter pass haute 570 nm

After 48 hours of incubation

Addition of MTT in the second plate, incubation for 4 hours

Removal of the milieu and addition of 200 µl DMSO

Reading with filter 570 nm "reproducible recent"

Estimation of doxorubicin lost during filtration

At 200 µM in PBS, filtration of one Part – Reading of 6 wells of each with the filter 'Pass – all '492 nm.

Observation of wells with inverted microscopy – presence of particles of doxo non – filtrated, so:

OD: - Moyenne or average

Before filtration: 0,632 ± 0,007

- Average after filtration: 0, 170 ± 0,002

Lost during filtration = 73%

True Doxorubicin = 2,7 µM concentration

B16F1 cells, a metastasic sub-line of B16 murine melanoma pulmonary cells (d-Dr M Gregoire, INSERM U419, Nantes, France) were cultivated in RPMI 1640 medium, supplemented with 5% foetal calf serum (FCS) in 25 cm^2 flasks (Nunclon, VWR Int, Strasbourg, France) in a humid atmosphere containing 5% CO_2.

UACC-903 cells, a melanoma cell line (Dr J Trent, Phoenix, Ariz.) were cultivated in DMEM medium containing 4.5 g/l glucose, supplemented with 5% FCS in 25 cm^2 flasks (Nunclon) in a moist atmosphere containing 5% CO_2.

B16F1 cells (10,000 cells per well in 96-well plates) were seeded in growth medium for 24 h and cultured at 37°C and 5% CO_2. Ferulenol at concentrations of 10 µM in 0.005% ethanol and added to the cells for 30 min, 2 and 4 h. Cell viability was determined with the WST-1 test using a Dynatech MR 4000 plate reader. All measurements were carried out in triplicate. The WST-1 test is based on the cleavage of tetrazolium salts to formazan by cellular enzymes. An expansion in the number of viable cells resulted in an increase in the overall activity of mitochondrial dehydrogenases in the sample. This augmentation in enzyme activity has led to an increase in the amount of formazan dye formed. This has directly correlated to the number of metabolically active cells in the culture.

B16F1 cells, were a generous gift from Dr. F Antonocelli (Unité Mixte de Recherche 6198, Institut Fédératif de Recherche 53 Biomolécules, Université de Reims Champagne-Ardenne, 51 rue Cognacq Jay, F-51095 Reims Cedex, France). They were grown in RPMI 1640 supplemented with 5% fetal bovine serum in 25-cm^2 flasks (Nunclon, Dutscher, Brumath, France) at 37°C in a humid atmosphere (5% CO_2, 95% air).

FAO cells (FAO) were obtained from rat hepatoma (Sigma Aldrich, France), a differentiated cell line derived from H4-11-E-C3 (ECACC catalog number 85061112). The cytotoxicity of ferulenol, lapiferin and coladin was estimated by the MTT assay. Cells were incubated with increasing concentration of each compound and then exposed for 48h in a 5% CO_2 incubator at 37°C. After incubation, 20 µl MTT (Sigma) (5mg/mL) solution was added in all wells, except in one well that served as blank, and incubated at 37°C for 4 h. Finally, the medium was removed and formazan salt crystals were dissolved by addition of 200µl of dimethylsulfoxide (Biobasic INC) to all wells. Plates were then analyzed in an ELISA plate reader (Labsystems multiskan RS-232C, Finland) at 570nm. Cell viability was defined relative to untreated control cells as follows:

Subculture: Split at 70-80% confluency, approx. 1:3 to 1:6 (2-4 × 10^4 cells/cm^2) Trypsinize using 0.25% solution, with or without EDTA, 37 °C and 5% CO_2.

Culture medium: Coon′s Modified Ham′s F12 + 2 mM L-Glutamine + 10% FBS or Kaign′s Modified Ham′s F12 + 2 mM L-Glutamine + 45 mg/L ascorbic acid + 18 mg/L myo-inositol + 10% FBS.

Statistical analysis : t – student has been used.

Results

VIII. Results (Toxicopharmacological study)

VIII. 1. Structural analysis

The structure of three compounds was confirmed by 1H NMR spectrum (250 MHz, CDCl3) and higher resolution chemical ionization mass spectrum analysis.

Ferulenol (LK1): The structure of this compound was in agreement with the molecular formula ($C_{24}H_{30}O_3$) which was previously reported from Ferula species Willet et al; **2000;** Bradford. **1976;** Morin et al; **2001**and *F. vesceritensis roots* Elimadi et al. **1998**.

Coladin (LK 8): Coladin was previously isolated from *F. vesceritensis* roots and its structure was confirmed by comparing its NMR data with those reported in literature Murray. **1989**.

Lapiferin (LK 51): This compound was also reported from *F. vesceritensis* roots and its structure was in agreement with those reported in literature.Gamal Eldeen et al; **2010.**

The structures of identified compounds are presented in figure 1

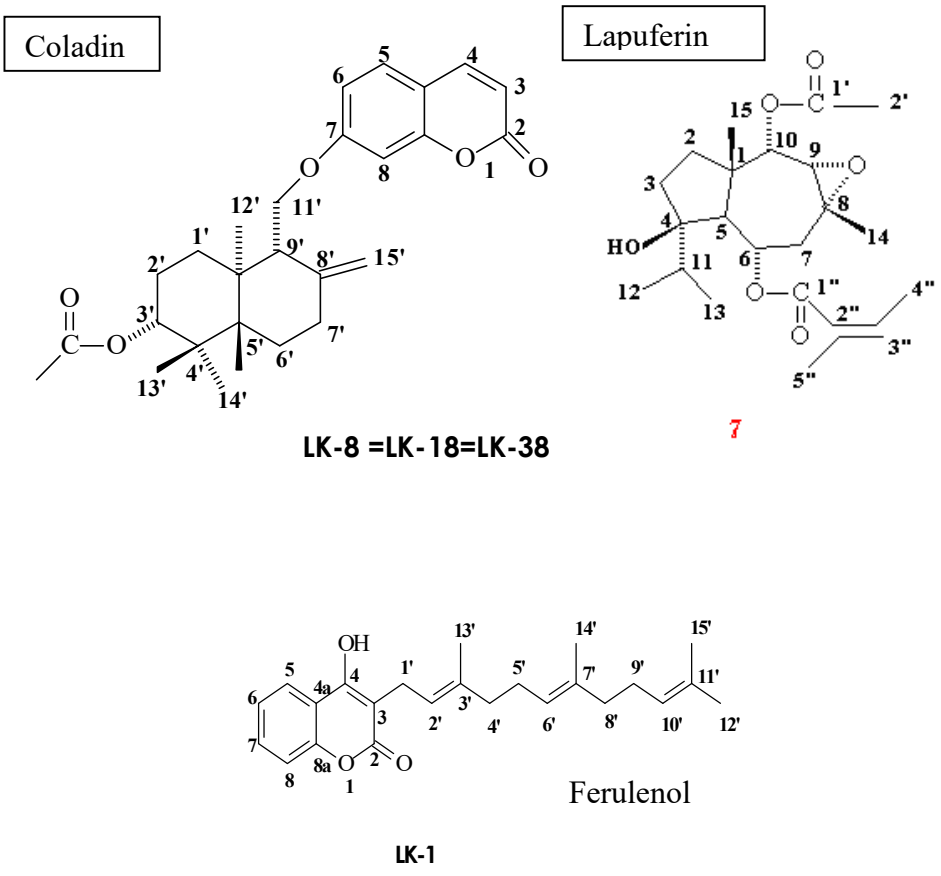


Figure 14: Chemical structures of compounds

VIII.2. Effect of Ferulenol of (Ferula roots) over mitochondrial swelling (Ferulenol modulates mitochondrial swelling)

We first studied the effect of ferulenol on mitochondrial swelling which is the consequence of an increase in membrane permeability. In another set of experiments, we explored the protocol described by Kobayashi. **2003** who has induced PTP by means of the addition of an uncoupler to mitochondria that has accumulated Ca^{2+} load became unable to induce PTP per se. This effect translates the fact that the pore could be opened by depolarization. Surprisingly, in these experimental conditions ferulenol did not inhibit mitochondrial swelling. On the contrary it was able to promote swelling when it was added instead of the well-known uncoupling agent carbonyl cyanide m-chlorophenylhydrazone (CCCP, Figure. 14*C*). This effect was concentration-dependent and was inhibited by CsA confirming the involvement of PTP in the swelling process. However, this result was not at variance with the inhibitory effect observed in Figure 14A. This could be explained by the fact that ferulenol could have been prevented the matrix mitochondrial Ca^{2+} accumulation which was required for swelling in this model. As ferulenol did not show any effect on mitochondrial calcium flux (not shown). This raised the possibility that ferulenol might act as an uncoupling agent.

Furthermore, ferulenol added at increasing concentrations along with carbonyl cyanide chlorophenyl hydrazone (CCCP) to isolated rat live has allowed mitochondrial membrane potential to reestablish as shown in Figure 14A. However, the rate of hydrogen superoxide production was declined as a result of ferulenol inhibition (figure 14B). This possibly has had a link with the mitochondrial antioxidant defense system that has possibly had prevented the oxidant cascade reactions.

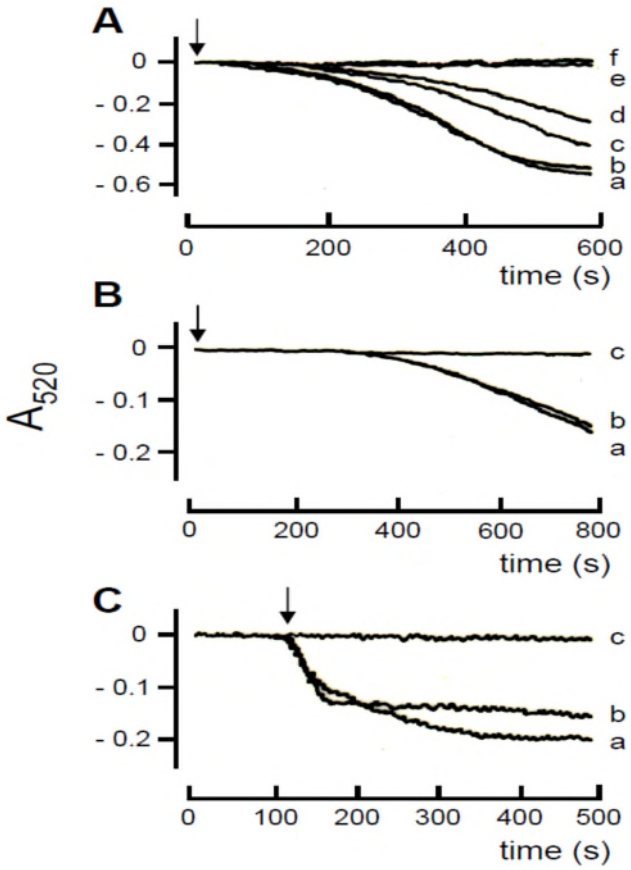


Figure 15: Effect of ferulenol on mitochondrial swelling.

A: ferulenol inhibited Ca^{2+} induced mitochondrial swelling under energized conditions.

Livermitochondrial1 mg/ml were preincubated for 2 min in a medium containing 250 mM sucrose, 5 mM KH_2PO_4, including 2 µM rotenone and 6mM succinate, pH 7.4 at 25°C, in the absebce (a) or in the presence of either increasing concentrations of ferulenol (0.1 µM (b), 1 µM (c), 10 (d), 100 (e)) or 1 µM CsA (f). Then, swelling was induced by the addition of 25 µM Ca^{2+} (arrow).

B: Mitochondria were preincubated for 6 min in a buffer containing 150 mM sucrose, 5 mM Tris-HCl, 2 µM rotenone, 1 µM antimycin A and 100 µM Ca^{2+}, pH=7.4 at 25°C, in the absence (a) or in the presence of either 100 µM ferulenol (b) or 1 µM CsA (c). Then, swelling was induced by the addition of 10 µM of tert-butylhydroperoxide (arrow).

C: ferulenol induced mitochondrial swelling. Liver mitochondrial (1 mg/ml), energized with succinate (6mM), were incubated in a medium containing 250 mM sucrose, 10 mM Tris pH=7.4, 1 mM KH_2PO_4, 20 µM EGTA-Tris, 2 µM rotenone and 1 mg/ml oligomycin.

After 1 min incubation, a pulse of 25 µM Ca^{2+} was added. Two minutes later, swelling was induced (arrow) by either 1 µM CCCP (line a) or 10 µM ferulenol (line b). 1 µM CsA (line c) inhibited the effect of ferulenol.

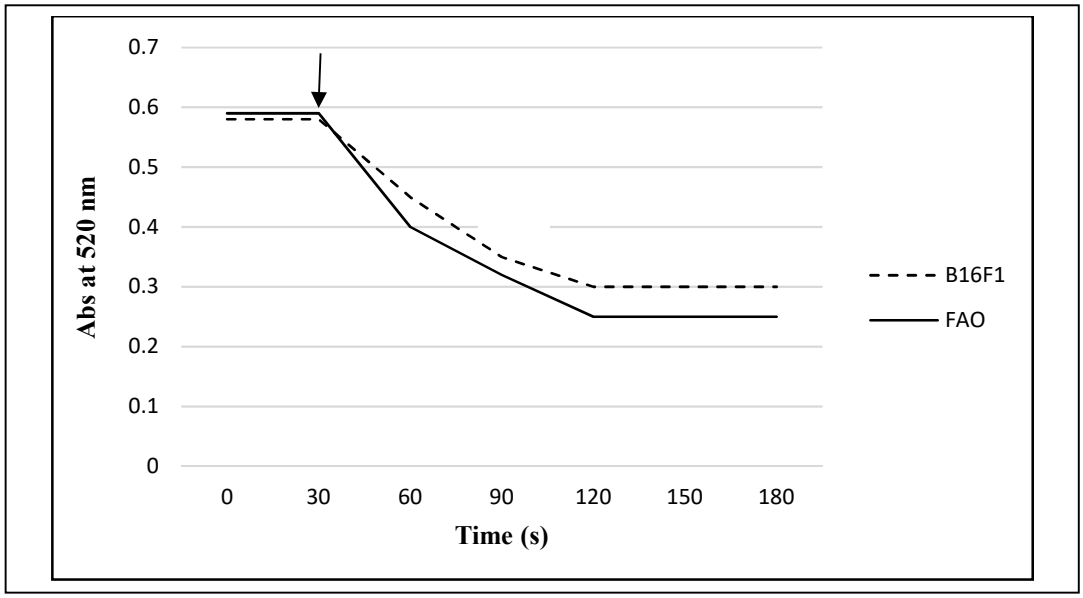


Figure 16 : Effect of coladin on mitochondrial swelling.

Coladin induced mitochondrial swelling. After 1 min incubation, a pulse of 25 µM Ca^{2+} was added. Two minutes later, swelling was induced (arrow) by either 1 µM coladin (line b). 1 µM CsA (line c) inhibited the effect of coladin.

VIII.3. Effects of ferulenol (Ferula roots) over mitochondrial superoxide anion production

The effect of "different doses" of ferulenol (50 mg/kg) was examined using enzymes involved in the antioxidant function, glutathione content and lipid peroxidation in the liver of wister albino rats (16 weeks old) after 12 and 24hrs). Significant changes were observed in the rat liver. The specific activities of superoxide dismutase (SOD) catalase (CAT), glutathione – S – transferase (GST), reduced glutathione content (GSH) content, were found to variably decrease in the liver. The mode and magnitude of change in the specific activities was seen to depend on dose and time of ferulenol administration. Ferulenol also decreased the reduced glutathione content and enhanced the lipid peroxidation in the liver.

An important role of ferulenol in "liver" cancer cells is further supported by the enhancement of lipid peroxidation status as a result; the generated free radicals which are significant factor associated with cancer and other diseases.

The results concerning the production of superoxide anion by mitochondria isolated from rat liver and treated with different concentrations of ferulenol are summarized in the figure 16. Comparing these results with the control, it is found that the ferulenol at different concentrations 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M 10^{-9}M induces the production of the anion superoxide, ranging from a higher percentage of an anion production at 10^{-4} M to a lower percentage of anion formation at 10^{-9} M. This increase is concentration dependent, which reflects the toxicity of the ferulenol at different concentrations and, therefore, clearly shows the pro-oxidant effect of ferulenol at both higher and lower concentrations.

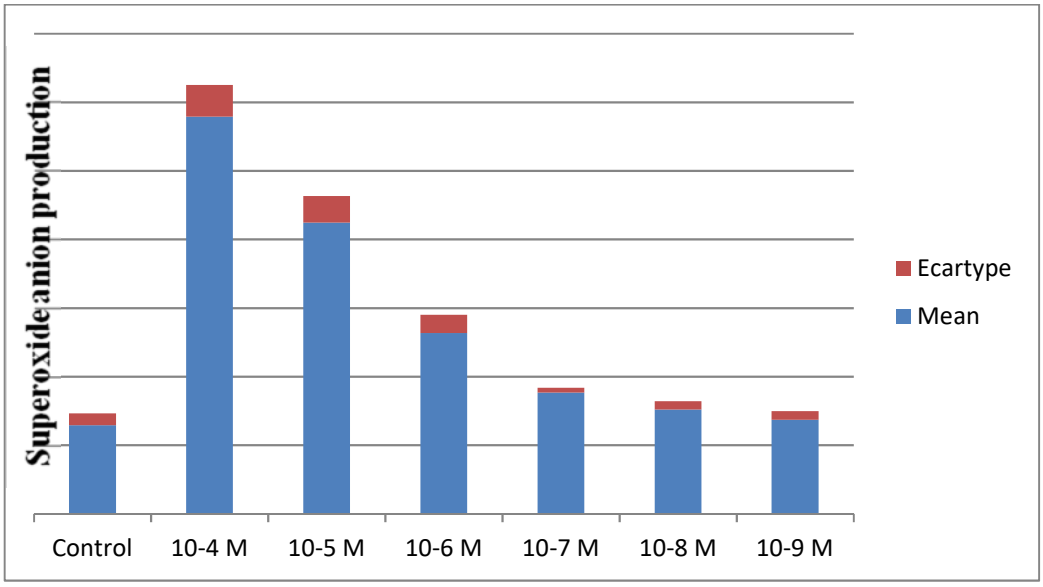


Figure 17: Production of superoxide anion by isolated mitochondria rat liver

The results are expessed as Means ± Standard deviation.

Control: Control rats, B (a) P: B (a) P alone treated rats, B (a) P + ferulenol treated rats.The results are expessed as Means ± Standard deviation.

*VIII.4. In vivo effects of ferulenol (*Ferula *roots) over mitochondrial lipid peroxidation (MDA)*

We have evaluated the production of malondialdehyde (MDA), which reflects lipid peroxidation due to excessive production of free radicals, in animals during different treatments. The figure below shows the effect of ferulenol on membrane lipid peroxidation at the cell level.

Variation of malonyldialdehyde (MDA) concentrations in different treated groups. The results are expessed as Means ± Standard deviation.

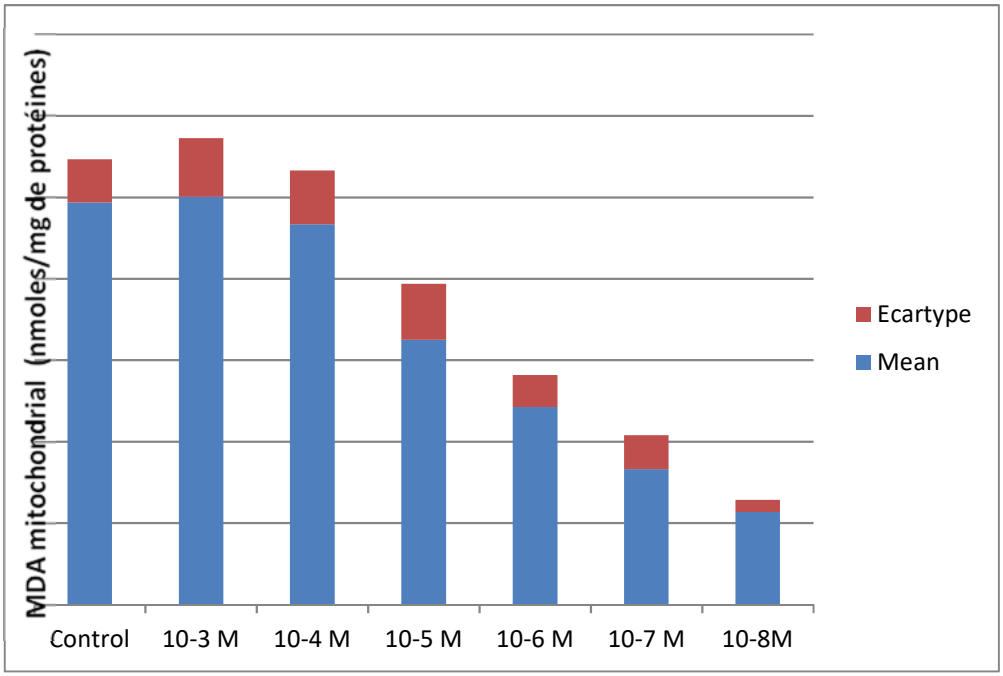


Figure 18 - a: Mitochondrial

Control: Control rats, B (a) P: B (a) P alone treated rats, ferulenol treated rats.The results are expessed as Means ± Standard deviation.

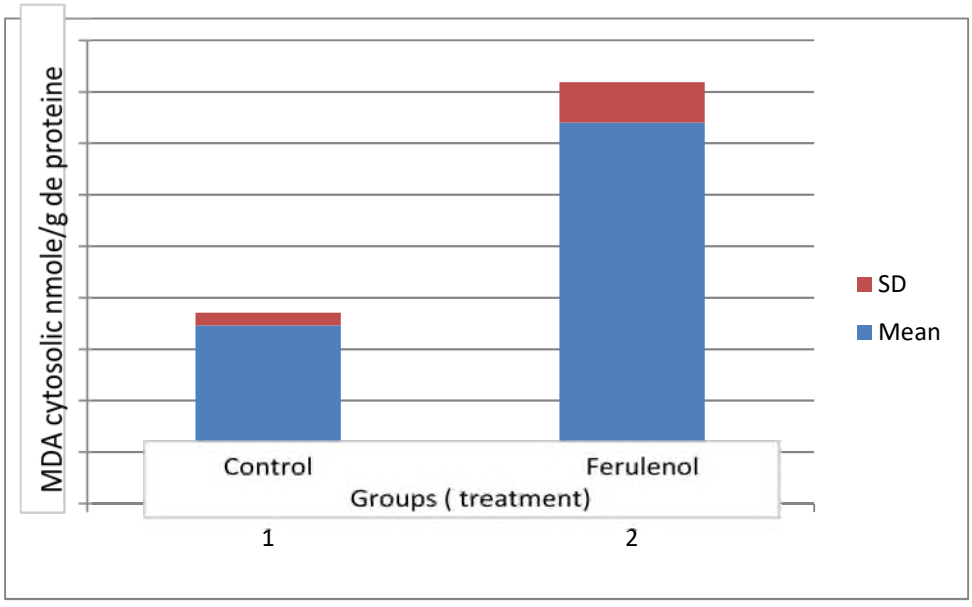


Figure 18 - b: Cytosol

Control: Control rats, B (a) P: B (a) P alone treated rats, ferulenol: rats treated with ferulenol (methanol extract of ferula roots) .The results are expessed as Means ± Standard deviation.

VIII.5. Evaluation of the enzymatic antioxidant activities

VIII.5.1. Influence of ferulenol on the enzymatic defense system in liver cells

ROS resulting from the administration of anticancer drugs have been implicated in the etiology of cardiotoxicity, pulmonary toxicity, nephrotoxicity, neurotoxicity and ototoxicity. These ROS, however, may contribute to gastrointestinal toxicity that contributes to nausea and vomiting and causes diarrhea and poor absorption of nutrients (Conklin, 2009) Conklin et al ; **2009** many researchs have reported the strong link of anticancer drugs to liver hepatotoxicity which translates the chemotherapeutic effects over liver cells DeLeve Laurie . **2003**. Most studies have shown that secondary malignancies develop in 5 to 10% of individuals who receive chemotherapy and that ROS generated during chemotherapy play an important role in the development of secondary malignancies (Guyton and Kensler, 1993). Antioxidants have been shown to prevent the toxic, mutagenic, and carcinogenic effects of antineoplastic agents.

These findings are suggestive of the prooxidant effect of ferulenol on the antioxidant defense system. It is likely that ferulenol generates free radicals which in turn lower the antioxidant status in animals. The increased lipid peroxidation provide support for the involvement of free radical process in the detrimental effects of ferulenol.

SOD and Catalase defend against ROS by catalyzing and decomposing the $O_2^{\circ-}$ and H_2O_2 radicals, respectively. GST is also involved in the reduction of ROS damage in different cells. In this study, based on the fact that anticancer drugs generate ROS in their metabolic process, we evaluated the effect of chemotherapy alone or associated with ferulenol treatment on the enzymatic activities of SOD, CAT and GST. The following figure shows the variations in the activity of the three enzymes.

Under higher concentration of ferulenol, the antioxidant enzymes activities seemed to decrease in comparison with the control due to possibly consumptionof these antioxidant enzymes or oxidation of the thiol groups present in those enzymatic proteins structures.

VIII.5.2. Variations in mitochondrial enzymatic activities in rat treated with ferulenol.

The results are expressed as Means±Standard deviation.

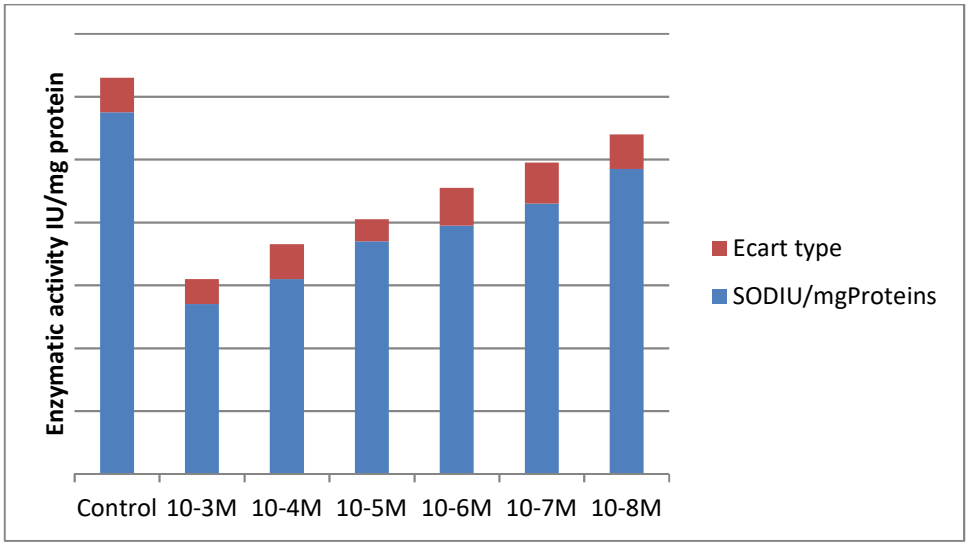


Figure 19 - a: Superoxide dismutase (SOD)

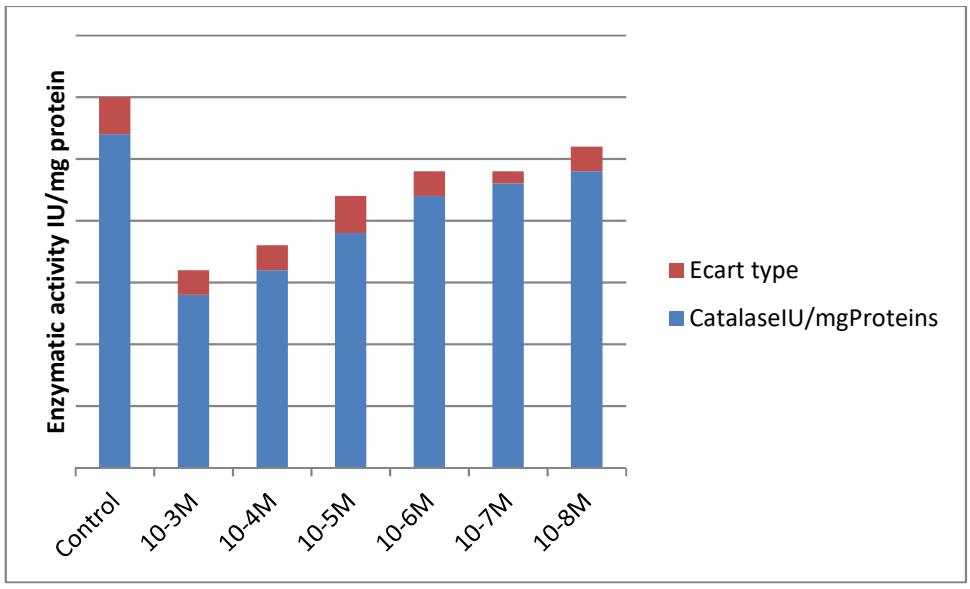


Figure 19 - b: Catalase (CAT)

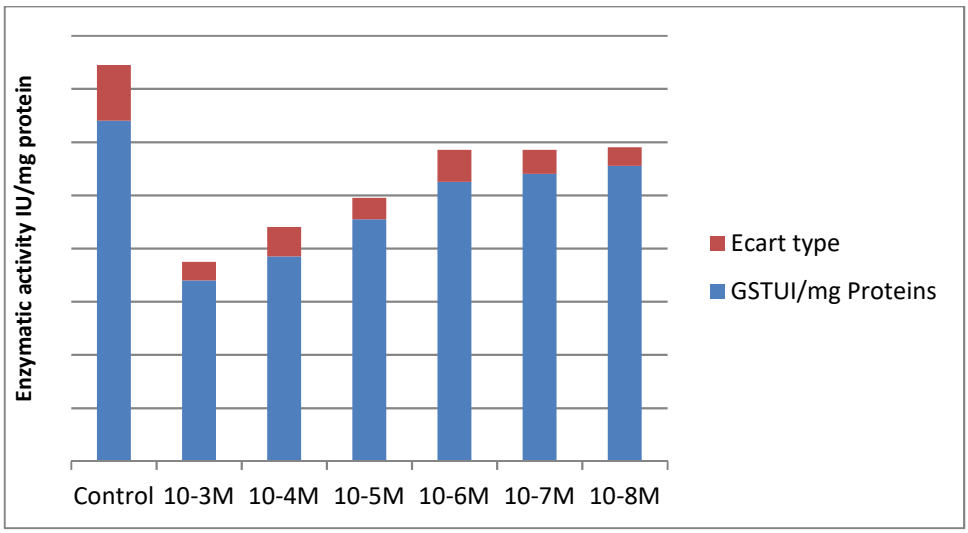


Figure 19 - c: Glutathione – s – transferase (GST).

Under higher concentrations of ferulenol ,there has been a decrease in the activities of the three enzymes mentioned above due to overproduction of superoxide ions and thus all the antioxidant enzymes could be overwhelmed and excessively consmmed as well as the possible oxidation of the thiol groups in the structures of the proteins of these enzymes whereas ,under lower concentrations of ferulenol ; the effect of ferulenol over the enzymatic activities waq less noticed.

VIII.5.3. Evaluation of the enzymatic activity of cytosolic SOD

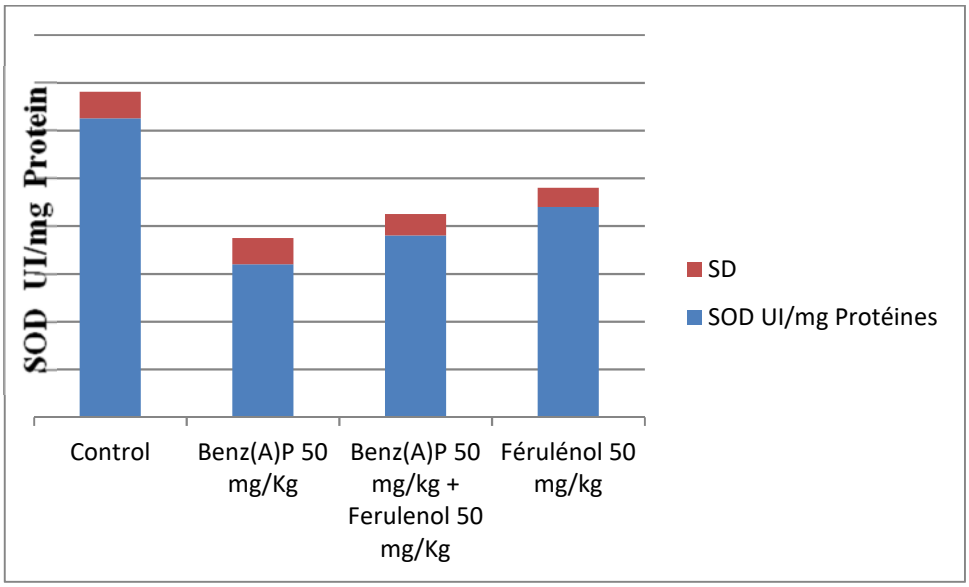


Figure 20 : Variation in the enzymatic activity of cytosolic SOD. The results are expressed as Means±Standard deviation.

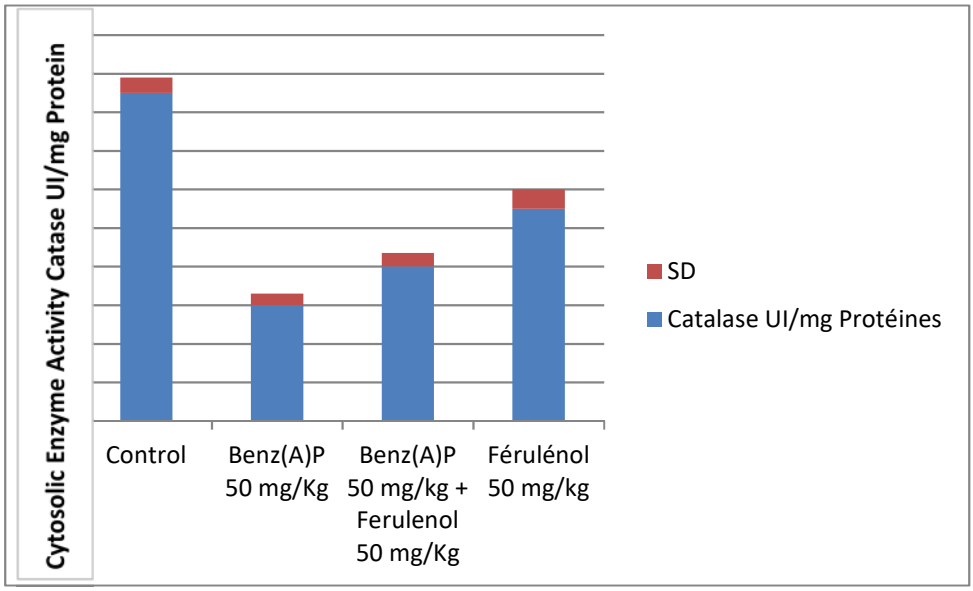


Figure 21: Variation in the enzymatic activity of cytosolic catalase. The results are expressed as Means±Standard deviation.

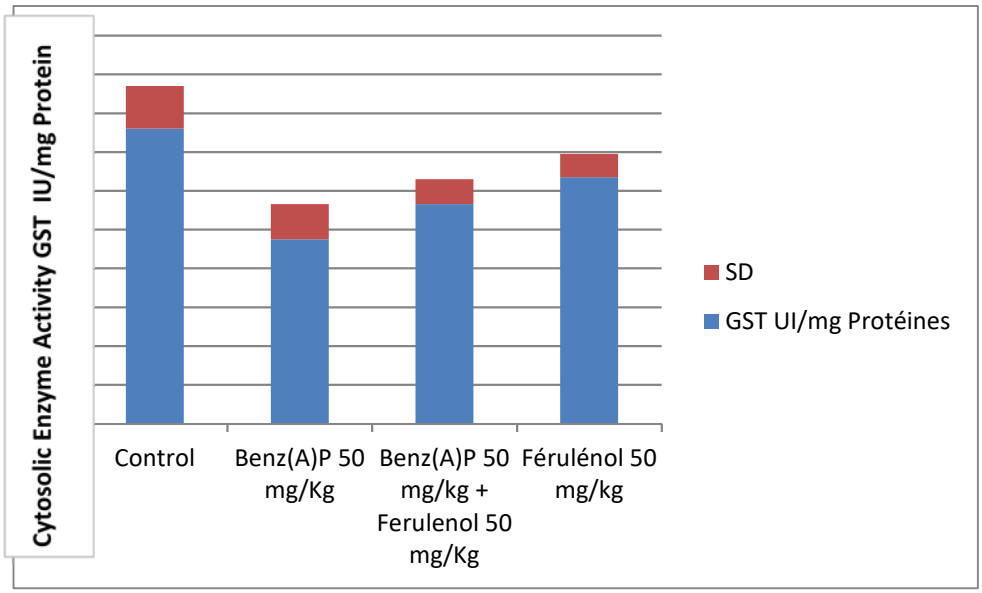


Figure 22: Variation in the enzymatic activity of cytosolic GST in rat treated with ferulenol, benzo(a) pyrene and the mixture of ferulenol + benzo(a)pyrene at 50mg/Kg .The results are expressed as Means±Standard deviation.

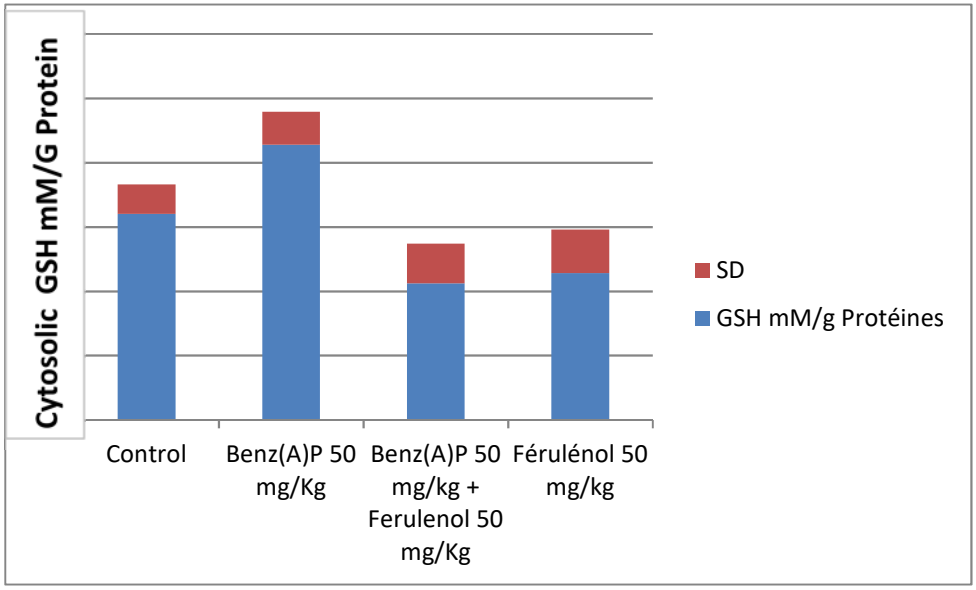


Figure 22 - bis: Variation in the cytosolic GSH (Glutathione) in rat treated with ferulenol, benzo(a)pyrene and the mixture of ferulenol + benzo(a)pyrene at 50mg/Kg. The results are expressed as Means±Standard deviation.

Generally, in order to overcome the toxic side effects of anticancer drugs, certain antioxidants are considered useful for modulating oxidative stress. As a result, treatment with potential anti-oxidants may be an approach to improve chemotherapeutic toxicity Cormier et al; **2003.** Feruulenol has been shown to potentiate chemotherapy Ghaderi et al; **2017**; Soliman Ali et al; **2018;** Patel et al; **2016.**

After treatment with ferulenol of rats with liver cancer provoked by B (a) P (receiving chemotherapy), MDA levels are increased.

The net result of this event is the increase in lipid peroxide and MDA.

Effects of free and encapsulated doxorubicin upon hepatocyte FAO cell line

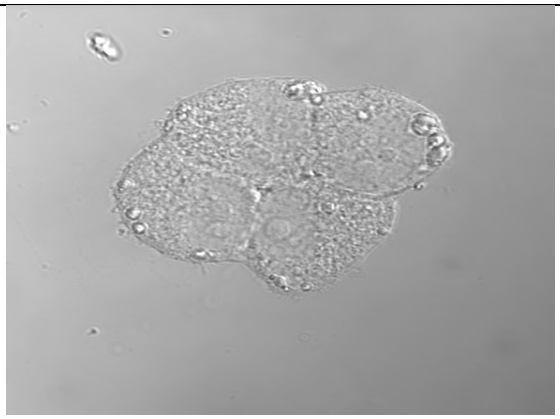


Image 7 hepato_3h control

Image 11 hepato_20h fluo

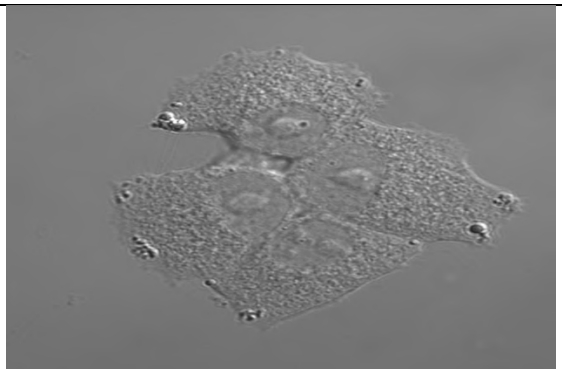


Image 35 hepato_3hrs_3dic

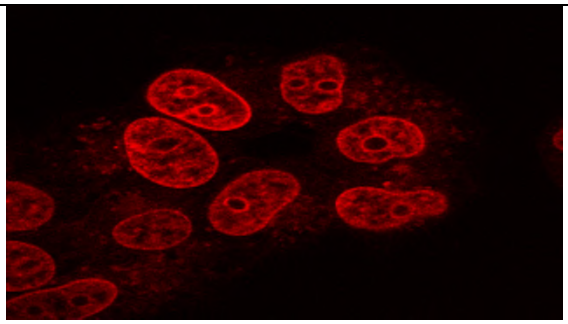


Image 37 hepato_3hrs fdoxo1

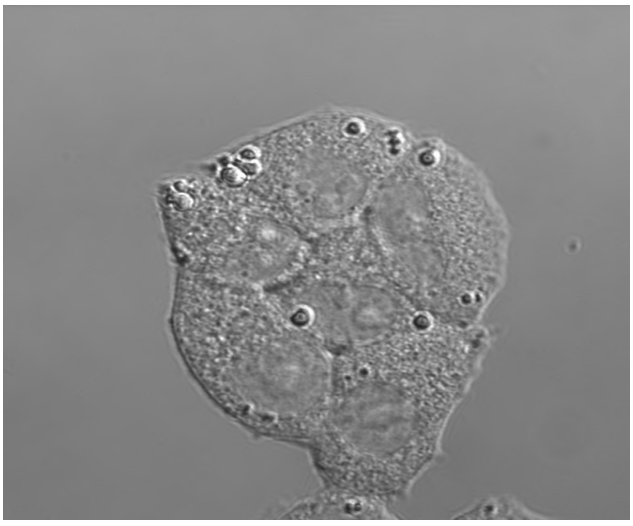


Image 45 hepato_3hrs fdoxoencap2dic

Image 7 control : after 3h no treatment

Image 11 control : after 20h

Image 37 hepato 3h free doxo treated cells diffusion of free doxo inside the cells as reflected by fluororesence.

Image 45 : hepato 3h encapsulated doxo treated cells poor absorbtion by hepato FAO cells as reflected by the absence of fluroresence. This could be attributed to the type of cells and probably the need for pH gradient.

*VIII.6. Cytotoxity evaluation of ferulenol, coladin and lapiferin (***Ferula roots***) over FAO hepatocytes and B16F melanoma cells*

Exploring the cytotoxic effect of lapiferin on cancer cell lines, B16F1 and FAO cells were treated with different doses of lapiferin, coladin and ferulenol then submitted to MTT assay, a metabolic cytotoxicity assay. The experiment indicated that ferulenol and coladin as well as lapiferin exhibited a dose-dependent cytotoxic effect at concentrations of 50µM and higher as shown in Figure 23. All the results are significantly different from the control by the Student's test. Therefore, we can assure that the effects of ferulenol, coladin and lapiferin were not due to the vehicle (P>5%, Student's two-tailed test). Moreover, the 24h treatment with coladin of both B16F1 and FAO cells hasreflectednearlly similar effect in both kinds of cell lines as indicated in Figure 25. On the other hand, the 24h treatment with ferulenol of both B16F1 and FAO cells as shown in Figure 24, reflected that ferulenol cytotoxic effect was markedly pronounced in B16F1 in comparison with FAO cells; which made us hypothesized that the antioxidant defense system was still relatively active in FAO cells.

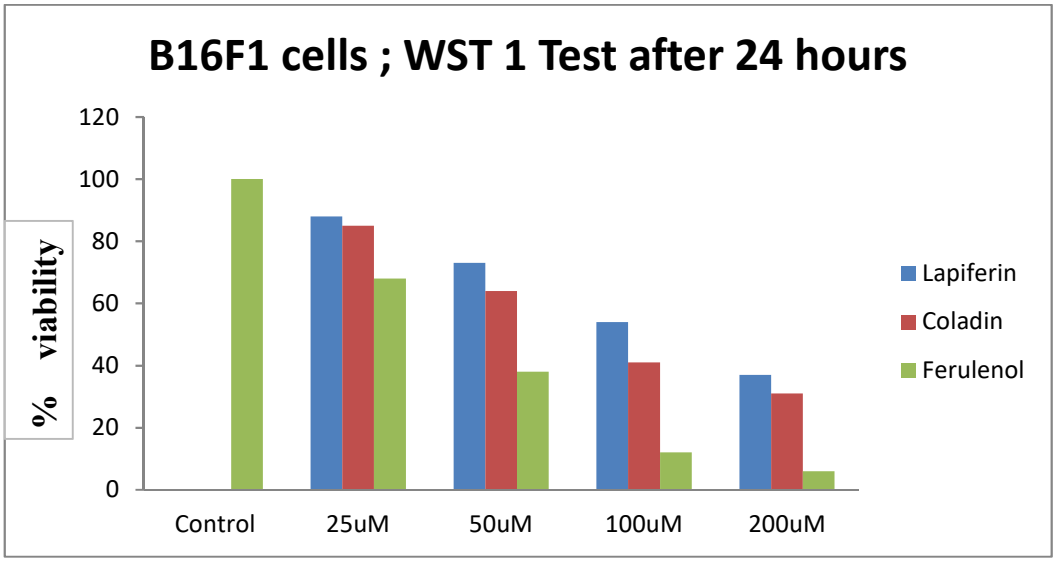


Figure 23: Ferulenol, coladin and lapiferin cytotoxicity *in vitro* on melanoma cell after 24h incubation at 37°C.Test de Student.

As it is among the three coumarins from *ferula vesceritensis* methanol extract of roots, ferulenol was the most potent in its cytoxic effect over B16F7 cells after 24h.

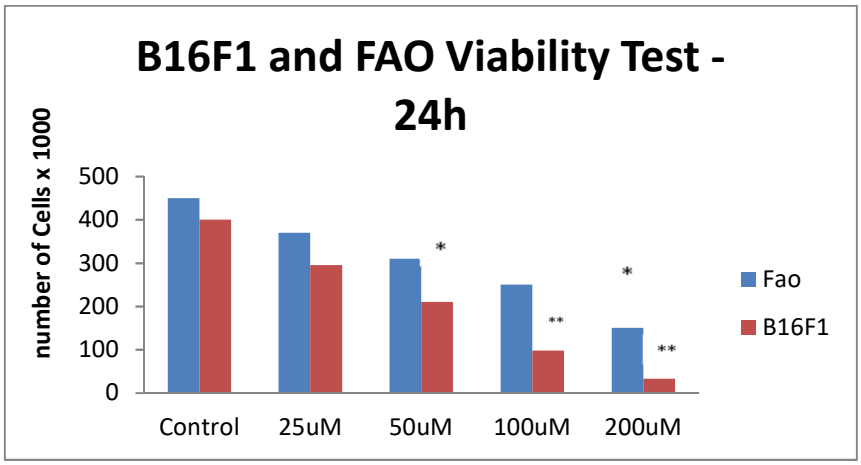


Figure 24: Ferulenol effect on B16F1 and Fao cells viability after 24h incubation at 37°C. Test de Student (* : p<0.05, ** : p<0.01, *** : p<0.001).

Ferulenol showed higher toxicity over B16F7 cells than that upon FAO cells

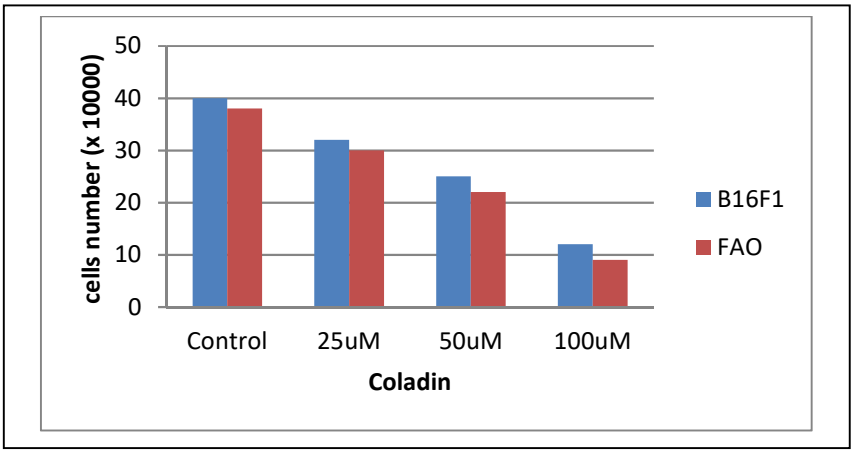


Figure 25: Coladin effect on B16F1 and Fao cells viability after 24h incubation at 37°C. Test de Student (* : p<0.05, ** : p<0.01, *** : p<0.001).

Coladin was more toxic towards FAO cells than B16F7 cells.

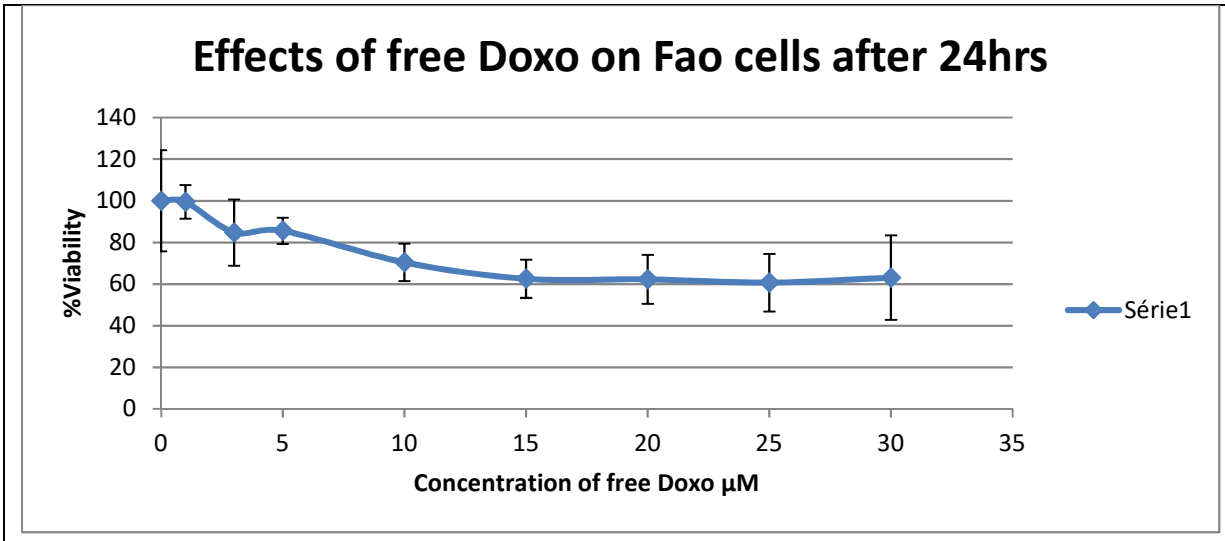


Figure 26: Effects of free doxorubicine on FAO cells after 24h 37°C

Data presented aspercentage of control cells (=100%) represent the mean ± S.D. of 3 independent experiments.

Doxirubicin in its conventional form was highly toxic over FAO cells.

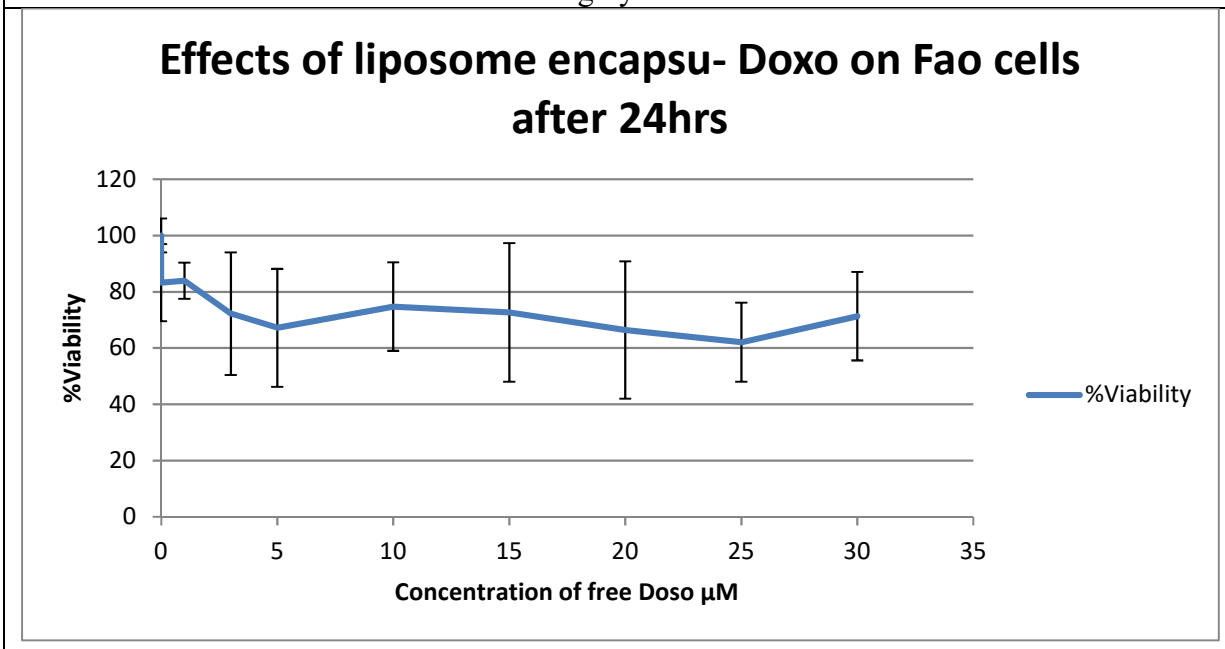


Figure 27: Effects of liposome encapsulated doxorubicine on FAO cells after 24h 37°C

Data presented aspercentage of control cells (=100%) represent the mean ± S.D. of 3 independent experiments.

Liposome encapsulated doxorubicin did not clearly affect FAO cells viability after 24h.

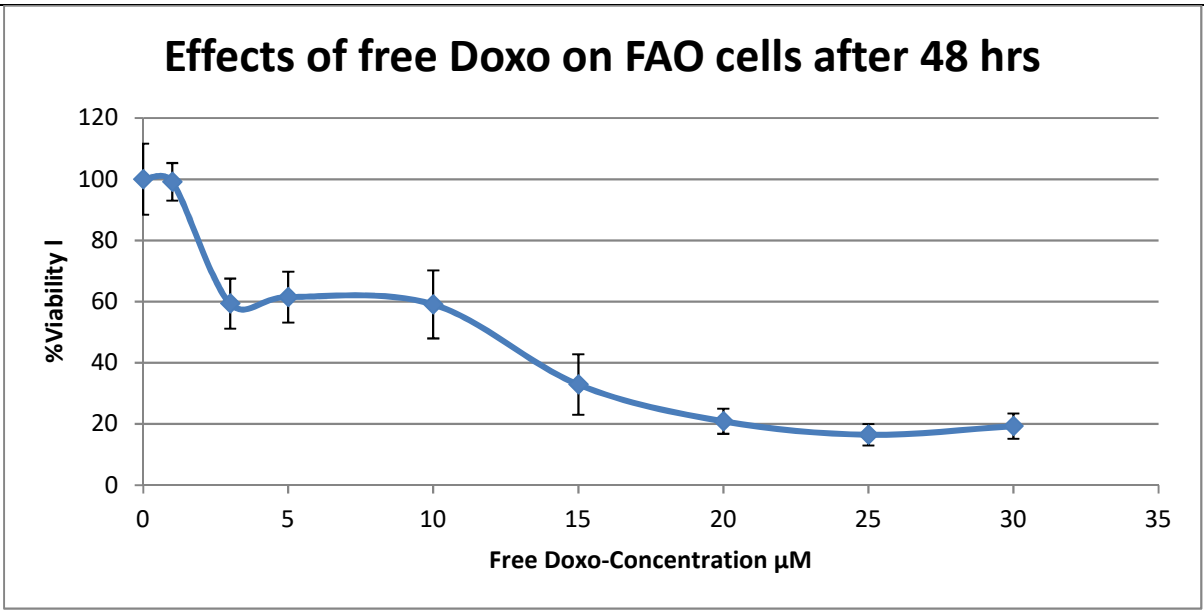


Figure 28: Effects of free doxorubicine on FAO cells after 48h 37°C

Data presented aspercentage of control cells (=100%) represent the mean ± S.D. of 3 independent experiments.

The conventional form of doxorubicin did show high toxicity towards FAO cell after 48h of treatment.

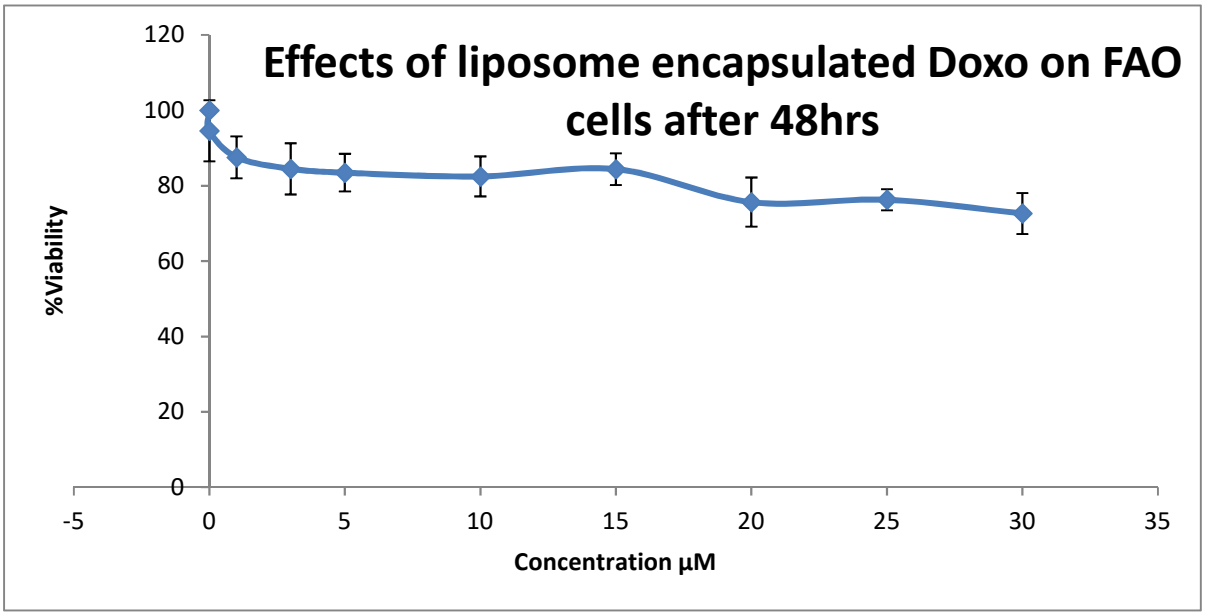


Figure 29: Effects of liposome encapsulated doxorubicine on FAO cells after 48h 37°C

Data presented aspercentage of control cells (=100%) represent the mean ± S.D. of 3 independent experiments.

Liposomal encapsulated doxorubicin did not show noticeable toxicity towards cultured hepatocytes FAO cells after 48h of exposure.

Cellular and mitochondrial viability, in other words the cytotoxicity and proliferation of treatments by our Ferulenol coladin and lapiferin alone or doxorubicine associated, on B16 F melanoma and FAO cells was determined using the MTT assay, a test commonly used *in vitro*. to measure the number of metabolically active cells. This assay is based on the transformation of yellow-colored MTT (3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) into formazan salt (purple coloration).

FIG 29 shows the percentage of viability of these cells (B16 F melanoma and FAO cells) studied in the absence or in the presence of bioactive substances at different concentrations () for a duration of incubation of 24 hours. The values are presented as average ± SD of three independent experiments.

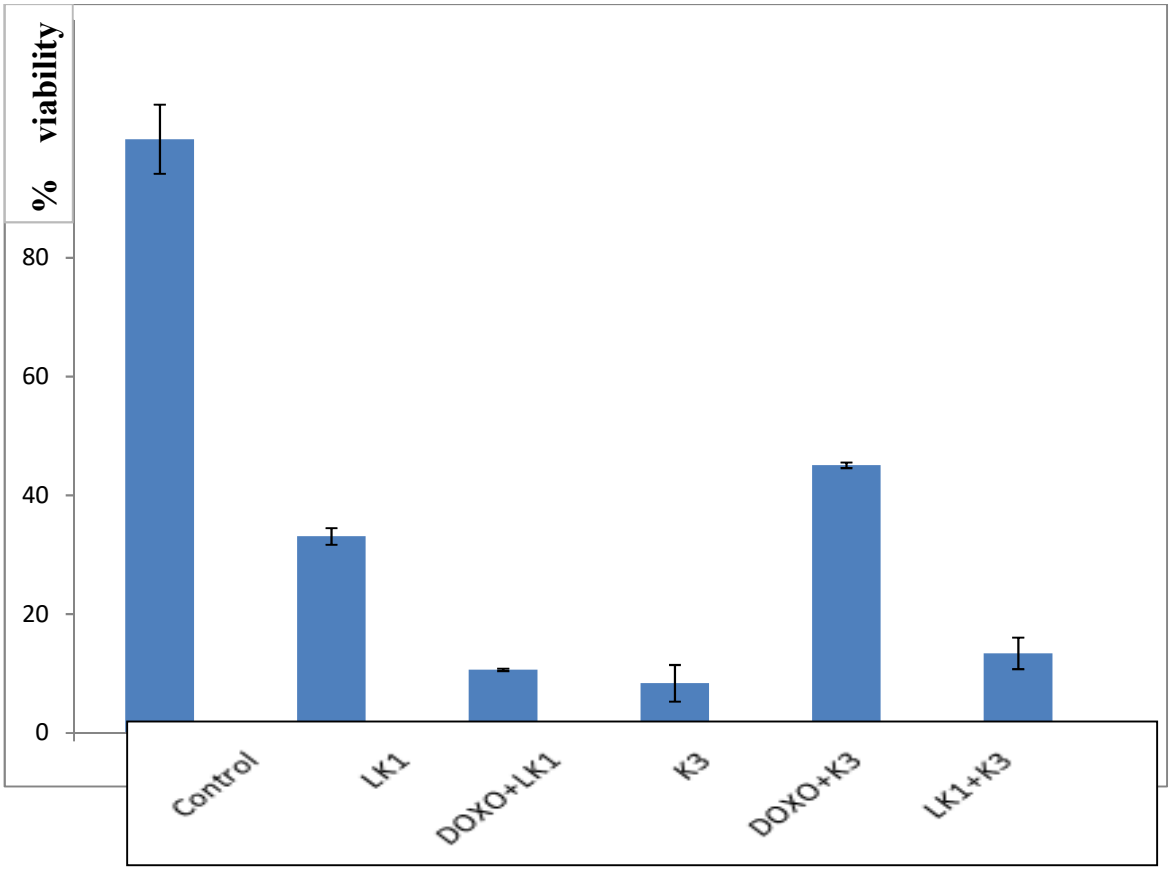


Figure 30: Effects of LK1 doxorubicine + LK1; K3; Doxo + K3 and LK1 + K3 on FAO cells after 24h 37°C. Data presented aspercentage of control cells (=100%) represent the mean ± S.D. of 3 independent experiments.

As we have mentioned previously, coladin (K3) was more toxic than ferulenol over FAO cells. Moreover; the combinaison of doxorubicin and ferulenol (LK1) reflected higher

synergetic effect over FAO cells as far as cytotoxicity is concerned while that combinaison of doxorubicin and coladin (K3) demonstraded lower synergetic effect after 24h.

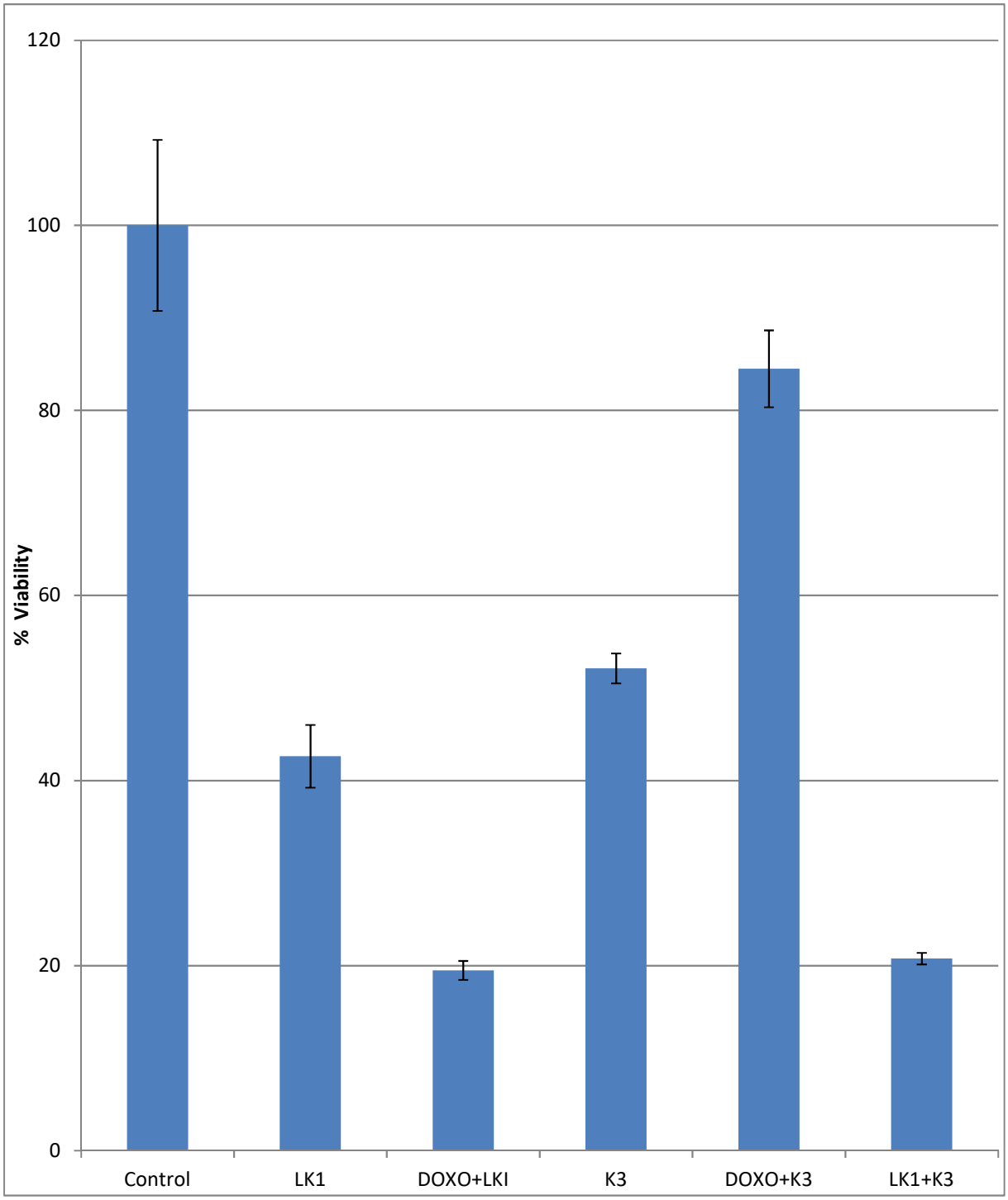


Figure 31: Effects of test compounds at 10 µM Ferulenol LK1 and K3, doxorubine after 24h 37°C. Data presented as percentage of control cells (=100%) represent the mean ± S.D. of 3 independent experiments.

Here, we noticed that both combinaison of ferulenol with either doxorubicin or coladin have had the same cytotoxic effect; possibly due to an artifact.

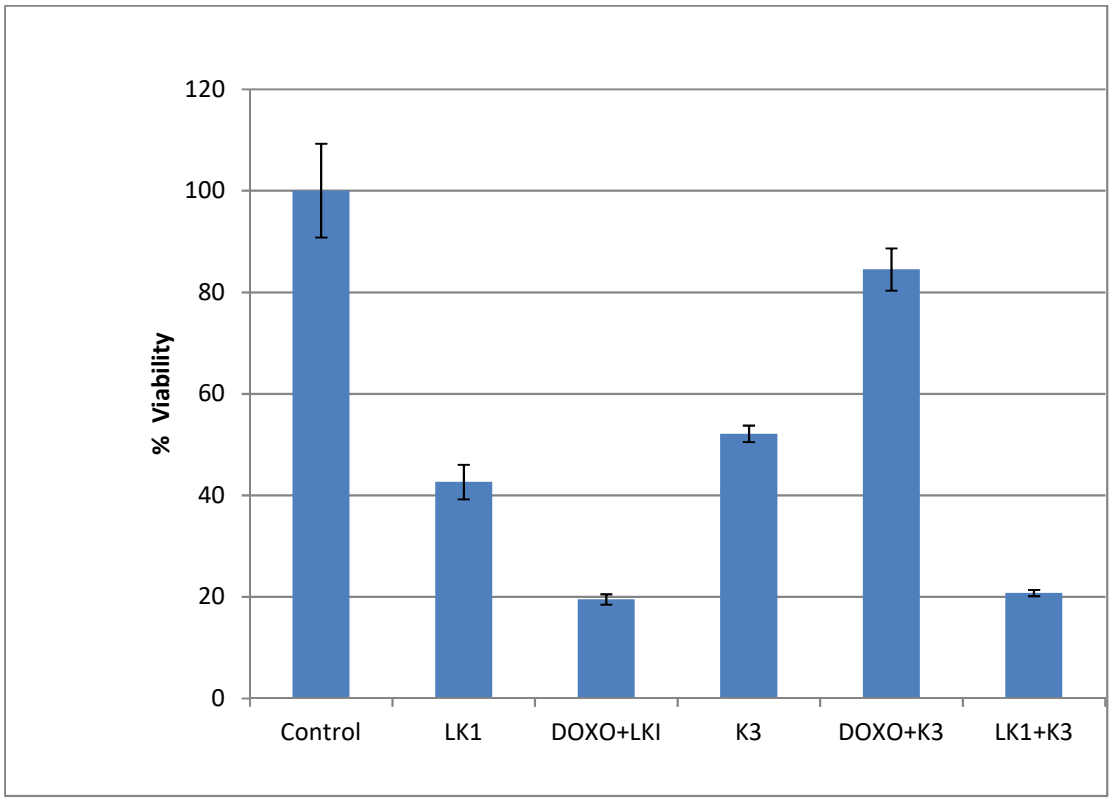


Figure 32: Effects of test compounds at 10 µM Ferulenol LK1 and K3 as well as doxorubine on FAO cells after 24h 37°C. Data presented aspercentage of control cells (=100%) represent the mean ± S.D. of 3 independent experiments.

In this figure, the toxicity of (LK1) < Doxo + K3 > K3 > Doxo + K3 < LK1 + K3 after 24h.

For the mixtures Doxo + LK1 and Doxo + K3 as well as LK1 + K3, we have noticed the following :

- There was some synergetic effect between Doxo and LK1 in comparaison to LK1,
- The same synergetic effect between LK1 + K3 as compared to K3.
- No synergetic effect has been observed in the case of Doxo + K3 as compared to K3

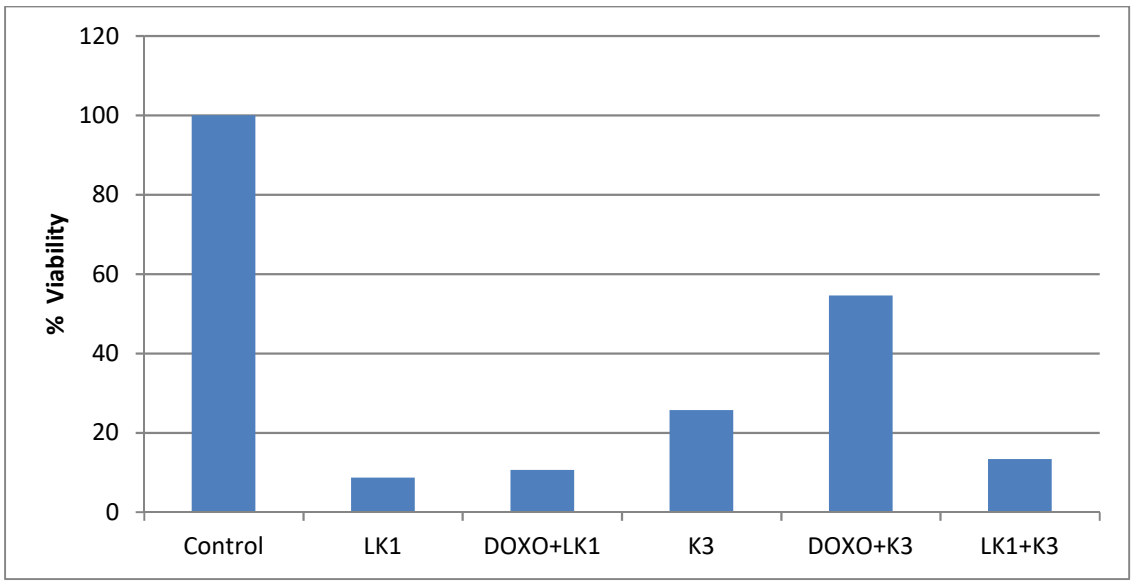


Figure 33: Test compounds at 10 µM Ferulenol LK1 and K3 as well as doxorubine on FAO cells after 24h 37°C. Data presented aspercentage of control cells (=100%) represent the mean ± S.D. of 3 independent experiments.

The cytotoxic effect of ferunlenol (LK1) > doxorubicin + ferulenol > coladin (K3) > Doxo + K3 < LK1 + K3 Fig 33.

As far as the synergetic effects of all the mixtures of tested compounds on the viability of FAO cells, are concerned; we have noticed the following:

- Both mixtures Doxo + LK1 and LK1 + K3 have reflected synergetic effects between Doxo and LK1on one hand,LK1 + K3 on the other hand; as compared to LK1 and K3 succesivelly.
- The mixture Doxo + K3 did not show any synergetic effect between Doxo and K3.

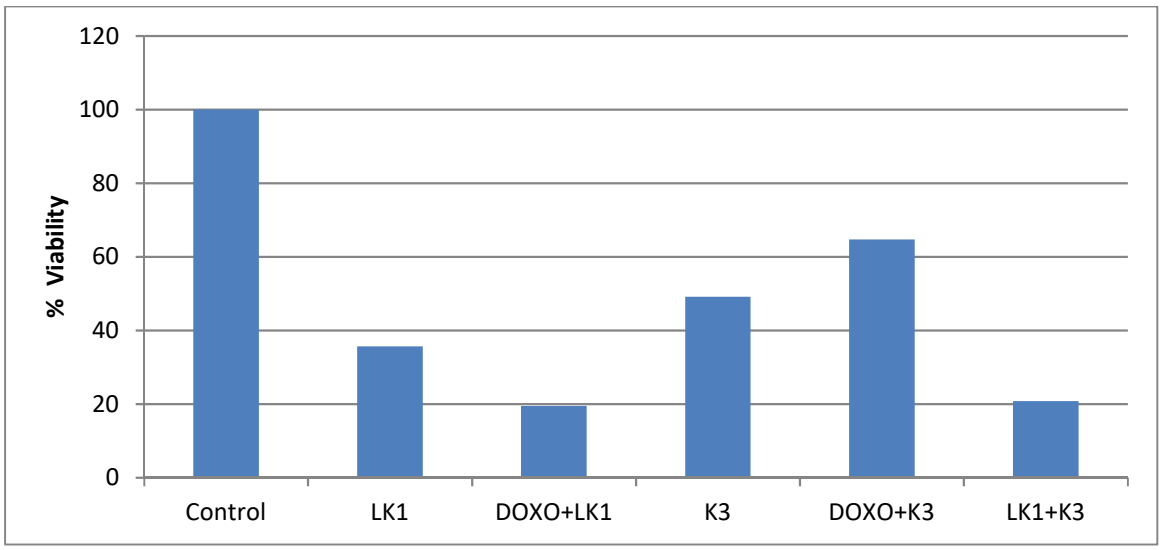


Figure 34: Test compounds at 10 µM Ferulenol LK1 and K3 as well as doxorubine on FAO cells after 24h 37°C. Data presented aspercentage of control cells (=100%) represent the mean ± S.D. of 3 independent experiments.

All tested compounds have reflected relatively mild toxic effect over FAO cells except for both mixtures in their free forms of Doxo + ferulenol and ferulenol + coladin wich have shown the highest cytotoxic effect after 24h Fig 34.

As far as the synergetic effects of all the mixtures of tested compounds over the viability of FAO cells, are concerned, the same effect that has been mentioned above could apply to this figure.

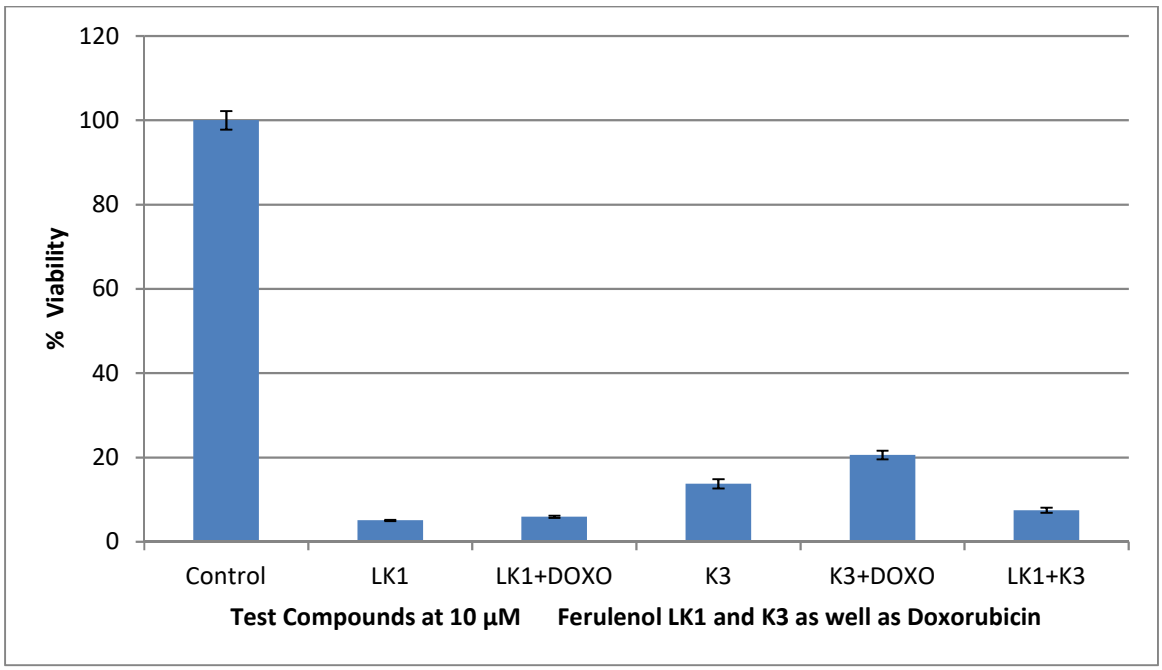


Figure 35: Test compounds at 10 µM Ferulenol LK1 and K3 as well as doxorubine on FAO cells after 48h 37°C. Data presented aspercentage of control cells (=100%) represent the mean ± S.D. of 3 independent experiments.

In this figure ferulenol was the most potent in FAO cells cytotoxicity compared with coladin and both mixtures ferulenol + Doxo as well as coladin + doxo while the last mixture of ferulenol and coladin after 48h Fig 35.

The synergetic effects of all mixtures over FAO cells viability were quite noticed after 48H which confirmed the time-dependency of the effects of tested compounds.

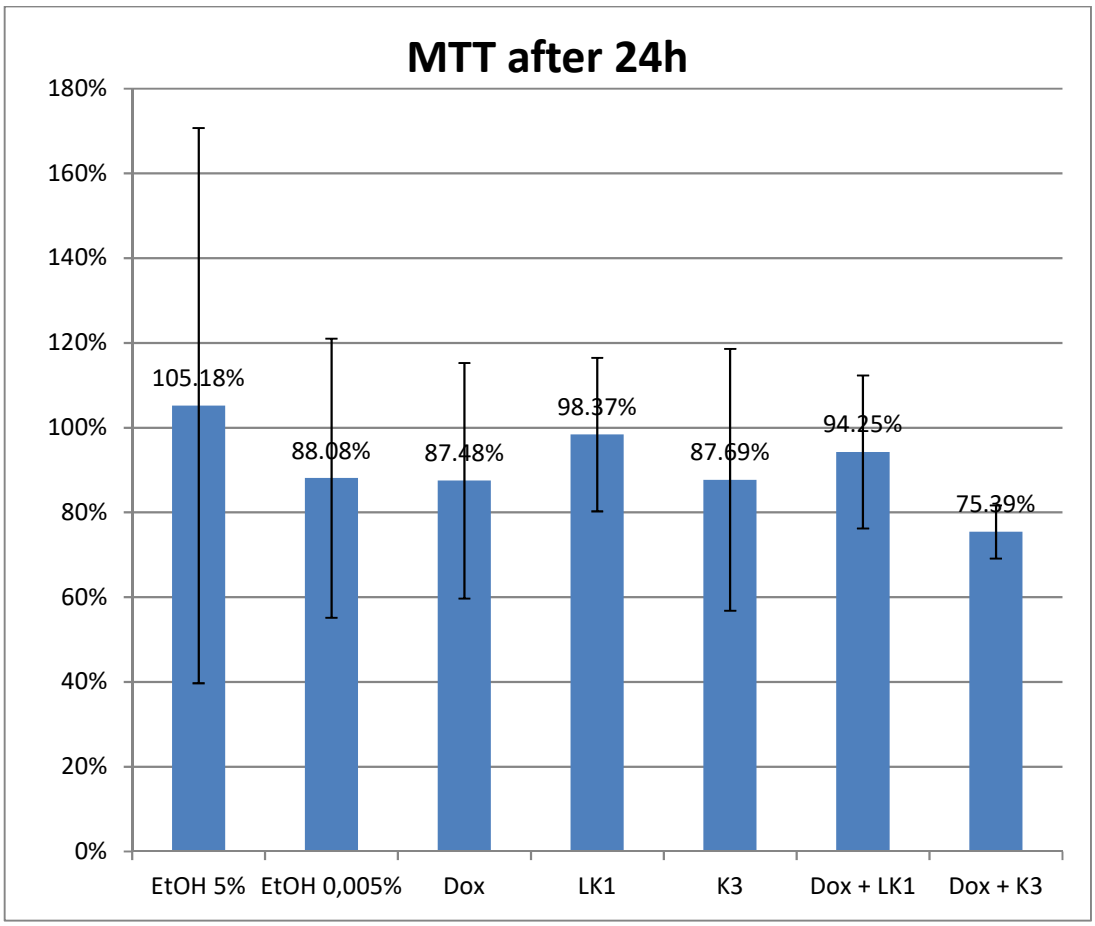


Figure 36: Effect of Ferulenol LK1 and K3 as well as doxorubine on FAO cells after 24h 37°C. Data presented as percentage of control cells (=100%) represent the mean ± S.D. of 3 independent experiments.

A very mild cytotoxic effect of all compounds tested over FAO cells after 24h.

In addition; a very mild synergetic effects among mixtures have been noticed after 24H.

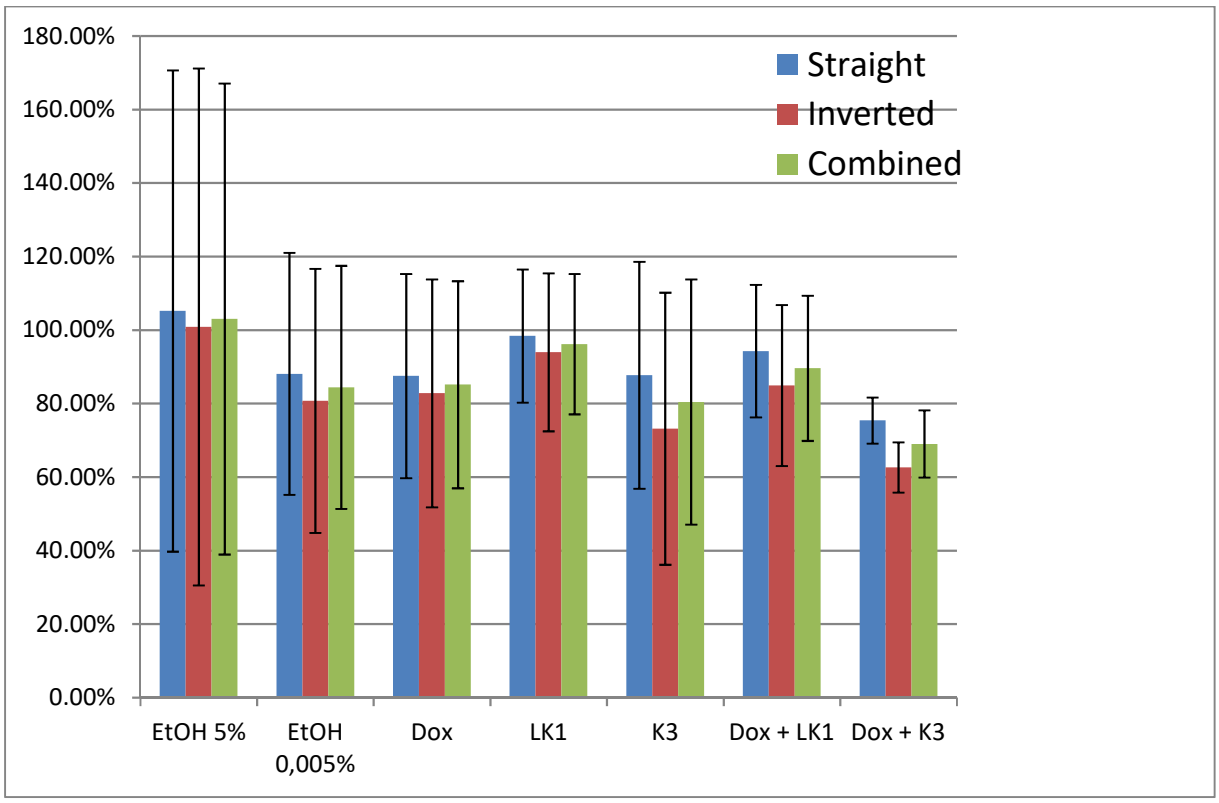


Figure 37: Effect of Ferulenol LK1 and K3 as well as doxorubine on FAO cells after 24h 37°C. Data presented as percentage of control cells (=100%) represent the mean ± S.D. of 3 independent experiments.

Mild cytotoxic effect of all compounds being tested over FAO cells after 24h in all directions of the 96 well – microplate.

Besides; a very mild synergetic effects among mixtures have been noticed after 24H.

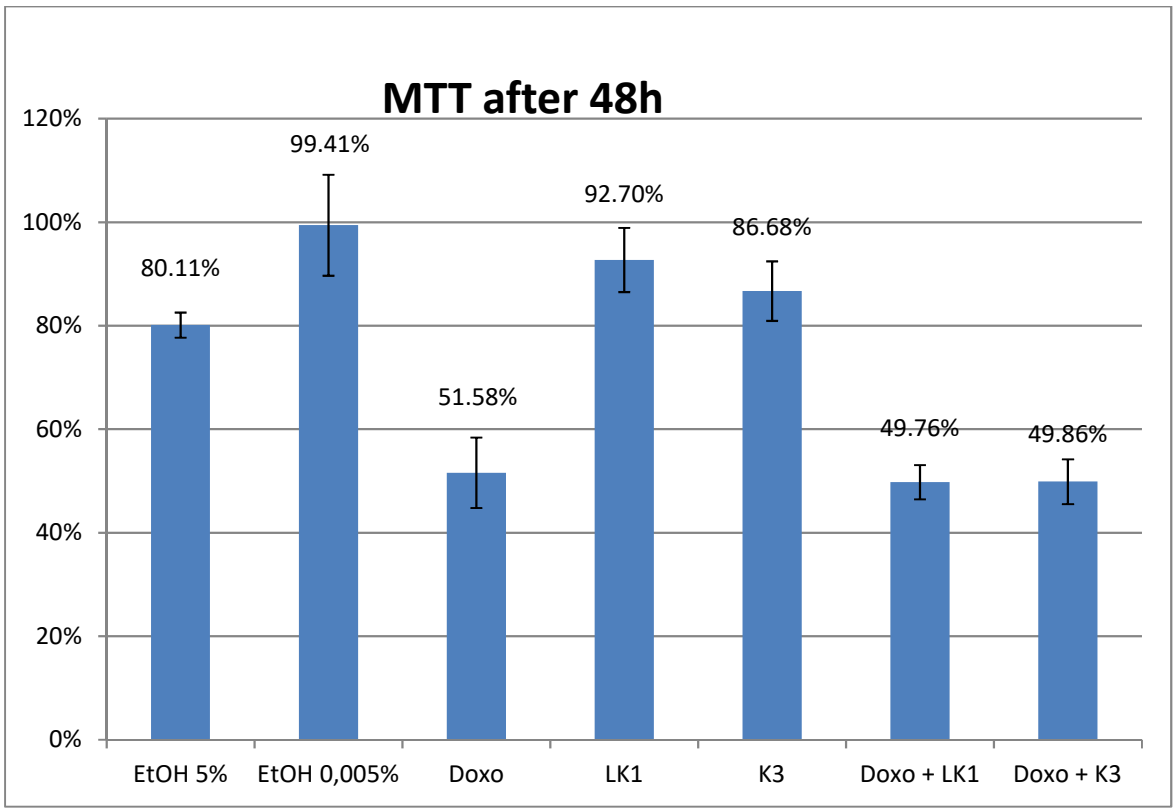


Figure 38: Effect of Ferulenol LK1 and K3 as well as doxorubine on FAO cells after 48h 37°C. Data presented as percentage of control cells (=100%) represent the mean ± S.D. of 3 independent experiments.

In this figure, the time dependence of all the tested compounds was so stressed and ferulenol alone was most potent in its cytotoxicity towards FAO cells after 48h.

Again; a pronounced synergetic effects between the concerned tested compounds have been observed after 48H.

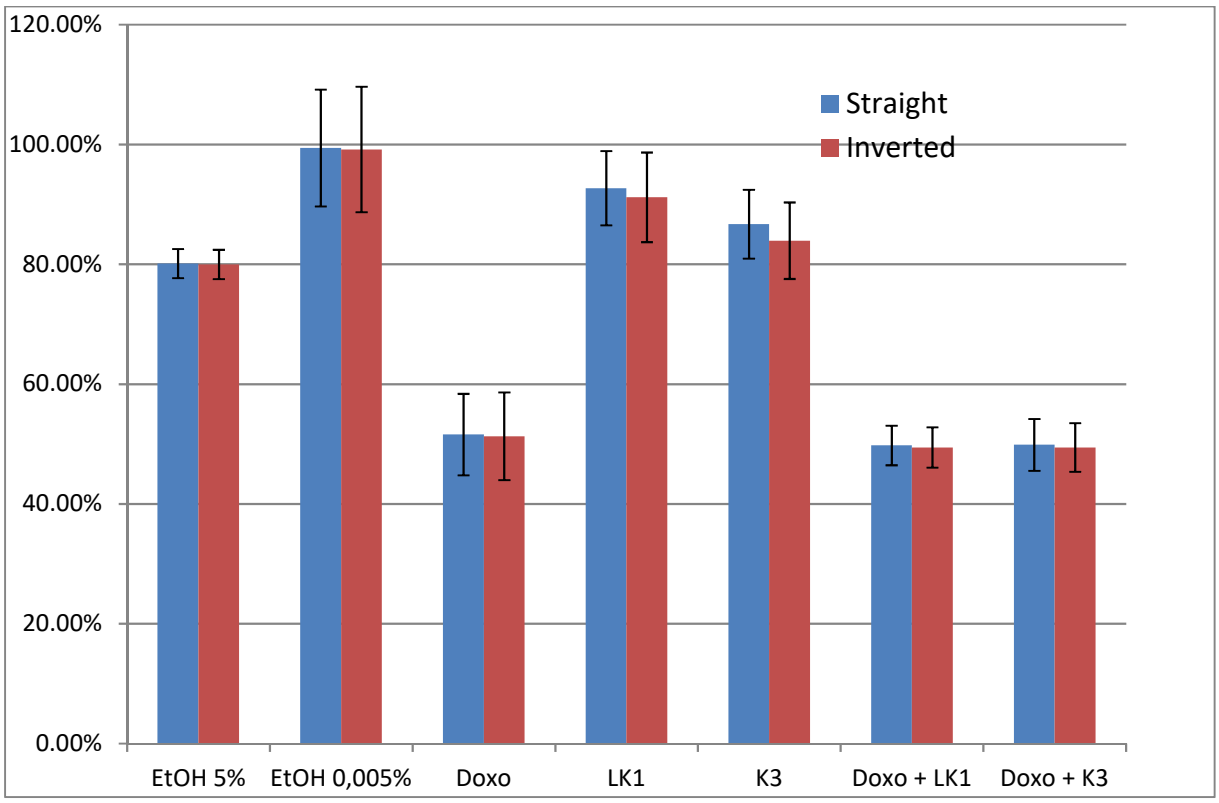


Figure 39: Effect of Ferulenol LK1 and K3 as well as doxorubine on FAO cells after 48h 37°C. Data presented as percentage of control cells (=100%) represent the mean ± S.D. of 3 independent experiments.

The direction of the 96 well – microplate did not have any effect on the cytotoxicity results of all the compounds being tested.

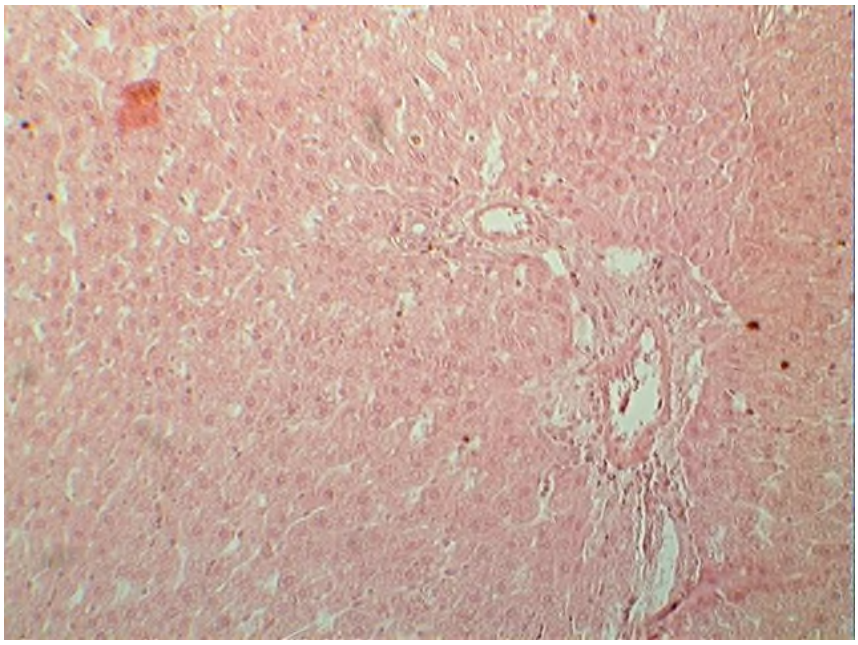
Moreover; we have noticed a pronounced synergetic effect among the concerned tested products after 48H.

Note: the effects of all tested products shown above are time – depended.

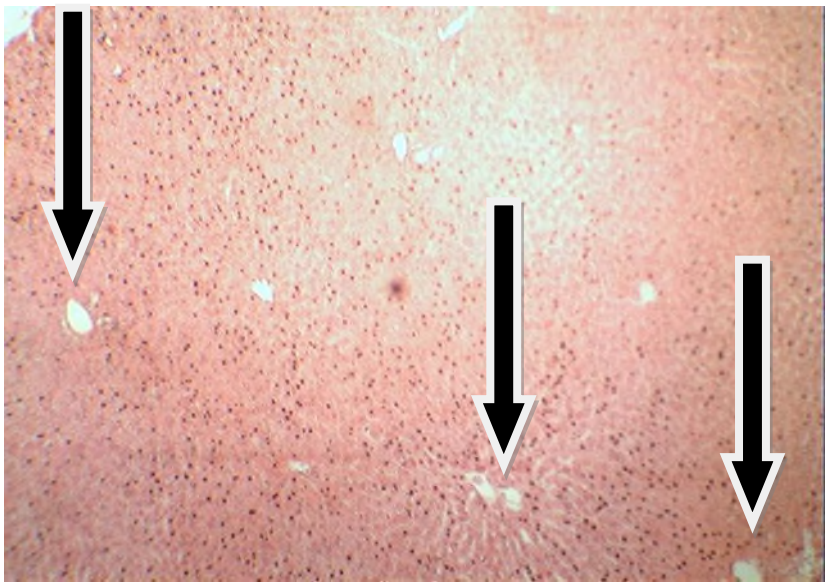
VIII.7. Histological study

Treatment of rats either with B(a) P alone or combined with ferulenol revealed prevalence of hepatic lesions. (A) normal liver cells, (B) B(a) P exposure resulted in hepatocelluar morphological alterations, apoptosis was evident and a few cell necrosis; indicating development of hepatocellular carcinoma. C B(a) P + ferulenol exposure, we observed that hepatocellular lesions were aggrevated which indicated irregular and invasive border of the carcinoma (Bars 100 µm).

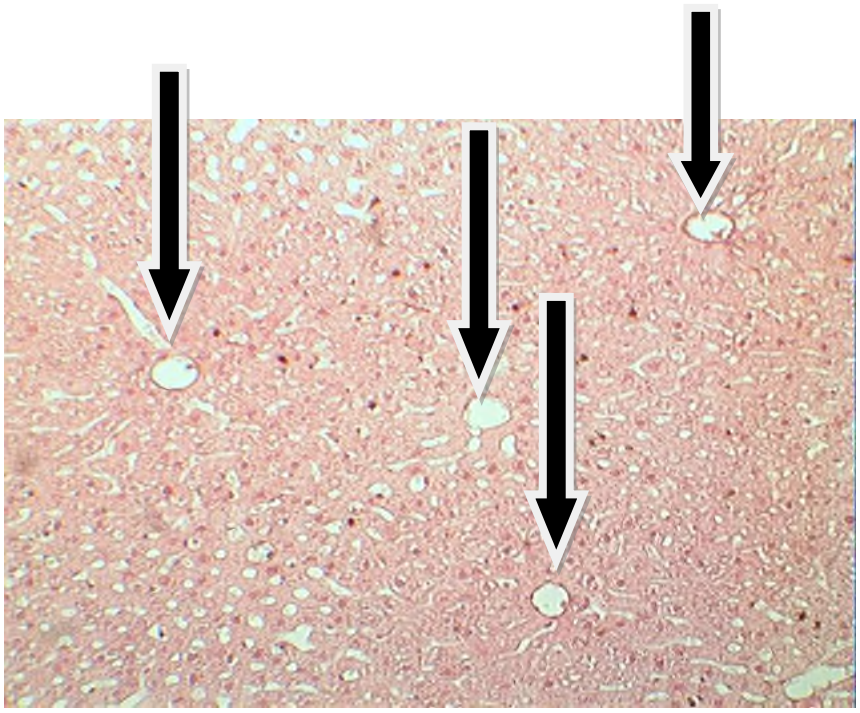
Histoligical test as shown in figures A, B and C

A 

Histology of the liver of control rat

B 

Histology of the liver of rat treated with benzopyrane

C 

Histology of the liver of rat treated with benzopyrane + ferulenol 50 mg/kg

Histologie study of rat liver after exposure to B(a) P alone and in combinaison with ferulenol

Discussion

DISCUSSION

Description of mitochondrial function has previously been evidenced as the target of many bioactive compounds including ferulenol and has been proposed to play a role in pathologies associated with cancer. Ferulenol has brought about mitochondrial membrane potential dissipation, altered mitochondrial membrane permiability and thus, triggered apotosid which ted to cell death. In another set of study, ferulenol played a role of uncapler. Lahouel et al; **2007.**

However, the prooxidant effect of ferulenol and the consequent impact on the antioxidant status has been indicated in many diseases Lariche et al; **2017**.

In this study we reported the isolation and structure elucidation of new sesquiterpene coumarin coladin, from an extract of *Ferulavesceritensis* and showed that sesquiterpenes ferulenol, lapiferin and coladin promoted efficient cytotoxic effects and anti-cancer activity. Extracts from different species of the genus Ferula have had various biomedical applications for many centuries and biological features of this genus such as cytotoxicity and antibacterial activity have been attributed to sesquiterpene coumarins as reported by Srinivasan et al; **2007;** Oliveira Silva et al; **2017.**

Besides this natural products are characterized by their P-glycoprotein (PgP) inhibitory effects, antioxidant and anti-inflammatory activities as well as anticancer agents. The importance of sesquiterpenes coumarins has been hiligthed by the presence of coumarin's group and sesquiterpene's moity Nazari et al; **2010;** Iranshahi et al; **2009.**

IX.1. Lipid peroxidation

Our results show an increase in lipid peroxidation in B (a) P-treated rats compared to control and ferulenol treated rats, as shown in figures 18-a and 18-b. However; this increase in lipid peroxidation was quite evidenced in the cytosol and concentration depended in the mitochondria of rat liver cells, indicating the prooxidant effect of ferulenol and the role of generated oxygen free radical in damaging rat liver mitochondrial membrane causing mitochondrial dysfunction. It seemed that ferulenol by brining about a high levels oxidative stress as prooxidant, could contribute to anticancer properties through apoptosis induction Lahouel et al; **2007.** Also, Rassouli et al, reported the apoptosis – inducing effects of Rassouli

et al; **2011**.These results are in agreement with those of other studies where LPO products include aldehydes such as MDA, were found with very high rates during treatment with B (a) P and were also found involved in the formation of tumors by interacting with DNA to form MDA-DNA adducts. The latter induce genetic alterations as well as inhibition of protective enzymes leading to carcinogenesis Hasanzadeh et al; **2017**; Zhanpeng et al; **2016**; Giuseppina Barrera. **2012**; Salem et al; **2018**. Some researchers showed that B (a) P-quinones were most effective in producing MDA in rat sera when they were treated with B (a) P or with any of its metabolites, including B (a) P-quinones. Their results strongly evidenced the involvement of quinones in targeting many cellular macromolecules including lipids, proteins and DNA Shuai et al; **2016;** Bolton et al; **2017.** Thus contributing to lipid peroxidation and cellular membrane damage. However, the other group treated with ferulenol have exhibited a variable increase in lipid peroxidation.

Superoxide anion O_2 • - is directlygenerated during a reaction of the electron transport chain with O_2 which will undergo disproportionation to form H_2O_2, which diffuses of the innermitochondrial membrane and can still react to form hydroxyepoxide radicals ($OH^{\bullet}$). Thus, it is recognized that the mitochondrial generation of $O_2^{\bullet-}$ is the main intracellular source of oxygenated radicals produced under physiological conditions from the O_2 used by the mitochondrial respiratory chain, with an estimate of 1% to 2% Cadenas et al; **2000.**

In eukaryotes, the electrons provided by NADH and FADH2 are transferred to complex I, II and III and possibly complex IV to reduce molecular oxygen to water Lu, Fiskum , Schubert. **2002.** However, up to 1 to 2% of oxygen consumed during mitochondrial respiration is subjected to an incomplete reduction giving $O_2^{\circ-}$(Cadenas et al; **2000).** Flavin in complexes I and II and ubiquinone binding sites in complexes I, II and III are the most important sites for mitochondrial $O_2^{\circ-}$production.

IX.2. In vivo effects of ferulenol (Ferula roots) over mitochondrial lipid peroxidation (MDA)

An increase in lipid peroxidation was observed in rats treated with B (a) P alone and after the treatment of these rats with ferulenol which resulted in an increase in MDA levels of both mitochondria and cytosol. Compared with controls (25.04 ± 3.58nmol / mg protein vs. 24.67±2.66 nmol / mg protein) and (14.80 ± 1.56 nmol/mg protein vs 6.91 ± 0.499 nmol/mg

protein) successively. This may be explained by an alteration of membrane lipids by ROS generated in liver cancer cells during drug metabolism. These are known to produce large amounts of RLOs, which in turn interact with membrane lipids and therefore induce an LPO, which is considered to be the most damaging process known to occur in all living organisms Montillet et al; **2005.**

The way in which oxidative stress and cytotoxic antineoplastic agents influence cell progression through the cell cycle is very important. For example, lipid peroxidation may prolong the G0 phase of the cell cycle, so tumor cells in the non-proliferative state G0 are little affected by anticancer drugs and can re-enter the dividing cycle after the end of chemotherapy, resulting in recurrence of the disease.

IX.3. Effects of ferulenol (Ferula roots) over mitochondrial superoxide anion production

Data showed that using benzo (a) pyrane provoked cancer in rat liver after 60 weeks. Moreover, our results reflected an increase in superoxide anion MDA in the hepatic tissue group treated with ferulenol or in combination with anticancer drugs compared to group I (control).Figures 17, 18a and 18b.

IX.4. Effects of ferulenol on mitochondrial swelling

Treatment with B (a) P produces large amounts of free radicals, which in turn interact with membrane lipids and consequently induce lipid peroxidation (LPO) in rat liver tissue, whether cellular or mitochondrial Asha et al; **2011;** Kim et al; **2000.**Progressive changes in cellular architecture due to oxidative stress and LPO generated during cytochrome p450s (i.e CYP1A1) dependent B (a) P metabolism were involved in liver carcinogenesis. The individual sensitivity to B(a)P ‘s carcinogenesis is related to P450s geneticpolymorphism since these P450 enzymes are the ones which control the stereoselectivity of B(a)P reactive metabolites and the balance between the activatin and detoxification B(a)P Hao jiang et al; **2007**.In fact, the extended peroxidation of the fatty acids associated with the membranes causes an alteration of the fluidity, an increased permeability of the ions and, possibly, induces rupture of the extended membrane until rupture, with the release of the organelles. Thus the LPO does not only affect the normal cellular functioning, but it also aggravates the

state of oxidative stress through the production of radicals derived from lipids. It has been proposed that these cumulative oxidative changes may mediate phenotypic and genotypic changes that lead to immune suppression and cellular mutation leading to neoplasia Samir et al ; **1999**.

The addition of concentrations 150 and 300 µg / ml to Mitochondrial suspensions obtained from the rat group with hepatocellular carcinomas (HCC) led to significant mitochondrial swelling within 60 min of incubation. Researchers have found a paradoxical effect of some antioxidants known as cytoprotective. These alter the mitochondrial function and particularly induce the opening of the PTP in the mitochondria of the liver. The authors explained this by the protection of mitochondria against the accumulation of ROS and against lipid peroxidation of membranes by involving a direct interaction with thiol proteins of mitochondrial membranes which supposed to control the opening of the PTP Morin et al; **2001.**

The changes in superoxide anion production and mitochondrial swelling shows us that ferulenol does modify the functionality of liver mitochondria, this finding is consistent with that of many studies Lahouel et al; **2007.**

The anticancer effects of sesquiterpene coumarins from ferula species roots has been previously elucidated. Several compounds from the later have been shown to be potent inhibitors of tumorigenesis. Among which we can distinguish our bioactive compounds, derivatives of cinnamic acid and some diterpenoids ... etc. Gholami et al; **2016 ;** Iranshahy et al; **2011.**

IX.5 Influence of ferulenol on the reserve of glutathione of liver cells

The antioxidant status of cancer patients may play an important role in their response to chemotherapy, in people with a deficient condition being relatively insensitive. However, nutritional therapy with antioxidants during chemotherapy can reduce the production of lipid peroxides resulting from treatment and overcome the inhibitory effects of oxidative stress and maintain responsiveness to antineoplastic agents Conklin et al;**2009**.

As the first line of defense against ROS, the GSH antioxidant system is a crucial defense mechanism. Liver toxicity induced by chemotherapy begins when GSH stores are depleted,

according to other study results or hepatotoxicity induced by the use of certain anti-cancer drugs such as EPI Wu et al; **2012.** Wondrak. **2009** or cyclophosphamide has shown a reduction in reserves in GSH. The metabolism of cyclophophomide produces highly reactive electrophiles and the decrease of the GSH value in the cyclophophamide-treated group probably due to the electrophilic charge on the cells and also because of the acrolein formation which decreases the GSH content Jain et al; **2017.** These results correlate well with our results wherer the GSH level decreased of cells compared to the control as indicated upon treatment with ferulenol as shown in figure 21 bis.

GSH levels were decreased as a result of ferulenol treatment of rat liver tissue, figures 21bis.

Many studies have shown that administration of antineoplastic drugs during chemotherapy results in a very high degree of oxidative stress. This is illustrated by the elevation of lipid peroxidation products, the reduction of the total capacity of radical trapping of blood plasma, the marked reduction of plasma levels of antioxidants such as vitamin E, vitamin C and β-carotene, and the marked reduction in tissue glutathione levels (GSH) that occurs during chemotherapy with essentially all antineoplastic drugs Conklin et al; **2009**.

IX.6. Influence of ferlenol on the ability of antioxidant enzymes

A decrease in the activities of antioxidant enzymes SOD, CAT, GST, as well as GPx in hepatic tissues, figures 18 (a, b, c mitochondrial), 19, 20, 21 (cytosolic).

SOD and Catalase defend against ROS by catalyzing and decomposing the O_2 ° - and H_2O_2 radicals, respectively. GST is also involved in the reduction of ROS damage in different cells. In this study, based on the fact that anticancer drugs could cause a rise in oxidative stress and increase free radicals production. Additionally; knowing that B(a)Pyrane generated ROS in its metabolic processeswhose dependence upon dose,time and routes of administration has been elucidated Hao et al; 2007; we have evaluated the effect of chemotherapy alone or associated with ferulenol treatment on the enzymatic activities of SOD, CAT and GST. Figures 18(a,b,c mitochondrial) Figure 19, 20,21 cytosolic) show the variations in the activities of the three enzymes among different treated groups. Although lower concentration of ferulenol have increased the antioxidant enzymes and higher concentrations decreased those activities of the antioxidant enzymes and hence; ferulenol ‘s effect was concentration-dependent.

Similarly; the administration of anthracyclines (EPI and doxorubicin) increased the sensitivity of myocytes to ROS by suppressing the activity of potentially protective antioxidant enzymes (SOD, CAT and GST). Anthracyclines also initiated toxic actions through changes in mitochondrial structure and function Lipshultz et al; **2013**.

Numerous studies have also shown that antioxidants do not inhibit, but actually enhance, the cytotoxic effect of antineoplastic drugs on cancer cells Conklin et al; **2009**. Ferulenol administration significantly decreased SOD, CAT and GST activity, suggesting that it had the ability at higher concentrations to depletethe activities of these three enzymes, but at lower concentrations it increased the activities of those three antioxidant enzymes confiming its concentration-dependent effects. Oxidative stress resulting from overproduction of ROS and degradation of antioxidant defenses is documented to induce chromosomal disruption. It has been suggested that clastogenic factors,also called chromosome breakage factors, are released by cells exposed to oxidative stress. Their chromosome damaging effects are mediated through superoxide anions since they are regularly inhibited by superoxide dismutase(SOD) Ingrid Emirit.**2007**.Free radical scavengers, including natural compounds such as terpenoids, elevate the activity of these reactive oxygen species. These results support the hypothesis that cyclophophomide-induced genotoxicity and liver cancer cell cytotoxicity may be in enhanced by reactive oxgen species production. Jain et al; **2017.** Decreased levels of GSH, SOD and CAT activities in ferulenol treated tissues at higher concentrations,possibly due to an increase in oxidative stress and thus enhance; free radicals productionall of that could be linked to ferulenol ‘s cytotoxic effects the later is in accordance with the results of Bocca et al;**2002**.While lower concentrations of this prenylated coumarins have had an inverse effects by increasing those enzymatic activities; again reflecting the less cytotoxic effects of this natural product under these circumstances; as far as cytotoxicity is concerned,this could be related to the findings of Valiahdi et al;**2013.** The anticancer drugs are reported to be able to induced oxidative stress in cancer cells, however; the precise mechanisms by witch anticancer drugs induce ROS’s generation remains obscure.Yocomaya et al; **2017**.

These few examples among many clearly underline that, in addition to the potential of ferulenol witch could be used as a therapeutic agent in the future, its co-administration with anticancer is an interesting area of investigation. Our results confirm that pre-treatment with natural prooxidants can potentiate chemotherapeutic drugs efficacy on tumor cells. In addition, the results of the present rat study have shown us that ferulenol and terpenoids may

enhance the oxidative stress, due to chemotherapeutic agents, and may be used in humans in the future as standard therapy against cancer.

The effect of different dose of ferulenol (50 mg/kg) was examined using enzymes involved in the antioxidant function, glutathione content and lipid peroxidation in the liver of wister albino rats (6 – 7 weeks old) after 12 and 24hrs. Significant changes were observed in the rat liver. The specific activities of superoxide dismutase (SOD) catalase, glutathione – S – transferase (GST) reduced glutathione content (GSH) content were found to decrease in the liver. The mode and magnitude of change in the specific activities was seen to depend on dose and time of ferulenol administration. Ferulenol also decreased the reduced glutathione content and enhanced the lipid peroxidation in the liver.

These findings are suggestive of the prooxidant effect of ferulenol on the antioxidant defense system. It is likely that ferulenol generates free radicals which in turn lower the antioxidant status in animals. The increased lipid peroxidation provides support for the involvement of free radical process in the detrimental effects of ferulenol.

An important role of ferulenol in liver cancer cells is further supported by the enhancement of lipid peroxidation status as a result, the generated free radicals are significant factor associated with cancer and other diseases.

In cancer, there is considerable evidence for mitochondrial changes and elevated oxidative stress that contributes to pathology and this has been in part ascribed to mitochondrial damage due toprooxidant agents Sha. **2015;** Ozgur et al; **2010;** Rosaria et al; **2010;** Tabet et al; **2007** futherrmore, the reactive oxygen species diserupt mitochondrial function *in vitro*Yildirim et al; **2010;** Syama et al; **2013;** Zhang et al; **2011;** Ben-Zhan et al; **2011.**

IX.7. Cytotoxic effects of ferulenol, lapiferin and coladin

The genus Ferula is a rich source of various biologically active phytochemicals among of which sesquiterpene coumarins.It is assumed that the most prominent features of the genus ferula are their cytotoxic effects.Moreover; the type of cell line used and their mechanisms of actions have been reported. Iranshahi et al ;**2018.**Phytochemicals obtained from the species of Ferula are used in traditional medicine for the treatment of various diseases Iranshahi et al; 2010.

This part of the study aims to show the effect of our Algerian *Ferula vesceritensis* roots methanol extract constituents on B16F melanoma and Fao cells used as liver cancer models. In fact the standard therapy of the liver cancers only marginally increases the survival rate with a very high recurrence rate associated with numerous cytotoxic effects.

The antitumor activity of ferulenol, lapiferin and coladin exhibited a dose-dependent cytotoxic effect depending on their ability to inhibit tumor cell proliferation *in vitro;* of these active compounds ferulenol had a strong antitumor activity on both FAO hepatocytes cells and metastasic melanoma cells, B16F1 cells. Many authors have reported sesquiterpenes compounds cytotoxicity.

Some species of the genus *Ferula* have therapeutic properties such as contraceptive, antipyretic, smooth-muscles relaxant and aphrodisiac activities Nazari and Iranshahi. 2010; Yaqoob et al; **2016**. Also, several *Ferula* species are well-known because of their applications in the treatment of various diseases. For example, *F. persica* root extract possesses antispasmodic, carminative, laxative and expectorant properties and has been used for the treatment of diabetes and high blood pressure Razavi and Janani. **2015.** *F. assa-foetida* exhibits anti-carcinogenic properties and has protective activities against free radical-mediated diseases Gamal-Eldeen and Hegazy. **2010**. Iranshahi et al. reported that *F. assa-foetida* has anti-leishmanial activity against promastigotes Iranshahi et al; **2007.**

Moreover ; A number of sesquiterpenes obtained from the species of *Ferula* roots, revealed antibacterial, antifungal, cytotoxic, antioxidant, and hormonal activities as well as P-glycoprotein inhibitory and immunomodulatory effects Miski. 2013.Sanandajin and ethyl galbanate, the two sesquiterpene coumarins isolated from *F. pseudalliacea* root extract have shown potent antibacterial activities and are being used in pharmaceutical and food industries Dastan et al; **2016.** Some compounds have reflected chemopreventive and / or cytotoxic activities towards various several cancer cell lines. Alkhatib. **2010.**

Furthermore ; many ferula species root extracts have reflected cytotoxic effects against various cancer cell lines. In our results both ferulenol and coladin have reflected certain cytotoxicity against melanoma B16F7 cells and FAO hepatocytes cells in a time-dependent manner. Ferulenol, a prenylated 4- hydroxycoumarin isolated from *F. communis,* exerted dose-dependent cytotoxicity against various human tumor cell lines. It stimulated tubulinpolymerization *in vitro*, inhibited the binding of radio-labeled colchicine to tubulin, re-arranged cellular microtubule network into short fibres and altered nuclear morphology Bocca

et al; **2002.** In another study, the cytotoxicity of ferulenol on human breast cancer (MCF-7), colon cancer (Caco-2), ovarian cancer (SKOV-3) and leukemic (HL-60)cells was evaluated; based on the results, ferulenol showed significant cytotoxic effects at concentrations of 10 nM, 100 nM and 1µM, against these cancer cell lines Nazari and Iranshahi. **2010.** Conferone is another sesquiterpene comarin isolated from *Ferula* root extract. Barthomeuf et al; **2006** showed that 10µM of conferone enhances the cytotoxicity of vinblastine in MDR1-transfected Madin-Darby canine kidney (MDCK-MDR1) cells Barthomeuf et al., (2006). Additionally, conferone enhanced the cytotoxicity of cisplatin and vincristine in 5637 cells Neshati et al; **2012;** Neshati et al; **2009.** In another study, sanandajin, farnesiferol B, and kamolonol acetate displayed cytotoxic activities against HeLa cells Dastan et al; **2014**a; Kasaian et al; **2015** revealed that sesquiterpene coumarins isolated from *Ferula* species exert different cytotoxic activities. Also, It has been reported that farnesiferol B, farnesiferol C and lehmferin reverse doxorubicin-resistance properties of MCF-7/Adr cells Kasaian et al; **2015.**Furthermore, conferone exhibited moderate cytotoxicity against CH1 (human ovarian carcinoma) and A549 (human nonsmall cell lung cancer) cells Valiahdi et al; **2013.** Also, umbelliprenin, a prenylated coumarin synthesized by various *Ferula* species, showed cytotoxic activity by inhibition of the growth of human M4Beu metastatic pigmented malignant melanoma cells through induction of cell cycle arrest in G1 and caspase-dependent apoptosis Lourenco et al; 2012. In our results and based on cellular and mitochondrial viability test using the MTT colorometric assay; both ferulenol and coladin as well as lapeferine have reflected different cytotoxic effects against melanoma B16F7 cells and FAO hepatocytes cells in a concentration-dependent manner (15 µM and up).Ferulenol's cytotoxic activity was markedly pronounced over melanoma B16F7 compared with coladin and lapiferin figure A. Inversely; coladin cytotoxic effect was profoundly noticed over FAO hepatocytes cells while lapiferine whose cytotoxic effect on human MCF7 breast cancer has been elucidated Gamal-Eldeen et al; **2010;** reflected moderate cytotoxicity as related to the other two sesquiterpene coumarins from ferula vesceritensis roots methanolextracts. Figure B. This observed cytotoxicity;in another word this affected cell viability in both types of cell lines either melanomaB16F7 or hepatocytes FAO cells could be attributed to the presence of the coumarin and prenyl moieties in the structures of the tested compounds as reported in the work of Soltani et al; **2009.** Mousavi et al; **2015.** Additionally; Methyl caffeate, a compound isolated from *F. lutea* showed cytotoxic effects against HCT-116 (human colon carcinoma cell line), IGROV-1 and OVCAR-3 (human ovarian cancer cell line), respectively Znati et al**; 2014b.** Umbelliprinin has not only refelected cytotoxic effect in two different types of lung cancer cell lines that are(i.e QU-DB and A549,but also showed antigenotoxic properities in

human peripheral lymphocytes , possibly due to its prenyl moiety.Soltani et al; **2009;** Khaghanzadeh et al; **2012.** Although farnesiferol B, farnesiferol C and lehmferin reverse doxorubicin-resistance properties of MCF-7/Adr cells Kasaian et al., 2015; ferulenol and coladin did not seem to affect doxorubicin cytotoxic effect either in its free or encapsulated forms. This could be explained by the poor absorption of doxorubicin by the hepatocytes FAO cells,hence; poor accumulation of doxorubicin inside those cells which led to difficulties of this anthracyclin to get to the nucleus. All that might explain the unaffected by this anticancer drug mainly in its encapsulated form. However; other sesquiterpene coumarins from different parts of ferula species have exhibited that synergetic cytotoxic effect over a variety of cancer cell lines. Furthermore, Feselol and mogoltacin are two biologically active sesquiterpene coumarins isolated from root extracts of *Ferula* species that showed cytotoxic properties. For example, a combination of 40 mg/mL vincristine and 16 mg/mL mogoltacin increased the cytotoxicity of vincristine by 32.8%, in human transitional cell carcinoma (TCC) cells (Rassouli et al; **2009**). Similar results were found for feselol, a sesquiterpene coumarin isolated from the fruits of *F. badrakema* (Mollazadeh et al; **2010**). Also, a combination of feselol and mogoltacin enhanced the cytotoxicity of cisplatin in 5637 cells (human bladder carcinoma cell line) (Mollazadeh et al; **2011;** Rassouli et al; **2011**). Hanafi-Bojd et al. 2011 showed that farnesiferol A and galbanic acid, two sesquiterpene coumarins isolated from *Ferula* species, increase verapamil cytotoxicity (Hanafi-Bojd et al; **2011**). It seemes that the plant source of bioactive substances, their tested concentrations and the type and concentration of the solvent affects the bioactive compounds cytotoxicity and mechanisms of actions as well as the type of the investigated cell lines and their time of exposure to the bioactive substances. Therefore; the cytoxicity of ferula species extracts are solvent type and concentration dependent. As a consequence of that; the cytotoxic activity of *F. assa-foetida* extract, the methanol extract possessed more marked cytotoxic effects than theethanol extract Shafri et al; **2015**. In another study, results of MTT assay of *F. assa-foetida* extract against an osteosarcoma cell line (HOSCRL-1543) showed that this activity is dependent on the type of solvent (methanolic>ethanolic) and its concentration (higher methanolic content>lower methanolic content) Shafri et al; **2015**. Also, the *F. gummosa* root extract reduced the viability of GP-293 cells at concentration of 750 mg/mL Ghorbani et al; **2016**. Generally, Bcl-2 family proteins such as Bcl-2 protein and Bax protein, have important regulatory roles in apoptosis. Iranshahi et al; **2018;** Aldaghi et al; **2016** indicated that farnesiferol C and microlobin, two sesquiterpene coumarins isolated from *F. szowitsiana*, have greater binding affinity to Bax protein in comparison to Bcl-2 protein. These researchers assumed that the interaction between drugs and hydrophobic groove of Bax protein might

result in conformational changes and insertion of Bax protein into mitochondrial membrane, consequently inducing Bax-dependent apoptosis (Aldaghi et al; **2016**).

Sesquiterpene coumarins isolated from the *Ferula* genus, showed both growth inhibitory and cytotoxic activities in different cancerous cell lines. Ryu et al; **2001.** *F. vesceritensis roots* methanolextract, as a new natural source of bioactive compounds,in particular sesquiterpenes and sesquiterpene coumarins; the later have showed promising specific cytotoxic activity against various cancer cells. Among those sesquiterpene coumarins; our bioactive substances ferulenol,and coladin inaddition to the daucane ester lapiferine which showed promising prooxidant and cytotoxic activities. The cytotoxic activity of those bioactive compounds could have a link to the prooxidant activity that brought about a rise in oxidative stress and free radicals productions in both rats cancerous liver models and melanomaB16F7 as well as Hepatocytes FAO cells leading to possibly Bax-dependent apoptosis through DNA damage. Lariche et al; **2017.** Thus; ferulenol, coladin like other sesquiterpne coumarins as well as lapiferine from ferula roots methanol extracts coul have exerted their effects through mitochondrial membrane potential dissipation, causing an alteration in mitochondrial membrane permeability; leading to mitochondrial PTP opening and DNA destruction, the later could lead to apoptosis which was reported to be upregulated by Bcl2 family-proteins such as Bcl2/Bax expression and modulation of caspases pathways. Gholami et al ; **2013;** Sadeghizadeh et al ; **2008 ;** Lariche et al ; **2017.**Ferulenol, a prenylated 4- hydroxycoumarin isolated from *F. communis,* exerted dose-dependent cytotoxicity against various human tumor cell lines. It stimulated tubulin polymerization *in vitro*, inhibiting tubulin‘s binding to cholchicine by tubules rearrangement; Bocca et al; **2002.** Conferone is another sesquiterpene comarin isolated from *Ferula* root extract. Barthomeuf et al; **2006** showed that 10µM of conferone enhances the cytotoxicity of vinblastine in MDR1- transfected Madin-Darby canine kidney (MDCK-MDR1) cells (Barthomeuf et al; **2006**). Additionally, conferone enhanced the cytotoxicity of cisplatin and vincristine in 5637 cells Neshati et al; **2012;** Neshati et al; **2009.** In another study, conferone exhibited moderate cytotoxicity against CH1 (human ovarian carcinoma) and A549 (human nonsmall cell lung cancer) cells Valiahdi et al; **2013.** Also, umbelliprenin, a prenylated coumarin synthesized by various *Ferula* species, showed cytotoxic activity by inhibition of the growth of human M4Beu metastatic pigmented malignant melanoma cells through induction of cell cycle arrest in G1 and caspase-dependent apoptosis Lourenco et al; **2012.** Khaghanzadeh et al; **2012.** Studied umbelliprenin cytotoxicity in two different types of lung cancer cell lines (i.e. QU-DB and A549). Their results revealed that IC50 values for QU-DB and A549 were 47±5.3 and 52±1.97 µM, respectively

Khaghanzadeh et al; **2012.** Also, an investigation on umbelliprenin nanoliposomes revealed that liposomal umbelliprenin possesses time and concentration-dependent cytotoxicity on melanoma cell line Ramezani et al; **2014.**

These results are in agreement with previous studies that showed that treatment with ferulenol could improve the side effects caused by CP injection, although it can not return their values to normal levels Hasanzadeh, Mahdavi, Dehghan, Charoudeh. **2017;**.Nwodo, Ibezim, Simoben, Ntie-Kang. **2016.** Other researchers have shown that the therapeutic dose of CP can cause liver, lung and serum toxicity in mice and that oxidative stress is responsible for this toxicity Selvakumar, Prahalathan, Mythili, Varalakshmi. **2005;** Ayhanci, Yaman, Appak and Gunes. **2009;** Jain, Ahmad, Cairo, Aronow.**2017;** Ludemann. **1999;** Ayhanci et al; **2009**, reported that CP undergoes metabolic activation by cytochrome P450 to produce the two metabolites, phosphoramide mustard and acrolein, which are responsible for the induction of oxidative stress Ludemann. **1999.**

Conclusion

Conclusion

*Ferula plants are rich sources of sesquiterpenes and sesquitrpene coumarins.*Considering all the previously mentioned elements, we speculated that the sesquiterpenes antitumor activity could de due to their ability to alter many signalling pathways including stimulation of necrosis and induction of apoptosis through DNA damage. As a consequence, ferulenol was able to induce mitochondrial swelling, which may have contributed to the induction of apoptotic process. Apoptosis is a natural process that regulates the cell death induced by an external signal or mediated by mitochondria.

To conclude, the beneficial properties of terpenes in liver cells, that could be attributed to suppression of proliferation, enhanced antioxidant status, pro-apoptotic effect, anti-inflammation, induction of mitochondrial and nuclear morphology alterations, inhibition of tubuling binding and tubules rearrangements to tubular fibers as well asthe enhancement of chemotherapy in various cancer cellswhich suggest them as potential candidates to be explored in improving the health of human beings, preventing and/or treating several degenerative diseases such as cancer.

These results are of major interest and open up great prospects:

As an extension of this thesis, it would be interesting to study and analyze the possible effects of *Ferula vesceritensis* roots methanol extract on the metastatic spread of liver cancer induced by several cancer provoking chemicals and anticancer agents since the latter also targets other organs. We can complete our results on cell invasion and how to influence cell to cell communication which is crucial for cancer migration, invasion and metastasis.

It is necessary to further support the results of our *Ferula vesceritensis* roots methanol constituents of Algeria on liver cancer by combining it with new therapies such as molecules targeting the DNA for tracing the oncogenes and cancer sensing, cancer suppressor genesand

how to take advantage of alternative, natural therapy alone or in combination with conventionalchemtherapy using nano based strategy. Hence; increasing the lifespan of patients.

Finally, Again; it recommendes to use encapsulated bioactive compounds in their administration to targeted cancerous organ or cells in order to diminish their cytotoxicities and rise their bioavailability by breaking barriers which could block their cell targets bioavailability. Furthermore, to deepen the angle of their effects over various types of cancer, pharmacokinetics and pharmacodynamics studies of those bioactive compounds are necessary for a better understanding of their metabolism and how to facilitate their clearance anf excretion out of the organisms to avoids their cell endogenous build ups and toxicity using different sets of *in vitro* and *in vivo* experiments to seeck the possibility of jumping from the pre-clinical to clinical trials without the risks of chronic side-effects.

Tables

The following tables containing some details about different ferula species, sources, diseases utilizations and mechanism of actions are taken from the thesis of Alkhatib **2010.**

Table 2: Production of superoxide anion by isolated mitochondria rat liver

		Ferulenol Concentration (Optical density OD)					
	Control	**10^{-4} M**	**10^{-5} M**	**10^{-6} M**	**10^{-7} M**	**10^{-8} M**	**10^{-9} M**
1	0,115	0,564	0,398	0,231	0,171	0,13	0,126
2	0,098	0,603	0,402	0,248	0,18	0,146	0,133
3	0,095	0,552	0,444	0,245	0,176	0,149	0,118
4	0,132	0,671	0,45	0,267	0,164	0,158	0,144
5	0,114	0,538	0,368	0,279	0,184	0,163	0,152
6	0,141	0,546	0,485	0,311	0,184	0,166	0,149
Mean	0,129	0,579	0,4245	0,2635	0,1765	0,152	0,137
Ecartype	0,017317759	0,04612302	0,03892621	0,02629163	0,00720532	0,01212436	0,01235584

Table 3- a: Ferulenol Effects on the mitochondrial MDA

	Molaire concentrations of extracts (M)						
MDA mitochondrial nMole/mg de proteine	**Control**	**10^{-3} M**	**10^{-4} M**	**10^{-5} M**	**10^{-6} M**	**10^{-7} M**	**10^{-8}M**
	24,67	25,04	23,34	16,24	12,11	8,31	5,68
SD	2,66	3,58	3,30	3,45	1,98	2,07	0,74

Table 3 - b: Ferulenol Effects on the cytosolic MDA

MDA cytosolique nMole/g de proteine	Control	Ferulenol 50mg/kg
1	6,5	14,4
2	7,7	17,3
3	6,8	13,8
4	7,1	13,2
5	7,1	14
6	6,3	16,1
Mean	6,916666667	14,8
SD	0,499666555	1,568438714

Table 4 - a: Mitochondrial Enzymes

	Ferulenol Concentration Molaire						
	Control	**10^{-3}M**	**10^{-4}M**	**10^{-5}M**	**10^{-6}M**	**10^{-7}M**	**10^{-8}M**
SOD IU/mgProteins	11,5 ± 1,1	5,4±0,8	6,2±1,1	7,4±0,7	7,9±1,2	8,6±1,3	9,7±1,1
CatalaseIU/mgProteins	2,7 ± 0,3	1,4±0.2	1,6±0.2	1,9±0,3	2,2±0,2	2,3±0,1	2,4±0,2
GSTIU/mg Proteins	12,8±2,1	6,8±0,7	7,7±1,1	9,1±0,8	10,5±1,2	10,8±0,9	11,1±0,7

Table 4 - b: Cytosolic Enzymes

	Control	Benzopyrene 50 mg/kg	Benzopyrene 50 mg/kg + Ferulenol 50 mg/kg	Ferulenol 50 mg/kg
SOD UI/mg Proteins	12,5± 1,12	6,4±1,1	7,6±0,9	8,8±0,8
Catalase UI/mgProteins	1,7 ± 0.08	0,6±0,05	0,8±0,07	1,1±0,1
GSTUI/mg Proteins	15,2±2,2	9,5±1,8	11,3±1,3	12,7±1,2

Table 5 : Cytosolic Glutathione

	Control	Benz(A)P 50 mg/Kg	Benz(A)P 50 mg/kg + Ferulenol 50 mg/Kg	Férulénol 50 mg/kg
GSH mM/g Protéines	16	21,38	10,6	11,4
SD	2,3	2,56	3,1	3,4

Table 6 : Effects of free doxorubicine on FAO cells after 24h

	[DOXO]µM	%viability	SD	% SD
	0	100	0,24335769	24,3357693
	1	99,4358326	0,08060131	8,06013116
	3	84,7212088	0,1593297	15,9329697
	5	85,5562477	0,06273595	6,27359546
	10	70,40168	0,09016361	9,01636079
	15	62,5104558	0,09210631	9,2106305
	20	62,2555126	0,11734739	11,7347393
	25	60,6631191	0,13852058	13,8520585
24hrs	30	63,0746231	0,20354205	20,3542051

Table 7: Effects of liposome encapsulated doxorubicine on FAO cells after 24h

	[Enc-DOXO]µm	%Viability	SD	% SD
24hrs	0	100	0,06042587	6,04258696
	0	83,2442689	0,13675832	13,6758324
	1	83,9580752	0,06414281	6,41428094
	3	72,2222222	0,21781906	21,781906
	5	67,1623296	0,20966992	20,9669922
	10	74,7315159	0,15718824	15,718824
	15	72,6559273	0,24633069	24,6330691
	20	66,4188352	0,24395657	24,3956573
	25	62,0714581	0,14030884	14,0308843
	30	71,3444857	0,15740775	15,7407751

Table 8 : Effects of free doxorubicine on FAO cells after 48h

	[DOXO] µM	%Viability	SD	% SD
48hrs	0	100	0,11578181	11,5781815
	1	99,1491086	0,06130355	6,1303548
	3	59,3192869	0,08205051	8,20505071
	5	61,4802809	0,08315207	8,31520695
	10	59,0877518	0,11130246	11,1302462
	15	32,9011345	0,09910432	9,91043221
	20	20,884464	0,04087321	4,0873207
	25	16,4852975	0,03531019	3,53101901
	30	19,2637185	0,04129914	4,1299141

Table 9: Effects of liposome encapsulated doxorubicine on FAO cells after 48h

	[Enc-DOXOµM]	%Viability	SD	% SD
48hrs	0	100	0,00753658	0,75365775
	0	94,5665446	0,08105965	8,10596488
	1	87,5457875	0,05569201	5,56920102
	3	84,4845631	0,06796743	6,79674292
	5	83,4641549	0,05006234	5,00623421
	10	82,4786325	0,05306097	5,30609712
	15	84,3956044	0,04201428	4,20142833
	20	75,6410256	0,06532993	6,53299319
	25	76,2820513	0,02800595	2,80059517
	30	72,6373626	0,05433507	5,43350715

Table 10: Effects of LK1 doxorubicine + LK1, K3 Doxo + K3 and LK1 + K3

	SD	SD%	%viability
Control	0,05810028	5,81002828	100
LK1	0,0140085	1,40085008	33,1043615
DOXO+LK1	0,00212132	0,21213203	10,628707
K3	0,03096288	3,0962881	8,35112693
DOXO+K3	0,00469042	0,46904158	45,0771056
LK1+K3	0,02653165	2,65316523	13,4045077

Table 11: Test compounds at 10 µM Ferulenol LK1 and K3 as well as doxorubine

24hrs	%Viability	SD	%SD
Control	100	0,09239009	9,23900891
LK1	42,6138614	0,03392197	3,39219693
DOXO+LKI	19,4851485	0,01018823	1,01882285
K3	52,1188119	0,01623884	1,62388423
DOXO+K3	84,4884488	0,04158686	4,15868569
LK1+K3	20,7524752	0,00622093	0,62209324

Table 12: Effects of LK1 doxorubicine + LK1, K3 Doxo + K3 and LK1 + K3

24hrs	%Viability	SD	%SD
Control	100	0,09239009	9,23900891
LK1	42,6138614	0,03392197	3,39219693
DOXO+LKI	19,4851485	0,01018823	1,01882285
K3	52,1188119	0,01623884	1,62388423
DOXO+K3	84,4884488	0,04158686	4,15868569
LK1+K3	20,7524752	0,00622093	0,62209324

Table 13: Test compounds at 10µM Feru K1 and K3 as well as Doxorubicine

EXPT 1 24hrs			
10µM			
	%viability	**SD**	**SD%**
Control	100	0,05810028	5,81002828
LK1	8,67649551	0,0140085	1,40085008
DOXO+LK1	10,628707	0,00212132	0,21213203
K3	25,717675	0,03096288	3,0962881
DOXO+K3	54,5670225	0,00469042	0,46904158
LK1+K3	13,4045077	0,02653165	2,65316523

Table 14: Test compounds at 10µM Feru K1 and K3 as well as Doxorubicine

EXPT 2 24hrs			
10µM			
	% Viability	**SD**	**% SD**
Control	100	0,09239009	9,23900891
LK1	35,6435644	0,03190611	3,19061123
DOXO+LK1	19,4851485	0,01018823	1,01882285
K3	49,1089109	0,01276715	1,276715
DOXO+K3	64,6864686	0,04692902	4,69290244
LK1+K3	20,7524752	0,00622093	0,62209324

Table 15: Real Calculation of Concentration of Doxorubicine.

Real calculation of concentratio doxo :			**Before filtration**		**After filtration**	**Before filtration**		**After filtration**
			Reading 1	0,624	0,173	**Reading 2**	0,619	0,172
Average over 12			(492 nm)	0,636	0,170	(492 nm)	0,632	0,170
Before	0,631			0,629	0,167		0,626	0,168
				0,626	0,168		0,627	0,169
After	0,170			0,644	0,172		0,642	0,174
				0,632	0,170		0,632	0,171
% loss :	73%		**Average**	**0,632**	**0,170**	**Average**	**0,630**	**0,171**
			ET	0,007	0,002	**ET**	0,008	0,002
Real conc added µM		2,70						
				Student T by pairs :			**7%**	**17%**
Real conc of stock µM		54,01						

Table 16: Test compounds at 10µM Feru K1 and K3 as well as Doxorubicine (48h)

10µM	EXPT1 48hrs		
	% Viability	**SD**	**% SD**
Control	100	0,02196907	2,19690692
LK1	5,04976581	0,00129904	0,12990381
LK1+DOXO	5,92798595	0,00277263	0,27726341
K3	13,7587822	0,01104536	1,1045361
K3+DOXO	20,5893833	0,01040299	1,0402991
LK1+K3	7,46487119	0,0060208	0,60207973

References

References

Achon M, Alonso-Aperte E, Reyed, Ubeda N, Verela Moréras G.Hight-dose folic acid supplementation in rats: effects on gestation and the methionine cycle. Br J Nutr. **2000;** 83: 177-183.

Aggarwal BB, Shishodia S. Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol. **2006**; 71:1397–1421.

Ahmed A, Mohamed Elamir F, Hegazy E F, Toshifumi H. Ferulsialic acid, a sesquiterpenes coumarin with a rare carbon skeleton from ferula species. Phytochemistry. **2007;** 68(5) : 680-686.

Ajikumar P K, Tyo K, Carlsen S, Mucha O, Phon T H. Stephanopoulos G. Terpenoids: Opportunities for biosynthesis of natural product drugs using engineered microorganisms. Mol- Pharm. **2008;** 5:167–190.

Alan L, Miller N D.Antioxidant flavonoids: Sturcture, function and clinical usage. Alt Med Rev. **1996;** 1(2): 103-111.

Aldaghi L, Rad A, Arab A, Kasaian J, Iranshahi M, Sadr A, Soltani F. In silico and *in vitro* evaluation of cytotoxic activities of farnesiferol c and microlobin on MCF-7, Hela and KYSE Cell Lines. Drug Res. **2016;** 66: 532–538.

Alexandre F.L'apiculture aujourd'hui. Dargaud ed. **1999 ;** pp 121-125.

Alkhatib R. Etude phytochimique et activité cytotoxique des metabolites secondaires de ferula elaeochytris korovin et ferula lycia boiss (apiacéec). Chem sci.univ droit - santé – Lille II, **2010.**

Alnemri E.S, Livingston D J, Nicholson D W, Salvesen G, Thornbery N A, Womg W W and Yuan J.Human ICE / CED-3 protease nomenclature. Cell. **1996;** 87: 171.

Alnemri T F, Armstrong R C, Krebs J, Srinivasula S M, Wang L J, Bullrich F, Fritz L C,Trapani J A, Tomaselli K J, Litwack G and EMAD S. Alnemri E S. *In vitro* activation of CPP32 and Mch3 by Mch4, a novel human apoptotic cysteine protease containing two FADD-like domains (granzyme B/Mch2/protease cascade/FAS/APO-I-receptor). Proc Natl Acad. Sci. **1996;** vol 93, pp 7464 – 7469.

Amoros M, Simoes M O, Girrel A and Cormier M.Synergetic effect of flavones and flavonols against Herpes simplex virus type 1 in coll culture. Comparaison with the antiviral activity of proplis. J Nad Prod. **1992;** 55: 1732-1740.

Anderson R, Amarasinghe C, Fisher L J, Mak W B and Paker J E.Reduction in free radical induced strand breaks and base damage through fast chemical repair by flavonoïd. Free Radic Res. **2000;** 33: 91-103.

Andreyev AY, Kushnareva YE, Starkov AA. Mitochondrial Metabolism of reactive oxygen Species. Biochem. **2005;** 70(2):200-214.

Antonio C, Santos A, Sergrio A, Uyenura A, Joav L, lapes J, Fabio E, Mingahoc.Effect of naturally occuring flavonoïds on lipid peroxidation and membrane permeability transition in mitochondria. Free Radic Biol Med. **1998;** 24 (9): 1455-1461.

Antonsson B and Martinou J C.The B-cl_2 protein family. Exp Res. **2000;** 256: 50- 57.

Antunes F, Han D and Cadenes E.Relative contribution of heart mitochondria glutathione peroxydase and catalase to H_2O_2 detoxification in *in vivo*. Free Rad Biol Med. **2002**; 20: 463-466.

Anu Rahal, Amit Kumar, Vivek Singh, Brijesh Yadav, Ruchi Tiwari, Sandip Chakraborty, andKuldeep Dhama. Oxidative Stress, Prooxidants, and Antioxidants: The Interplay. Biomed Res Int. **2014;** 761264.

Arsenijevic D, Onuma H, pecqueur C, Raimbault S, Manning B S, Mirousc B, Couplan E, Alvers Guevra M C, Goubern M, Survit R, Bouillaud F, Richard D, Collins S, Riquier D. Disruption of the uncoupling protein -2 gene in mice revealsa role in immunity and reactive oxygen species production. Nat Genet. **2000;** 26: 435-439.

Asha S and Girija D. Benzo[a]pyrene induced liver and kidney cancer in swiss albino mice. Res- Pharm.**2011;** Vol 1, Issue 4.

Astridillo L, Avila C.Biologically active compounds from Chilian propolis.Bol Soc Chil.Quim. **2000 ;** 45(4).

Aurousseau B, Durand D, Gruffat D.Contrôle des phénomènes oxydatifs pendant la gestation chez les monogastriques et les ruminants. INRA Prod Anim. **2004;** 17(5) : 339-354.

Ayhanci A, Yaman S, Appak Sand Gunes S. Hematoprotective effect of seleno-L-methionine on cyclophosphamide toxicity in rats. Drug and Chem Toxicol. **2009;** 32(4): 424–428

Bagchi D, Ihm Z J, Stohs S J, Qkubue P I. Excretion of MDA, acetaldehyde cetone in the urine of rats given an acute dose of MDA. Arch Toxicol. **1994;** 6: 259-266.

Barrera G. Oxidative Stress and Lipid Peroxidation Products in Cancer Progression and Therapy. ISRN Oncol. **2012;** Volume 2012, Article ID 137289, 21pages.

Barthomeuf C, Demeule M, Grassi J, Saidkhodjaev A, Beliveau R. Conferone from Ferula schtschurowskiana enhances vinblastine cytotoxicity in MDCK-MDR1 cells by competitively inhibiting P-glycoprotein transport. Planta Med. **2006;** 72:634-639.

Beauchamp C, Fridovich I.Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem. **1971;** 44(1):276-87.

Beecher GR.Overiew of dietary flavonoïds: Nomenclature, occurrence and intake. J Nutr. **2003 ;** 133(10):32485-32545.

Benskota A H, Tezuka Y, Adryana I K.Hepatoprotective and anti-Helicobacter pylon activities of constituents from Brazilian proplis. Phytomed. **2001;** 8:16-23.

Ben-Zhan Zhu, …Li Mao. Metal-Independent Pathways of Chlorinated Phenol/Quinone Toxicity. Mol Toxicol (J& Books Sci Direct). **2011;** vol 5 pp1-143.

Bertrand F.Le vieillissement moléculaire et cellulaire et ses futurs enjeux. Actua chim Rev. **2003;** Vol 270 pp 103-107.

Bishayee A, Darvesh AS. Oxidative stress in cancer and neurodegenrative diseases: prevention and treatment by dietary antioxidants. In: Kozyrev D, Slutsky V, editors. Free radicals: formation, types and effects. Hauppauge: Nova Science Publishers, Inc; **2010;** pp. 1–

Bocca C, Gabriel L, Bozzo F, Miglietta A. Microtubule-interacting activity and cytotoxicity of the prenylated coumarin ferulenol. Planta Med. **2002;** 68:1135-1137.

Boharun T, Gressier B, Trotin F, Bruner C, Dine T, Vasseur J, Gazin J C, Pinkas M, Luyckx M, Gazin M.Oxygen species scavenging activity of phenolic extract form Hawthorn fresh plant organs and pharmaceutical preparatios. Arzeim-Forsch/Drug Res. **1996;** 1-6.

Bohr V A. Repair of oxidative DNA damage in nuclear and mitochondrial DNA, and some changes with aging mammalian cells. Free Radic Biol Med. **2002a;** 32: 804- 812.

Bohr V A, Stevnsner T and De Souza- Pinto N C.Mitochondrial DNA repair of oxidative damage in mammalian cells. Gene. **2002 b;** 286: 127-134.

Bolton J L and Dunlap T. Formation and Biological Targets of Quinones: Cytotoxic versus Cytoprotective Effects. Chem Res in Toxicol. **2017;** 30(1):13-37.

Borner C. The Bcl-2 protein family: sensors and checkpoints for life-or-death decisions. Mol Immunol. **2003;** 39. 615-647.

Boussenane H N, Kebsa W, Zellagui A, Alyane M, Rhouati S and Lahouel M. Disruption of mitochondrial membrane potential by Ferulenol and Restoration by Propolis extract : A apoptotic role of propolis , Acta Biol Hung. **2009;** 60 (4): 385-398.

Boyle S P, Dobson V L, Duthies S J, Kyle J and Collins A R.Absorption and DNA protective effects of flavonoïd glycosides from an onion meal. Eur J Nutr. **2000;** 39: 213-223.

Bradford M M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. **1976;** 7,72:248-54.

Bridger S, Deaths from low dose paracetamol poisoning. Br Med J. **1998;** 316: 1724-1725.

Brijesh Kumar Singh, Madhulika Tripathi, Bhushan P Chaudhari, Pramod K Pandey and Poonam Kakkar. Natural Terpenes Prevent Mitochondrial Dysfunction, Oxidative Stress and Release of Apoptotic Proteins during Nimesulide-Hepatotoxicity in Rats. PLoS One. **2012;** 7(4): e 34200.

Brossy-Wetzel E, Neumeyer D D, Green D R.Mitochondrial C- release in apoptosis occurs apstream of DEVD – Specific Caspase activation and indepently of mitochondrial transmembrane depolarisation. EMBO. **1998;** 17: 37-49.

Brown J E, Khodr H, Hider R C and Rice-Evans.Structural dependence of flavonoïd interactions with copper ions: Implications for their antioxidant properties. Biochem J. **1998;** 330: 1173- 1178.

Brunet S.Vue sur les mécanismes de contrôle du cholestérol hépatique suite au stress peroxydatif induit par le fer. Thèsede doctorat**,** Montréal Canada.**1999**; pp. 4-24.(2b)

Bruneton J. Pharmacognosie, Phytochimie, Plantes médicinales, (2ème éd.). Paris: éditions Tec & Doc Lavoisier. **1993 ;** pp 266-293.

Bruneton J.Pharmacognosie, Phytochimie, Plantes médicinales, (3ème éd.). Paris: éditions Tec & Doc Lavoisier. **1999 ;** p1120.

Burdick G A. Review of the biological properties and toxicity of bee propolis. Food Chem Toxicol.**1998;** 36: 347-363.

Cadenas E, Davies K J. Mitochondrial free radical generation, oxidative stress, and aging. Free Rad Biol and Med. **2000;** 29(3–4):222-230.

Cadet J, Delatour T, Douki T, Gasparutto D, Pouget J P, Ravanat J P, Ravanat J L and Sauvaigo S. Hydroxyl radicals and DNA bases damage. Nutr Res. **1999 ;** 424: 9- 21.

Cadet L, Harrington B and Ordonez S. The Metabolism and toxicity of quinones, quinonimines, quinone methides, and quinone-Thioethers. Synapse. **2000;** 35: 228-233.

Cadet J, Bbellon S, Bberger M, Bbourdat A G, Ddouki T, Duarte V, Frelon S, Grasparu Ho D, Muller E, Revanat E J L and Sauvaigo S.Recent aspects of oxidative DNA dange. Biol Chem. **2002;** 282(6): 3.

Chacon E, Acosta D. Miochondrial regulation of superoxide by Ca^{2+} : an alternate mechanism for the cardiotoxicity of doxorubicin. Toxicol and appl pharm .Elsevier. **1991;** 107(1):117-28.

Chadge G D, Lee J P, Bindokas V P, Jordan J, Ma L, Miller R J and Ross R P. Mutant superoxyde dismutase -1 linked FaL S. Molecular mechanism of neuronal death and protection. J Neurosis. **1997;** 17: 8756-8766.

Chanvallon C, Blanchema Son P, Cance-Sanchez B.Les flavonoides. Act Med Angiology. **1994;** 179: 3846- 3850.

Chehma A, Djebar M R, Hadjaiji F, Rouabeh L. The floristic composition of the Saharan rangelands of South East Algeria. Sc Chang Plan/Sécheresse. **2005;** 16(4) : 275–285.

Cheng E H Y, Kirsch D G, Clem R J, Ravi R, Kastan M B, Bedi A, Ueno K and Hardwick J M. Conversion of Bcl-2 to a Bax-like death effector by caspases. Sci. **1997;** 278:1966–1968.

Cheng I F, Breen K.On the ability of form flavonoid, bacilien, luteolin, naringenin and quercetin, to supress the Fenton reaction of the iron-ATP complex. Biometals.**2000;** 13(1): 77- 83.

Chin O K, Kim D O, Lee C Y.Superoxide radical scavenging activity of the polyphenols in frush plums. J Agric Food Chem. **2003;** 51(27):8067-8072.

Christov R, Bankova V, Hegazi A, Abd El Hady F and Popov S. Chemical composition of Egyptian propolis. Z Natur Foresh. **1998;** 53c: 197-200.

Claiborne A. Catalase activity. In: CRC handbook of methods for oxygen radicalresearch. Ed Greenwald RA, CRC Press, Boca Raton. **1985;** 283-284.

Clark D P, Pazdernik N J. Aging and apoptosis. Biotech (second edition). **2016.**

Clifford M N.Chlogenic acids and other cinnamitales, nature, occurrence and dietary burden. JSC Food Agric. **1999;** 79: 362- 372.

Comhair S A and Erzurum S C.Antioxidant responses to oxidant-mediated lung diseases. AmJ physiol. **2002;** 283: L 246-255.

Conklin Daniel J, Haberzettl Petra, Prough Russell A and Bhatnagar Aruni. Glutathione-*S*-transferase P protects against endothelial dysfunction induced by exposure to tobacco smoke. Amer Physiol Soci. **2009;** 296(5).

Coogh A C, Miles J S, Spurr N K, Moss J E, Gaedigk A, Wolf C R. Identification of the primary gene defect at the cytochrom P450. Nat. **1990**; 347: 773-776.

Cook N C, Samman S.Flavonoids-chemitry, metabolism, cardioprotectiveeffects and dietary sources. J Nutr Biochem. **1996;** 66- 76.

Corbiere C. Comparaison de l'effet anti-prolifératif de trois stéroïdes végétaux (diosgénine, hécogénine, tigogénine) sur la lignée 1547 d'ostéosarcome humain Implication de la mitochondrie et de la cyclooxygénase-2 dans l'apoptose induite par la diosgénine sur les lignées 1547, HEp-2 (laryngocarcinome) et M4Beu (mélanome).Thèsede doctorat univ de Limoge. **2003 ;** pp 22-250.

Cormier A, Morin C, Zini R, PTillementJ, Lagrue G.Nicotine protects rat brain mitochondria against experimental injuries.Neuropharm **2003;** vol 44, issue 5.

Crespy V, Morand C, Besson C, Manach C, Denigne C and Remesy C.Comparaison of the intestinal absorption of quercetin , phloretin and their glucosides in rats.J Nutr. **2004;** 131: 2109-2114.

Crowell PL.Prevention and therapy of cancer by dietary monoterpenes.J Nutr.(2a).**1999;** Volume 129, Issue 3, Pp 775S–778S.

Dastan D, Salehi P, Ghanati F, Gohari AR, Maroofi H, Alnajar N. Phytotoxicity and cytotoxicity of disesquiterpene and sesquiterpene coumarins from Ferula pseudalliacea. Ind Crops Prod, **2014a;** 55:43-48.

Dastan D, Salehi P, Gohari AR, Ebrahimi SN, Aliahmadi A, Hamburger M. Bioactive Sesquiterpene Coumarins from Ferula pseudalliacea. Planta Med. **2014b;** 80:1118-1123.

Dastan D, Salehi P, Aliahmadi A, Gohari AR, Maroofi H, Ardalan A. New coumarin derivatives from Ferula pseudalliacea with antibacterial activity. Nat Prod Res. **2016;** 30:2747-2753.

Day A J, Dupont M S, Ridley S, Rhodes M T C, Morgan M R A and Williamson G.Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and livre beter glucosidase activity. FERS Lett. **1998;** 436: 71-75.

Decastro S L, Higashi K B. Effect of different formulations of propolis on mice infeeted with Erypaosolna cruzi. J of Ethopharm. **1995;** 46: 55-8.

Deleve Laurie D. Hepatotoxicity by Anticancer Therapy.Cancer med, 6th edition. **2003.**

Deprey S, Brizillon C, Rabot S, Phillipe C, Milla I, Lapierre C and Scalbert A.Polymeric Proanthocyanidines are catabolused by a human colonic microfloa into low molecular weight phenolic acids. J Nutr. **2000;** 130: 2733- 2738.

Drog W.Free radicals in the physiological control of cell function. Cell Physiol Rev. **2002 ;** 82: 42.

Dugas A J, Castaneda-Acosta J, Boni G C, Price K L, Fisher N H, Winston G W. Evaluation of the total peroxyl radical Scavenging capacity of flavonoids: Sturcture-Activity reletionship. J Nat Prod.**2000;** 63: 327- 331.

E/ Razek MAA, Ohta S , Hirata T. Terpenoid coumarins of the genus Ferula. Heterocycles. **2003;** 60: 689-716

Echtay K S, Esteves T C, Pakay J L, Jekabsons M B, Lamber A J, Porterotin M, Pamplona R, Vidal-Puig A J, Wang S, Roebuck S J and Brand M D. A signalling role for 4HNE I regulation of mitochondrial uncompling. Embo J. **2003;** 22: 4103-4110.

Elicoh-Middleton Jr, Chithan K and Theoharis C.Effect of plant flavonoids on mammalian cells: implications for inflammation, heart diseases and cancer. Pharma and Exp therap. **2000;** 4(52): 673-751.

Elimadi A and al. Dose-related inversion of cinnarizine and flunarizine effects on mitochondrial permeability transition.Eur J of Pharm.**1998;** 348(1):115-121.

EllmanGL. Tissue sulfhydryl group. Arch Biochem Biophys. **1959;** 82: 70-77

English W, green way W, whatly F R.Analysis of phenolics in the bud exudates of propolis by GS-MS. Phytochemistry. **1992;** 31: 1255-1260.

Erica weinstin T, Giuseppina N, Renata M, Dejair M, Antonio S.Plant origin of green propolis: Bee behavior, plant anatomie and chemistry. Evid based complement alternat Med. **2005;** 2(1): 85- 92.

Estrela JM, Ortega A, Obrador E. Glutathione in cancer biology and therapy. Crit Rev Clin Lab Sci. **2006;** 43: 143-81

Evangelist-Rodrigus A, Carneiro P M.Analise comparative da qualidade da propolis colectado atravers de calços de medeira etela plasticana regiao do byo paraeibao. Mensagem Doce. **2001;** 63(bis).

Felgines C, Texier O, Morand C, Manach C, Scalbert A, Regerat F and Remesy C. Bioaviolability of the flavonone naringenin and its glycosides in rat. Am J physiol. **2000;** 279G: 148-1154.

Fesik S W, Shi Y.Structural biology. Controling the caspases. Sci. **2001;** 294: 1477-1478.

Friguet B, Szwed L I. Inhibition of the multicatalytic proteinase (proteasome) by 4- hydroxyl-2- nonenal -Gross linked protein. FEBS Lett. **1997;** 405: 21.

Gamal Eldeen AM, Hegazy M E. A crystal lapiferin derived from Ferula vesceritensis induces apoptosis pathway in MCF-7 breast cancer cells. Nat Prod Res. **2010;** 24 (3): 246-257.

Gardner P R, Raineri I, Epstein L B and Whitec W.Superoxide radical and iron modulate aconitase in mammalian cells. J Biol Chem. **1995;** 270: 13399- 13405.

Gardner R R. Aconitase. Sensitive target and measure of superoxide. Meth Enzymol. **2002;** 349: 9-23.

Gee J, Dupont M S, Day A J, plumb G W, Williamson G and Johnson I J. Intestinal transport of quercetin glucosides in rats involves both deglycosylation and interaction with the hexose transport pathways. J Nutr. **2000;** 130: 2765-2771.

Gerhard R.Métabolisme des végétaux, physiologie et biochimie. Lavoisier Tec & Doc. **1993 ;** pp 333-339.

Ghaderi A, Khaghanzadeh N, Samiei A, Mojtahedi Z. Umbelliprenin induced both anti-inflammatory and regulatory cytokines in C57/BL6 mice.I J B M. S **2017;** 20(7):829-834

Ghazal S A, Abuzarqua M and Mahernserh A M.Effect of plant flavonoïds on immune and inflammatory cell function. Phytothera Res. **1992;** 6: 265- 271.

Ghisalberti E L.Propolis, a review. Bee Wold. **1997;** 60: 59-84.

Gholami O, Jeddi-Tehrani M, Iranshahi M, Zarnani AH, Ziai SA.Umbelliprenin from Ferula szowitsiana activates both intrinsic and extrinsic pathways of apoptosis in jurkat T-CLL cell line. Iran J Pharm Res. **2013;** 12:371-376.

Gholami O, Shamsara J. Comparison of the cytotoxic effets of umberlliprenin and auraptene. Inter J of Pharm and Pharmaceu Sci. **2016;** Vol 8, Issue 1.

Ghorbani A, Mohebbati R, Jafarian AH, Vahedi MM, Hosseini SM, Soukhtanloo M, Sadeghnia HR, Hosseini A. Toxicity evaluation of hydroalcoholic extract of Ferula gummosa root. Regul Toxicol Pharmacol.**2016;** 77:35-41.

Giorgio Minotti, Pierantonio Menna, + 2 authorsLuca Gianni.Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity.Pharmacol Rev. **2004;** Volume 56 2, pages 185-229.

Gliszczynska A, Brodelius P E. Sesquiterpene coumarins. Phytochem Rev. **2012;** 11:77–96.

Grasfe E U, Witting J, Mueller S, Rie thling A K, Uehlek B, Drewelow B P, forte H, Jacobaxh G, Derenodorf H and Veit M. Pharmacokinetic and bioavailability of quercetin glycosidesin lumans. J Clin Pharmacol.**2001;** 41: 492-499.

Green D, Kromer G. The central executioners of apoptosis: Caspases or mitochondria?. Cell Biol. **1998;** 8: 267- 271.

Gross A, Mc Donnell J M, Koromeyer S J.Bcl-2 family members and the mitochondria in apoptosis. Genes Dev. **1999;** 13: 1899-1911.

Gu M, Gash M T, Mann Y M, Javoy-azid F, Cooper J M and Schapia A H. Molécular caracterization of mitochondrial poptosis-inducing factor Ann. Neurol. **1996;** 39:385-389.

Guan Z, Xue D, Abdallah I I, Dijkshoorn L, Setroikromo R, Guiyuan L, et al. Metabolic engineering of Bacillus subtilis for terpenoid production.Appl Microbio and Biotech. **2015;** 99:9395–9406.

Gupta S, Hastak K, Ahmad N, Lewin J S and Mukthen H.Inhibtion of procancinogenessis in TRAMP mice by oral infusion of green tea polyphenol. Natl Acad Sci.**2001;** 98(18): 10350-10355.

Gupta R A, Tejada L V, Tong B J, Das S K, Morrow J D, Dey S K and DuBois R N.Cyclooxygenase-1 is overexprossed and prometes angrogenic growth factor production in ovarien cancer. Cancer Res. **2003;** 63: 906-991.

Guyton KZ, Kensler TW. Oxidative mechanisms in carcinogenesis. Br Med Bull.**1993;** 49(3):523-44.

Habibi Z, Aghaie H R, Ghahremanzadeh R, Masoudi S. Composition of the Essential Oils of Ferula szowitsiana DC., Artedia squamata L. and Rhabdosciadium petiolare Boiss. & Hausskn.ex Boiss. Three Umbelliferae Herbs Growing Wild in Iran. J of Essent Oil Res. **2016;** 18(5):503-505.

Habig WH, Pabst M J, Jacoby W B. Glutathione S – transferases. The first Enzymaztic step in mercapturic acid formation. J Bio Chem. **1974;** 249(22):7130-9.

Halliwell B. Free Radical and antioxidants: a personal view. Nutr Rev. **1994;** 52: 253- 265.

Hang Cui, Yahui Kong and Hong Zhang. Oxidative Stress, Mitochondrial Dysfunction, and Aging. J Signal Transduct. **2012;** 646354.

Hanazaki Y, Ogawa S, Fukui S. The correlation beetweren active oxygen scavenging and antioxidative effects of flavonoids. Free Radical Biol Med. **1994;** 16:845- 850.

Hao jiang, Stacy L Gelhaus, Dipti Mangal, Ronald G Harvey,IaA.Blair and Trevor M.Penning. Metabolism of Benzo(a)pyranein Human BroncoalveolarH358 cells Using Liquid Chrpatography-Mass Spectrometry. ChemRes Toxicol. **2007;** 20(9):1331-1341.

Hansford R G, Hogue B A and Mildaziane V. Dependance of H_2O_2 formation by rat heart mitochondria on substrate availability and donor age. J Bioenerg Biomembr. **1997;** 29: 89-95.

Harada H, Kan Y, Fukui Y, Kageyama N, Nakai M, Miki W and Kiso V. Identification of the major antioxidative metabolites in biological fluids of the rat with ungasted catechin and epicatechin. Bio Biotech. **1999;** 63: 973-977.

Harborne, J B and Williams C A.Advances in flavonoides research Since 1992.Phytochemistry. **2000;** 55: 481-504.

Hasanzadeh D, Mahdavi M, Dehghan G, Charoudeh H N. Farnesiferol C induces cell cycle arrest and apoptosis mediated by oxidative stress in MCF-7 cell line. Toxicol Reports. **2017**; 4(420–426).

Hanafi-Bojd M Y, Iranshahi M, Mosaffa F, Tehrani SO, Kalalinia F, Behravan J. Farnesiferol A from Ferula persica and galbanic acid from Ferula szowitsianainhibit P-glycoprotein-mediated rhodamine efflux in breast cancer cell lines. Planta Med. **2011;** 77:1590-1593.

Hauptmann N, Grimsby J, Shuh J C and Cadenas E. The metabolism of tyramine by monoamine oxidase A/B causes oxidative damage to mitochondrial DNA. Arch. Biochem. Biophys. **1996**; 335: 295-304.

Heijnen G G, Haenen G R, Van Aker F A, Vander Vijgh W J. Bast AFlavonoïds as peroxinitrite scavengers: the role of the hydroxyl groups. Toxicol.**2001;** 15(1): 3- 6.

Hengarther 2000. Hengartner NO. The biochemistry of apoptosis. Nat . **2000;** 407: 770- 776.

Herrero A and Barja G. Localization of the Site of Oxygen Radical Generation inside the Complex I of Heart and Nonsynaptic Brain Mammalian Mitochondria. J Bioenerg. Biomembr. **2000;** 32: 609-615.

Hirano R, Osakabe N, Iwamoto T, Matsumoto A, Natsume M, Takizawa T, Igarashi O, Itakura H and Kondo K. Antioxidant effects of polyphonols in chocholate on low density lipoprotein both invitro and invivo.J Nutr Sa.Vitaminol. **2000;** 46: 199- 204.

Ho Y S, Xiong Y, M a W, Spector A and Ho D S.Mice laking catalase developped normallybut slow differencial sensitively to oxidant tissue injury. J Biol Chem. **2004**; 297: 32804- 32812.

Hofer O, Widhalm M, Greger H. Circular dichroism of sesquiterpene umbelliferone ethers and structure elucidation of new derivatives isolated from the gum resin asa4feloda. Monatsh Chem. **1984;** 115 (10): 1207-1213.

Hollman P C, Van Tijp J M, Brysman M N, Van der Gaag M S, Mengelers M J, De Vries J H and Katan M B.Relative bioavailability of the antioxidant flavonoid quercetin form various food in man. FEBS Lett. **1997;** 418: 152-156.

Hollman P C, Bijsman M N, Van-Gameren V, Gnossen E P, De Vris J H and Hatan M B. The suguar moiety is a major determinant of the absorption of dietaryflavonoids glycosides in man. Free Radic Res. **1999;** 31: 569-573.

Huang M T, Xie J C,Wang Z Y.Effect of tea, decaffeinated tea, caffeine on UVB light-iducedcompletecaecemogensisin SHK-1 micedemonstration of caffeine as a biologically important constituent of tea. Biol Res. **1997;** 13: 2623-2629.

Ikeda K, Arao Y, Otsuka H, Nomoto S, Horiguchi H and Kato S. Terpenoids found in the umbelliferae family act as agonists/ antagonists for ERa and ERb: differential transcription activity between ferutinine-liganded ERaand ERb. Biochem Biophys Res. **2002;** Commun 291:354–60.

Ingy I. Abdallah and Wim J. Quax. A Glimpse into the Biosynthesis of terpenoids.NRLS Conf Proceedings. **2017.** v3i5.981.

Ingrid Emirit. Clastenogenic Factorsas Potential Biomarkersof Increased Superoxide Dismutase. Biomark Insights. **2007;** 2;429-438

Iranshahi M, Arfa P, Ramezani M, Jaafari MR, Sadeghian H, Bassarello C, Piacente S, Pizza C. Sesquiterpene coumarins from Ferula szowitsiana and in vitro antileishmanial activity of 7-prenyloxycoumarins against promastigotes. Phytochemistry, **2007;** 68:554-561

Iranshahi M, Askari M, Sahebkar A, Dimitra Hadjipavlou L. Evaluation of antioxidant, anti-inflammatory and lipoxygenase inhibitory activities of the prenylated coumarin unberlliprenin.DARU J of Pharmaceu Sci.**2009;** Vol 17: 2, 99 - 103

Iranshahi M, Masullo M, Asili A, Hamedzadeh A, Jahanbin B, Festa M, Capasso A, and Piacente S.Sesquiterpene Coumarins from Ferula gumosa. J Nat Prod. **2010;** 73 (11), pp 1958–1962.

Iranshahi M, Rezaee R, Najafi M N, Haghbin A, Kasaian J. Cytotoxic activity of the genus Ferula (Apiaceae) and its bioactive constituents.Avicenna J Phytomed**, 2018;** 8 (4): 296-312.

Iranshahy M, Iranshahi M. Traditional uses, phytochemistry and pharmacology of asafoetida (Ferula assa-foetida oleo-gum-resin). A review J Ethnopharm. **2011;** 134:1–10.

Ishige K, Schubert D and Sagara Y.Flavonoïds protect neuronal cells from oxidative stress by three distinct mechanisms. Free Radic Biol Med. **2001;** 30: 433- 446.

Isla M, Moreno M, Sampietro A and Vattune M A.Antioxidant activity of Argentina propolis extract.J Ethnopharma. **2001;** 76: 165- 170.

Jabrane, A., Ben Jannet, H., Mighri, Z., Mirjolet, J.F., Duchamp, O., Harzallah-Skhiri, F. & Lacialle-Dubois, M. A. Two new sesquiterpene derivatives from the Tunisian endemic Ferula tunetana Pom. Chem. Biodivers. **2010;** 7, 392-399.

Jain D, Ahmad T, Cairo M, Aronow W.Cardiotoxicity of cancer chemotherapy: identification, prevention and treatment. Ann Transl Med.**2017;** Vol 5, No 17. 5(17): 348

J P Pal frey H C, Bindokas V P, Ghadge G D, Ma L, Miller R J. The role of immuno philins in mutant superoxide dismutase 1- linked FALS. Proc Natl Acad Sci. **1999;** 69: 3251-3256.

Julies A, Christin M.Dietry flavonoids: Bioavailabity, metabolic effects and safety. Annuel Rev of Nutr. **2002 ;** 22: 19-44.

Junemann S, Heathcote P and Rich P R.On the mechanism of quind oxidation in the bc1 complex. J Biol Chem. **1998;** 273: 21603- 21607.

Kaefer CM, Milner JA. The role of herbs and spices in cancer prevention. J Nutr Biochem. **2008**; 19:347–361

Kalkavan H and Douglas Green R. MOMP, cell suicide as a BCL-2 family business. Cell Death Differ. **2018;** 25(1): 46–55.

Karimi G, Iranshahi M, Hosseinalizadeh F, Riahi B, Sahebkar A. Screening of Acetylcholinesterase Inhibitory Activity of Terpenoid and Coumarin Derivatives from the Genus Ferula. Pharmacol online. **2010;** 1: 566-574.

Karmasyn M. Ischemic and reperfusion injury in the heart cellular mechanisms and pharmacilogical in tervention. J Physiol Pharmacol. **1991;** 69: 719-730.

Kasaian J, Mosaffa F, Behravan J, Masullo M, Piacente S, Ghandadi M, Iranshahi M. Reversal of P-glycoprotein-mediated multidrug resistance in MCF-7/Adr cancer cells by sesquiterpene coumarins. Fitoterapia. **2015;** 103:149-154.

Kawaltoxski A J, Castilho R E, Vercesi A E.Mitochondrial permeability transition and oxidative stress. FEBS Lett. **2001;** 495:12-15.

Kelner M J and Montoya M A.Structural organization of the human glutathione reductase gene. Determination of correct c DNA sequence and identification of a mitochondrial leader sequence. Biochem Biophys Res Commun. **2000;** 269: 366- 368.

Kessler M, Ubeau G, Jung L. Anti-and pro-oxydant activity of rutin and quercetin derivatives. J Pharm Pharmacol.**2002;** 55: 1-11.

Khaghanzadeh N, Mojtahedi Z, Ramezani M, Erfani N, Ghaderi A. Umbelliprenin is cytotoxic against QU-DB large cell lung cancer cell line but anti-proliferative against A549 adenocarcinoma cells. DARU J Pharm Sci. **2012;** 20:69-75.

Kim H S, Kwack S J,Lee B M.Lipid peroxidation, antioxidant enzymes, and benzo[a]pyrene-quinones in the blood of rats treated with benzo[a]pyrene.Chem-biol Interactions.**2000;** 127(2):139-150] .

Kluch R M, Bossy E, Green D R, Neumeyer D D.The release of cytochrom-C- from mitochondria. Sci. **1997;** 275: 1132- 1136.

Kobayashi T.Calcium-induced mitochondrial swelling and cytochrome c release in the brain: its biochemical characteristics and implication in ischemic neuronal injury. Brain Res. **2003;** 960(1-2):62-70.

Kogure K, Yamauchi I, Tokumura A, Kondou K, Tanaka N, Takaishi Y, et al. Novelantioxidants isolated from plants of the genera Ferula, Inula, Prangosand Rheum collected inUzbekistan. Phytomed. **2004;** 11: 645-51.

Kokaleci A, Marina B, Pepel S. Analysis of propolis from the continental and adreatic regions of Croatia. Acta Pharm. **2003;** 53: 275-285.

Korshunov S S, Skulachev V P and Strakov A A.High prolonic potential actuates: a mechanism of the production of reactive oxygen species in mitochondria.FEBS Lett. **1997;** 416: 15-18.

Koza R A, Kozak U C, Brown L J, Leiter E H, Macdonald M J and Kozak L P. Sequence and tissue-dependent RNA expression of mouse FAD-linked glycerol-3-phosphate dehydrogenase. Arch Biochem Biophys. **1996;** 336: 97-104.

Krajewski S, Takayama S, Schilber Fenton W and Pxeed J C.Investigation of the subcellular distribution of the b-cl_2 encoprotein. Cancer Res. **1993;** 53: 4701-4714.

Krinsky N E.Mechanism of Action of Biological antioxidants. Proc Soc Exp Biol Med. **1992;** 200: 248- 254.

Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griel AE, Etherton TD. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med. **2002**; 113 Suppl 9B:71S–88S.

Kroemer G, Dallaporta B, Resche-Rigon M. The mitochondrial death/life regulator in apoptosis and necrosis, Annu. Rev. Physiol. **1998;** 60619–642.

Kroemer G and Reed J C.Mitochondrial control of cell death. Nat Med. **2000;** 6: 613-519.

Kujumgier A, Tsvetkova J, Serhdjieva Y, bankova V, christov R and Popov S. Antibacterial, antifungal and antiviral activity of propolis of differet geographic origin. J Ethno Pharmacol. **1999;** 64: 235-240.

Kumazawa S, Yoneda M, Shibata I, Kanaeda J, Hamasaka T, Nakayama T. Direct evidence of Brazilian propolis by the observation of honeybee behavior and phytochemical analysis. Chem Pharm Ball. **2003;** 203: 740 – 742.

Kunduzova O R, Bianchi P, Parini A and Cambon C.Hydrogen peroxide production by monoamine oxidase during ischemia-reperfusion.Eur J Pharmacol. **2002;** 448(2-3): 225-30.

Kuresh A, Youdim A, Jeremy P E, Spencer, Hangen S and Rice-Evans C.Dietary flavonoids as potential neuroprotectants. Biol Chem. **2002;** 383: 503-519.

Kushnareva V, Murphy A N and Andreyev A.Complex I mediated reactive oxygen species generation: modulation by cyt-C and NAD(P) oxidation- reduction state**.** J Biochem. **2002;** 368: 545-553.

Lahouel M, Zini R, Zellagui A, Rhouati S, Carrupt P A, Morin D. Ferulenol specifically inhibits succinate ubiquinone reductase at the level of the ubiquinone cycle. BBRC. **2007;** 35 (1): 252-257.

Lahouel M. Interaction flavenoïdes-mitochondrie et rôle de la propolis dans la prévention de l'apoptose induite par certains médicaments anticancéreux. Thèse de doctorat univ Mentouri de Constantine, Algérie. **2005 ;** pp 61-80.

Lambert A J and Brand M D.Superoxide production by NADH: ubiquinone oxidoreductase depends on the pH gradient across the mitochondrial inner membrane. Biochem J. **2004;** 382: 511- 517.

Lamnaouer D B, Bodo M, Martin D. Molho, Ferulenol and xhydroxyferulenol, toxic coumarins from Ferula communis var. genuine. Phytochem. **1987;** 26: 1613–1615.

Lariche N, Lahouel M, Benguedouar L, Zellagui A. Ferulenol, a Sesquiterpene Coumarin, Induce Apoptosis via Mitochondrial Dysregulation in Lung Cancer Induced by Benzo[a]pyrene: Involvement of Bcl2 Protein. Anti-Cancer Agents in Med Chem. **2017;** Vol 17 , Issue 10

Lee J P, Pal frey H C, Bindokas V P., Ghadge G D, Ma L, Miller R J. The role of immuno philins in mutant superoxide dismutase 1- linked FALS. Proc Natl Acad Sci. **1999;** 69: 3251-3256.

Lenaz G A. Role of mitochondria in oxidative stress and aging. Biolphys Acta. **1998;** 1366: 53-67.

Ligeret H, Barthelemy S, Zini R T, tillement J P, Labidalle S and Morin D.Effects of curcumin and curcumin derivatives on mitochondrial permeability transition pore. Free Radical Biol& Med. **2004;** 36. 919- 929.

Likhachev A J, Benioashvili S D, Bykov V J, Dikun P P, Tyndik L, Sabouchkina I V, Yarmilov V B and Zabezhinskai M A. Biomarkers for individual susceptibility to carcinogenic agents: Excretion and carcinogenic risk of benzo(a)pyrene metabolites. Environ Health Perspectives. **1992;** 98, pp. 211-214.

Lipshultz S E, Cochran T R, Franco V I and Miller T L. Treatment-related cardiotoxicity in survivors of childhood cancer. Nat Rev Clin Oncol. **2013;** 10(12).

Liu Y, Liu J, Iszard M B, Andrews G K, Palmitert R D and Klaasen C D.Transgenic mice that overexpresse metallothioneine-I are protected from cadmium lethality and hepatotoxicity. Toxicol Appl pharmacol. **1995 ;** 135(2): 222-228.

Liu Y, Fiskum G, Schubert D. Generation of reactive oxygen species by the mitochondrial electron transport chain**.**J of Neurochem (JNC). **2002;** Vol80, Issue 5 pp 780-787.

Lofler M, Becker C, Wegerle E and Schuster G. Catalytic enzyme histochemistry and biochemical analysis of dihydroorotate dehydrogenase/oxidase and succinate dehydrogenase in mammalian tissues, cells and mitochondria. Histo Chem Cell Biol, **1996;** 105: 119-128.

Lopez-Lazaro M. A new view of carcinogenesis and an alternative approach to cancer therapy. Mol Med. **2010;** 16: 144-53.

Lourenco AM, M Ferreira L, S Branco P. Molecules of natural origin, semi-synthesis and synthesis with anti-inflammatory and anticancer utilities. Curr Pharml Des. **2012;** 18:3979-4046.

Lowry O H, Rosebrough N J, Farr A L, Randall R J. Protein measurement with the Folin phenol reagent. J Biol Chem. **1951;** 193: 265-75.

Ludemann S M. The chemistry of the metabolites of cyclophosphamide. Current Pharmaceu Design. **1999;** 5, 627 - 643

Maceijeuvicz W, Danieuski M, Bal K, Markouski W.CG – MS identification of the flavonoïde aglycones isolated from propolis. Chromatographia ed. **2001;**5 3: 343- 346.

Maggi F, Cecchini C, Cresci A, M Coman M, Tirillini B, Sagratini G, Papa F. Chemical composition and antimicrobial activity of the essential oil from *Ferula glauca* L. (*F. communis*L. subsp. *glauca*) growing in Marche (central Italy).Fitoterapia**2009;** Vol 80, Issue 1, Pages 68-72.

Małgorzata Nita and Andrzej Grzybowski. The Role of the Reactive Oxygen Species and Oxidative Stress in the Pathomechanism of the Age-Related Ocular Diseases and Other Pathologies of the Anterior and Posterior Eye Segments in Adults. Oxid Med Cell Longev. **2016;** 3164734.

Manach C, Morand C, Texier O, Favier M L, Agullo G, Regerat F and Remesy C.Quercetin metabolites in plasma of rats fed diets contaming rutin or quercetin. J Nutr. **1995;** 125: 1911-1922.

Manach C, Morand C, Demigne C, Texier O, Regerat F and Remesy C.Bio availabity of rutin and quercetin in rast. FEBS Lett. **1997;** 409: 12-16.

Manach C, Scalbert A, Morand C, Remesy C, Jimenez L.Polyphenols: Sources and bioavailability. Amj chin Nutr. **2004;** 79(5):727-747.

Marfak A. Radicaux issus des alcools : formation des depsides. Thèse de doctorat univ - Limoges. **2003 ;** pp 24-42.

Marfak A. Radicaux issus des alcools : formation des depsides. Thèse de doctorat univ de Limoges. **2003;** pp 175-176.

Martin Cordero C, Leon González A J , Calderon Montaño J M , Burgos Morón E and Lopez Lázaro M. Pro-Oxidant Natural Productsas Anticancer Agents. Bentham Sci Publishers **2012;** Vol. 13, No. 8

Martin F. Vannin-1, un nouveau régulateur moléculaire du stress oxydant et du l'inflammation. Thèse de doctorat univ de la méditerranée centre de l'immunologie de Marseille – Luminy NSERM U 136- CNRSU MR6102. **2003;** pp 17.

Marzo I, Brenner C, Zamzami, Susin S A, Beutner G, Brdiczka D, Rémy R, Xie Z H, Reed J C and Guido Kroemer. The Permeability Transition Pore Complex: A Target for Apoptosis Regulation by Caspases and Bcl-2–related Proteins. J Exp Med. **1998;** Vol 187, Number 8, 1261–1271

Matsriski T, Sakai T, Naito E, Nagamitsu S, Kuroda Y, Iwashita H and Kato H. Elevated cerebrospinal fluid malate / pyruvate ration in Machado – Joseph disease. Acta. Neurol. Scand, **1996;** 93: 72-75.

Mayer L D, Tai L C L, Bally M B, Mitilenes G N, Ginsberg R S, Cullis P R. Characterization of liposomal systems containing doxorubicin entrapped in response to pH gradients. Biochim Biophys Acta. **1990;** 1025, 143–151.

Mclennan H R and Degli -Eposti M. The contribution of mitochondrial respiratory complexes to the production of reactive oxygen species. J Bioenerg Biomembr. **2000;** 32: 153-162.

Melina V, Bankova V, SorkumK, Saadi H.Propolis from the Mediterranean region, Chimical composition and antimicrobial activity. Z Naturforsh. **2000;** 55c, 790-793.

Meloo S, Cokun P, Patel M, Tuinstra R, Cottrell B, Jun A S, Zastaway T H, Dizdaroglu M, Goodman S I, Huang T T, MIziorko H, Epstein C J and Wallace D C.Mitochondrial disease in 2 superoxide dismutase 2-mutant mice. Proc Nath. Acad SCI USA.**1999;** 96: 846- 851.

Mira L, Ferrandeg M T, Sontos M, Rocha R.Interaction of flavonoids with iron and copper ions: a mechanism for the antioxidant activity. Free Radic Res. **2002;** 36(11):1199-1208.

Miski M. Biologically Active Sesquiterpenes from the Indigenous Ferula species(Apiaceae) of Turkey.Planta Med, **2013;** 79:PN32

Mivabella M, Di Giovanni S, Silvesti G, Tonali P and Servidei S. Apoptosis in mitochondrial encephalomyopathies with mitochondrial DNA mutations: a potential pathogenic mechanism. Brain.**2000;** 123: 93-104.

Miyak T, Shibamato K. Antioxidative activites of natural compounds fond in plants. Food Chem. **1997;** 45: 1819- 1822.

Mollazadeh S, Matin MM, Iranshahi M, Bahrami AR, Neshati V, Behnam-Rassouli F. The enhancement of vincristine cytotoxicity by combination with feselol. J Asian Nat Prod Res, **2010;** 12:569-575.

Mollazadeh S, Matin MM, Bahrami AR, Iranshahi M, Behnam-Rassouli M, Rassouli FB, Neshati V. Feselol enhances the cytotoxicity and DNA damage induced by cisplatin in 5637 cells. Z Naturforsch C. **2011;** 66:555-561.

Montillet J L, Chamnongpol S, Rustérucci C, Dat J, van de Cotte B, Agnel J P, Battesti C, Inzé D, Van Breusegem F, Triantaphylidès C. Fatty acid hydroperoxides and H_2O_2 in the execution of hypersensitive cell death in tobacco leaves. Plant Physiol. **2005;** Vol. 138 (3): 1516-26.

Morin D, Thierry H, Spedding M, Tillement J P.Mitochondria as target for antiischémic drugs. Adv Drug Deliv Rev.**2001;** 13:22-67.

Morin D, Zini R, Tillement S P and Burdeau APrevention of cell domage in ischemic-reperfusion: mitochondrial respiratory chain as a pharmacological target. Letters in drugs & Discovery. **2004**; 1:279-284.

Moris C J, Earl J R, Trenam C W and Blake D R.Reactive oxygen species and iron-a dangerons part- nership in inflammation. J Biochem Cell Biol. **1995;** 27: 109-122.

Morrissay C, Watson R.Phytoestrogens and prostate cancer .Curr Drug Targets. **2003;** 4: 231-241.

Mousavi SH, Davari A-S, Iranshahi M, Sabouri-Rad S, Najaran ZT. Comparative analysis of the cytotoxic effect of 7-prenyloxycoumarin compounds and herniarin on MCF-7 cell line. Avicenna J Phytomed. **2015;** 5:520-530.

Muchmore S W, Sattler M, Liang H.X-ray and NMR structure of human Bcl-xL, an inhibitor of programmed cell death. Nature. **1996;** 381: 335-41.

Murad J M, Calvi S A, Sorares A, Benkova V and Sforcin J M.Effect of propolis from Brazil and Bulgaria on fungucidal activity of macrophages against Paracoccidioïde brasiliensis. Ethnopharm. **2002;** 79: 331-334.

Murat K, Kaya S and Kurucu. SGC – MS analysis of propolis samples fromtwo different Regions of turkey. Z Naturforsch. **2002;** 570: 905 – 909.

Murphy M P.Investigating mitochondrial radical production using targets probes. Biochem Soc Trans. **2004;** 32: 1011-1014.

Murray RDH. Coumarrins. Nat Prod. **1989;** 6 : 591-624.

Mytitinedu C, Werner P, Molinari S, Di Rocco A, Cohen G and Yahr M D. J Neural Transn. Park. Dis Dement. Sect. **1994;** 8: 223-228.

Naithani R, Huma LC, Moriarty RM, McCormick DL, Mehta RG. Comprehensive review of cancer chemopreventive agents evaluated in experimental carcinogenesis models and clinical trials. Curr Med Chem. **2008;** 15:1044–1071

Nakagawa T, Yuan J.Activation of caspase -12 by calpain in apoptosis. J Cell Biol. **2000;** 150: 887- 894.

Nazari Z, Iranshahi M**.** Biologically active sesquiterpene coumarinsfrom ferula species. Phytothr Res. **2010;** 25: 315-323.

Neshati V, Matin MM, Iranshahi M, Bahrami AR, Behravan J, Mollazadeh S, Rassouli FB. Cytotoxicity of vincristine on the 5637 cell line is enhanced by combination with conferone. Z Naturforsch C. **2009;** 64:317-322.

Neshati V, Matin MM, Bahrami AR, Iranshahi M, Rassouli FB, Saeinasab M. Increasing the cisplatin cytotoxicity andcisplatin-induced DNA damage by conferone in 5637 cells. Nat Prod Res. **2012;** 26:1724-1727.

Neger- Salvayre A, Hirtz C, Carrera G, Cazenave R, Troly M, Slvayre R, Penicaud L and Casteilla L. A role for uncoupling protein-2 as a regulator of mitochondrial hydrogene peroxide generation. Fased J. **1997;** 11: 809- 815.

Nestel P. Isoflanvines: Their effect on cardiovascular risk and functions. Curr Lipidol. **2003;** 14:3-8.

Nwodo J N, Ibezim A, Simoben C V, Ntie-Kang F. Exploring Cancer Therapeutics with Natural Products from African Medicinal Plants, Part II: Alkaloids, Terpenoids and Flavonoids. Anti-Cancer Agents in Med Chem. **2016;** Vol 16 , Issue 1.

Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. **1979;** Volume 95, Issue 2, Pp 351-358

Oliveira Silva E and Batista R. Ferulic Acid and Naturally Occurring Compounds Bearing a Feruloyl Moiety: A Review on Their Structures, Occurrence, and Potential Health Benefits. Comprehensive Rev in Food Sci and Food Safety· **2017;** Volume16, Issue 4: Pp 580-616

Olson M and Kornbluth S. Mitochondria in Apoptosis and Human Disease. Curr Mol Med. **2001;** 1(1): 91-122.

Ong K C, Khoo H E. Efects of Myrecitin on glycemia and glycogen metabolism in diabetic rats. Life Sci. **2000;** 67: 1695-1705.

Ouglissi Dehhak K, Lawton P, Michalet C B, Derbour S N, Hadj Mahammed M, Badjah Hadj A, Dijoux Franca M G, Guilet D. Sesquiterpenes from aerial parts of Ferula vesceritensis. Phytochemistry. **2008;** 69: 1933-1938.

Ozgur E, Guler G, Seyhan N. Mobile phone radiation-induced free radical damage in the liver is inhibited by the antioxidants *N*-acetyl cysteine and epigallocatechin-gallate. Int J Radiat. Biol. **2010;** 86:935–945.

Papa S and Skulachev V P.Reactive oxygen species, Mitochondria, apopotsis and aging. Mol Cell Biochem. **1997b;** 17: 23-31.

Paris R, Jacquemin H, Linard A. Plantes de Madagascar Leptolaena Paciflora Baker, L. DiospyroideaCavaco Var, Tampoketsensis etSarcolinaMultiflora Dup: Présence d'hétérosides de myricétol.Plantes médicinales et 'phytotothérapie.**1975;** IX (3):230-237.

Park Y K, Alenear SM, Aguiar CL. Botaical origin and chemical composition of Brazilian propolis. J Agric Food Chem. **2002;** 50: 2502-2506.

Park V K, Paredes G, Aguiar C L, Alenear S M.Chemical constituents in Bacharus dracunculifolia as the main botanical origin south eastern Brazilian propolis. J Agric Food Chem.**2004;** 52: 1100 – 1103.

Patel S, Peretz J, Pan Y X, Helferich W G and Flaws J A. Genistein exposure inhibits growth and alters steroidogenesis in adult mouse antral follicles. Toxicol Appl Pharmacol. **2016;** 293, 53–62.

Pelletier M, Vallette F M.Apoptose et maladies neurodégénératives. Pharmacol. **2001;**15: 9.

Pham T K, Vinh P T, Nong H D and Nuguyen D K.Flavonoïds as antimicrobial agents. Tap Duoc Hoc. **1993;** 6:14-21.

Pincemail J. Antioxidants and human diseases. In: Favier A et al, editors. Analysis of Free Radicals in Biological Systems. Basel, London, Boston: Berlin Birkhäuser. **1995;** pp 83-98.

Pincemail J. Espèces oxygénées en médecine humaine: une approche didactique. Vaisseaux, Cœur, Poumon. Free radic.Biol. **1998;** 3: 133–8.

Poljsak B. Strategies for Reducing or Preventing the Generation of Oxidative Stress.Oxid Med Cell Longev. **2011;** 194586.

Pollack M and Leeuwenburgh C. Apoptosis and Aging: Role of the Mitochondria.Gerontology. **2001 ;** 56A 11: B475-B482.

Powis G, Mustacich D, Coon A. The role of the redox protein thioredoxin in cell growth and cancer. Free Radic Biol Med**. 2000;** 29: 312-22

Pre J.Lipid peroxidation. Path Biol. **1991;** 39. 716-736.

Pré J.Radicaux libres et peroxydation lipidique: I. Aspects biologiques généraux. Sem Hôp Paris. **1992;** 98(41): 1430- 1437.

Raj Narayana K, Sripal Reddy M, Chaluvady M R, Krishma D R.Bioflavonoids: classification pharmacological, Biochemical effects and therapeutic potential. Indian J of Pharmacol. **2001;** 33: 2-16.

Ramezani A, Iranshahi M, Hanafi-Bojd MY, Malaekeh-Nikouei B. Preparation, characterization and cytotoxic effects of nanoliposomes containing umbelliprenin.Intern J Pharm Res. **2014;** 6:79-84.

Rassouli FB, Matin MM, Iranshahi M, Bahrami AR, Neshati V, Mollazadeh S, Neshati Z. Mogoltacin enhances vincristine cytotoxicity in human transitional cell carcinoma (TCC) cell line. Phytomed. **2009;** 16:181-187.

Rassouli FB, Matin MM, Iranshahi M, Bahrami AR, Behravan J, Mollazadeh S, Neshati V, Kalalinia F. Investigating the enhancement of cisplatin cytotoxicity on 5637 cells by combination with mogoltacin. Toxicol *in Vitro*. **2011;** 25:469-474.

Ravagnan et al 2002. Ravagnan L, Ronnier T, Kroemer G.Mitochondria: the killer organelles. J Cell physiol. **2002;** 3: 839-843.

Razavi S M, Janani M. A new ester coumarin from Ferula Persica wild, indigenous to Iran. Nat Prod Res. **2015;** 29:717-721.

Rechner A R, Spencer J P E, Kuhine G and Rice-Evans C.Novel biomarkers of the metabolism of caffeic acid derivectives *in vivo*. Free Radic Biol Med. **2000;** 30: 1213-1222.

Reichmann A, Florke S, Hebenstreit G, Schrubar H and Reiderer P, Respiratory chain and mitochondrial deoxyribonucleic acid in blood cells from patients with focal and generalized dystonia. J Neurol. **1993;** 240: 377- 380.

Remesy C, Manach C, Demigne C, Texier O, Regerat F.Intérêt nutritionnel des flavonoïdes. Méd Nut. **1996;** 32(1):17-27.

Riboli E, Norat T. Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. Am J Clin Nutr. **2003**; 78:559S–569S

Rice-Evans C, Spencer J E, Schroter H and Rechner A R.Biovaibilty of flavonoids and potential bioactive forms invivo. Drug Metab. Drug Interact. **2001;**17,291-31.

Ritta P G.Flavonoids as antioxidants. J Nat Prod. **2000;** 63: 1035- 1042.

Rosaria Varì,…Roberta Masella. Extra Virgin Olive Oil Biophenols and mRNA Transcription of Glutathione-related Enzymes. Olives and Olive Oil in Health and Disease Prevention (J& Books Sci Direct). **2010.**

Russo A, Acquavira R, Campisi A, Sorrenti V, Di Giacormo C, Virgata, Bancellona M L and Vanella A.Bioflavonoïds as antiradicals, antixodants and DNA cleavage protectors. Cell Biol Toxicol. **2000;** 16: 91- 98.

Russo GL. Ins and outs of dietary phytochemicals in cancer chemoprevention. Biochem Pharmacol. **2007**; 74:533–544.

Rustin P, Chretien D, Bourgeron T, Gerard B, Rotig A, Sandubray J M, Munich A. Biochemical and molecular investigations in respiratory chain deficiencies. Clin Chim Acta. **1994;** 228: 35-51.

Ružička Leopold. The isoprene rule and the Biogenesis of terpenic compounds. Cellular and Mol Life Sci. **1953;** 9 (10): 357–367.

Ryu S-Y, Lee C-O, Choi S-U, Park S-h, Kim Y-S, Kim S-K, Kim S-K, Kang S-K. Anticancer composition comprising sesquiterpenes isolated from Resina ferulae. In: Google Patents. Publication number : US. **2001;** 20040043083 A1.

Sadeghizadeh M, Ranjbar B, Damaghi M, Khaki L, Sarbolouki MN, Najafi F, ParsaeeS, Ziaee AA, Massumi M, Lubitz W. Dendrosomes as novel gene porters-III. J Chem Technol Biotech, **2008;** 83:912-920.

Saleem M, Alam A, Sultana S. *Asafoetida*inhibits early events of carcinogenesis: A chemopreventive study. Pharmacology letters. **2001;** Volume 68, Issue 16, 9, Pages 1913-1921.

Saleem M , Afaq F , Adhami V M and Mukhtar H. Lupeol modulates NF-jB and PI3K/Akt pathways and inhibits skin cancer in CD-1 mice. Oncogene. **2004;** 23, 5203–5214.

Salem G A, Shaban A, Diab H A, Elsaghayer W A, Sahu R P. Phoenix dactylifera protects against oxidative stress and hepatic injury induced by paracetamol intoxication in rats. Biomed & pharmacothera. 2018; Vol 104, pp 366 – 374.

Samir M, and Kholy N M. Thiobarbituric acid reactive substances in patients with laryngeal cancer. Clin Otolaryngol Allied Sci.**1999;** 24(3):232-4.

Sato M, Fujuwara S, Tsuchiya H, Fujii T, Linuma M, Tosa H and Ohkwa Y. Role of flavonoids in microbial diseases. J Ethopharmacal. **1996;** 54: 171-178.

Serkerov S V. A sesquiterpene lactone-semopodine-from the seeds of Ferula oopodine. Chemistry of Natural Compounds. **1969**; Vol 5, Issue 4, Pp 205 – 207.

Serkerov, S. A new sesquiterpene hydroxylactone from Ferula oopoda. Chemistry of Natural Compounds 7, 817-818 (1971). Serkerov, S. The structure of semopodin and a hydroxylactone from Ferula oopoda. Chemistry of Natural Compounds **1976;** 12, 343-344.

Scandalias J G.. Oxygen stress and superoxide dismutase. Plant Physiol. **1993;** 101: 7-12.

Schendel S L, Montal M, Reed J C.Bcl-2 family proteins as ion-channels. Cell DeathDiff. **1998**; 5: 372-80.

Selvakumar E, Prahalathan C, Mythili Y, Varalakshmi P. Beneficial effects of DL-α-lipoic acid on cyclophosphamide-induced oxidative stress in mitochondrial fractions of rat testis**.** Chem-Biol Interactions. **2005;** Vol 152, Issue 1, Pages 59-66

Shahverdi A R, Saadat F, Khorramizadeh M R, Iranshahi M and Khoshayand M R. Two matrix metalloproteinases inhibitors from *Ferula persica* var. persica. Phytomed. **2006;** 13 : 712 – 717.

Sha Li, Hor-Yue Tan, Ning Wang, Zhang-Jin Zhang, Lixing Lao, Chi-Woon Wong, and Yibin Feng. The Role of Oxidative Stress and Antioxidants in Liver Diseases.Int J Mol Sci. **2015;** 16(11): 26087–26124.

Shen J and Cookson M R. Mitochondria and dopamine: new insights into recessive parkinsonism. Neuron. **2004;**43:301-304.

Shafri MAM, Yusof FA, Zain AZM. *In vitro* cytotoxic activity of Ferula assafoetida on osteosarcoma cell line (HOS CRL). J Teknol. **2015;** 77:7-11.

Shimazawa M, Chikamatsu S, Morimotov N, Mi shima S.Neuroprotection by Brazilian green propolis agaits *in vitro* and *in vivo* ischemic neuronal damage.E CAM. **2005;** 2(2): 201- 207.

Shimazu S, Eguch Y, Kamike W, Eumahashi A, Lacraique V, Matsuda H, Tsujimoto Y.Bcl-2 prevents apoptotic mitochondrial dysfunction by regulation protonflusc. Proc Natl Sci. **1998;** 95: 1455-1459.

Shuai Wang, Tareisha L. Dunlap, Caitlin E. Howell, Obinna C. Mbachu, Emily A. Rue, Rasika Phansalkar, Shao-Nong Chen, Guido F. Pauli, Birgit M. Dietz, and Judy L. Bolton.Hop (Humulus lupulus L.) Extract and 6-Prenylnaringenin Induce P450 1A1 Catalyzed Estrogen 2-Hydroxylation. Chem. Res. Toxicol.,***2016;*** 29 (7), pp 1142–1150.

Simonson S G, Zhang J, Canada A T, Benveniste H and Piantadosis C A. Hydrogen peroxide production by monoamine oxidase during ischemia-reperfusion in the rat brain. J Ceredr Blood Flow Metab. **1993;** 13: 125-134.

Singal P K and Iliskovic N.Doxorubicin-induced cardiomyopathy. N.Engl. J. Med.**1998;**339: 900-905.

Sinha AK, Colorometric assay of catalase. Anal Biochem. **1972;** 47(2):389-94.

Smolka M B, Zoppi C C, Alves A A, Silveira L R, Maragnoni S, Rereira-Da-Silva L, Novello J C, Macedo D V. HSP72 as a complementary protection against oxidative stress induced by exercise in the muscle of rats. Am J Physiool.**2000;** 279R: 1539- 1545.

Soliman Ali M, Knany H, Elgazar A A. Insights on the molecular mechanism of anti-inflammatory effect of formula from Islamic traditional medicine: An in-silico study.J T C M.**2018.**

Spencer J E, Choivrimootoo G, Chondhury R, Debnam E S, Srai S and Rice E.The small intestine can both absorb and glucuronidate luminal flavonoids. FEBS Lett. **1999;** 458: 224-230.

Srinivasan M, Sudheer A R and Menon V P. Ferulic Acid: Therapeutic Potential through Its Antioxidant Property. J Clin Biochem Nutr**. 2007;** 40(2): 92–100.

Starkov A A and Robinson B H**.** Superoxide from mitochondrial complex III: the role of manganese superoxyde dismutase. Free Radic Biol Med. **2000;** 29:170-180.

Starkov A A and Fiskum G. Myxothiazol induces H_2O_2 production from mitochondrial respiratory chain. Biochem Biophys Res Commun. **2001**; 281: 645-650.

Starkov A A, Fiskum G, Chinopoulos, Lorenzo B J, Brovene S E, Patel M S and Beal M.F. Mitcondrial ketoglutarate dehydrogenase complex generates reactive oxygen species. J Neurosci. **2004;** 24: 7779-7778.

Stohs S and Bagchi D.Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med. **1995;** l8 (2): 321 -366.

Subbroto Kumar Saha, Soo Bin Lee, Jihye Won, Hye Yeon Choi, Kyeongseok Kim, Gwang-Mo Yang, Ahmed Abdal Dayem, and Ssang-goo Cho* . Correlation between Oxidative Stress, Nutrition, and Cancer Initiation. Int J Mol Sci. **2017**; 18(7): 1544.

Su F, Hayami S, Origi V, Tanaka K, Yanada Y, Ikeda K, Yamada H.*In vivo* antioxidative activity of propolis evaluated by the interaction with vit -C and vit-E and the level of lipid hydroperoxid in rats. Food chem. **2000;** 48: 1462-1465.

Suzuki, K., Okasaka, M., Kashiwada, Y., Takaishi, Y., Honda, G., Ito, M., Takeda, Y., Kodzhimatov, O. K., Ashurmetov, O., Sekiya, M. & Ikeshiro Y. Sesquiterpene Lactones from the Roots of Ferula varia and Their Cytotoxic Activity. Journal of Natural Products. **2007;** 70, 1915-1918.

Syama S, Reshma S C, Sreekanth P J, Varma H K, Mohanan P V. Effect of zinc oxide nanoparticles on cellular oxidative stress and antioxidant defense mechanisms in mouse liver. Environ. Toxicol. Pharmacol. **2013;** 95:495–503.

Tabet F, Rhian M Touyz. Reactive Oxygen Species, Oxidative Stress, and Vascular Biology in Hypertension. Comprehensive Hypertension (J& Books Sci Direct). **2007;** (6).

Terschuk M L, Riera M Q, Casteo G R and Afdala L R.Antimicrobial activity of flavonoïds from leaves of tagetes minuta. J Ehopharmacol. **1997;** 56: 227- 236.

Thoppil R J, Bishayee A. Terpenoids as potential chemopreventive and therapeutic agents in liver cancer. World J Hepato. **2011**; 3:228–249.

Thorberry N A.Caspases: Key mediators of apoptosis. Chem Biol. **1998;** 5: 97-103.

Tretter L and Adam-Vizi V.Generation of reactive oxygen species in the reaction catalysed by Alpfa- ketoglutarate dehydrogenase. J Neuro Sci. **2004;** 24: 7771-7778.

Turmpower B L.The protonative Q cycle energy transduction by coupling of proton tranlocation to electron transfer by the cytochrome bc_1 complex. J Biol Chem. **1990;**265: 11409-11412.

Valiahdi S M, Iranshahi M, Sahebkar A. Cytotoxic activities of phytochemicals from Ferula species. DARU J Pharm Sci; **2013;** 21(39).

Vander Heiden M G, Chandal N S, Williamson E K, Schumacker P T and Thompson C B. Bcl-XL regulates the membrane potential and volume homeostasis of mitochondria. Cell. **1997;** 91:627–637.

Vander M S. Bcl-XL prevents cell death following growth factor with drawal by facilitaling mitochondrial ATP/ADP exchange. Mol Cell. **1999;** 3: 159- 167.

Vanessa C, Chritine M, Claudine M, Cathrine B, Christian D and Christian R. Part of quercetin absorbed in the small in testine is conjugated and further secreted in the intestinal lumen. Am J phlysiol Gastrain. **1999;** 277G: 120-126.

Vasquez-Vivar J, Kalynaraman B and Kennedy M L.Mitochondrial aconitaseis asource of hydroxyl radical: An electron spin resonance investigation. J Biol Chem, **2000;** 275: 14064-14069.

Vaux D L & Korsmeyer S J.Cell death in development. Cell, **1999;** 96: 245-54.

Velikova M, Bankova V T, svetkova I, Kujungiv A and Marcucci M C.Antimicrobial activity of Brazilian propolis of native stingless bees. Phytototherapia.**2000;** 71: 693-696.

Vidal-Puig A J, Grujic D, Zhang C Y, Hagen T, Bosso A, Ido Y, Szezepanik A, Wade J and Mootha V**.** Energy metabolism in uncoupling protein 3 gene knockout mice. J Biol Chem. **2000;** 275: 16258-16266.

Wall U K, Galija T A and Wall T.Transport of the flavonoid chrysin and its conjugated metabolited by the human intestinal cell line Caco-2. Biochem Pharmacol. **1999;** 58:431-438.

Wallace D C. Mitochondrial DNA variation in human evolution, degenerative disease, and aging. Am. J. Hnm. Gent. **1995;** 57: 201-223.

Wang G, Tang W, Bidigare R R. Terpenoids as therapeutic drugs and pharmaceutical agents. Natural products: Drug discovery and therapeutic medicine. In: Zhang L, Demain AL (Eds.). Totowa, NJ. Humana Press. **2005**; p. 197–227.

Wathley S A, Curti D and Marchbanks R.M. Mitochondrial involvement in schizophrenia and other functional psychoses. Neur chem Res. **1996;** 21:995- 1004.

Wei Y H and lee H C. Oxidative stress, Mitochondrial DNA. Mutation and impairment of antioxidant enzymes in aging. Exp. Biol. Med. **2002;** 277: 671-682.

Willet K, Detry O and Sluse F E. Resistance of isolated pulmonary mitochondria during *in vitro* anoxia/reoxygenation, Biochimica et Biophysica. Acta-Bioenergetics, **2000;** 1460.

Williams R I, Spencer J P, Rice-Evans C.Flavonoids: Antioxidents or signalling molecules. Free Radic Biol Med. **2004;** 36(7): 838- 849.

Wilson D W, Metz H N, Graver L M, Rao P S. Direct method of quantification of free malondialdehyde with high-performance capillary electrophoresis in biological samples. Clin Chem.**1997;** 43:1982-1984.

Wollenweber E and Buchma S L.Propolis sources other that poplar (Populus sp). Z.Naturforshm. **1997;** 52s: 530-535.

Wondrak G T. Redox-Directed Cancer Therapeutics: Molecular Mechanisms andOpportunities. Comprehensive Invited Rev. **2009;** Vol 11, Number 12.

Wondrak G T. Redox-directed cancer therapeutics: molecular mechanisms andopportunities. Antioxid Redox Signal.**2009;** 11: 3013-69.

WuC Y, Yi-Ju Chen, Hsiu J Ho, et alYao-Chun Hsu, Ken N Kuo, Ming-Shiang Wu, Jaw-Town Lin . Association Between Nucleoside Analogues and Risk of Hepatitis B Virus–Related Hepatocellular Carcinoma Recurrence Following Liver Resection. **2012**; 1913-1906 : (18) 308.

Xiaodong W. The expanding role of mitochondria in apoptosis**.** Gene & Develop. **2001;** 15:2922–2933.

Yamagishi M, Natsume M and Osakabe N.Effects of cacao liquor proantho-cyanidins on induced mutagenesis *in vitro*, and *in vivo* pancreatic tumonigenesis in female Sprague-Dawley rats. Cancer L. **2002;** 185(2): 123-130.

Yang C S, Yang G Y, Landan J M, Kim S and Liao J.Tea and tea polyphenols inhibit cell hyperproliferation, lung tumorigenenis, and tumor progression. Lung Res. **1998;** 24(4): 629-639.

Yang G, Liu X, Bhalla K, Kim C N, Ibrado A M, Cai J, Peng T I, Jones D P, Wang X.Prevention of apoptosis by B-cl-2: released of cyt-C from mitochondria. Sci. **1997;** 275: 1129-1132.

Yaqoob U, Nawchoo IA, Ahmad M. Phytochemical screening of the root tuber extracts of Ferula jaeschkeana vatke. J Essent Oil Bear Pl, **2016;** 19:208-211.

Yen G C, Chen H Y, Peng H H.Antioxidant and pro-oxidant effects of various tea extracts. J Agr Food Chem. **1997;** 45: 30-34.

Yildirim N C, Yurekli M, Yildirim N. Investigation of some antioxidant enzymes activities depending on adrenomedullin treatment and cold stress in rat liver tissue. Turk J Biochem. **2010;** 35:138–142.

Yocomaya C, Ema M, Takaishi K and Hisatomi H. Induction of oxidative stress by anticancer drugs in the presence and absence of cells. Oncol Lett. **2017.** 14(5):6066-6070

Zhang L Yu L and Yu C A. Generation of superoxide anion by succinate cytochrome c reductase from bovin heart mitochondria.J Biol Chem.**1998;** 273: 33972-33976.

Zhang X, Strakovsky R, Zhou D, Zhang Y, Pan Y X. A maternal high-fat diet represses the expression of antioxidant defense genes and induces the cellular senescence pathway in the liver of male offspring rats. J Nutr. **2011;** 141:1254–1259.

Zhanpeng W, Zhuonan L, Yanshuo Y, Lijuan X and Wei L. Oxidative Stress and Liver Cancer: Etiology and Therapeutic Targets. Oxid Med Cell Longev. **2016;** (5):1-10.

Zhou Q, Nie R, Prins G, Saunders P T K, Katzenellenbogen B S and Hess R A. Localization of androgen and estrogen receptors in adult male mouse reproductive tract. J Androl. **2002;** 23:870–81.

Zini R, Morin C, Bertelli A, Bertelli A E, Tillement J P. Effects of resveratrol on the rat brain resperatory chain. DrugsExpt Clin. Res. **1999 ;** 25:87-97.

Znati M, Jannet H B, Cazaux S, Bouajila J. Chemical composition, biological and cytotoxic activities of plant extracts and compounds isolated from Ferula lutea. Molecules. **2014b;** 19:2733-2747.

Zucker D, Marcus D, Barenholz Y, Goldblum A. Liposome drugs' loading efficiency: a working model based on loading conditions and drug's physicochemical properties. J Control Release. **2009;** 139, 73–80.

Summaries

Abstract

Ferula vesceritensis roots are potential sources of natural antioxidant and prooxidant sesquiterpenecoumarinsthat could potentially have antiproliferative, cytotoxic effects against many types of cancers including liver cancer which is the principle cause of death worldwide. The aim of this work is to investigate the biological activity of sesquiterpenecoumarins, ferulenol, coladin and lepiferin obtained from the *Ferula* roots crude methanol extract. These bioactive compounds whose structures determined by extension NMR analysis are explored *in vivo* and *in vitro* studies by following the prooxidant effects, tested on experimental liver cancer induced by Benzo(a)pyrane,and cytotoxic effects on B16F1 melanoma and FAO cell lines. The impact of these bioactive compounds over the mitochondrial function has also been evaluated in liver mitochondria.
Ferulenol, coladin and lapiferin have increased the oxidative stress by variably decreasing the enzymatic and non enzymatic antioxidants (SOD CAT, GST) and increasing the lipid peroxidation as well as diminishing the mitochondrial dehydrogenases activities tested on Bl6F1 melanoma and FAO cells with MTT and WST-1 assay after 24 and 48 hrs throughout an apoptotic pathway.At the level of mitochondria, those bioactive substances have caused mitochondrial swelling and a variable diminution in the mitochondrial superoxide anion $O^{\cdot}_2$productionin isolated mitochondrial liver in a dose dependant manner. The prooxidant, cytotoxic and/or chemopreventiveeffects of *Ferulavesceritensis* roots sesquiterpenecoumarins have permitted us to suggest the possible potential exploration of those bioactive compounds for combating and/or preventing many diseases such as liver cancer.

Key words : FAO , Bl6F1 melanoma , benzo(a)pyrane, liver cancer, oxidative stress , ferulenolcoladin , lapiferin.

ملخص

تعتبرجذور نبات *Ferulavesceritensis*مصدر هام في المستقبل لمضادات تأكسد و مواد أولية مؤكسدة طبيعية مثل.Sesquiterpenecoumarins هذه المواد الحيوية النشطة لها تأثيرات سامة و / أو كيميو وقائية ضد مجال واسع من السرطانات بما في ذالك سرطان الكبد الذي يعتبر السبب الرئيسي للوفيات عبر العالم. إن هدف أطروحاتنا هو دراسة النشاط البيولوجي لـ : Sesquiterpenecoumarins(Ferulenol, Coladin and lapiferin) المعزولة من مستخلص الميثانول لجذور *Ferulavescevitensis*. هذه المركبات التي حددت صيغتها *بواسطة تقني* RMN و تم إختبارها على سرطان تجريبي محفز بواسطة benzopyrène*in vivo* عند الجرذانwistar albinosو تم اختبار تأثيراتها الأولية المؤكسدة و السامة *in vitro* على خلايا Bl6F1de mélanomeو خلايا سرطان الكبد FAOباستخدام اختبارات MTTو WST-1. زيادة على ذالك تم اختبار هذه المواد النشطة sesquiterpene coumarinsعلى عمل المتاكوندريا *invitro*. Ferulenol ،coladin و lapiferinأدوا إلى ارتفاع التوتر التأكسدي المتحصل عليه بواسطة B(a)P و/ أو الأدوية المضادة للسرطان متمثلا في تخفيض المضادات الأنزيمية و غير الأنزيمية(SOD,CAT,GST et GSH)و زيادة التأكسد الدهني lipidic peroxidation . بالمثل لوحظ انخفاض نشاطات أنزيمات المتاكوندية النازعة الهيدروجينactivities mitochondrialdeshydrogenase، تورم متاكوندري و زيادة ملحوظة لأنيون superoxide من خلال عملية proapoptosis. هذا التأثير السام و الكيميو وقائي يقترح إمكانية استخدام تلك المواد النشطة مستقبلا لمكافحة و / أو منع العديد من الأمراض بما في ذالك سرطان الكبد.

الكلمات الدالة : خلاياB16F, melanoma1FAOhepatocytes، الإجهاد التأكسدي ، بنزوبيران، سرطان الكبد فيريلينول، كولادين لا,بيرفيرين،*Ferulavesceritensis*.

Résumé

Les racines de *Ferulavesceritensis* représentent une source potentielled'antioxydants et prooxidantsde sesquiterpène comarines. Ces substances bioactives ont des effets cytotoxiques et/ouchimiopréventifs, contre une large gamme de cancers dont le cancer du foie qui est la principale cause de décès par cancer dans le monde. Le but de notre thèse est d'étudier l'activité biologique des sesquitérpène coumarines Ferulenol, coladine et lapiferine isolés de l'extrait brutméthanoliquedes racines de *Ferulavesceriteusis*. Ces composés dont leurs structuresont été déterminés par RMN, ont été évalués sur un cancer expérimental induit par le benzopyrènein vivo chez les ratswistar albinos et leurs effetsprooxidants,et cytotoxiques *in vitro* surles cellules Bl6F1de mélanome et les cellules FAO de cancer du foie en utilisant les tests MTT et WST-1. De plusces sesquiterpènes coumarines ont été évalué sur le fonctionnement mitochondrial *invitro*. LeFerulenol, coladine et lapiferine ont augmentés le stress oxydatif généré par le B(a)P et/ou les anticancéreux objectivé par la réduction des antioxydants enzymatiques et non enzymatiques (SOD,CAT,GST et GSH) et l'augmentation de la péroxidation lipidique. De même qu'une diminution des activités déshydrogénases mitochondriales, un gonflement mitochondrial et une augmentation importante de la production d'anion superoxideà travers un processusproapoptotique. L'effet cytotoxique, et/ou chimiopréventif des composés de *Ferulavesceritensis* suggère la possibilité de leurs utilisation potentielle pour combattre et / ou prévenir plusieurs maladies telle que le cancer du foie.

Mots clés : Bl6 F1melanoma, FAO hepatocytes , benzol (a) pyrène , cancer du stress oxydatif, ferulenol, coladine , lapiférine, *Ferulavesceritensis*.