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Dedication

It is with deep and sincere gratitude that I dedicate this work:

To my parents, for all the efforts they have made for me, for their encouragement, trust, moral and financial support. It is thanks to their prayers and blessings that this work was possible. I hope that one day I can repay all the goodness they have shown me. I love you.

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International publications

Khelfi S, Zerizer S, Foughalia A, Tebibel S, Bensouici C, and Kabouche Z. (2023). The antioxidant activity and the anti-inflammatory effect of *Citrus sinensis* L. fruit on intestinal inflammation induced by hyperhomocysteinemia in mice. Natural and Life Sciences Communications. **22**(1): 1-14.

Khelfi S, Zerizer S, Bensouici C, Tebibel S, and Kabouche Z. (2023).The antioxidant activity and the protective effect of *Rosa canina* L. fruit against intestinal inflammation caused by L-methionine induced hyperhomocysteinemia in murine model. Pharmaceutical Chemistry Journal. ISSN: 1573-9031 and Print ISSN: 0091-150X.

International communications

Khelfi S**,** Zerizer S, Bensouici C, and Kabouche Z. Determination du contenu phenolique et evaluation de l'activitè anti oxydante *in vitro* d'eglantier (*Rosa canina*)**;** 1er Séminaire International (Webinaire) Perturbateurs Endocriniens Environnement et Reproduction, Université SAAD DAHLAB Blida1 (USDB) (Algeria), 7_8 Juillet 2021.

Khelfi S, Zerizer S. The anti-inflammatory effect of *Citrus sinensis* L. on intestinal inflammation induced by hyperhomocysteinermia. International Seminar on Valorization of Agronomic, Ecological and Food Resources (ISVAEFR 2022)- 18,19& 20 October 2022.

List of abbreviations

AA Ascorbic Acid

ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid

$AlCl_3$ Aluminum Chloride

ANOVA Analysis of Variance

ATP Adenosine Triphosphate

BHA Butylated Hydroxy Anisole

BHMT Betaine Homocysteine Methyltransferase

BHT Butylated Hydroxytoluene

BSA Bovine Serum Albumin

CAT Catalase

CBS Cystathionine-β-synthase

CSE Cystathionine-γ-lyase

CD Crohn's Disease

CRP C-Reactive Protein

CUPRAC CUPric Reducing Antioxidant Capacity

CV Cyclic Voltametry

DC Dendritic Cell

DHFR Dihydrofolate Rreductase

DNA Deoxyriboncleic Acid

DPPH 1,1-Diphenyl-2-Picryl-Hydrazyl free radical

DTNB 5,5′-dithiobis-2 nitrobenzoic acid

DSS Dextran Sulfide Sodium

EDTA Ethylene Diamine Tetra Acetic Acid

GT Gastrointestinal Tract

GSH Reduced Glutathione

GSSG Glutathione Disulphide

H_2O_2 Hydrogen Peroxide

Hcy Homocysteine

HHcy Hyperhomocysteinemia

IBD Inflammatory Bowel Diseases

IC_{50} The Half Maximal Inhibitory Concentration

IFN-g Interferon-gamma

IL-1 Interleukin 1

IL-2 Interleukin 2

IL-6 Interleukin 6

LD_{50} Medial Lethal Dose

MS Methionine Synthase

MTHFR Methylene Tetrahydrofolate Reductase

MTT 3-(4,5-dimethythiazol 2-yl)-2,5-diphenyl tetrazoliumbromide

NADPH Nicotinamide Adenine Dinucleotide Phosphate

NF-Kb Nuclear Factor-Kappa B

NO Nitric Oxide

$O2^-$ Superoxide Anion

$^1O_2$ Singlet Molecular Oxygen

O_2 Diatomic Oxygen Gas

$^{\bullet}OH$ Hydroxyl Radicals

RDA Recommended Dietary Allowance

ROS Reactive Oxygen Species

SAH S-Adenosyl-L-Homocysteine

SAM S-Adenosyl-Methionine

SPSS Statistical Package for Social Science

TBS Tris-Buffered Saline

THF Tetrahydrofolate

TNF-α Tumor Necrosis Factor α

UC Ulcerative Colitis

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Introduction

Introduction

Inflammatory bowel diseases (IBD) is becoming more and more common worldwide, placing a heavy burden on the health care system (Robbe Masselot et al., 2023) and affects 6.8 million people worldwide (Kudelka et al., 2020).

It is, a chronic relapsing-remitting disease of gastrointestinal inflammation (Chen et al., 2023). IBD is characterized by chronic idiopathic inflammatory episodes of the gastrointestinal tract, resulting in altered structure and function (Kumarapperuma et al., 2023). It is an immune disease of unknown etiology, involving complex interactions between the gut microbiome and the host immune response. Dysbiosis disrupts the intestinal barrier by transferring bacterial symbionts into the intestinal mucosa, initiating an aberrant immune response, resulting in an imbalance between pro- and anti-inflammatory molecules (Upadhyay et al., 2023).

Homocysteine (Hcy) is a serum containing amino acid synthesized by its demethylation during the metabolism of methionine, an essential amino acid for humans. When levels of Hcy are above normal limits, hyperhomocysteinemia (HHcy) occurs (Son and Lewis, 2021).

The risk of HHcy is significantly elevated in patients with IBD (Keshteli et al., 2015). Also Jiang et al. (2012) reported that patients with inflammatory bowel disease had high levels of homocysteine.

There is a potent association between HHcy and the induction of inflammatory determinants, including the expression of adhesion molecules and oxidative stress. HHcy, activates NFκB, a transcription factor that regulates the transcription of various genes involved in inflammatory and immune responses inducing the increase of pro-inflammatory cytokines and downregulation of anti-inflammatory cytokines that lead to cell apoptosis (Almutayri, 2020). Moreover, HHcy promotes inflammation and leads to oxidative stress and is associated with several disease states such as gastrointestinal disorders (Yakovleva et al., 2020).

Oxidative stress and free radicals are implicated in the pathogenesis of IBD, studies have proposed the use of antioxidants as a therapeutic approach (Vaghari-Tabari et al., 2021).

Vitamin C, an antioxidant, decreases oxidative stress related to inflammation (Gęgotek et al., 2022) and lipid peroxidation and also inflammation. The anti-inflammatory property of vitamin C could be attributed to its ability to modulate NF kB (Ellulu et al., 2015). The previous literature on vitamin C in IBD is limited. Vitamin C deficiency in patients with IBD is likely multifactorial, but potential risk factors include non-consumption of vitamin C-rich fruits and vegetables (Dunleavy et al., 2021).

Anti-inflammatory drugs and immunosuppressants have been the main treatment choices for IBD patients (Zhang et al., 2023), however, these drugs are limited in their use due to their adverse side effects. It is essential to investigate new treatment methods that can improve efficacy and reduce side effects (Zhang et al., 2023).

For a very long time, natural resources have been the principal source of remedy to treat different diseases and infections, and remains until now, the key source to obtain new active molecules in the pharmaceutical field (Kemassi et al., 2014).

Natural product molecules have been gradually developed to become an important source of anti-inflammatory drugs for the treatment of IBD due to their strong anti-inflammatory activity, well recognized safety, and the diversity of their therapeutic mechanisms (Zhou et al., 2023).

Plant polyphenols have attracted much more attention in recent years and have become an important focus of research, due to their antioxidant activities and various beneficial effects on human health (Belščak-Cvitanović et al., 2018).

A diet rich in fiber, fruits and vegetables has long been associated with a lower risk of chronic diseases. Several mechanisms may explain these associations, but an important factor is likely the production of bioactive molecules from plant foods by colonic bacteria. This relates to our understanding of the role of the gut microbiome in promoting health (Edwards et al., 2017).

Plant fruits are rich in ascorbic acid and are the primary source of human intake of ascorbic acid (Zheng et al., 2022; Muñoz et al., 2023). For this reason and considering the content, efficacy, richness and diversity of *Citrus sinensis* and *Rosa canina* fruits in biologically active constituents. The present study aims to provide new research on the possible mechanism of action of the preventive effect of these fruits against L-methionine-induced experimental intestinal inflammation in a mouse model to confirm their pharmacological use as a remedy for IBD.

For this purpose, two studies were conducted in parallel: an *in vitro* study and an experimental *in vivo* study in order to achieve the objective of this research which focuses on:

- Preparation of the ethanolic extracts of *Citrus sinensis* and *Rosa canina* fruits;
- To quantify the phenolic and flavonoid contents of fruits extract;
- Evaluation of the antioxidant activity of fruits extracts *in vitro*;

- Identification of the chemical composition of *Rosa canina* fruit extract by GC/MS;
- Amperometric detection, electrochemical characterization and antioxidant capacity determination *Rosa canina* fruit extract;
- Evaluation of the antioxidant activity of *Rosa canina* fruit extract on human fibroblast using MTT test;
- Evaluation of the anti-inflammatory activity of fruits extracts *in vitro*;
- Assess the toxicity of the extract by step-down method on mice;
- Evaluation of the antioxidant effect of *Citrus sinensis* and *Rosa canina* fruits *in vivo*;
- Examination of the effect of L-methionine on intestinal inflammation by measuring the levels of plasma hs-CRP, and homocysteine markers;
- Evaluation the anti-inflammatory activity in a mouse model of pure fruits of *Citrus sinensis*, *Rosa canina* and vitamin C on intestinal inflammation by measuring the levels of plasma hs-CRP, and homocysteine markers,
- Confirmation of the induction of intestinal inflammation by L-methionine and the protective effect of *Citrus sinensis*, *Rosa canina* fruits and vitamin C on intestinal inflammation by a histological investigations.

Chapter I

Literature Review

I -Inflammatory bowel disease

I.1. Definition

Inflammatory bowel disease (IBD) has become a global disease with accelerating incidence worldwide in the 21st century (Ananthakrishnan et al., 2020; Liu et al., 2021). IBD affects 6.8 million people worldwide (Kudelka et al., 2020).

Inflammatory bowel disease is a complex group of disorders involving alterations in gastrointestinal physiology with relapsing-remitting phases (Nakase et al., 2021; Gampierakis et al., 2021). Those are a chronic immune-mediated inflammatory diseases characterized by damage to the epithelial barrier and disruption of immune homeostasis in the gastrointestinal tract and mucosal immunity by a complex inflammatory process (Yuan et al., 2019; Triantafyllou et al., 2020; Vuyyuru et al., 2022).

The etiology of IBD is unclear, but it appears that a variety of factors have been implicated in the pathogenesis of it (Alenazi et al., 2021; Di Re et al., 2021). Although the development and approval of immunosuppressants and biological treatments, but due to the complex etiology complications are common and remain difficult to manage. Current treatments for IBD are quite limited and mainly focus on remission of the disease (Amiot et al., 2021; Cai et al., 2022).

I.2. Anatomical reminder of the architecture of gastrointestinal tract

The principal functions of the gastrointestinal tract including digestion and absorption of nutrients, secretions and immune response. The unique architecture of the gastrointestinal tract facilitates these functions.

The wall of the intestine is generally defined in terms of the layers that make up the intestine. These layers are not entirely separate from each other, but are connected by connective tissue and by neural and vascular elements. The GI tract segments are divided into four layers: mucosa, submucosa, muscularis propria, and serosa (Rao and Wang, 2010).

1.2.1. Small intestine

The intestinal tract following the stomach includes the small intestine, which includes the duodenum, jejunum, and ileum (Montgomery et al., 1999; Rao and Wang, 2010).

1.2.2. Large intestine

The large intestine, or colon, surrounds the small intestine and begins in the right ileal region. In human adults, the colon is approximately 1.5 m long.

The anatomical divisions of the large intestine include the cecum, ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon, sigmoid colon, rectum and anus (Figure 1) (Antonioli and Madara, 1998; Montgomery et al., 1999; Rao and Wang, 2010).

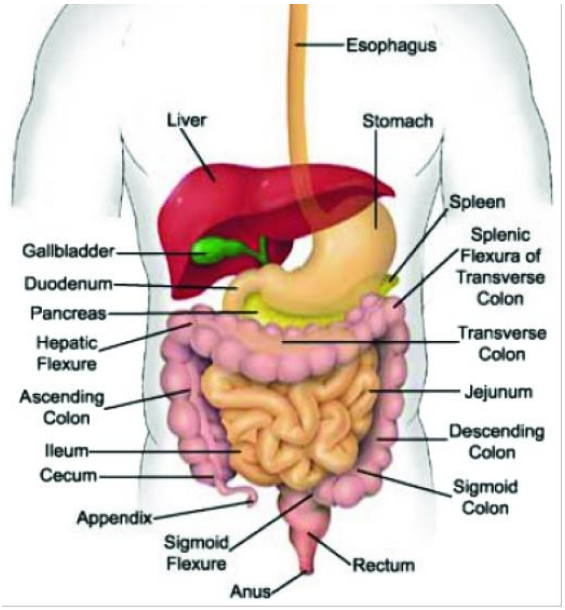

Figure 1. Architecture of human digestive system (Rao and Wang, 2010).

1.3. Types of inflammatory bowel disease

Inflammatory bowel disease encompasses two types of idiopathic intestinal disease that are differentiated by their location and depth of involvement in the bowel wall (Elhag et al., 2022; Vennou et al., 2020). The two main subtypes of IBD are: Crohn's disease (CD) and ulcerative colitis (UC). In UC, inflammation generally affects the large intestine, and CD, which can affect any part of the gastro intestinal tract from the mouth to the anus (James et al., 2020; Chiu et al., 2021).

Clinically, CD and UC share similar symptoms, while the location and extent of inflammation, as well as complications and prevalence, differ (Colombel et al., 2019; Vennou et al., 2020).

1.3.1. Crohn disease

Crohn's disease was first described by Dr Burrill B. Crohn and colleagues in 1932 (Feuersteina and Cheifetz, 2017). CD represents one of the main IBD forms. Although the disease can involve any part of the gastrointestinal tract, it most often affects the terminal ileum and the colon (Kline et al., 2020; Schmitt et al., 2021).

Patients with Crohn's disease have symptoms that are often variable and may include diarrhea, abdominal pain, weight loss, nausea, vomiting, and in some cases fever or chills (Feuersteina and Cheifetz, 2017; Reyt, 20219; Colombel et al., 2019).

1.3.2. Ulcerative colitis

Ulcerative colitis, a subtype of IBD, is characterized by relapsing and remitting mucosal inflammation, starting in the rectum and extending to the proximal segments of the colon (Gajendranet al., 2019; Noda et al., 2021).

Typical presenting symptoms include bloody diarrhea, abdominal pain, urgency, and tenesmus, should trigger the examination of a diagnosis of UC (Figure 2) (Rubin et al., 2019; Mak et al., 2019; Rostamani et al., 2021).

Figure 2. Different localization in ulcerative colitis and Crohn's disease (Longo et al., 2020).

I.4. Epidemiology

Inflammatory bowel disease is a global disease of the 21st century with evolving epidemiology in which the incidence is rising in newly industrialized countries (Borg-Bartolo et al., 2020; Lohning et al., 2021). IBD is much more prevalent in North America and Europe than in Asia or Africa (Su et al., 2019).

Inflammatory bowel disease is a pathology of young adults, usually between 20 and 30 years of age. There is a second peak in incidence at around 60 years for CD and UC patients probably because of the aging. Both sexes are equally affected (Park et al., 2019; Mak et al., 2020). Although 25% of patients will develop IBD in adolescence (Su et al., 2019).

I.5. Pathogenesis of inflammatory bowel disease:

The currently most accepted hypothesis of the pathogenesis of IBD considers it to be an interaction between genetics, abnormal intestinal microbiota, environmental factors, dysfunction of the intestinal epithelial barrier and immune dysregulation (Figure 3) (Gubatan et al., 2021; Xie et al., 2021).

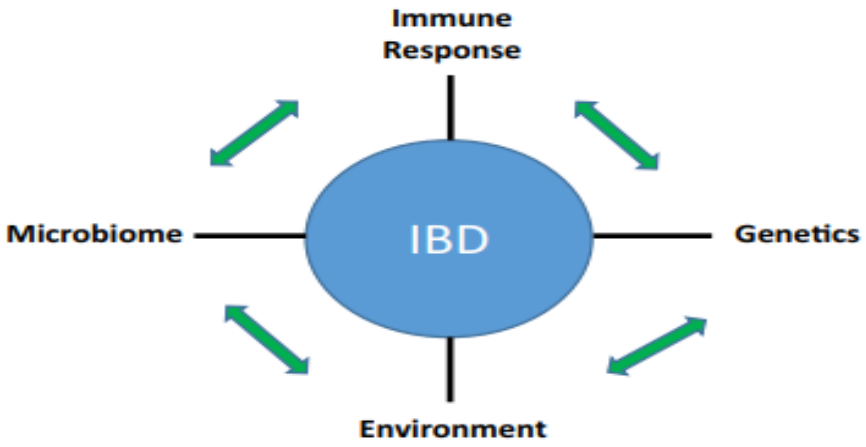

Figure 3. Schematic representation of the pathophysiology of inflammatory bowel disease.

1.5.1 Gut microbiota in inflammatory bowel disease

The adult human gut is colonized by over 1000 microbial species, collectively known as gut microbiota. The intestinal microbiota is associated with many functions essential for the host physiology (Kovatcheva-Datchary et al., 2019; Kastl et al., 2020).

The gut microbiota co-evolves with humans, and a variety of symbiotic interactions between the human host and the microbiota are required to maintain human health. An adverse alteration in the composition and function of the gut microbiota is known as dysbiosis, which impairs the host-microbiota interaction and the host immune system (Nishida et al., 2018; Guan, 2019).

The microbiota is an important contributing factor in the pathogenesis of IBD. Fundamental role of an altered microbiome or an aberrant immune response to the microbiome in the development of intestinal inflammation (Glassner et al., 2020).

The dysbiosis observed in IBD has been well described as being characterized by reduced microbial diversity and disturbances in microbial transcription (Borg-Bartolo et al., 2020; Qi et al., 2022). This dysbiosis results in impaired barrier function, pathogen translocation, and

imbalance of Treg and Th17 cells, which result in increased pro-inflammatory cytokines (Chen and wang, 2022).

1.5.2. Genetic factors

Over the past decade, genome-wide association studies (GWAS) have identified more than 200 genetic susceptibility loci associated with the pathophysiology of IBD. These loci are involved in microbiological sensing, microbiome-host interactions, innate immunity, and T-cell regulation.

In CD, disease-related genes were found primarily in innate immunity, including NOD2/ and autophagy-associated genes such as ATG16L1. For UC, deficiencies in ECM1, HNF4a, and LAMB1 have adverse effects on epithelial defense function. Interleukin (IL)-23R, IL12B, STAT3, JAK2, and TYK2, which are involved in adaptive immunity of interleukin-23 and T-helper 17 cell signaling, as well as IL-10 in interleukin-10 signaling, appear to be involved in CD and UC (Glassner et al., 2020; Dal Buono et al., 2021; Qi et al., 2022).

1.5.3 Imuunopathogenesis

Interactions between the immune system and the intestinal epithelium play an important role in the pathogenesis of IBD (Lu et al., 2022; Bharti et al., 2022). A defective gut barrier and microbial dysbiosis induce such accumulation and local activation of immune cells, which results in a pro-inflammatory cytokine loop that overrides anti-inflammatory signals and causes chronic intestinal inflammation (Neurath, 2019; Holtrop, 2023).

The immune response in IBD patients results in inflamed intestinal tissue infiltrated by large numbers of immune cells which secrete pro-inflammatory mediators, such as cytokines and nitric oxide (NO). The persistent recruitment of large numbers of leukocytes from the blood to the intestinal mucosa is largely mediated by adhesion molecules - integrins and their ligands (Jovani et al., 2013; Lohning et al., 2021).

The intestinal epithelium is composed of a monolayer of columnar epithelial cells that continuously communicate with the luminal microbiota and an underlying network of innate and adaptive immune cells (Turner, 2009; Jergens et al., 2021).

Cells of both the innate and adaptive immune system play a role in IBD pathogenesis. Macrophages, dendritic cells; B cells; and T helper (th) 1, Th2, Th17, Th9, and Treg cells have all been implicated in the initiation or propagation of intestinal inflammation in IBD (Knutson et al., 2013; Dharmasiri et al., 2021).

Recently, research on IBD pathogenesis has focused on T helper (Th17 cells, which secrete IL-17). It is well documented that Th17 inhibition can decrease the development of acute colitis by reducing inflammation. Additionally, innate lymphoid cells (ILCs) were recently discovered to be novel pathogenic effector lymphocytes in IBD (Lee et al., 2018; Bharti et al., 2022). Adaptive immunity consisting of humoral immunity, effector T cells, regulatory T (Treg) cells, natural killer (NK) cells and innate lymphoid cells (ILCs) were also revealed to produce inflammatory molecules involved in the pathogeneses of IBD (Park et al., 2017).

Several pro-inflammatory cytokines are involved in the progression of IBD, the activated lamina propria cells produce high levels of proinflammatory cytokines in the local tissue, including IL-1β, IFN-γ, TNF-α, TGF-β , and cytokines of the IL-23/Th17 pathway (Lee et al., 2018; Guan, 2019). Various studies have implicated the pro-inflammatory cytokines IL17 and IL23 in the pathogenesis of IBD (Schmitt et al., 2021), IL17 has a critical role, which activates STAT3 and stimulates a strong chronic immune inflammatory response (Figure 4) (Hou et al., 2020; Lee et al., 2020; Lee et al., 2018).

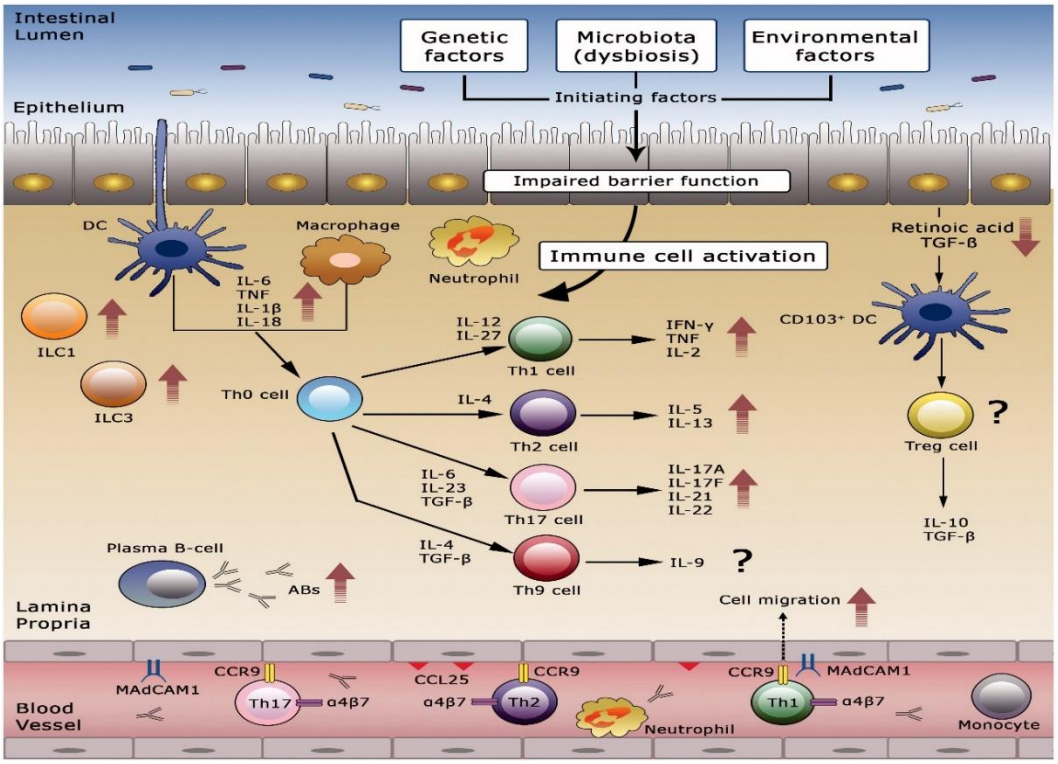

Figure 4. Immunopathogenesis of inflammatory bowel disease (Ahluwalia et al., 2018).

1.5.4. Environmental risk factors

Environmental factors are known to play a role in the development of IBD, these include the hygiene hypothesis, exposure to gastroenteritis, breast-feeding, early antibiotic use, cigarette smoking, pollution and diet (Borg-Bartolo et al., 2020; Mentella et al., 2020).

1.6. Histopathology

Microscopic evaluation in active IBD patients reveals pronounced infiltration of the lamina propria with a mix of neutrophils, macrophages, dendritic cells, and natural killer T cells. The histopathology in UC will show the involvement of only the mucosa and submucosa with the formation of cryptic abscesses and mucosal ulcers.

Biopsy specimens show neutrophilic infiltrate, granulomas are not seen in UC. The disease is contiguous and usually involves the rectum. In CD, the entire intestinal wall is involved and granulomas may be seen. Inflammation in CD is transmural characterized by lymphocytic infiltrate (Mc Dowell et al., 2022).

1.7. Vitamin C deficiency and inflammatory bowel disease

Patients with inflammatory bowel disease are at risk for nutritional deficiencies, including vitamin C deficiency (Amani, 2009; Alicja ewa, 2020).

Vitamin C deficiency is due to malabsorption, active inflammation and reduced consumption of fresh vegetables and fruits, which are the main sources. IBD patients have elevated levels of tumor necrosis factor α (TNF-α), which causes decreased absorption of various nutrients, such as vitamin C. As a result, IBD patients have lower vitamin C concentration (Subramanian et al., 2018; Ratajczak et al., 2020).

In the gastrointestinal tract, vitamin C plays an important role as an essential micronutrient and antioxidant that protects intestinal cells from inflammatory stimuli. However, in the inflamed mucosa of patients with IBD, mucosal concentrations of vitamin C are greatly reduced. In addition, vitamin C has been shown to have potent immunomodulatory activity during gastrointestinal inflammatory diseases (Mousavi et al., 2019).

II: Hyperhomocysteinemia

II.1. Homocysteine

II.1.1. Definition

Homocysteine (Hcy) has garnered much attention in the medical community and has been under a lot of speculation since its discovery in 1932 by Du Vignaued and Butz. Its chemical properties showed a similarity to cysteine, hence the name homocysteine (Figure 5)(Ganguly and Alam, 2015; Kaye et al., 2020).

Hcy is a non-proteinogenic sulfur amino acid that is not acquired from the diet, synthesized in the human body during the metabolism of methionine, an essential dietary amino acid (Koklesova et al., 2021; Tiwari et al., 2022; Brennerová et al., 2022). It is physiologically essential for such processes as cell cycle progression and maintenance of cellular homeostasis (Koklesova et al, 2021).

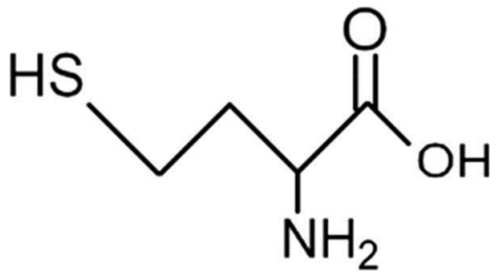

Figure 5. Homocysteine structure (Ganguly and Alam, 2015).

II.1.2.Biosynthesis

Methionine is an essential sulfur-containing amino acid present in various protein foods such as meat, eggs, dairy products, and legumes (Mc Isaac et al., 2016; Azzini et al., 2020).

Humans cannot acquire homocysteine through their food rather, homocysteine is biosynthesized from methionine through multiple steps. Hcy is produced in all human tissues through the transmethylation of methionine with three steps (Kim et al., 2018). In the first S-adenosyl-L-methionine (SAM) synthase catalyzes the reaction of methionine with ATP to form SAM. Secondly, SAM is converted into S-adenosyl-L-homocysteine (SAH) via a methyltransferase-catalyzed methyl transfer reaction, donating the methyl group to acceptor molecules (DNA, RNA, amino acids, proteins, phospholipids etc). Finally, SAH is rapidly metabolized by SAH hydrolase to adenosine and Hcy (Kim et al., 2018; Mohammad and Kowluru, 2020).

Hcy is synthesized by the demethylation of methionine via formation of two intermediate compounds, S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH). Methionine is first converted to SAM through the catalytic action of methionine adenosyltransferase. Different methyltransferases remove the methyl group from SAM generating SAH, which is then converted into Hcy and adenosine by SAH hydrolase. The formation of Hcy from methionine is the only pathway of Hcy biosynthesis in humans (Seminotti et al., 2021).

II.1.3. Metabolism

When methionine is available in sufficient quantity, Hcy is coupled to serine and then catabolized into cysteine, the latter a main precursor of the antioxidant compound, glutathione. This reaction is called transsulfuration. In the case where the oral intake of methionine is low, the Hcy is mainly transformed into methionine. This reaction is called remethylation (Herrmann et al., 2022).

Hcy can be converted to cysteine or remethylated to regenerate methionine through enzymatic reactions that depend on the presence of cofactors, such as folic acid and vitamins B6 and B12 (Garcia-Alfaro et al., 2022). Both these pathways require vitamin-derived cofactors, including pyridoxine (vitamin B6), for transsulfuration pathway mediated synthesis of cysteine as well as folate (vitamin B9), cobalamin (vitamin B12), and riboflavin (vitamin B2) in the methionine synthesis cycle (Koklesova et al., 2021; Garcia-Alfaro et al., 2022).

Homocysteine is converted to methionine by the addition of a methyl group from 5-methyltetrahydrofolate (MTHF) or betaine homocysteine methyltransferase (BHMT), which requires betaine as a methyl donor. MTHF is produced by the conversion of folic acid to 5,10-methyltetrahydrofolate and then to MTHF by the enzyme 5,10-methyltetrahydrofolate reductase (MTHFR).

After absorption, folic acid is reduced to tetrahydrofolate (THF) by dihydrofolate reductase (DHFR) (Parkhitko et al., 2019; Azzizi et al., 2020; Lee et al., 2021).

In the transsulfuration pathway, Hcy is converted to cystathionine by cystathionine β-synthase (CBS) and then to cysteine using vitamin B6 as a cofactor (Figure 6) (Lee et al., 2021; Herrmann et al., 2022).

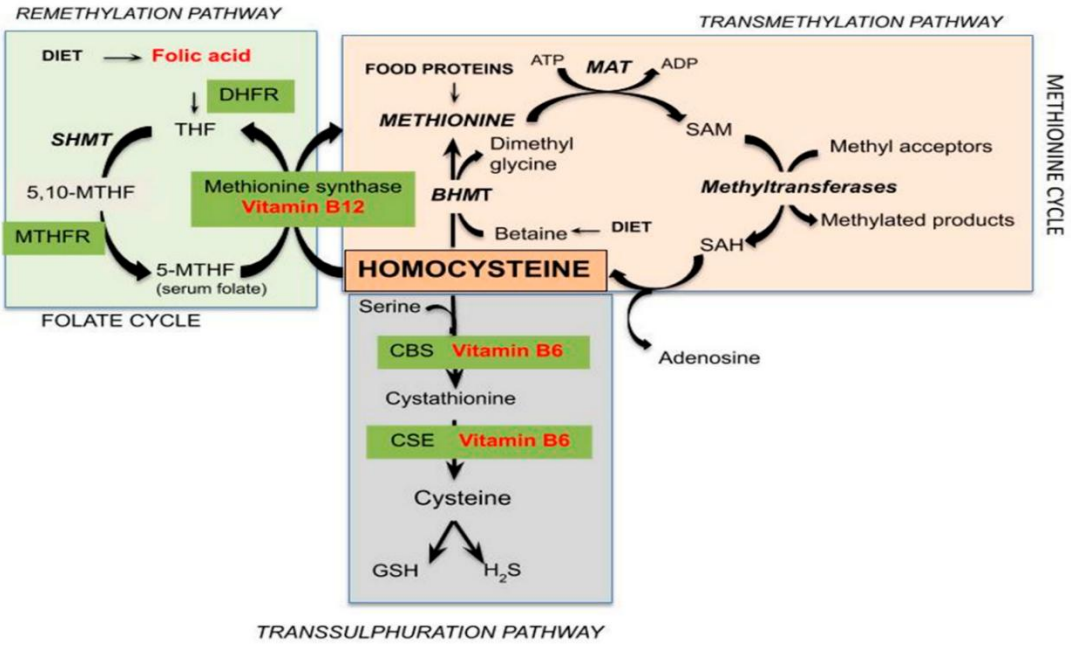

Figure 6. Schematic representation of pathways of homocysteine metabolism (Azzizi et al., 2020).

II.2. Hyperhomocysteinemia

II.2.1. Definition

An increase in the concentration of plasma homocysteine, called hyperhomocysteinemia (HHcy), is a pathological condition resulting from impaired metabolism of sulfur amino acids as a result of mutations in genes coding for enzymes, a deficiency in coenzymes, namely folic acid, B12 and B6 (Ledda et al., 2020; Moretti et al., 2021; Gerasimova et al., 2022).

II.2.2. Types of hyperhomocysteinemia

Homocysteine is present in plasma, with normal concentrations between 5 and 15 µmol/L, slightly elevated concentrations between 15 and 30 µmol/L, moderate between 30 and 100 µmol/L and a value > 100 µmol/L classified as severe hyperhomocysteinemia (Al Mutairi, 2020; Régis Guieu et al., 2022).

II.2.3. Homocysteine status in inflammatory bowel disease

Homocysteine may also have pathogenetic implications in IBD, demonstrating that it is a pro-inflammatory and immunostimulating molecule (Lamda et al., 2017; D'Amico et al., 2022).

II.3. Hyperhomocysteinemia and inflammatory bowel disease

Hyperhomocysteinemia is a risk factor for several pathological disorders (Cordaro et al., 2021; Ji et al., 2022). Previous studies have confirmed that HHcy is a risk factor associated with cardiovascular disease and possibly a significant independent risk factor for inflammatory bowel disease (Vezzoli et al., 2020; Chen et al., 2021). In 1996, Lambert and its collaborators were the first to report elevated Hcy levels in patients with CD (Keshteli et al., 2015).

Furthermore, HHcy promotes inflammation and leads to oxidative stress, and is associated with several disease states such as gastrointestinal disorders (Yakovleva et al., 2020).

II.4. Oxidative stress

II.4. 1. Definition

Oxidative stress is a metabolic condition in which the cellular antioxidant capacity does not counterbalance the oxidative damage induced by various aggressions such as free radicals (Al Alawi et al., 2021). Oxidative stress is the consequence of the inequality between reactive oxygen species (ROS) and antioxidants leading to cellular damage and the pathophysiology of many diseases (Baudin, 2020; Abdullah et al., 2021).

II.4. 2. Reactive oxygen species

Reactive oxygen species (ROS) are highly active moieties, some of which are direct oxidants, and some of which have oxygen or electronegative oxygen-like elements produced in the cell during cell metabolism or under conditions pathological. Some of the reactive species are free radicals such as hydroxyl radical and superoxide radical, and some are non-radicals such as hydrogen peroxide (Nandi at al., 2019; Hamma, 2019; Uchida, 2003).

The term ROS encompasses oxygen free radicals, such as superoxide anion radical ($O_2^{\cdot}$) and hydroxyl radical ($^{\cdot}OH$), and nonradical oxidants, such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O2$) (Zorov et al., 2014; Perillo et al., 2020).

Hydrogen peroxide is produced from O_2 mainly by NADPH oxidases in conjunction with superoxide dismutases, by the mitochondrial electron transport chain and by numerous other enzymes. H_2O_2 is a strong two-electron oxidant, but its high activation energy confines its reactivity to a few biological targets. H_2O_2 is relatively stable. It reacts very slowly with glutathione, cysteine and methionine, but its reactivity towards cysteine in specific proteins can increase greatly depending on the particular protein structure and environment, providing a basis for selectivity and specificity of H_2O_2 in redox signaling (Dagnell et al., 2019; Sies and Jones, 2020).

II.5. Antioxidant defense system

Oxidative stress has been implicated in the pathophysiology of many diseases, including cancer, cardiovascular disease, and inflammatory disease. To protect themselves from oxidative stress, organisms have developed an arsenal of antioxidants (Nandi et al., 2019; Baudin, 2020).

Antioxidants have a primary role in the body, as they are generated in normal physiological processes, and their production and intake must be balanced (Škugor Rončević et al., 2022).

The antioxidant system includes endogenous antioxidants (enzymatic and non-enzymatic) such as superoxide dismutase, glutathione peroxidase, catalase, glutathione and antioxidants provided to the body via nutrition such as vitamins C and E, carotenoids, secondary plant compounds (polyphenols, flavonoids) (Nibbe et al., 2023).

Recently, the antioxidant activity of plants has been assessed with analytical systems, mainly chromatographic or spectrophotometric, capable of measuring indirectly, with a single assay, the sum of the antioxidant activities of many molecules (Sadowska-Bartosz and Bartosz, 2022). The electrochemical method has advantages over these analytical techniques. The direct electrochemical determination of antioxidants in plants or plant extracts (Alam et al., 2022; Zheng et al., 2022) has been widely used since ionization potential is the key factor that determines the effectiveness of antioxidants (Percevault et al., 2020; Zheng et al., 2022).

II. 5. 1. Vitamin C

II.5.1.1. Definition

Vitamin C (L-ascorbic acid) was discovered by Albert Szent Györgyi in 1912 (Drouin et al., 2011). The human body is unable to synthesize vitamin C endogenously due to a mutation in the L-gulono-γ-lactone oxidase gene, which codes for an enzyme involved in the biosynthesis of this vitamin. Therefore, fruits represent the main source of vitamin C intake for humans, but it can also be produced industrially (Yin et al., 2022; Beton-Mysur and Brozek-Pluska, 2023; Bedhiafi et al., 2023).

Vitamin C is a water-soluble micronutrient and a potent reducing agent (Pehlivan, 2017; Parab et al., 2023).

II.5.1.2. Sources and recommended intake

The recommended Dietary Allowance (RDA) of vitamin C in males and females is 75 and 90 mg/day, respectively (Monsen, 2000; Paciolla et al., 2019). Therefore, human's nutritional source of vitamine C is mainly dependent on plant foods. The primary dietary sources of vitamin C are *Citrus* fruits, kiwi, nuts, vegetables, sprouts, capsicum, guava and strawberries (Zulfiqar et al., 2016; Chaturvedi et al., 2022).

II.5.1.3. Biological role

Vitamin C is notably known for its dominant antioxidant properties, and for its ability to neutralize ROS and free radicals, protecting cellular elements from oxidative stress damage by donating electrons (Zylinska et al., 2023; Zhang et al., 2023).

It plays a major role in the synthesis of catecholamines, collagen, cortisol, neurotransmitters, and peptide hormones, immune cell functions, maintenance of vasodilation and the endothelial barrier, and metabolism of iron and folic acid (Berger et al., 2015; Dresen et al., 2023).

Vitamin C participates in immune defense, contributes to epithelial barrier function against pathogens (Carr and Maggini, 2017). In addition, this vitamin has immunomodulatory effects by strengthening innate and adaptive immune responses promoting the differentiation and proliferation of B and T cells (Bedhiafi et al., 2023).

II.5.1.4. Therapeutic use

In recent years, there has been significant interest in the use of vitamin C in chronic diseases and it represents an inexpensive, safe, and promising therapeutic approach to improving clinical outcomes (Dresen et al., 2023).

Vitamin C is a therapeutic agent for many diseases (Chambial et al., 2013) and is used in the prevention and treatment of a broad spectrum of conditions, including scurvy, diabetes, atherosclerosis, the common cold, and cancer. Recently, scientists have also begun to focus on the function of vitamin C in the prevention and treatment of inflammation and immunity (Mussa et al., 2022). Vitamin C has anti-inflammatory effects and may optimize immune defense mechanisms (Carr et al., 2015; Amrein et al., 2018; Dresen et al., 2023).

II.5.2. Polyphenols

II.5.2.1. Definition

The polyphenols or "phenolic compounds" includes a large group of more than 8000 molecules, devided into about ten chemical classes (Hennebelle et al., 2004; Élie, 2022).

Polyphenols are characterized by the presence of at least one benzene nucleus to which at least one hydroxyl group is directly linked, free or engaged in another function: ether, ester, glycoside (Bruneton, 1999). They are commonly subdivided into phenolic acids (benzoic acid derivatives or cinnamic acid derivatives), coumarins, stilbenes, flavonoids, lignans, lignins, tannins (Cheynier, 2005).

II.5.2.2. Source, location and interest

Polyphenols are most abundant antioxidants in our diet and are widespread constituents in fruits (Landete, 2012; Palaska, 2013).

At the cellular level, phenolic compounds are mainly distributed in two compartments: the vacuoles and the cell wall. In the vacuoles, the polyphenols are conjugated with sugars or organic acids, which increases their solubility and limits their toxicity for the cell. At the wall level, we find mainly lignin and flavonoids linked to the parietal structures. Phenolic compounds are synthesized in the cytosol. Some of the enzymes involved in phenylpropanoid biosynthesis are bound to endoplasmic reticulum membranes, where they are organized into metabolons (Bénard, 2009).

Polyphenols contribute to the organoleptic quality of plant foods (color, astringency, aroma, bitterness). These molecules play an important role by acting directly on the nutritional quality of fruits and vegetables and their impact on health (Visioli et al., 2000).

II.5.2.3. Classification of polyphenols

Polyphenols can be grouped into two large groups: Non-flavonoids, the main compounds of which are: phenolic acids, stilbenes, lignans, lignins and coumarins and flavonoids, of which we mainly characterize: flavones, flavanones, flavonols, isoflavonones, anthocyanins, proanthocyanidins and flavanols (Pincemail et al., 2007).

II.5.2.4. Therapeutic use

Polyphenols have been used in herbal medicine for a long time and have been implicated in defense against several stressful conditions (Asensi et al., 2011).

These compounds are known for their high bioactivity which translates at the organism level into a wide range of biological properties such as: antioxidant, anti-inflammatory, antimicrobial and anticancer (Morand and Milenkovic, 2014; Zillich et al., 2015).

Epidemiological, clinical and animal research confirms the role of polyphenols in the prevention of various chronic diseases, including cardiovascular diseases, inflammatory diseases, neurodegenerative diseases and some cancers (Mitjavila and Moreno, 2012; Palaska, 2013).

III: Plant fruits

III.1. *Rosa canina* L.

III.1.1. Description

Rosa canina L., belonging to the Rosaceae family, mainly distributed in Europe, North Africa, Algeria, Tunisia, Morocco, West Asia (Iancu et al., 2021; Ikhsanov et al., 2021; Sapkota et al., 2023). It is a deciduous perennial shrub with spiny and thin stems and can reach 2-3 meters in height. The *Rosa canina* L. fruit are called rosehips and are pseudo-fruit of oval shape. Ripe fruits are orange red (Figure 7) (Pehlivan et al., 2018; Sapkota et al., 2023).

Figure 7. *Rosa canina* L. fruit (Rosehips)

III.1.2. Chemical composition of *Rosa canina* fruit

Rosehips are rich in natural antioxidants such as phenolic compounds such as phenolic acids, flavonoids, tannins, coumarins, lignans, quinones, stilbens, and curcuminoids and considered the richest fruit in vitamin C of all the most popular, vitamins, B, D, A and E, and

carotenoids (Marmol et al., 2017; Tabaszewska and Najgebauer-Lejko, 2020; Igual et al., 2021). The fatty acids are determined by (Vlaicu et al., 2022; Lustrup and Winther, 2022).

III.1.3. Botanical classification (Zahara et al., 2020).

Reign	Plantae
Sub-kingdom	Tracheobionta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Rosales
Family	Rosaceae
Gender	*Rosa*
Species	*Rosa canina* L.

III.1.4. Utilisation

Rosehips are consumed as a natural source of vitamin C, in the form of tea, nectar, and dried pulp, and are also incorporated as ingredients in the preparations of several food products such as soups, jams, syrups, and soft drinks. These fruits are of considerable interest especially in the food, pharmaceutical and cosmetic industries (Turan et al., 2018; Hendrysiak et al., 2023).

III.1.5. Therapeutic properties

The *Rosa canina* L. fruit has been used for a long time in traditional medicine and it has many biological powers including anti-inflammatory, anti-tumor, immunomodulatory, and anti-oxidant (Khazaei et al., 2020; Lakka et al., 2021).

Numerous studies have confirmed the prophylactic and therapeutic effects of rosehips against various inflammatory disorders such as osteoarthritis, rheumatism, cancer, and dysfunction of the gastrointestinal system (Ouerghemmi et al., 2016; Wanes et al., 2021; Maloupa et al., 2021; Özdemir et al., 2022). Rosehip extracts have also been shown to have the potential to act as antioxidants, inhibiting cell proliferation and migration in breast (MCF-7), colon (HT-29) and cervical (HeLa) cancer cell lines (Cagle et al., 2012).

R. canina have medicinal properties, being recommended in the prevention and curative treatment of some inflammatory affections of gastric mucosa as well as against the gastric ulcer (Ciornea et al., 2018).

III.2. *Citrus sinensis* L.

III.2.1. Description

Citrus sinensis L., commonly known as orange or sweet orange, belongs to the Rutaceae family. This species is the most cultivated and commercialized species in the world (Figure 8) (Sathiyabama et al., 2018; Juibary et al., 2021).

Figure 8. *Citrus sinensis* L. fruit (Orange).

III.2.2. The composition of *Citrus sinensis* fruit

The fruit of *Citrus sinensis* L. is an excellent source of vitamin C, as well as other phytochemicals such as phenolics, flanovoids, and carotenoids, which are known to have beneficial health effects (Liu et al., 2012; Oikeh, 2020).

The nutritional composition of *Citrus sinensis* is based on the following elements: flavonoids, steroids, hydroxyamides, alkanes and fatty acids, coumarins, peptides, carbohydrates, carbamates, alkylamines, carotenoids, volatile compounds and nutritional elements such as potassium, magnesium, calcium and sodium (Grosso et al., 2013; Favela-Hernández et al., 2016). Other nutrients are provided in table 1.

Table 1. Nutrient composition of *Citrus sinensis* fruit (Etebu and Nwausoma, 2014).

Composition	Amount	Composition	Amount
Energy	197 kJ (47 kcal)	Vitamin B6	0.06 mg (5%)
Sugars	9.35 g	Folate (vit. B9)	30 μg (8%)
Dietary fiber	2.4 g	Choline	8.4 mg (2%)
Protein	0.94 g	Vitamin C	53.2 mg (64%)
Fat	0.12 g	Vitamin E	0.18 mg (1%)
Water	86.75 g	Calcium	40 mg (4%)
Vitamin A equiv.	11 μg (1%)	Iron	0.1 mg (1%)
Thiamine (vit. B1)	0.087 mg (8%)	Magnesium	10 mg (3%)
Riboflavin (vit. B2)	0.04 mg (3%)	Manganese	0.025 mg (1%)
Niacin (vit. B3)	0.282 mg (2%)	Phosphorus	14 mg (2%)
Pantothenic acid	(B5) 0.25 mg (5%)	Potassium	181 mg (4%)
-	-	Zinc	0.07 mg (1%)

III.2.3. Botanical classification

Reign Planta

Class Magnoliopsida

Subclass Magnoliidae

Super-order Rosanae

Order Sapindales

Family Rutaceae

Gender *Citrus*

Species *Citrus sinensis* L.

III.2.4. Utilisation

The orange fruit is usually consumed whole or processed into juice (Oikeh et al., 2020). *C. sinensis* is an excellent source of secondary metabolites which have been identified in the fruits, peel, leaves, juice, and roots that contribute to the pharmacological activities attributed to this plant (Favela-Hernández et al., 2016).

III.2.5. Therapeutic properties

C. sinensis has been used in traditional medicine to treat bowel disorders, respiratory disorders, cardiovascular disease, stress (Mannucci et al., 2018), inflammation (Maugeri et al., 2019) and gastrointestinal inflammatory disorders (Musumeci et al., 2020). The genus *Citrus* has been recognized for its antibacterial, antioxidant, and anti-inflammatory properties (Nawrin et al., 2021; Niazi et al., 2023). In addition, it has demonstrated a protective role against oxidative stress and gastric ulcer (Selmi et al., 2018; Nawrin et al., 2021).

Chapter II

Materials and Methods

I.1. *In vitro* experiment

I.1.1. Plant material

I.1.1.1. Sample collection

- The ripe *Rosa canina* fruits (rosehips) were collected in October 2019 from the region of Djbel El Ouahch Constantine, (North-Eastern Algeria).
- The fresh *Citrus sinensis* fruits (orange) was purchased from a local market at Constantine (North-Eastern Algeria) in November 2019. The fruits were fresh, of taste quality, and free from damage.

I.1.1.2. Preparation of fruits extracts

The preparation of the ethanol extracts was carried out according to the protocol used in the L.O.S.T laboratory (Laboratoire d'Obtention des Substances Thérapeutiques).

The fruits of *Rosa canina* and *Citrus sinensis* were washed, peeled, cut into small pieces and then crushed before being macerated in a mixture of ethanol-water (8/2) for 48 h at room temperature. The macerate of each fruits was filtered; and evaporated using Buchi R-215 vacuum evaporator at 40 °C. Each fruit extract was placed in a crystallizer, then frozen for two days and dried using a laboratory freeze dryer (ALPHA 1-4 LD then processed into fine powder, then ground again into fine powder . The extract obtained were stored in an airtight bottle for *in vitro* assays (Figure 09).

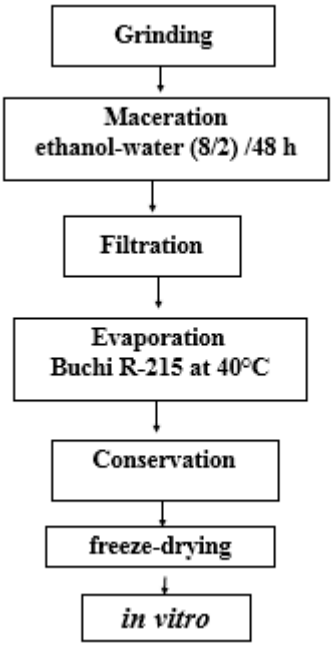

Figure 9. Preparation of fruit extracts.

I.1.2. Qualitative phytochemical analysis of fruits extracts

I.1.2.1. Determination of total phenolic content (TPC)

The TPC was determined using the Folin-Ciocalteu reagent (Singleton and Rossi, 1965) according to the method described by Muller et al. (2010). The solution was prepared by mixing 20 µL of each fruit extract (1 mg/mL) with 100 µL of Folin Ciocalteu reagent (0.2 N) and 75 µL of sodium carbonate (75 g/L) in a 96 wells microplate. The microplate was incubated in the dark for 2 h at room temperature. Absorbance was then measured at 765 nm using a microplate reader (Perkin Elmer Enspire, Singapore). The concentration of total phenolic compound was calculated from a standard curve of gallic acid.

I.1.2.2. Total flavonoid content (TFC)

The TFC was determined following the described method of Topçu et al. (2007) with slight modifications. A volume of 50 µL of each extract (1 mg/mL) was mixed with 130 µL of methanol, 10 µL of potassium acetate (1 M) and 10 µL of aluminum nitrate (10%). The absorbance was read spectrophotometrically at 415 nm after 40 min incubation at room temperature and quercetin was used as standard.

The formation of a yellow complex between flavonoids and aluminum trichloride ($AlCl_3$), the intensity of which indicates the importance of the content of the extract in flavonoids.

I.1.3. Evaluation of antioxidant activity of fruits extracts

I.1.3.1. DPPH free radical scavenging assay

The capacity of ethanolic extracts of both fruits to scavenge the DPPH- (2,2-diphenyl-1-picrylhydrazyl) radical was evaluated by the method of Blois, (1958). DPPH solution was prepared by solubilization of 6 mg of 2,2-Diphenyl-1-picrylhydrazyl in 100 mL of methanol. The reaction was started by adding 160 µL of DPPH solution to 40 µL of each extract at different concentrations. The microplate was incubated in the dark for 30 min and the absorbance measurement was taken at 517 nm. DPPH solution in methanol was used as a control, α-tocopherol, BHT, and BHA were used as antioxidant standards.

Upon reaction of the antioxidant with DPPH, the DPPH accept the hydrogen donor, and the solution loses its color from purple to pale yellow and discoloration acts as an indicator of antioxidant activity. The antioxidant concentration necessary to decrease the initial DPPH concentration by 50% inhibition is used for the comparison of antioxidant activity of different

compounds. The calculation of IC_{50} value requires the determinatin of kinetics of the reaction between DPPH and different concentrations of the antioxidant.

The inhibition percentage was calculated using the following equation:

% Inhibition = [(Abs (c) — Abs (E)) /Abs (c)] × 100.

Abs (c) is the absorbance of the control reaction; Abs (E) is the absorbance of the extract or standard.

I.1.3.2. ABTS scavenging activity assay

The ABTS scavenging activity was determined by the method of Re et al. (1999). In this assay, 160 µL of diluted $ABTS^+$ solution (5 mL of water was mixed with 19.2 mg of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid and 3.3 mg of potassium persulfate, the resulting mixture was incubated in the dark for 16 h) was added to 40 µL of each extract at different concentrations. After 10 min the absorbance was measured spectrophotometrically at 734 nm. $ABTS^+$ solution in ethanol was used as a control, BHT and BHA were served as antioxidant standards.

The antioxidants capacity scavenging of stable $ABTS^+$ radical, the reduction causes the color loss of ABTS, consequently, the intensity of discolouration can be expressed as the inhibition percentage of ABTS. The percentage of inhibition was calculated using the previous formula.

I.1.3.3. Galvinoxyl radical scavenging assay (GOR)

The GOR assay was assessed by the method of Shi et al. (2001). 40 µL of different concentrations of each extract in methanol was mixed with 160 µL of 0.1 mM methanolic solution of galvinoxyl. The mixture was incubated for 120 min in dark at room temperature and the absorbance was measured at 428 nm. BHT and BHA are used as antioxidant standards.

Galvinoxyl solution in methanol was used as a control, BHT and BHA are used as antioxidant standards.

The color of galvinoxyl radical is degraded from dark yellow to pale yellow in the presence of an antioxidant and its absorbance decreases. The following equation was used to calculate the galvinoxyl radical scavenging:

% Inhibition = [(Abs (c) — Abs (E)) /Abs (c)] × 100.

Abs (c) is the absorbance of the control reaction; Abs (E) is the absorbance of the extract or standard.

I.1.3.4. Cupric reducing antioxidant capacity (CUPRAC)

Cupric reducing antioxidant capacity was determined by the method adapted by Apak et al. (2004). The reaction consisted of mixing 50 µL of copper (II) chloride solution (10 mM), 50 µL of neocuproine (7.5 mM in ethanol) and 60 µL of ammonium acetate buffer (1 M) with 40 µL of each extract at different concentrations.

Reduction of Cu^{2+}, in the presence of neocuproine by a reducing agent, gives a Cu^{+} complex with a color change. A larger absorbance indicates a higher reducing capacity of antioxidants.

The absorbance was measured at 450 nm after 1h. BHT and BHA were used as antioxidant standards.

I.1.3.5. Reducing power assay

The reducing power of each extract was measured according to the method previously described by Oyaizu (1986) with a slight modification. A volume of 10 µL of fruits extracts at various concentrations was added to 40 µL of phosphate buffer (0.2 M, pH 6.6) and 50 µL of potassium ferricyanide (1%) were mixed and incubated at 50 °C in a water bath for 20 min. Then 50 µL of 10% trichloroacetic acid (TCA) and 10 µL of ferric chloride (0.1%) were added to the mixture and completed with 40 µL of distilled water. Reducing power is based the ability of antioxidants present in the extracts to reduce ferric ion ($Fe3^{+}$) to ferrous ion ($Fe2^{+}$), the increase in absorbance is proportional to the reducing power of the tested fraction. The absorbance was read spectrophotometrically at 700 nm. Ascorbic acid and α-tocopherol were used as standards.

I.1.4. GC-MS analysis of *Rosa canina* fruit extract

The chemical composition of *Rosa canina* fruit extract was analyzed by GC-MS.

GC analysis was performed using an Agilent technologies GC 17A gas chromatograph equipped with a gross-linked HP 5MS column (30 m*0.25 mm, flm thickness 0.25 µm). The oven temperature was programmed as isothermal at 60 °C for 8 min, helium was used as the carrier gas at a rate of 0.5 ml/min. GC/MS was performed using a HP Agilent technologies 6800 plus mass selective detector, the operating conditions were the same as for the analytical GC the MS operating parameters were as follows: ionization potential, 70 eV; ionization current, 2 A; ion source temperature, 280 °C; resolution,1000 scan time,5 s; scan mass range,

34–450 u; spit ratio, 50 :1; injected volume, 1.0 µL. The identifcation of compounds of the *R. Canina* fruit extract was based on their retention times in comparison with matching spectral peaks available with NIST and Wiley mass spectral libraries.

I.1.5. Amperometric detection, electrochemical characterization and antioxidant capacity determination of *Rosa canina* fruit extract

1.1.5. 1. Amperometric detection of ascorbic acid in *Rosa canina* fruit extract

The ascorbic acid (AA) content in *Rosa canina* extract was electrochemically detected by Constant Potential Amperometry (CPA); the screen-printed sensors used in this work (Figure 9) were purchased by GSI Technologies (311 Shore Drive Burr Ridge, IL, USA - www.GSITech.com).), and consist of a 4 mm working electrode (WE), an Ag/AgCl pseudoreference electrode (RE) and a carbon auxiliary electrode (AE) according to Spissu et al. (2021) with some modification.

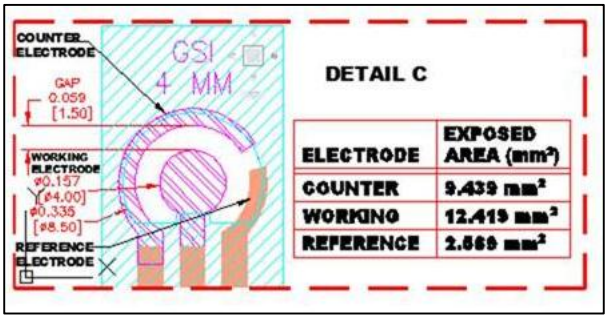

Figure 10. Carbon screen-printed sensor schematic drawing with details of working, counter and reference electrodes exposed area.

The ascorbic acid calibration was carried out as follows: a first aliquot of 70 µL, containing only PBS (used as supporting electrolyte), was deposited on the sensor surface with a graduated micropipette in order to obtain a baseline. A positive potential of +150 mV was applied vs. Ag/AgCl pseudo-RE. Once the baseline current was recorded, the PBS drop was dried with absorbent paper without touching the surface of the sensor. Five subsequent 70 µL aliquots of increasing AA concentrations (5, 10, 20, 50, a100, 250 and 500 µM) were deposited on, and removed from, the sensor surface by the same technique. The current values were read out every three minutes.

Similarly, the electrochemical detection of AA in the *R. canina* fruit extract was made by simply exposing the screen-printed sensors surface to 70 µL of solution of 2 mg/mL of extract.

1.1.5.2. Electrochemical characterization and antioxidant activity determination

The electrochemical characterization of *R. canina* fruit extract and the antioxidant activity (AAox) determination was achieved by cyclic voltammetry as previously reported (Spissu et al., 2021a; 2021b) with some modifications. Measures were acquired by the screen-printed sensors described in the previous paragraph. Cyclic voltammograms (CVs) were obtained from -0.2 V to +0.8 V (vs. Ag/AgCl pseudo-RE) at a scan rate of 0.1 V/s. A first 70 µL aliquot, containing only PBS (used as a supporting electrolyte), was deposited on the WE in order to obtain a baseline current; then, 70 µL aliquots of a 2 mg/mL *R. canina* extract solution was deposited on the sensor surface.

I.1.6. Determination of antioxidant activity of *Rosa canina* fruit extract on human fibroblasts

The antioxidant activity of *R. canina* fruit extract was determined on normal human fibroblasts (WI-38).

I.1.6.1. Cell culture

- **Preparation of cell culture medium**

Human fibroblasts was obtained from American Type Culture Collection (ATCC) and was maintained on DMEM supplemented with 10% FBS, 1% penicillin/streptomycin, 2 mM Glutamine, and 1X MEM non-essential amino acid at 37 °C under 5% CO_2 and 95% humidity.

I.1.6.2. Viability assays

The effect of the extract on the viability of fibroblasts was evaluated by the MTT assay. The latter is based on the measurement of the reduction of the yellow tetrazolium salt, 3-(4,5-dimethylthiazoly-2)-2,5-diphenyltetrazolium bromide (MTT), to purple formazan crystals by mitochondrial succinate deshydrogenase, which is only active in living cells. The intracellular purple formazan can be solubilized and quantified by spectrophotometric means.

Cells were plated in 96 wells at a concentration of 1 x 10^4/100 µL and the following experiments have been carried out:

- 1) a dose response test for 24 hours with increasing concentrations (1-10-50-100-250-500 µg/mL) of *R. canina* fruit extract was performed to assess the toxicity of the extract versus normal cells;

- 2) the second experiment aimed to evaluate the ability of *R. canina* fruit extract to protect fibroblasts from oxidative stress induced by hydrogen peroxide. Cells were firstly treated with the lowest concentrations of extract, among those tested, which had a significantly higher

viability than the control (1 and 10 µg/mL of extract according to experiment 1) for 24h; then they were treated with 500 µM H_2O_2 for an additional 24 hours.

At the end of the experiments, cells were incubated with 100 µL of MTT (0.5 mg/mL), and the cultures were allowed to incubate at 37 °C for 3 h. The MTT was removed and the formazan crystals were dissolved in 100 µL of 2-propanol. The color was read at 570 nm using a microplate reader (Sunrise™ Absorbance Reader - TECAN). The percentage of cells growth was calculated by normalizing the absorbance of treated cells to corresponding control.

I.1.7. Evaluation of anti-inflammatory activity of fruits extracts

I.1.7.1. Inhibition of the denaturation of albumin

The *in vitro* anti-inflammatory activity of extracts was assessed by using the inhibition of the bovine serum albumin (BSA) denaturation method of Kandikattu et al. (2013) with slight modifications.

A dose of 500 µL of each concentration of fruits extracts or the standard (diclofenac sodium) was transferred to a glass test tube with 500 µL of 0.2% w/v BSA prepared in Tris-Hcl buffer (pH = 6, 6, C = 0.05 M).

All tubes were incubated at 37°C for 15 min then heated to 72°C for 5 min in a water bath and then cooled for 10 min. Each experiment was performed in triplicate, and the mean absorbance was recorded. The absorbance of these solutions was determined at a wavelength of 660 nm.

Percent inhibition was determined using the following equation:

Denaturation inhibition % = Control Abs − Sample Abs/Control Abs × 100

I.2. *In vivo* experiments

I.2.1. Preparation of fruit samples

The fresh fruits of *Citrus sinensis* and *Rosa canina* were washed, cut into small pieces and crushed. Once crushed, each fruit was placed in a crystallizer, then frozen for two days and dried using a laboratory freeze dryer (ALPHA 1-4 LD plus, Martin Christ), then transformed into a fine powder, then crushed again into a fine powder and each fruit was stored in an airtight bottle for the *in vivo* experiment.

I.2.2. Animals

Adult male *Mus Musculu*s albino mice (2-3 months old), weighing between (25–35 g) come from the faculty of Pharmacy, University Constantine, Algeria. These mice were used for all *in vivo* experiments. They were provided with food (standard pellet diet- SARL Production

Locale, Bouzareah. Algeria) and water adlibitum, maintained in Animal house and housed in plastic cages at temperature room 25°C with 12 h light-dark cycle.

I.2.3. Evaluation of the toxicity of *Rosa canina* fruit

- **step-down method**

An acute toxicity study was performed using a step-down method according to (Lim et al., 2020) with slight modifications. A dose of (2000 mg/kg) of *Rosa canina* fruit was prepared in distilled water and administered to seven male mice, the dose was orally administered to the first mouse and this mouse was observed for mortality and clinical signs during the first hour then every hour for three hours, then the animal was checked occasionally for up to 48 hours. If the animal survived, six additional mice received the same dose of *Rosa canina* fruit. Mice were observed for toxic signs and mortality for 14 days. The LD_{50} was predicted to be above 2000 mg/kg if three or more mice survived.

I.2.4. Evaluation of the anti-inflammatory activity of fruits and vitamin C

The anti-inflammatory activity was realized by four experiments, on adult male mice divided into eight experimental groups (each group, includes 5-7 mice), during a treatment period of 21 days (Table 2).

The experimental groups were as follow:

- in the first experiment, the control group (C) received a normal diet and the second group (M) was administered a high dose of L-methionine (200 mg/kg);
- in the second experiment, the group (OM) received a dose of 200 mg/kg of L-methionine then treated with *Citrus sinensis* fruit (200 mg/kg), and the group (O) was administered by a dose of 200 mg/kg of *Citrus sinensis* fruit only;
- in the third experiment, the group (RM) received a dose of 200 mg/kg of L-methionine then treated with *Rosa canina* fruit (200 mg/kg), and the group (RC) was administered by a dose of 200 mg/kg of *Rosa canina* fruit only;
- in the fourth experiment, the group (VM) received a dose of 200 mg/kg of L-methionine then treated with a dose of (500 mg/kg) of vitamin C, and the group (VC) was administered by a (500 mg/kg) dose of vitamin C only.

Throughout the treatment period, the weight and the food of the mice are measured daily at the same time.

The dose of each treatment is calculated according to the average weight of the mice of each batch and given to the mouse once a day.

The *Citrus sinensis, Rosa canina* fruits and vitamin C doses were chosen based on previous references (Orhan et al., 2007; Amirshahrokhi et al., 2019), (Matuka et al., 2020) and of (Samantaray et al., 2021) respectively.

Each treatment dose was prepared in distilled water and administered orally as a single daily dose *via* an orogastric tube.

Table 2. Treatment of mice during 21 days.

Experimental groups	Administered substance	Daily dose	Number of animal	Period of treatment
C	(distilled water)	-	5-7	21
M	L-methionine	200mg/Kg		
OM	L-methionine + *C.sinensis* fruit	200mg/Kg+200mgKg		
O	*C. sinensis* fruit	200mg/Kg		
RM	L-methionine + *R. canina* fruit	200mg/Kg+200mg/Kg		
R	*R. canina* fruit	200mg/Kg+200mg/Kg		
VM	L-methionine+ vitamin C	200mg/Kg+500mg/Kg		
VC	vitamin C	500mg/Kg		

After 21 days of treatment, the animals are fasted for 12 h all night long, the animals' blood was taken from the retro-orbital sinus using glass capillaries and collected in EDTA tubes. After centrifugation at 3000 g/m for 15 minutes, the plasma was recovered in eppendorf tubes and stored at -20° C for the determination of certain biological parameters.

I.2.5. Biochemical analysis

The plasma was treated to determine the levels of inflammatory markers: measurement of C-reactive protein and homocysteine in plasma.

I.2.5.1. Homocysteine test assay

The plasma values of homocysteine was measured by competitive solid phase chemiluminescance immunoassay on an (IMMULITE 2000 XPi). The assay consists of two fully automated steps: a sample pre-treatment step, followed by an immunological step based on the competition principle. The values of homocysteine was expressed in (µmol/L).

I.2.5.2. C-reactive protein test

The principle consists in measuring the concentrations of C-reactive protein (CRP) in the plasma by an immunoturbidimetric method using a sensitized latex (Onraed and Hennache, 2005). The plasma values of hs-CRP was measured by an analyzer (ARCHITECT ci8200SR). The values of hs-CRP was expressed in (mg/L).

I.2.6. Dissection and removal of organs

After the blood samples collection, the animals were dissected after anaesthesia with chloroform, and the removed samples organs and tissues of the experimental groups were obtained for:

- the determination of oxidative stress parameters;
- histological study.

I.2.7. Determination of oxidative stress parameters

After sacrifice, the livers were quickly collected and frozen at -20˚C until processed.

I.2.7.1. Preparation of the homogenate

0.5 g of liver from each mouse was homogenized using an Ultra-Turrax homogenizer in 2 mL of TBS (50 mM Tris, 150 mM NaCl, pH 7.4), centrifuged for 15 min at 4˚C, and then the supernatant was used for GSH and catalase measurement.

- **Determination of proteins**

The protein concentration in the supernatant was determined colorimetrically by a spectrophotometer according to the Bradford method (Bradford, 1976) using bovine serum albumin as protein standard and coomassie blue as reagent. The principle is based on the formation of a blue complex between coomassie blue and the amine groups ($-NH_2$) of proteins in solution.

The procedure consists to mixing 0.1 mL of the homogenate with 5 mL of Bradford reagent. After 5 min the optical density was measured at 595 nm.

The protein concentration is determined by comparison with a standard of bovine serum (BSA) (1mg/mL) previously realized in the same conditions.

The protein concentration is obtained by the following formula:

$$\text{X mg of proteins} = \frac{\text{y} - 0{,}069}{1{,}293}$$

Y : Optical density.

I.2.7.2. Reduced glutathione (GSH) assay

The reduced glutathione value was estimated spectrophotometrically by using 5,5′-dithiobis-2 nitrobenzoic acid)(DTNB) as a coloring reagent, according to the method (Moron , 1979) with slight modifications. Its principle is based on the measurement of the optical absorbance of 2-nitro-5- mercapturic acid which results from the reduction of (DTNB) by the thiol (-SH) groups of GSH.

Sample of liver homogenate 800 µL was deproteinized with 200 µL of 5-sulfosalicylic acid (0.25%) and centrifuged at 1000 rpm for 5min. 1 mL of buffer tris-EDTA (0.4 M, pH 9.6 containing 20 mM EDTA) and 25 µL of DTNB (0.01M 5,5′- dithiobis-2 nitrobenzoic acid) were mixed with 500 µL of the supernatant and left at room temperature for 5 min. The increase in absorbance due to the formation of TNB was measured at 412 nm. The value of GSH was expressed in (nmol/mg) of protein.

The glutathione concentration was calculated by the following formulae:

$$\text{GSH}\left(\text{nmol}\frac{\text{GSH}}{\text{mg}}\text{of proteins}\right) = \frac{\text{DO} \times 1 \times 5{,}525}{13100 \times 0{,}8 \times 0{,}5 \times \text{mg proteins}}$$

- DO: optical density.

- 1: total volume of the protein decomposition solutions (0.8 mL the homogenate + 0.2 sulfo-salicylique acid).

- 1.525: volume of the total solutions used for the GSH dosage at the supernatant level (0.5 mL supernatant + 1 mL Tris-EDTA + 0.025 mL DTNB).

- 13100: group –SH absorbance coefficient at 412 nm.

-0.8: homogenate volume.

- 0.5: supernatant volume.

I.2.7.3. Catalase assay

Catalase (CAT) activity was determined according to the method of (Aebi, 1974) with slight modification. The principle of the assay was based on the determination of the rate of H_2O_2 decomposition at 240 nm. The reaction medium (mixture) contains homogenate, phosphate buffer (100 Mm, pH 7.5) and H_2O_2 (500 mM) at an incubation temperature of 25°C (Table 3).

The results were expressed as μmol of H_2O_2 per minute per mg of protein. The decrease in optical density is due to H_2O_2 decomposition, which was measured against a blank at 240 nm.

The table below represents the concentrations and quantities of the reagents required for the catalase activity assay.

Table 3. Protocol used for the catalase activity assay.

Reagents	assay	Blank
Phosphate buffer 100 Mm, pH= 7.5	780 µL	800 µL
H_2O_2 500 mM	200 µL	200 µL
Homogenate	20 µL	0 µL

The reading of the absorbance is done after 15 seconds of delay and during 30 seconds of measurement. CAT activity is calculated according to the following equation:

$$\text{CAT } (\mu\text{mol } H_2O_2/\text{min/mg proteins}) = \Delta \text{ Do}/\varepsilon \text{x L x } \gamma \text{ mg of proteins}$$

ε: molar linear extinction coefficient in Mm.cm is the molar extinction coefficient of the absorbing species in solution

L: width of the measuring cell in cm = 1cm

Y : protein content in mg.

I.2.8. Histological study

After dissection, the collected colon were well cleared of any adjacent tissue, washed, and then placed in petri dishes containing physiological water (NaCl 0.9%), cut into fragments and directly they were preserved in tubes filled by formalin diluted to 10%.

Photomicrographs of the results were obtained using a digital photographic microscope (OPTECH MICROSCOPE).

Statistical analysis

All results are given as mean ± standard errors of the mean (S.E.M). The *in vitro* experiments were performed in triplicate.

The data from *in vivo* study was analyzed by SPSS 20.0 statistics software with one-way ANOVA followed by Turkey's post hoc test for multiple comparisons and $P < 0.05$ was considered as statistically significant.

Chapter III

Results & Discussion

Part I: *In vitro* study

I.1. Total phenolic and flavonoid contents of fruits extracts

Colorimetric assays of Folin–Ciocalteu and aluminum chloride were used to determine the total phenolic and flavonoid contents of extracts.

The calculation was performed using the regression equation of the gallic acid calibration curve: $y = 0.0034x + 0.1044$ with $R^2 = 0.9972$ for polyphenols and the quercetin calibration curve: $y = 0.0048x$, $R^2 = 0.997$ for flavonoids.

The results showed that the *R. canina* fruit extract exhibited the highest total phenolic content (222.907 ± 1.03 µg GAE/mg extract) comparatively to *C. sinensis* fruit extract (173.41 ± 3.06 µg GAE/mg extract). Our data shows that the *R. canina* and *C. sinensis* fruits extracts gave the value of (28.682 ± 2.643 µg QE/mg extract) and the value of (17.01 ± 0.96 µg QE/mg) for the total flavonoid content respectively. The results are shown in table 4.

Table 4. The total phenolic and flavonoid contents of fruits extracts.

	TPC (µg GAE/mg extract)	TFC (µg QE/mg extract)
***R. canina* fruit extract**	222.907 ± 1.03	28.682 ± 2.643
***C. sinensis* fruit extract**	173.41 ± 3.06	17.41 ± 0.96

NB. Values are expressed as means ± SEM of three parallel measurements.

The majority of plants used in traditional medicine are rich in polyphenols and flavonoids. Polyphenols and flavonoids have been used as nutraceuticals in human medicine to treat many modern lifestyle diseases. These phytochemicals have great pharmacological potential (Jaime et al., 2013; Garg et al., 2019).

The quantitative analysis of the *R. canina* fruit extract showed interesting result about the determination of total phenolic content, this result is in agreement with literature (Koczka et al., 2018; Ousaaid et al., 2020; Kilinc et al., 2020) with few variation in values. However, the extract presents a moderate content of the flavonoid compounds. Previous studies have confirmed the presence of flavonoids in the fruits of *Rosa canina* (Shameh et al., 2019; Medveckienė et al., 2021; Bozhuyuk et al., 2021).

The variation in those results can be attributed to diverse factors that affect the total phenolic content in fruits like; plant genotype, cultivation area, extraction technique, and differences in fruit ripeness (Demir et al., 2014).

On the other hand, the *C. sinensis* fruit extract also revealed a good content of total phenolic compounds with a moderate amount of total flavonoids. Our results are in agreement with Campone et al. (2020) and Abd Ghafar et al. (2021).

I.2. Antioxidant activity of fruits extracts

The *in vitro* antioxidant activity of the fruits extracts was evaluated by five different spectrophotometric methods: DPPH, ABTS, GOR, CUPRAC and reducing power. The results were given as IC_{50} (µg/mL) and $A_{0.50}$ (µg/mL), corresponding to the concentration indicating an absorbance of 0.50.

The antioxidant activity evaluation of the extracts showed the highest antioxidant activity in ABTS and reducing power assays. According to table 5, the extracts showed good activity in DPPH and in GOR.

CUPRAC is based on the reduction of neocuproin-copper complex and *R. canina* fruit extract showed high copper reducing capacity but the inhibitory capacity of *C. sinensis* fruit extract was moderate.

The scavenging of the ABTS radical was revealed to be stronger than that of the DPPH radical in both extracts. However, these results are consistent with the phenolic content of each fruit mentioned in the previous table

Table 5. Antioxidant activity of fruits extracts.

	DPPH assay	ABTS assay IC_{50} (µg/mL)	GOR assay	CUPRAC assay $A_{0.5}$ (µg/mL)	Reducing power assay
***R. canina* fruit extract**	121.94 ± 1.85	27.07 ± 0.28	107.51 ± 1.70	43.02 ± 0.50	90.79 ± 2.43
***C. sinensis* fruit extract**	153.48 ± 0.98	76.27 ± 0.07	114.86 ± 1.64	161.81 ± 1.50	80.16 ± 0.89
BHA	6.14 ± 0.41	5.35 ± 0.71	5.38 ± 0.06	5.35 ± 0.71	NT
BHT	12.99 ± 0.41	1.29 ± 0.30	5.35 ± 0.71	8.97 ± 3.94	NT
α-Tocopherol	12.99 ± 0.41	NT	NT	NT	34.93 ± 2.38
AA	NT	NT	NT	NT	6.77 ± 1.15

NB. Values are expressed as means ±S.E.M of three parallel measurements. BHA: butylated hydroxyanisole; BHT: butylated hydroxytoluene; AA: ascorbic acid; NT: not tested.

Antioxidant capacity tests can be broadly classified as tests based on electron transfer (ET) and hydrogen atom transfer (HAT). ET based tests measure an antioxidant's ability to reduce an oxidant, which changes color when reduced (Apak et al., 2007).

The DPPH assay measures the antioxidant activity of compounds capable of transferring hydrogen atoms, while the ABTS assay is focused on the inhibition of ABTS formation by one-electron oxidants (Sridhar and Charles, 2018). The findings indicated that the ethanolic extract of *C. sinensis* fruit presented the greatest activity in the ABTS assay and good activity against the DPPH assay. These results are in line with the study of (Campone et al., 2020).

The *R. canina* fruit extract has showed also an interesting free radical scavenging and reducing potential. The scavenging properties of the antioxidant compounds are often associated with their ability to form stable radicals, the best activity for the free radical scavenging potential was obtained in the ABTS test. Our results agree with literature (Jemaa et al., 2017; Javid et al., 2021) that have found difference in the scavenging ability of *R. canina* fruits extract between DPPH and ABTS tests.

The study of Tahirović and Bašić (2017) demonstrated that the antioxidant activity by the use of DPPH and ABTS assays was low for ethanol extract and high about 50% for methanol extract. Likewise, the antioxidant activity of *R. canina* fruits in DPPH and ABTS assays was correlated to the amount of phenolic compounds (Choudhary, 2005).

Reduction capacity is defined by the ability of substances, which have a reduction potential, to react with potassium ferricyanide to form potassium ferrocyanide, which then reacts with ferric chloride to form a ferric complex (Singhal et al., 2014). The extract of *C. sinensis* fruit revealed the highest reducing power, which is consistent with the study of Bentahar et al. (2020) that showed that the extract from *C. sinensis* fruit exhibited the same effect.

Similarly, the *R. canina* fruit extract has a good reducing power activity, the previous investigation of (Kılıçgün and Altıner, 2010) has reported that the fruit extract of *R. canina* fruit possesses high antioxidant capacity in the reducing power test and demonstrated that this activity is correlated with the total phenol content in this fruit.

The CUPRAC assay is a method that relies on the reduction of copper (II)-neocuproin complex to copper (I)-neocuproin chelate (Akar and Burnaz, 2019). Our results proved that *C. sinensis* extract exhibited interesting copper-reducing antioxidant power.

The previous results are probably due to the richness of *C. sinensis* fruit extract in total phenolic compounds and the presence of flavonoids that mainly contribute to the antioxidant capacity of the extract. Studies have shown that there is a good correlation between antioxidant activity and polyphenol and flavonoid contents (Canan et al., 2016; Bentahar et al., 2020).

Likewise, results revealed that the *R. canina* fruit extract has a strong copper reducing activity which agrees with the study of (Kılıçgün and Altıner, 2010), this latter reported that the ethanol extract of rosehip fruits evaluated by CUPRAC assay has the highest values.

The antioxidant properties of *R. canina* fruit are mainly attributed to polyphenols due to their chemical structure bearing hydroxyl groups, which allow them to act as hydrogen donors, reducing agents, activators of antioxidant enzymes, and oxidase inhibitors. Various reports have confirmed that rosehip extracts contain active compounds, well known for their excellent antioxidant properties (Shahidi et al., 2015; Kerasioti et al., 2019; Cardoso-Avila et al., 2021).

I.3.Chemical composition of *Rosa canina* fruit extract by GC-MS analysis

In this study, sixty-six components were identified with their percentage, characterized at 91.77 % of the total *Rosa canina* fruit. GC-MS analysis indicated that the main compositions were n- hexadecanoic acid (palmitic acid)(15.41%), oleic acid (10.69%), and stearic acid (4.64%). The chemical composition found in the *Rosa canina* fruits extract is given in table 6.

Table 6. Chemical composition of *Rosa canina* fruit extract.

pic	Compounds[a]	RT (min)	MF	MW	%
1	Furfural	5.488	$C_5H_4O_2$	96,0846	0.080
2	Ethene, 1,2-bis(4-bromophenyl)-1,2-bis(trimethylsilyl)-	18.880	$C_{14}H_{10}Br_2$	338.04	0.242
3	1,6-Anhydro-.alpha.-d-galactofuranose	20.400	$C_6H_{10}O_5$	162.14	0.601
4	Cyclononasiloxane, octadecamethyl-	20.629	$C_{18}H_{54}O_9Si_9$	667.4	0.199
5	Heneicosane (CAS)	21.480	$C_{21}H_{44}$	296.6	0.396
6	trans-1,3-Diethylcyclopentane	21.603	C_9H_{18}	126.24	0.368
7	Hexadecanoic acid, methyl ester	21.803	$C_{17}H_{34}O_2$	270.4507	0.391
8	cyclodecasiloxane, eicosamethyl-	22.184	$C_{20}H_{60}O_{10}Si_{10}$	741.5	0.185
9	n-Hexadecanoic acid (Palmitic Acid)	22.665	$C_{16}H_{32}O_2$	256.4241	15.417
10	Decanoic acid	23.051	$C_{10}H_{20}O_2$	172.26	0.277
11	9-Octadecenoic acid (Z)- (CAS)	23.143	$C_{19}H_{36}O_2$	296.48	0.455
12	Docosanoic acid (CAS)	23.263	$C_{22}H_{44}O_2$	340.6	0.210
13	Undecanoic acid (CAS)	23.333	$C_{11}H_{22}O_2$	186.29	0.437
14	9-octadecenoic acid (z)- methyl ester	23.516	$C_{19}H_{36}O_2$	296.5	0.289
15	9-Desoxo-9-xi-hydroxy-3,7,8,9,12-pentaacetate ingol	23.596	$C_{28}H_{40}O_{10}$	536.6	0.348
16	2H-Pyran, 2-(7-dodecynyloxy)tetrahydro-	23.761	$C_{17}H_{30}O_2$	266.4	0.509
17	Z-11-Hexadecenoic acid	24.101	$C_{16}H_{30}O_2$	254.41	0.187
18	Oleic Acid	24.340	$C_{18}H_{34}O_2$	282.5	10.692
19	Stearic acid	24.490	$C_{18}H_{36}O_2$	284.5	4.641
20	Cyclodecasiloxane, eicosamethyl-	24.881	$C_{20}H_{60}O_{10}Si_{10}$	741.5	0.402
21	Cyclohexylpropionic acid	25.806	$C_9H_{16}O_2$	156.22	0.413
22	Cyclodecasiloxane, eicosamethyl-	26.050	$C_{20}H_{60}O_{10}Si_{10}$	741.5	0.206
23	4-Ethyloctanoic acid	26.135	$C_{10}H_{20}O_2$	172.26	0.212
24	7,11-Hexadecadienal	26.415	$C_{16}H_{28}O$	236.39	0.179
26	Oleic Acid	26.759	$C_{18}H_{34}O_2$	282.5	0.255
27	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	27.150	$C_{13}H_{40}O_5Si_6$	444.96	0.187

28	Octadecane	27.374	$C_{18}H_{38}$	254.5	1.091
29	(Trimethylsilyl)acetylene	27.757	$C_5H_{10}Si$	98.22	0.523
30	2H-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-5-phenyl-1-(trimethylsilyl)-3-[	28.182	$C_{19}H_{21}ClN_2O_2Si$	372.9	0.630
30	Cyclodecasiloxane, eicosamethyl-	28.182	$C_{20}H_{60}O_{10}Si_{10}$	741.5	0.630
31	C[Si](C)(C)OCCS[Si](C)(C)C	28.301	$C_{10}H_{17}ClO_5$	252.691	0.213
32	Cyclohexanepropanoic acid	28.463	$C_9H_{16}O_2$	156.22	0.627
33	Cycloheptane, methyl-	28.760	C_6H_{12}	84.1595	0.647
34	Quinoclamine	28.843	$C_{10}H_6ClNO_2$	207.61	0.374
35	Octadecanoic acid (CAS)	29.007	$CH_3(CH_2)_{16}COOH$	284.5	0.423
36	Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl-	29.149	$C_{16}H_{48}O_7Si_8$	577.2	1.041
37	Trimethylsilyl-di(timethylsiloxy)- silane	29.281	$C_9H_{27}O_2Si_4$	279.65	0.319
38	Adamantane-1-(3,3-dichloropropyn-1 -yl)	29.681	$C_{16}H_{48}O_7Si_8$	577.2	1.839
39	Silicic acid, diethyl bis(trimethylsilyl) ester	29.762	$C_{10}H_{28}O_4Si_3$	296.58	0.541
40	14.alpha.-Cheilanth-12-enic Methyl Ester	29.880	C26H42O2	386.6	0.639
41	1-Monolinoleoylglycerol trimethylsilyl ether	30.000	$C_{27}H_{56}O_4Si_2$	500.9	1.037
42	Cyclobarbital	30.250	$C_{12}H_{16}N_2O_3$	236.27	0.860
43	l-Limonene	30.387	$C_{10}H_{16}$	136.23	1.600
44	Propanamide, N-(3-methoxyphenyl)-2,2-dimethyl-	30.550	$C_{12}H_{17}NO_2$	207.27	1.440
45	2-Thiopheneacetic acid, 2-methyloc t-5-yn-4-yl ester	30.909	$C_{15}H_{20}O_2S$	264.4	3.157
46	Hexanoic acid, 2,7-dimethyloct-7-en-5-yn-4-yl ester	31.119	$C_{16}H_{26}O_2$	250.38	0.710
47	10-Cyclohexylnonadecane	31.229	$C_{25}H_{50}$	350.66	1.757
48	Cheilanth-13(14)-enic Methyl Ester	31.355	C26H42O2	386.6	1.712
49	Ergosta-4,7,22-trien-3-one	31.501	$C_{28}H_{42}O$	394.6	1.705
50	(Z)-1,3-Pentadiene	31.569	C_5H_8	68.1170	1.563
51	trans-3-Ethoxy-b-methyl-b-nitrostyrene	31.839	$C_{11}H_{13}NO_3$	207.23	2.622
52	Cyclononasiloxane, octadecamethyl-	32.050	$C_{18}H_{54}O_9Si_9$	667.4	1.463
53	1,6-Octadiene, (E)-	32.228	C_8H_{14}	110.20	2.144
54	Eicosamethylcyclodecasiloxane	32.406	$C_{20}H_{60}O_{10}Si_{10}$	741.5	2.715
55	N-Butylacetamide	32.649	$C_6H_{13}NO$	115.17	3.418
56	5-Methyl-2-Phenylindole	32.900	$C_{15}H_{13}N$	207.27	0.918
57	1,1,3,3,5,5,7,7,9,9,11,11,13,13-TetradecamethylL-heptasiloxane	33.101	$C_{14}H_{42}O_6Si_7$	503.07	1.574
58	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15 ,15-Hexadecamethym-octasiloxane	33.299	$C_{14}H_{42}O_6Si_7$	503.07	2.159
59	Decamethyltetrasiloxane	33.767	$C_{10}H_{30}O_3Si_4$	310.68	3.271
60	Cyclohexane, 1,1-dimethyl	34.155	C_8H_{16}	112.21	1.198
61	3-Methylhenicosane	34.705	$C_{22}H_{46}$	310.6	2.309

62	4a,7a-Epoxy-5H-cyclopenta[a]cyclop ropa[f]cycloundecen-4(1H)-one, 2,7 ,10,11-tetra	34.990	$C_{28}H_{40}O_{12}$	568.6	1.177
63	6-Amino-5-cyano-4-(3-iodo-phenyl)- 2-methyl-4H-pyran-3-carboxylic acid ethyl este	35.193	$C_{19}H_{22}N_2O_3$	290.34	1.103
64	A-NOR-DINOSTEROL, ME-15-4R 5-Methyl-2-phenylindole	35.725	$C_{15}H_{13}N$	207.27	1.830
65	3-Quinolinecarboxylic acid, 6,8-di fluoro-4-hydroxy-, ethyl ester	37.241	$C_{10}H_5F_2NO_3$	253.2	0.667
66	5-Methyl-2-phenylindole	39.563	$C_{15}H_{13}N$	207.27	0.348
	Identified compounds (%)	total			91,772

[a] Compounds listed in order of their RT; RT: retention time; MF: molecular formula; MW: molecular weight.

GC/MS is a technique for separating a mixture of volatile and semi-volatile compounds in order to analyze them in a qualitative and quantitative way. This technique was used to identify and quantify the compounds present in *Rosa canina* fruit extract.

The GC-MS analysis of *Rosa canina* fruit extract allows the identification of a wide range of compounds. It has already documented that the fruit of *Rosa canina* contains valuable substances such as fatty acids (Kayahan et al., 2022). Many studies have been conducted on the chemical composition of *Rosa canina* fruit and they indicated that fatty acids are the main components in rosehip fruit. Palmitic acid, oleic acid and stearic acid were found as the main components of *Rosa canina* fruit (Kizil et al., 2018; Çömlekcioğlu et al., 2022). In this study, although the main components are the same, but they are proportionally different.

In our study, palmitic acid is the main fatty acid, this agrees with the results of the study of (Murathan et al., 2016). Inversely, Ercisli (2007) reported that linoleic acid was the main fatty acid in *R. canina* fruits. The studies of (Wenzig et al., 2008) and of (Babalau-Fuss et al., 2021) found the major fatty acids in rosehip fruits are linoleic acid, oleic acid and palmitic acid.

Palmitic acid, is a saturated fatty acid. Numerous fatty acids are well-known for their antibacterial and antifungal properties. Fatty acids can modulate immune responses by acting directly on T cells, exerting an anti-inflammatory effect by decreasing the production of inflammatory mediators such as prostaglandin E2, IL-6, IL-1b, TNFa and nitric oxide (Aparna et al., 2012).

I.4. Amperometric detection, electrochemical characterization and antioxidant activity of *Rosa canina* fruit extract

I.4.1. Amperometric detection of acid ascorbic in *Rosa canina* fruit extract

The electrochemical detection of ascorbic acid in *R. canina* fruit extract at 2mg/mL has been reported in the figure 11.

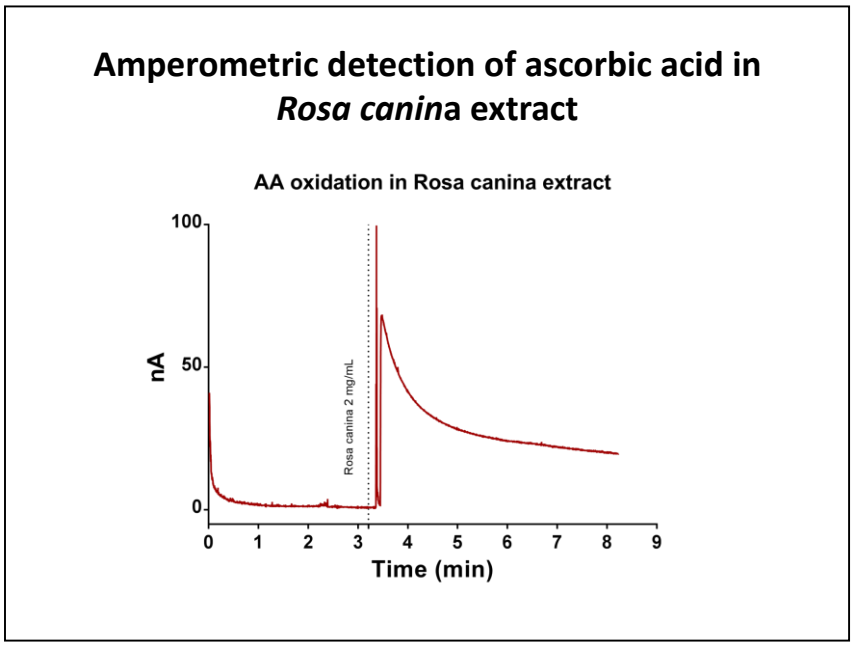

Figure 11. Amperometric response obtained by the addiction of a 70 µL aliquot of *R. canina* fruit extract to the sensor surface.

The amperometric response to the addiction of the extract indicated that *R. canina* fruit contains about 5,3 mg/g of ascorbic acid.

The analytical results are in good agreement with literature values determined by other methods (Taei et al., 2015).

The study by Freeman et al. (2020) determined that the concentration of ascorbic acid in *Rosa canina* was equal to 716.8 mg/100g.

I.4.2 Electrochemical characterization and antioxidant capacity of *Rosa canina* fruit extract

Figure 12 showed the cyclic voltammogram, with a scanned potential range (Eapp) comprised between –0.2 V and +0.8 V. The black arrow indicates the point where the voltammogram of the extract separates from the baseline: at that moment the polyphenols present in the extract begin to oxidize.

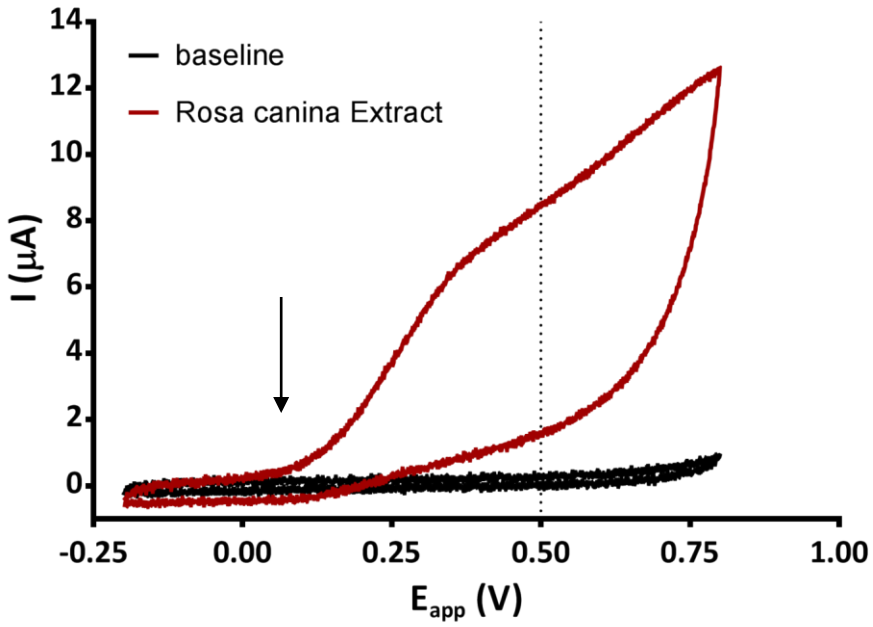

Figure 12. Cyclic voltammogram, with a scanned potential range (Eapp) comprised between –0.2 V and +0.8 V vs. carbon pseudoreference, in the absence (PBS black line) and in the presence of 2 mg/mL of *R. canina* fruit extract (red line).

A quantitative analysis of the CV pattern of the extract was performed by integrating the voltammogram. The area under curve (AUC) was calculated at +0.5 V and expressed in microcoulombs (µC) (Figure 13), as previously reported (Spissu et al., 2023). The redox potential of +0.5 V was used as a threshold to detect the antioxidant capacity of *R. canina* fruit extract, in accordance with previous studies (Piljac-Žegarac et al., 2010; Cotoras et al., 2014). As already reported (Barberis et al., 2015; Barberis et al., 2020; Bouzabata et al., 2022), oxidation potentials higher than +0.5 V refer to polyphenols with low reducing power which, for this reason, were not accounted as antioxidants.

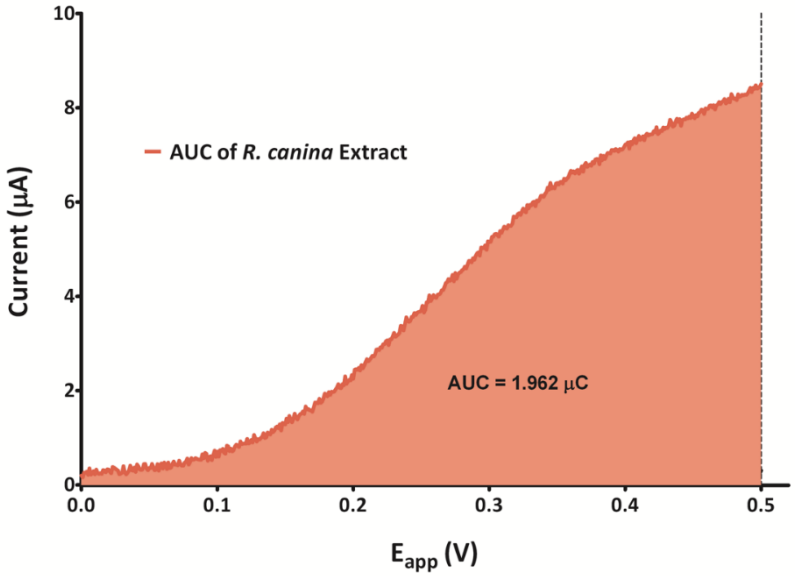

Figure 13. Antioxidant capacity of *Rosa canina* fruit extract calculated as area under curve (AUC) at +0.5 V and expressed in microcoulombs (µC).

The black arrow indicates the point where the voltammogram of the extract separates from the baseline (see figure 12): all the polyphenols which oxidize inside the AUC accounted as antioxidants.

Cyclic voltametry was obtained in the potential range –0.2 V +0.8 V in order to cover all groups of antioxidant compounds but only the AUC values at +0.5V refer to AAox. The voltammograms split from the baseline at around +0.1 V (black arrow), indicating the presence of polyphenols with low redox potential; as the applied potential increases, the AUC of the voltammogram increases, indicating a large polyphenol component that ionizes in that potential range.

Cyclic voltametry has been used to assess antioxidant capacity because the potential at which oxidation begins is an indicator of antioxidant capacity (Pedotti et al., 2016).

In this study, we used voltammetry to assess the antioxidant capacity of *R. canina* fruit extract. According to our results, *R. canina* fruit extract exerts a good antioxidant capacity. This capacity is probably due to the polyphenols which have an oxidation potential lower than 0.5V.

Sun-Waterhouse et al. (2008) found the antioxidant capacity of onions with a threshold at 0.5V, another study by Piljac-Žegarac et al. (2010) raised this threshold to 0.6V in tea infusions.

A study on Cabernet Sauvignon, Carmenere, and Syrah pomace suggests that polyphenols with an oxidation potential below 0.45 V exhibit antioxidant activity (Cotoras et al., 2014).

Kılıçgün and Altıner. (2010) examined the correlations between phenolic compound contents and the mechanisms of antioxidant effect of different concentrations of *R. canina* fruit extract and confirmed that *R. canina* acts as a powerful antioxidant. Several studies have attributed the antioxidant properties of *R. canina* to polyphenols (Czyzowska et al., 2015; Mansouri et al., 2020).

I.5. Antioxidant activity of *Rosa canina* fruit extract on human fibroblasts

I.5.1. Effect of *Rosa canina* fruit extract on the viability of the human fibroblasts

The results indicate that *R. canina* fruit extract did not reduce fibroblast viability even at the highest concentrations. On this basis, the lowest concentrations of extract which had a significantly higher viability than the control, were used to evaluate the ability of the extract to protect normal cells from hydrogen peroxide induced oxidative stress.

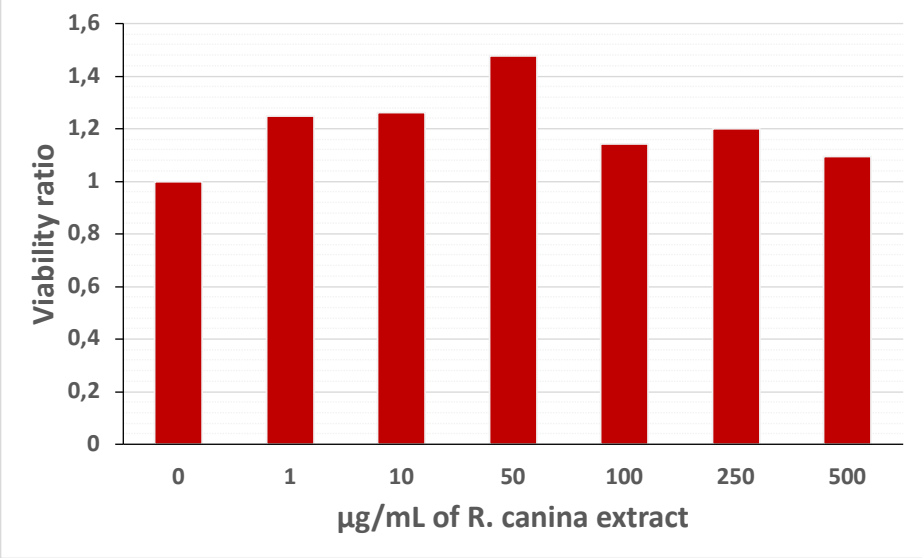

Figure 14. MTT viability assay on fibroblasts treated with 1, 10, 50, 100, 250 and 500 µg/mL of *Rosa canina* fruit extract for 24 hours.

Several studies have investigated the cytotoxic effects of *R. canina* on different cancer cell lines using MTT assay.

Turan and his team (Turan et al., 2018) examined the potential effect of *R. canina* on cell viability, cell cycle, apoptosis and telomerase expression in human colon cancer (Widr), their results demonstrate that *R. canina* may have considerable developmental potential as a novel

natural product-based anticancer agent. Another study evaluated the antiproliferative and antioxidant effects of *Rosa canina* on human colon cancer cell lines (Caco-2) and demonstrated that rosehip extracts have high cytotoxicity after 72 h, both at low and high concentrations and are a potent antioxidant that produces an antiproliferative effect in Caco-2 cells of (Jiménez et al., 2016). Olsson et al. (2004) reported that ethanolic extract of rosehip exhibits cytotoxic effect on human colon (HT-29) and breast (MCF-7) cancer cells.

Fujii et al. (2011) demonstrated that ethanolic extract of *R. canina* has a cytotoxic effect on mouse melanoma cells by inhibiting tyrosinase activity. The study of Jimenez et al. (2016) reported that *R. canina* extracts had antiproliferative effects on human colon cancer cells (Caco-2) by increasing the number of apoptotic cells and arresting the cell cycle in S phase. Cagle et al. (2012) evaluated the effect of rosehip extracts on proliferation and apoptosis of human brain tumor cells, demonstrating that rosehip extracts have an inhibitory effect on cell proliferation but without appoptosis and can serve as an alternative to current chemotherapeutic regimens.

Acar et al. (2019) reported that *Rosa canina* extract has anticancer and anti-metastatic activity on human colon adenocarcinoma cell line HT29. A previous study confirmed the antiproliferative and proapoptotic effects of *Rosa canina* extract on human lung and prostate cancer cells (Kilinc et al., 2020).

On the other hand, no study has been able to examine the toxic effect of *R. canina* fruit extract *in vitro* on normal human cell lines and in particular on fibroblasts. In this study the toxicity of the extract was analyzed and the results reveal that all the concentrations used are not toxic and that no variation in cell viability was observed when the fibroblasts were treated with the extract of fruit of *R. canina*.

Nam et al. (2012) evaluated the effect of rosehip extract on the survival of rat cartilage cells by MTT assay and demonstrated that rosehip extract improved the survival of cartilage cell cultures, which is in agreement with our results regarding the non-toxic effect of *R. canina* on normal cells.

Our results diverge with the study of Farhadi et al. (2015), which reports that high concentrations of *Rosa canina* fruit extracts are the most toxic on the cell line U937.

I.5.2. Protective effect of *Rosa canina* fruit extract on the viability of human fibroblasts stressed with H_2O_2

The treatment with 500 µM H_2O_2 alone reduced fibroblast viability by 23%. The combined treatments with *R. canina* extract reversed this trend according to the concentration of extract used. The preventive treatment with 1 mg/mL of extract protected the fibroblasts from the stress induced by H_2O_2 restoring the vitality above the control values; the treatment with 10 mg/mL of extract also protected cells from oxidative stress, but to a lesser extent than the previous combination.

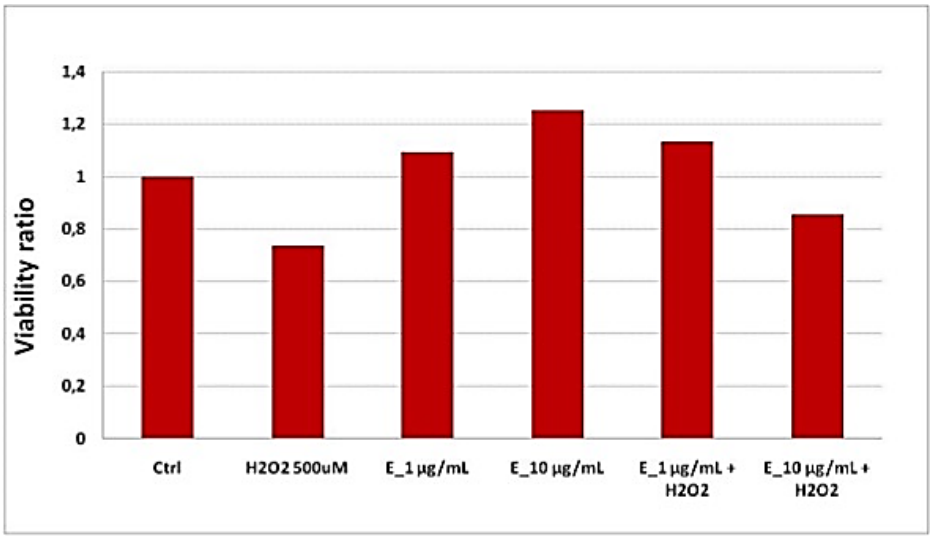

Figure 15. Protective effect of *Rosa canina* fruit extract from hydrogen peroxide-induced oxidative stress on fibroblasts. MTT viability assay on cells treated with 1 and 10 µg/mL of extract for 24 hours, and with 500 µM H_2O_2 for an additional 24 hours.

Numerous investigations have well established that oxidative stress is the major cause of various pathologies, including dysfunction of the inflammatory response, which promotes the development and progression of several chronic diseases (Durand et al., 2013; Debbeche, 2016; Bensakhria, 2018).

Although ROS such as, hydrogen peroxide is generated as a natural by product of normal oxygen metabolism, it can create oxidative damage via interaction with biomolecules (Saeidnia and Abdollahi, 2013).

Hydrogen peroxide is generally used as a generator of oxygen radicals, which can enter the interior of cells and cause cellular damage (Liang et al., 2019).

Fibroblasts are targeted by ROS, especially H_2O_2, which is able to convert them into active cancer-associated fibroblasts (CAFs). CAFs are essential for the increased levels of ROS

observed in cancer can also promote cancer growth and invasiveness (Chan et al., 2018; Perillo et al., 2020).

The antioxidant capacity of compounds can be well assessed using an intracellular oxidized cell model by testing their ability to scavenge intracellular ROS. H_2O_2 measurement is a very good indicator of oxidative stress in living cells (Zhou et al., 2019).

The protective effect of *Rosa canina* fruit has been proven by a number of studies (Nowrouzi et al., 2019; Sajadimajd et al., 2020).

Ashtiyani et al. (2013) confirmed the protective effects of *R. canina* against renal function disorders, Sadeghi et al. (2016) and Taghizadeh et al. (2018), reported its hepatoprotective effect and Nasrolahi et al. (2020) showed its cardioprotective effect.

The present study demonstrated the ability of 1 µg/mL of extract (minimal non-toxic dose to fibroblasts), to interact with hydrogen peroxide, a stress inducer widely used in bioassays. Our results reveal an increase in fibroblast viability compared to the control and that *Rosa canina* fruit extract has an effect against hydrogen peroxide-induced oxidative stress on normal human fibroblasts and thus remains a potent antioxidant with a cytoprotective effect.

Our study confirmed the results of Nam et al. (2012) revealing that rosehip extract has inhibitory effects on cell death by oxidative stress mediated by H_2O_2.

A recent study, evaluated the antioxidant capacity and protective property of *Rosa canina* fruit against hydrogen peroxide induced toxicity *in vivo*, concluding that *R. canina* fruit are effective against the detrimental effect of oxidative stress (Ousaid et al., 2022).

Our results are in agreement with (Jiménez et al., 2016) who showed a reduction in the production of oxidative species in Caco-2 cells treated with (20 mM) H_2O_2 and rosehip extract compared to the positive control (with 20 mM H_2O_2).

I.6. Anti-inflammatory activity of fruits extracts

The present results noted that the *R. canina* and *C. sinensis* fruits extracts, compared to the reference molecule (diclofenac), have concentration-dependent inhibitory activity against heat-induced protein denaturation (Figure 16). *R. canina* and *C. sinensis* fruits possessed a significant anti-inflammatory effect with a percentage of maximum inhibition at 79.79%, 59.58% respectively retained at the concentration of 2000 µg/mL. *R. canina* fruits extract was very close to the diclofenac standard (with a percentage inhibition of 84.23%).

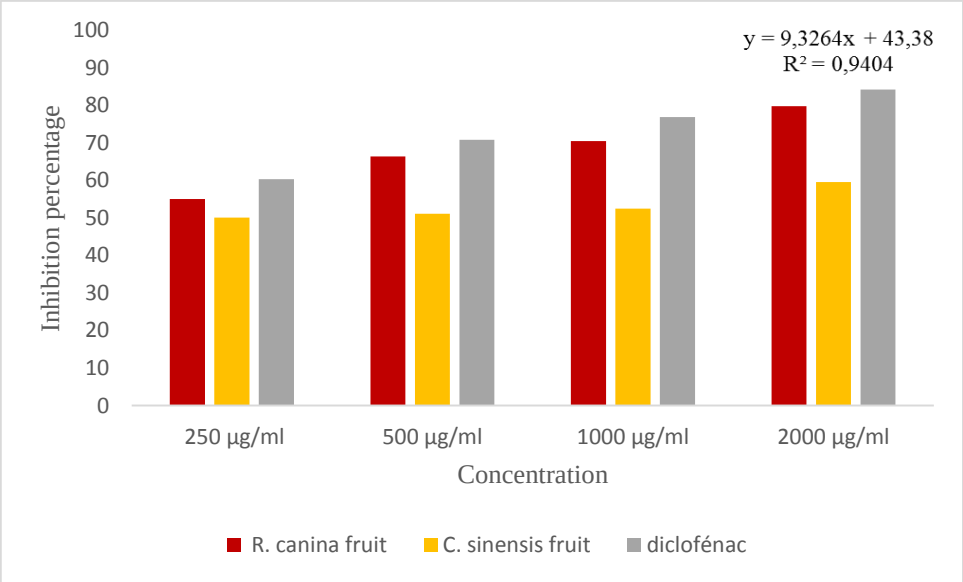

Figure 16. Inhibitory effect against BSA denaturation of fruits extracts.

Protein denaturation can be caused by the application of an external stress such as heat. It is a process in which proteins lose their tertiary and secondary structures as well as their biological functions, this can lead to the generation of autoantigens (Sivaraj et al., 2017; Belkhodja et al., 2022). Protein denaturation is a well-documented cause of inflammation (Medina, 2011).

In this study, the results show that the two fruits have an anti-inflammatory effect resulting in the inhibition of protein denaturation induced by heat (72°C). The effect was comparable to that of diclofenac, a classic anti-inflammatory, used as a reference. A potent anti-inflammatory effect with a maximum percentage inhibition was recorded at the concentration of 2000 µg/mL for the *R. canina* fruit extract. This is in perfect agreement with the results of Fetni et al. (2020) who showed the inhibitory power of *Rosa canina*. They noted that the methanolic extract at the concentration of 150 µg/mL was 93.41% inhibitory of protein denaturation.

On the other hand, the *C. sinensis* fruit extract showed an inhibitory capacity against the denaturation of bovine albumin proteins. These results are in agreement with Denaro et al. (2021) who confirmed by the anti-inflammatory activity of *Citrus* fruit flavonoids, assessed by the inhibition assay the heat-induced denaturation of BSA *in vitro*. They showed a significant inhibition of protein denaturation. Highlighting the role of *Citrus* fruits in combating oxidative stress and inflammatory response in intestinal diseases.

These results indicate that the fruits of the plants used are able to inhibit protein denaturation and may be beneficial during inflammatory diseases, in particular those associated with protein denaturation, such as IBD.

Part II: *In vivo* study

II.1. Acute toxicity test of *Rosa canina* fruit

In the acute toxicity study of *R. canina* fruit, mice that have received a dose of (2000 mg/kg) of *R. canina* fruit appeared normal, neither abnormal behavior nor mortality was detected during the experimental period. Thus, the minimum lethal dose of fruit tested in our study is greater than 2000 mg/kg.

Although the fruits of medicinal plants have beneficial biological activities for human health, the potential toxicity of these substances must be studied to optimize their good effect.

In pharmacology, it is very important to carry out toxicity tests for new drugs. The test was performed to assess the safety or hazards of several substances, including consumer products. The OECD 425 Up-and-Down procedure (UDP) is one of the LD_{50} acute toxicity test methods. UDP allows for a significant reduction in animal numbers, which is necessary to determine LD50 values (Atik et al., 2019).

As the dose of 2000 mg/kg induced no clinical signs of toxicity, the estimated LD_{50} of *R. canina* fruit is greater than 2000 mg/kg, demonstrating that *Rosa canina* fruit is safe for oral administration in mice.

This is in agreement with those of Wanes et al. (2020) who reported that the use of *Rosa canina* fruit at 2000 mg/kg as a limit dose is lacked any toxic effect in mice.

II.2.The anti-inflammatory study of fruits and vitamin C

II.2.1. Effect of fruits and vitamin C on hs-CRP values

C-reactive protein is a plasma protein that is one of the most widely expressed proteins in acute phase inflammation. In contrast, a continuous increase in CRP levels is a marker of chronic inflammation (Hosseinniya et al., 2023; Long et al., 2023).

- **Effect of *Citrus sinensis* fruit**

The current data show that there isn't any significant difference in the means for the plasma hs-CRP concentration between groups ($P > 0.05$). The figure 17, showed that plasma hs-CRP concentration in group (M) increased but not significantly when compared to the control group and the groups (OM) and (O) respectively.

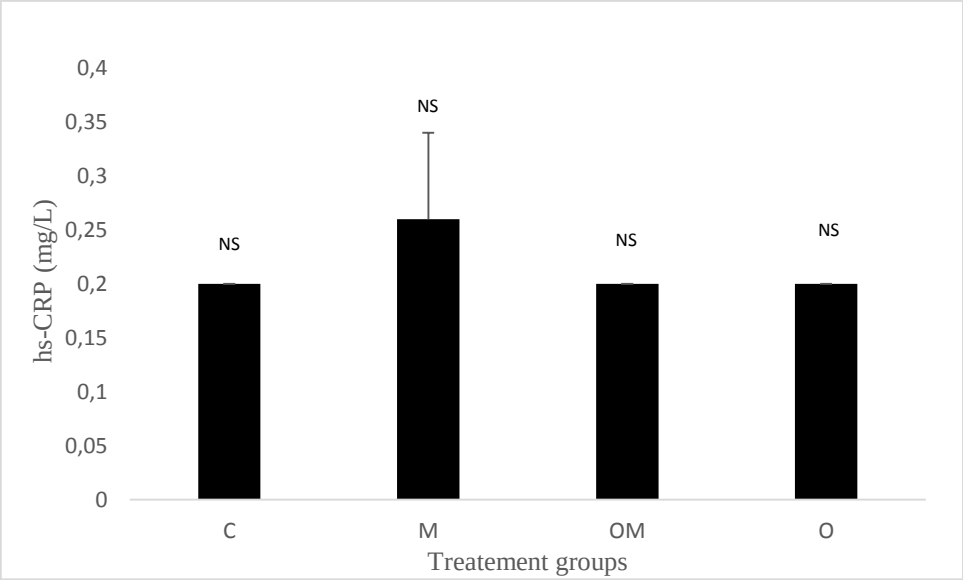

Figure 17. Effect of *Citrus sinensis* fruit on hs-CRP values.

Results are shown as mean ± S.E.M (n = 4) and ^{NS}: not significant (P > 0.05). (C): control group; (M): group administered with L-methionine (200 mg/kg); (OM): group administered with L- methionine (200 mg/kg) and treated with *C. sinensis* fruit (200 mg/kg); (O): group treated with *C. sinensis* fruit (200 mg/kg).

In our data we obtained that the treatment with *Citrus sinensis* fruits could reduce inflammation, this result is confirmed by (Khan et al., 2016) which reports that *C. sinensis*, promoted a reduced level of plasma CRP in a model of acute colitis and may be effective for the treatment of inflammatory bowel diseases. The effect of *Citrus* fruits on the inflammatory process may be associated with their antioxidant and anti-inflammatory properties. These anti-inflammatory effects could be due to the presence of flavonoid contents since flavonoids have strong antioxidant and radical scavenging activities.

Our results are in agreement with the work of (Abou Baker et al., 2022) who experimentally evaluated the hepatoprotective/gastroprotective effects of *Citrus* peel extracts in rat ulcer models. They obtained that the concentration of hs-CRP was significantly reduced in rats treated with *Citrus sinensis*. Thus confirming that *C. sinensis* has the best hepatoprotective, antioxidant, anti-inflammatory and gastroprotective effects.

- **Effect of *Rosa canina* fruit**

Our data showed that there is a difference in the values of hs-CRP between different tested groups but they were not significant (P > 0.05). The values of hs-CRP in the (M) group was increased comparatively to the rest groups (Figure 18).

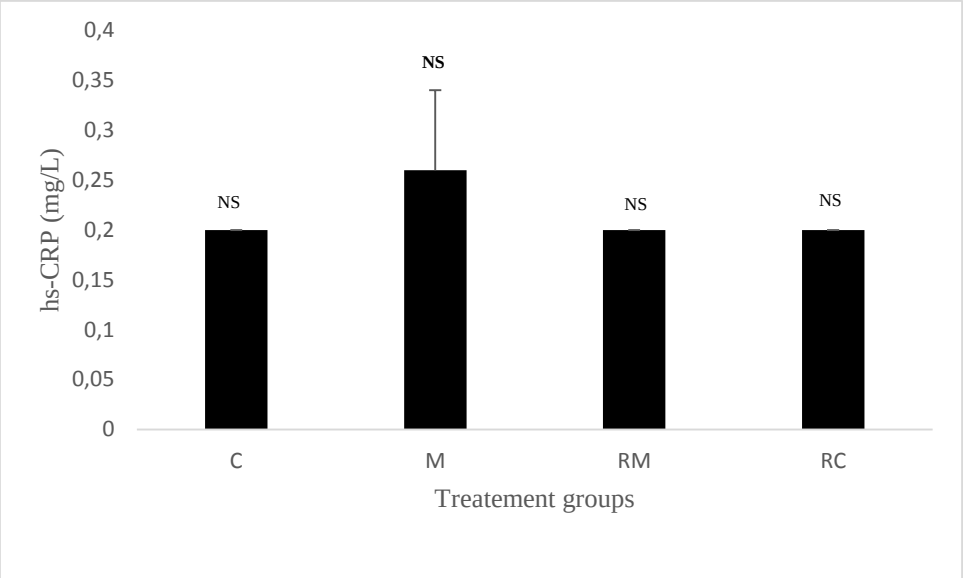

Figure 18. Effect of *Rosa canina* fruit on hs-CRP values.

Results are shown as mean ± S.E.M (n = 4) and ^{NS}: not significant (P > 0.05). (C): control group; (M): group administered with L-methionine (200 mg/kg); (RM): group administered with L-methionine (200 mg/kg) and treated with *R. canina* fruit (200 mg/kg); (R): group treated with *R. canina* fruit (200 mg/kg).

C-reactive protein is a recognized biomarker of inflammatory diseases.It is synthesized by hepatocytes in response to pro-inflammatory cytokines, in particular, interleukin-6 (Shrivastava et al., 2015; Sproston and Ashworth, 2018).

In a study conducted by Langhorst et al. (2008), it was found that IBD patients with active inflammation have high CRP levels.

İn the present survey the *R. canina* fruit decreased the level of hs-CRP in the (RM) group compared to the (M) group. It has been reported that rosehip reduces CRP levels in patients with rheumatoid arthritis (Kirkeskov et al., 2011).

- **Effect of vitamin C**

The present data show that there isn't any significant difference in the means for the plasma hs-CRP concentration between groups (P > 0.05). As shown in the figure 19, plasma hs-CRP concentration in group (M) increased but not significantly when compared to the control group and the groups (VM) and (V) respectively.

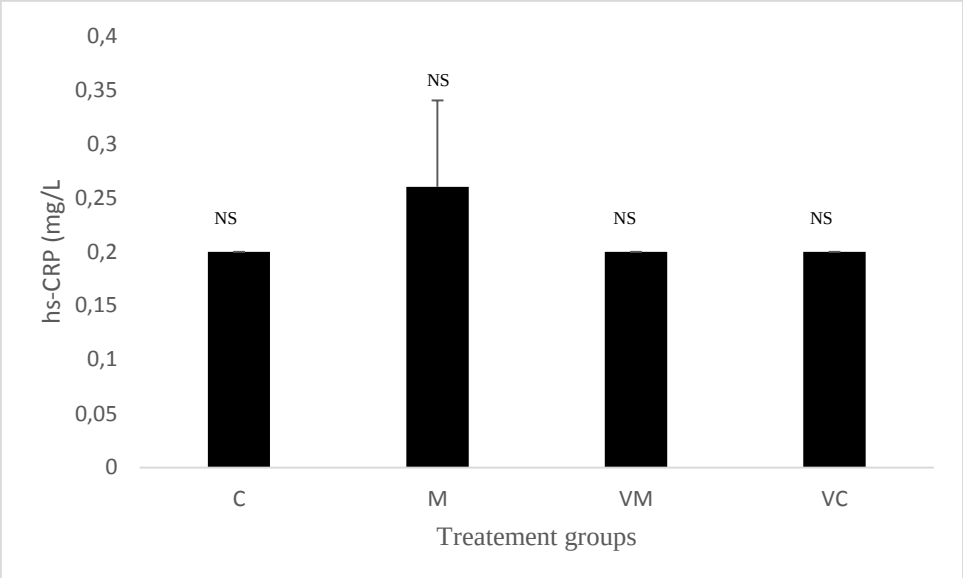

Figure 19. Effect of vitamin C on hs-CRP values.

Results are shown as mean ± S.E.M (n = 4) and ^{NS}: not significant ($P > 0.05$). (C): control group; (M): group administered with L-methionine (200 mg/kg); (VM): group was administered L-methionine (200 mg/kg) and treated with (500 mg/kg) of vitamine C; (V): group received (500 mg/kg) of vitamin C.

In our results we obtained that the treatment with the vitamin C may decrease CRP levels , this results is confirmed by (Block et al., 2009) who reported that vitamin C treatment (at dose 1000 mg/day) reduces elevated C-reactive protein .

In the current study, vitamin C decreased the level of hs-CRP in the (VM) group compared with the (M) group. Nevertheless, the (VM) group revealed a lower plasma level of hs-CRP than the M group. This decrease is due to the fact that vitamin C has anti-inflammatory properties (Yussif et al., 2016; Fesahat et al., 2022).

II.2.2. Effect of fruits and vitamin C on the homocysteine values

Patients with IBD have increased oxidative damage caused by elevated ROS production that cannot be managed by antioxidant defenses. Glutathione-dependent enzymes such as glutathione peroxidase, glutathione reductase, and glutathione S-transferase are antioxidant enzymes responsible for controlling ROS. Cysteine is a major contributor to Glutathione synthesis that can be obtained from the transsulfuration pathway from homocysteine to cysteine (Olaso-González et al., 2021).

High level o homocysteine is considered as a marker of intestinal inflammation. Previous work links increased levels of homocysteine in patients with IBD (Jiang et al., 2O12; Lurz et al., 2020).

To study the pathogenesis of HHcy-related metabolic disorders, methionine feeding has often been used to increase serum and tissue Hcy levels (Yalçınkaya et al., 2009).

The results of the current study will show that administration of L-methionine (200 mg/kg) to mice for 21 days clearly caused a significant increase in plasma homocysteine levels.

However, treatment with the fruits are reduced significantly the levels of inflammatory markers.

- **Effect of *Citrus sinensis* fruit**

The present study showed that there was a significant difference in homocysteine concentration between the groups (C, M, OM and O) at p = 0.05. In further, we found that the level of homocysteine decreased significantly in the (OM) group when compared to the (M) group administered with L-methionine, and the concentration of homocysteine in the (M) group is increased significantly when compared to the control group (p <0.05) (Figure 20).

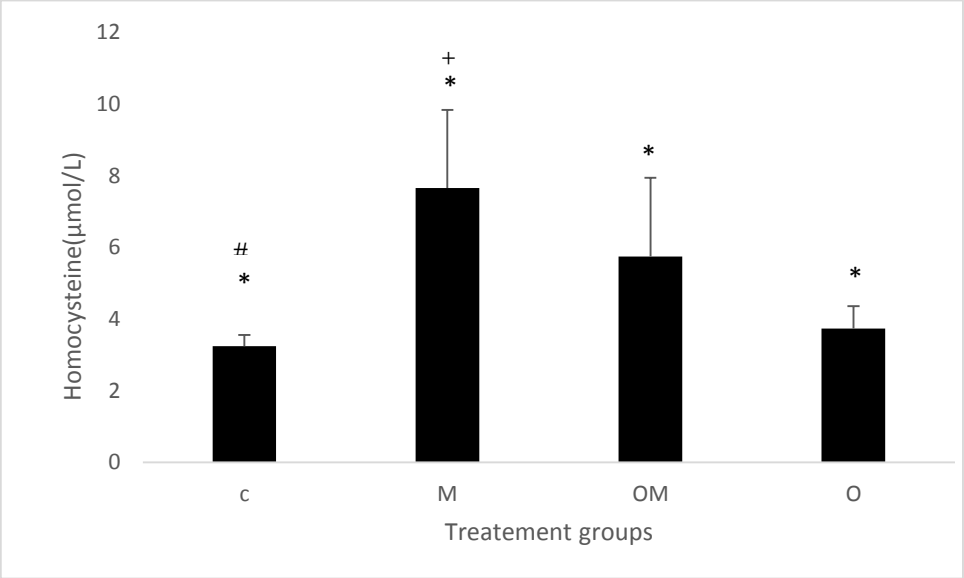

Figure 20. Effect of *Citrus sinensis* fruit on homocysteine values.

Results are shown as mean ± S.E.M (n = 4) and significant difference between groups is shown as (*p = 0.05). ($^{\#}$p = 0.05) compared to (M) group, ($^{+}$p = 0.05) compared to (OM). (C): control group; (M): group administered with L-methionine (200 mg/kg); (OM): group administered with L-methionine (200 mg/kg) and treated with *C. sinensis* fruit (200 mg/kg); (O): group treated with *C. sinensis* fruit (200 mg/kg).

In our investigation, we proved that treatment with *C. sinensis* fruit significantly reduced plasma homocysteine levels in animals receiving a high dose of L-methionine.

The *Citrus* genus has been shown to reduce inflammatory response via inhibition of NF-κB (Impellizzeri et al., 2015). Studies have also confirmed that *Citrus* fruits have anti-inflammatory potential (Leguizamón et al., 2019; Denaro et al., 2021).

Citrus fruits are a good source of vitamin C, folate, vitamins B6 and, flavonoids (Rauf et al., 2014; Rao et al., 2021). Through this, their consumption has beneficial effects on human health due to the antioxidant and anti-inflammatory properties (Ma et al., 2020; Rao et al., 2021) and their gross use reduces the risk of gastric and colorectal diseases (Roussos et al., 2011; Rauf et al., 2014).

Given the richness of *Citrus* fruits in vitamin C, folate, vitamin B6 and flavonoids (Rauf et al., 2014; Rao et al., 2021). Their use has beneficial effects on human health due to their antioxidant and anti-inflammatory properties (Ma et al., 2020; Rao et al., 2021 and their consumption reduces the risk of gastric and colorectal diseases (Roussos et al., 2011; Rauf et al., 2014).

A study by Gholap et al. (2012) who reports that the combination of *Moringa oleifera* root and *Citrus sinensis* fruit peel extract is effective in mice with acetic acid (AA)-induced UC by decreasing malondialdehyde (MDA) content and myeloperoxidase (MPO) activity. According to the study by (Fusco et al., 2017) orange juice decreased oxidative stress and inflammatory response in a mouse model of IBD.

After testing the effect of *Citrus sinensis* L., *Citrus paradisi* L. and their combinations on rats with experimental trinitrobenzene sulfonic acid (TNBS)-induced colitis, Khan and colleagues found that these fruit juices exerted antioxidant and anti-inflammatory activities (Khan et al., 2016).

It has been shown that the use of *Citrus aurantium* L. and its flavonoids exhibited anti-inflammatory activity, reduced weight loss and diarrhea, and suppressed isolated jejunal contraction in TNBS-induced IBD rats (He et al., 2018).

- **Effect of *Rosa canina* fruit**

In our results, we have obtained an interesting difference in the values of homocysteine between groups that was very large and significant ($p = 0.001$). The Tukey test showed that the value of homocysteine was increased very highly ($p = 0.002$) in the (M) group compared to the (C) group, and (RM) group as shown in figure 21.

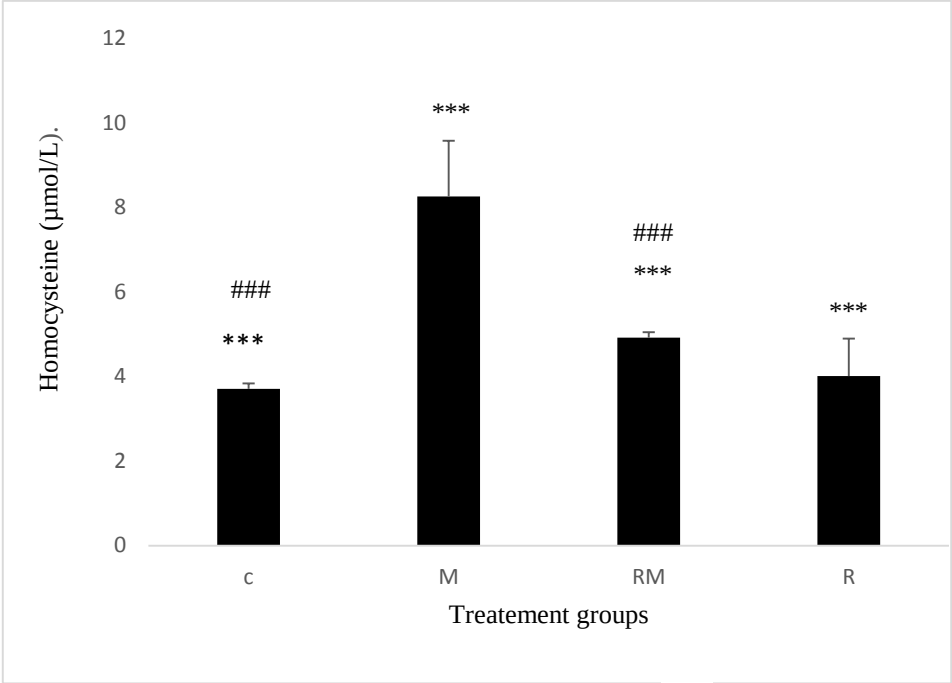

Figure 21. Effect of *Rosa canina* fruit on homocysteine values.

Results are shown as mean ± S.E.M (n = 4) and significant difference between groups is shown as (***p = 0.001). ($^{###}$p = 0.002) compared to (RM) group. (C): control group; (M): group administered with L-methionine (200 mg/kg); (RM): group administered with L-methionine (200 mg/kg) and treated with *R. canina* fruit (200 mg/kg); (R): group treated with *R. canina* fruit (200 mg/kg).

After 21 days of treatment, we detected an inflammation due to a high level of homocysteine in the (M) group and a decrease level in the (RM) group treated with *R. canina* fruit. These results are in accordance with (Aklil et al., 2017) that have noticed that plasma homocysteine achieved higher levels in the group of mice administered with L-methionine (500 mg/kg), otherwise, the levels of homocysteine were decreased in the mice receiving L-methionine (500 mg/kg) and treated by *Argania spinosa* (150 mg/kg).

These results are in line with the results obtained by Zerizer (2006), showing an increase in homocysteine levels in rats administered for 21 days with 200 mg/kg/d of L-methionine.

Our experiment approved the correlation between the elevated values of the hs-CRP and the homocysteine high level in the group administered with methionine.

- **Effect of vitamin C**

As shown in figure 22, there was a highly significant difference in homocysteine value between groups (P = 0.000) over the 21 days of the experiment. The homocysteine value increased very strongly (p = 0.000) in the (M) group compared to the (C) group and the (VC)

group. A highly significant decrease in homocysteine value was also observed in the (VM) group (P = 0.000) compared with the (M) group.

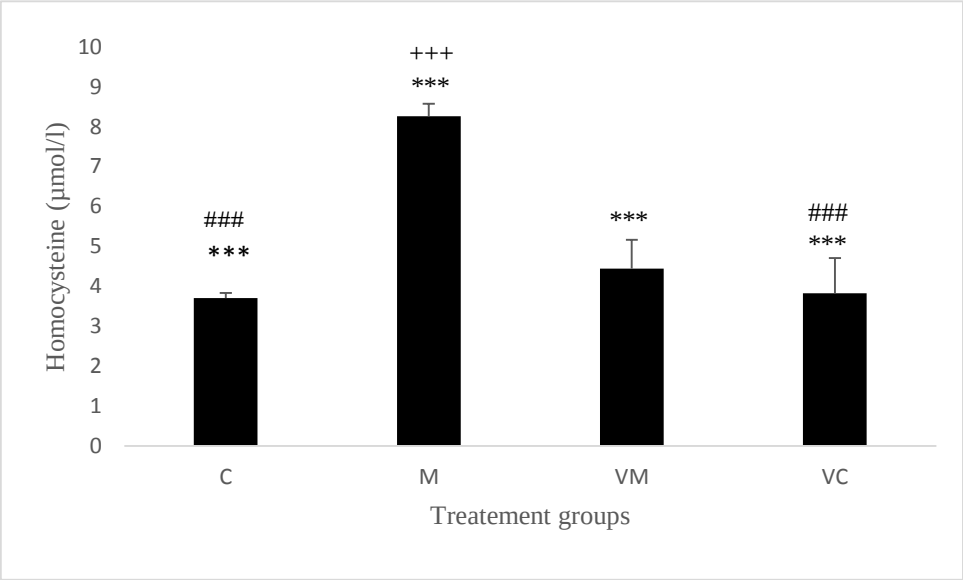

Figure 22. Effect of vitamin C on homocysteine values.
Results are shown as mean ± S.E.M (n = 4) and significant difference between groups is shown as (***p = 0.000). ($^{###}$p = 0.000) compared to (M) group, ($^{+++}$p = 0.000) compared to (VM). (C): control group; (M): group administered with L-methionine (200 mg/kg); (VM): group was administered L-methionine (200mg/kg) and treated with (500 mg/kg) of vitamine C; (V): group received (500 mg/kg) of vitamin C.

Previous work links increased levels of homocysteine to IBD, indicating a major role for vitamin B deficiency in intestinal damage and inflammation (Lurz et al., 2020).

The results of our experiment demonstrate that administration of vitamin C at the dose of 500 mg/kg has decreased the level of homocysteine in the group (VM). Our result is in accordance with the study of (Breilmann et al., 2010), which reported that vitamin C intake and supplementation should be considered in attempts to reduce plasma homocysteine levels.

Also the study of (Alul et al., 2003), demonstrated that vitamin C protects low-density lipoprotein from homocysteine-mediated oxidation.

Previous study examined the effects of high-dose folic acid and vitamin C supplementation on homocysteine levels and they confirmed that folic acid and vitamin C supplementation at a dose of 500 mg/day reduced homocysteine levels in Italian blood donor subjects (Cafolla et al., 2002).

It has been shown that most patients with IBD diagnosed with vitamin C deficiency can be treated with an oral supplement (500 mg 2/day for 4 weeks) which may lead to improvement of symptoms and prevent side effects to long term (Dunleavy et al., 2021).

Given the results obtained, we can confirm a real relationship between hyperhomocysteinemia and inflammation. We suggest that ascorbic acid, flanovoids and phenolic contents prevent the oxidation process thereby protecting the intestine from damage caused by ROS and vitamin B supplementation provided by studied fruits corrected the deficiency of B vitamins resulting in lower levels of homocysteine.

II.3. Evaluation of the *in vivo* antioxidant activity of fruits and vitamin C

The following results indicate that the fruit and vitamin C treatment used significantly decreased liver GSH and catalase levels.

II.3.1. Effect of fruits and vitamin C on GSH values

Reduced glutathione is an intracellular tripeptide with low molecular weight, hydrophilic properties, and strong antioxidant effects (Locigno and Castronovo, 2001; Atakisi et al., 2022).

- **Effect of *Citrus sinensis* fruit**

According to our results, there is a highly significant difference in GSH values between groups at p=0.001 (Figure 23). The Tukey test showed that the GSH values was decreased highly and significantly in group (M) when it is compared to the groups (C) and (OM) ($p < 0.01$).

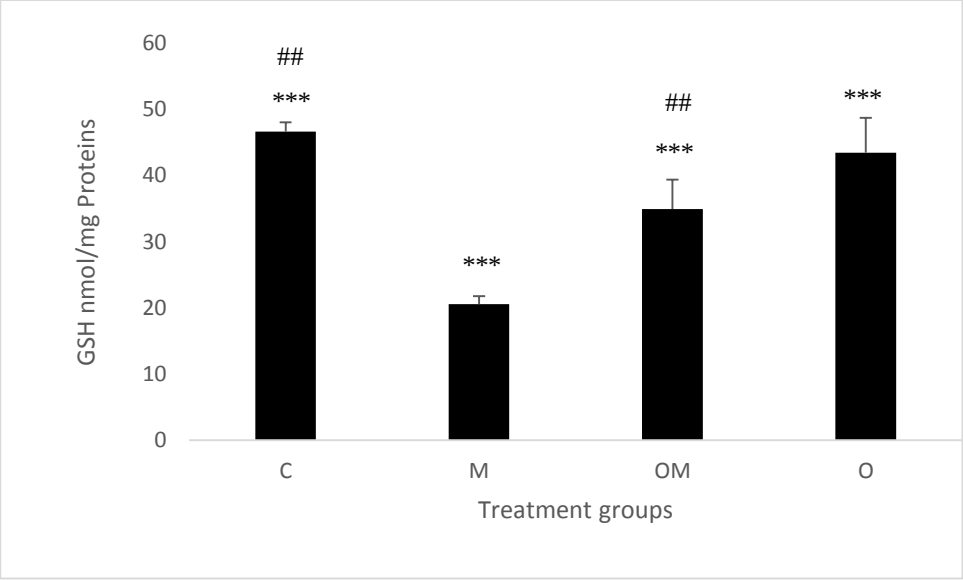

Figure 23. Effect of *Citrus sinensis* fruit on GSH values.

Results are shown as mean ± S.E.M (n = 4) and significant difference between groups is shown as (***p = 0.001). ($^{\#\#}$p < 0.01) compared to (M) group. (C): control group; (M): group administered with L-methionine (200 mg/kg); (OM): group administered with L- methionine (200 mg/kg) and treated with *C. sinensis* fruit (200 mg/kg); (O): group treated with *C. sinensis* fruit (200 mg/kg).

This study shows that treatment with *C. sinensis* fruit resulted in a significant increase in hepatic GSH levels, however, it is suggested that C. sinensis fruit increased glutathione reductase enzyme activity and GSH levels and improved ROS scavenging capacity by inhibiting ROS overexpression and oxidative damage.

After studying orange peel (*Citrus sinensis*) extract, Abdelghffar et al. (2021) found an increase in GSH levels in tissue homogenates and confirmed its protective efficacy against chemotherapy-induced toxicity in male rats.

- **Effect of *Rosa canina* fruit**

The data showed that there is a highly significant difference in the means for the reduced glutathione values between groups (P = 0.001) and the GSH value was decreased highly and significantly in group (M) when it is compared to the other groups (Figure 24). Thus, the GSH value was increased very highly and significantly in group (RM) when it is compared to group (M) (P = 0.001).

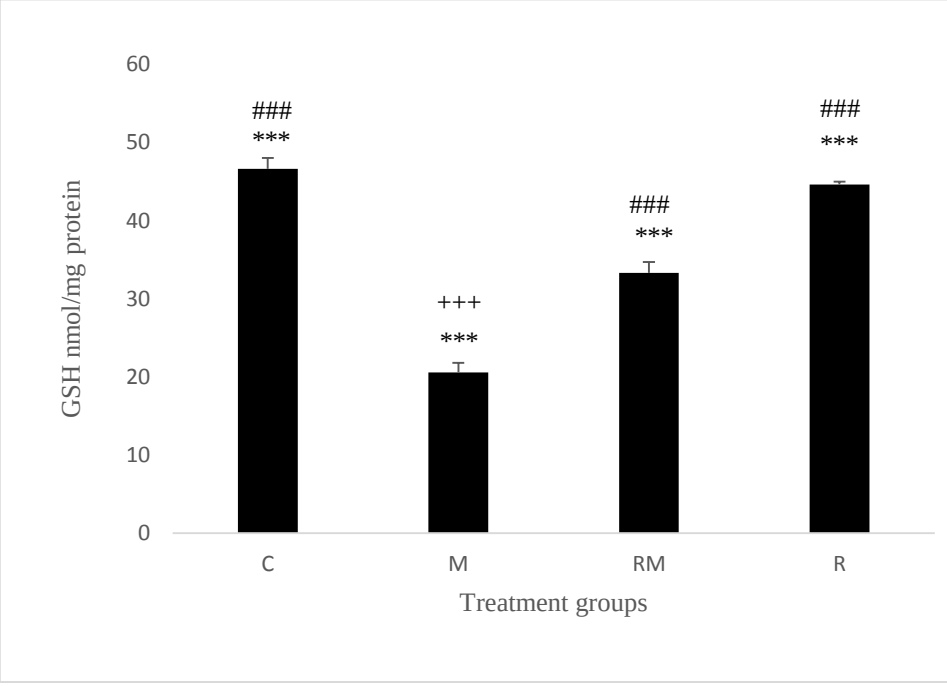

Figure 24. Effect of *Rosa canina* fruit on GSH values.

Results are shown as mean ± S.E.M (n = 4) and significant difference between groups is shown as (***p = 0.001). ($^{\#\#\#}$p = 0.000) compared to (M) group, ($^{+++}$p = 0.001) compared to (RM). (C): control

group; (M): group administered with L-methionine (200 mg/kg); (RM): group administered with L-methionine (200mg/kg) and treated with *R. canina* fruit (200 mg/kg); (R): group treated with *R. canina* fruit (200mg/kg).

Treatment with the *Rosa canina* fruit presented a very highly significant increase in the level of GSH. This confirms that rosehips improved the antioxidant activity and protected the intestine from inflammation.

Researchers have turned out that *R. canina* fruit increases the GSH levels which leads to a decrease of ROS in human endothelial cells (Kerasioti et al., 2016). Sadeghi et al. (2021) have also confirmed that *R. canina* fruit is a very ROS effective scavenger.

Vitamin C is a well-known antioxidant that effectively scavenges ROS. Insufficient levels of vitamin C inhibit collagen synthesis in cellular basal membranes and destroy the mucosal epithelium (Zerin et al., 2010). According to Karastogianni et al. (2021) and Aldemir et al. (2021) who reported that *R. canina* fruits is an abundant source of vitamin C. That is way, we strongly beleive that vitamin C in *R. canina* fruit was the main candidate of the positive effect obtaind in our results.

GSH is involved in various metabolic activities, including the scavenging of ROS (Lőrincz et al., 2017; Lv et al., 2019). Under conditions of low B vitamins, Hcy accumulates and leads to its associated HHyc and low biosynthesis of glutathione, the main intracellular antioxidant (Waly et al., 2016; Al-Alawi et al., 2021).

- **Effect of vitamin C**

Avery highly significant difference in the concentration of reduced glutathione in the (C), (M), (VM) and (VC) groups ($p = 0.000$) as presented in figure 25. The means showed that the value of GSH in liver increased very highly significantly in the (VM) group when compared with the (M) group ($p= 0.000$).

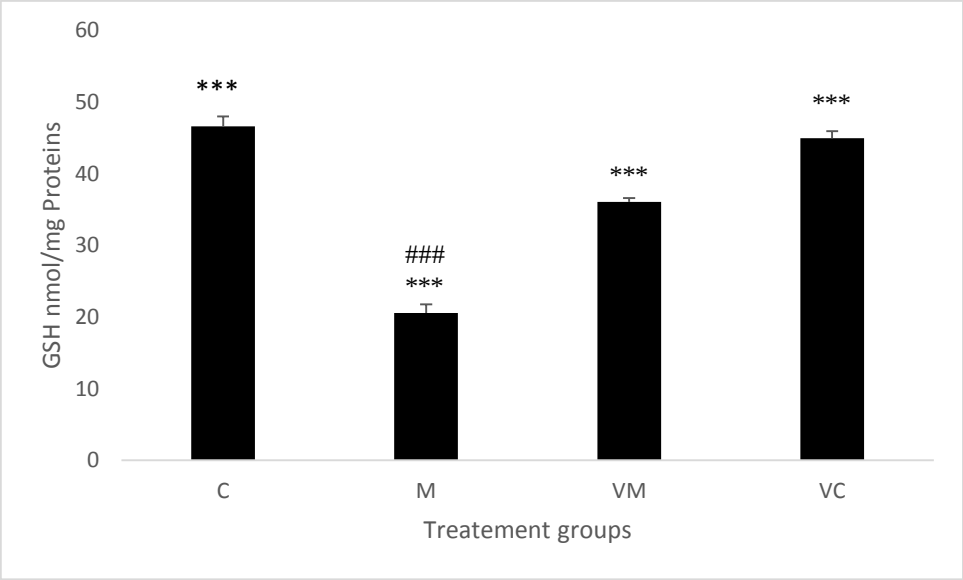

Figure 25. Effect of vitamin C on GSH values.

Results are shown as mean ± S.E.M (n = 4) and significant difference between groups is shown as (***p = 0.000). ($^{###}$p = 0.000) compared to (VM). (C): control group; (M): group administered with L-methionine (200 mg/kg); (VM): group was administered L-methionine (200mg/kg) and treated with (500 mg/kg) of vitamine C; (V): group received (500 mg/kg) of vitamin C.

These results are in agreement with the study of (Adaramoye et al., 2008) which evaluated the protective effects of vitamin C against radiation-induced liver damage in rats, they brought that the treatment with vitamin C at a dose of 800 mg/kg increased significantly ($p<0.05$) the levels of GSH.

Nath et al. (2022) confirmed the protective effect of vitamin C against oxidative stress induced by metane yellow in rat ovarian tissues.

It has been shown that under conditions of low B vitamins, Hcy accumulates and leads to its associated HHcy and low biosynthesis of glutathione, the major intracellular antioxidant (Waly et al., 2016; Al-Alawi et al., 2021).

GSH is involved in various metabolic activities including ROS scavenging (Lőrincz et al., 2017; Lv et al., 2019).

According to the results obtained, the administration of plant fruits and vitamin C increased the concentration of GSH very highly significantly. This increase can be explained by the effect of vitamin C and polyphenols on glutathione recycling.

II.3.2. Effect of fruits and vitamin C on catalase values

To trap the harmful effects of ROS, aerobic organisms have developed protective mechanisms that involve antioxidant enzymes, such as catalase. Catalase is one of the main enzymes of the antioxidant defense system, it catalyzes the decomposition of hydrogen peroxide into water and molecular oxygen (Krych-Madej and Gebicka, 2017; Marilisa Galasso et al., 2021).

However, catalase deficiency or dysfunction is linked to the pathogenesis of many diseases (Nandi et al., 2019).

We demonstrate by the present results the effect of the use of plant fruits and vitamin C in improving the activity of catalase.

- **Effect of *Citrus sinensis* fruit**

The enzymatic activity of the CAT shows a highly significant difference between the groups (P = 0.00). The value of CAT was highly and significantly decreased in the (M) group compared to the groups (OM) and (O) (p = 0.00), also, the CAT value was highly and significantly increased in the (OM) group compared to the (M) group (p = 0.01) (Figure 26).

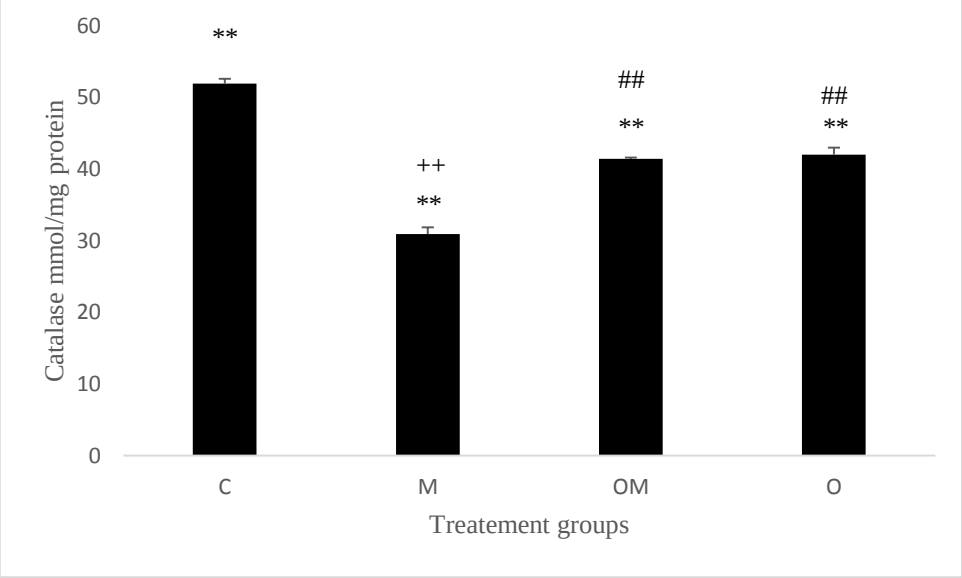

Figure 26. Effect of *Citrus sinensis* fruit on catalase values.
Results are shown as mean ± S.E.M (n = 4) and significant difference between groups is shown as (**p = 0.00). ($^{\#\#}$p = 0.00) compared to (M) group, ($^{++}$p = 0.01) compared to (OM). (C): control group; (M): group administered with L-methionine (200 mg/kg); (OM): group administered with L-methionine (200 mg/kg) and treated with *C. sinensis* fruit (200 mg/kg); (O): group treated with *C. sinensis* fruit (200 mg/kg).

Our data are in agreement with the study of (Abou Baker et al., 2022) who reported that a significant elevation of CAT levels in rats treated with *Citrus sinensis* L.

In the same context, administration of *Citrus sinensis* extract significantly elevated serum CAT enzyme levels in a dose-dependent manner and ameliorated CCl 4-induced liver injury in rats. The results of this study demonstrate that *Citrus sinensis* possesses antioxidant and hepatoprotective activity against oxidative stress, these biochemical observations were confirmed by histopathological studies **(**Unanma et al., 2021).

- **Effect of *Rosa canina* fruit**

The results of the figure 27 showed a very highly significant difference between the groups (P = 0.000). The value of CAT was very highly and significantly decreased in the (M) group compared to the control group and (RM) group (p = 0.000), also, the CAT value was very highly and significantly increased in the (RM) group compared to the control group (p = 0.001).

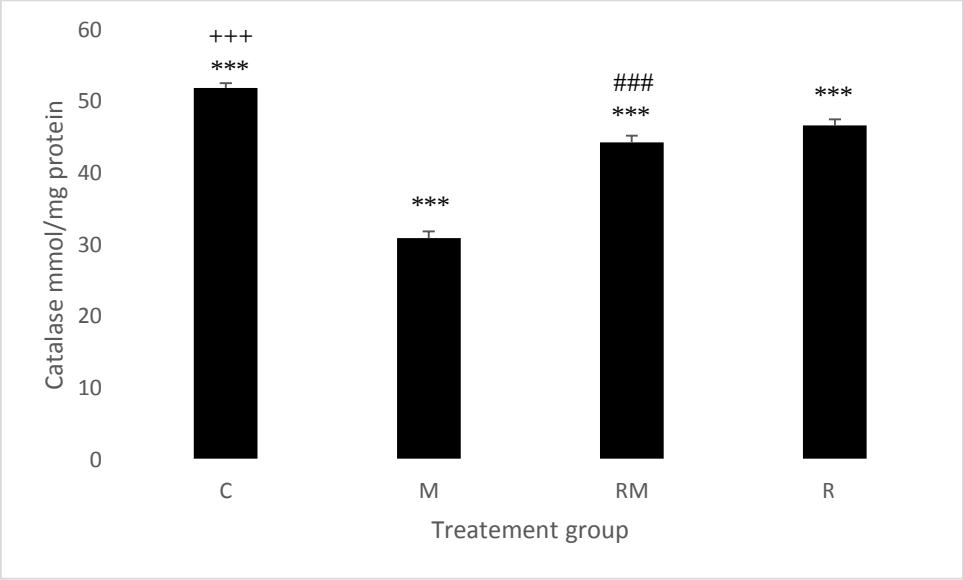

Figure 27. Effect of *Rosa canina* fruit on catalase values.

Results are shown as mean ± S.E.M (n = 4) and significant difference between groups is shown as (***p = 0.000). ($^{\#\#\#}$p = 0.000) compared to (M), ($^{+++}$p = 0.001) compared to (RM) group. (C): control group; (M): group administered with L-methionine (200 mg/kg); (RM): group administered with L-methionine (200mg/kg) and treated with *R. canina* fruit (200 mg/kg); (R): group treated with *R. canina* fruit (200mg/kg).

The levels of catalase activity in liver of animal administered with high-dose of methionine and *Rosa canina* fruit pretreatment exposed to H_2O_2, although reduced, were close to those of group (C), suggesting that pretreatment with *Rosa canina* fruit relatively restored normal antioxidant levels. The result obtained from this part of the study where compatible with the results of (Özmen et al., 2004; Şahan et al., 2017).

A different study investigated catalase activity levels in splenocytes and confirmed the enhanced cytoprotective and antioxidant activity of *Rosa canina* L. against H_2O_2-induced oxidative stress in primary mouse splenocytes (Soualeh et al., 2019).

- **Effect of vitamin C**

The present data showed a highly significant difference between the groups (P = 0.00). Figure 28 shows that the CAT value increased highly and significantly in the (VM) group compared to the group (M) group (p = 0.00).

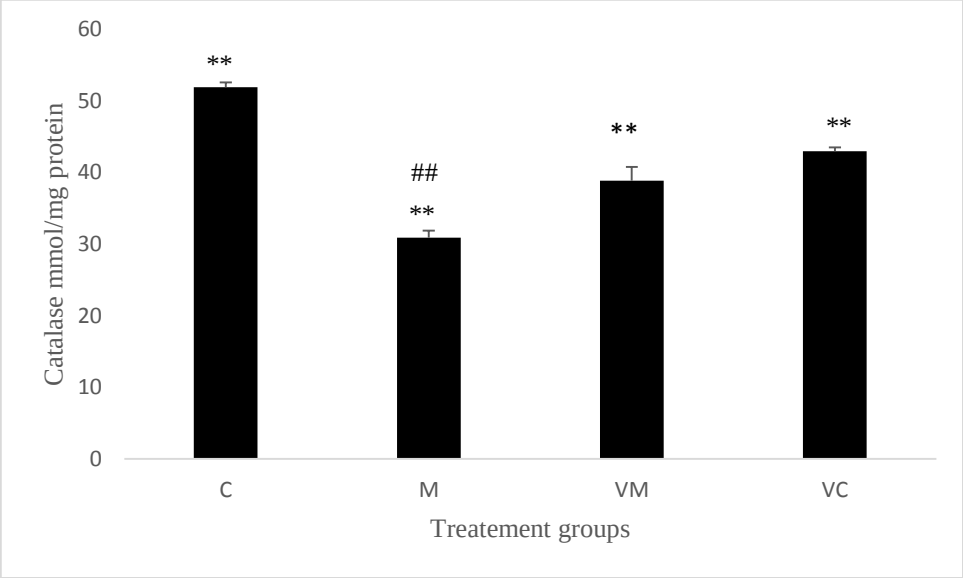

Figure 28. Effect of vitamin C on catalase values.

Results are shown as mean ± S.E.M (n = 4) and significant difference between groups is shown as (**p = 0.00). ($^{\#\#}$p = 0.00) compared to (VM) group. (C): control group; (M): group administered with L-methionine (200 mg/kg); (VM): group was administered L-methionine (200mg/kg) and treated with (500 mg/kg) of vitamine C; (V): group received (500 mg/kg) of vitamin C.

The team of (Dunleavy et al., 2021) reported a case series of IBD patients with vitamin C deficiency and concluded that vitamin C deficiency should be considered in patients with IBD, in especially those with reduced fruit/vegetable intake, as it can lead to significant signs

and symptoms. However, they recommended that all patients with IBD consume enough fruits and vegetables rich in vitamin C.

we agree with (Esmaeilizadeh et al., 2019) who reported that the concentration of CAT in hepatic tissues was increased in rats administered vitamin C confirming that vitamin C improves liver functions and in hypothyroid rats by reducing oxidative damage to test tissues.

The study of (Ibuki et al., 2020) examined the effects of vitamin C in preventing oxidative stress in the salivary glands of streptozotocin-induced diabetic rats, they confirmed that vitamin C caused an increase in the levels of MDA and CAT and a reduction in the activity of SOD, therefore, they concluded that vitamin C supplementation improved the antioxidant system.

The above results show that L-methionine significantly decreased liver catalase activity in group (M) compared to other groups. On the other hand, there was a significant increase in hepatic catalase concentration liver in mice treated with plant fruits and vitamin C. In the light of the results presented, we think that this antioxidant activity *in vivo* could be due to the synergistic action of the bioactive compounds present in the fruits which are probably vitamin C and polyphenols, underlining the influence of these antioxidants in the food for human health.

II.4. Effect of *Citrus sinensis, Rosa canina* fruits and vitamin C on the microscopic appearances in mice bowel with inflammation induced by hyperhomocysteinemia

The histological study of the intestines of the experimental groups are represented in the figures below. The groups (C), (O), (RC) and (V) showed normal architecture of the intestinal membrane (Figures: 29, 33,35 and 37).

In the (M) group that have been administered with L-methionine (200 mg/kg), showed a granuloma with significant lymphocytic infiltration and degeneration in the plasma enterocytes membrane cells (Figures 30 and 31).

However, the group animal (OM) showed a normal structure with distinct villi, markedly reduced lymphocyte infiltration, and restoration of enterocyte membrane cell integrity (Figure 32).

Whereas the group (RM), animal shad no evident histological changes, restoration of the integrity enterocytes membrane cells, structure with distinct long villi and markedly reduced lymphocyte infiltration (Figure 34). In addition, there was a restoration of the integrity of the colonic epithelium in the animal of group (VM) (Figure 36).

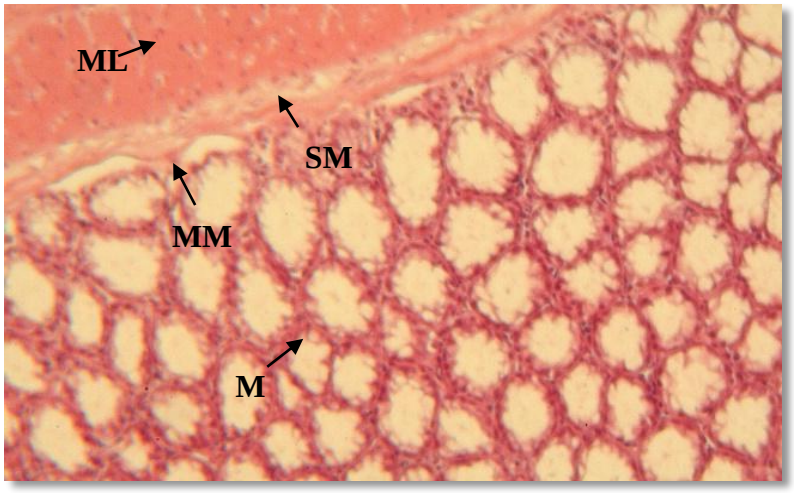

Figure 29. Histological section of colon of mouse received distilled water.

Hematoxylin – eosin staining (×100).

Mucosa (M). Muscularis (ML). Submucosa (SM). Muscularis Mucosa (MM).

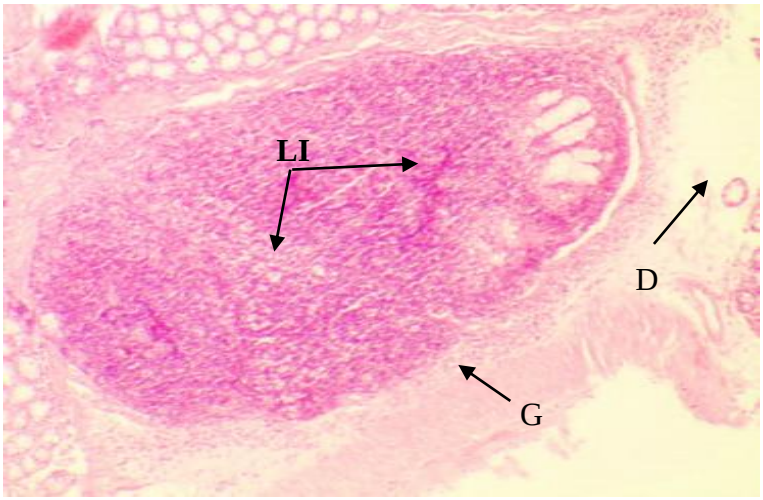

Figure 30. Histological section of colon of mouse administered with L-methionine (200 mg/kg).

Hematoxylin - eosin staining (×100).

Granuloma (G). Degeneration (D). Lymphocytic Infiltration (LI).

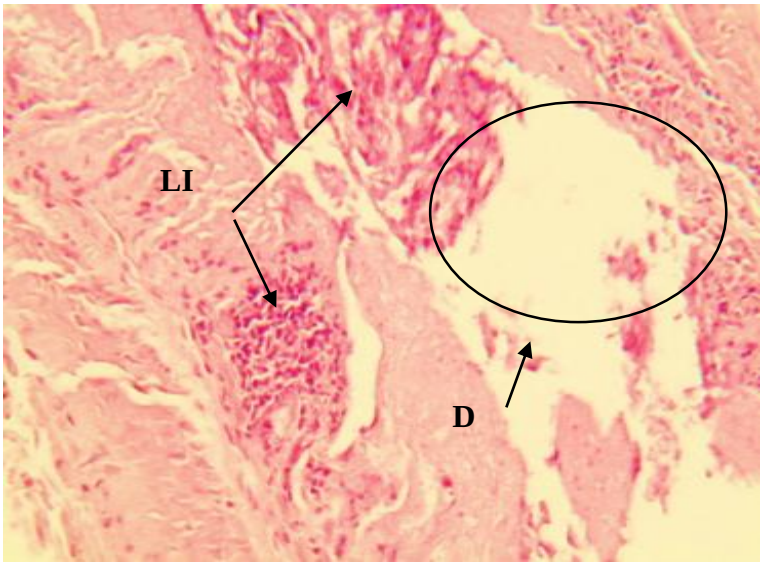

Figure 31. Histological section of colon of mouse administered with L-methionine (200 mg/kg). Hematoxylin - eosin staining (× 400).
Degeneration (D). Lymphocytic Infiltration (LI).

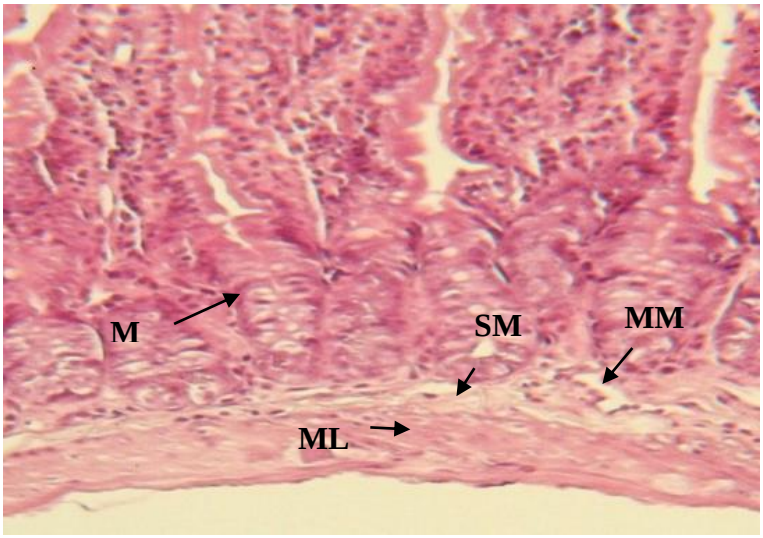

Figure 32. Histological section of colon of mouse administered with L- methionine (200 mg/kg) and treated with *C. sinensis* fruit (200 mg/kg). Hematoxylin - eosin staining (× 100).
Mucosa (M). Muscularis (ML). Submucosa (SM). Muscularis Mucosa (MM).

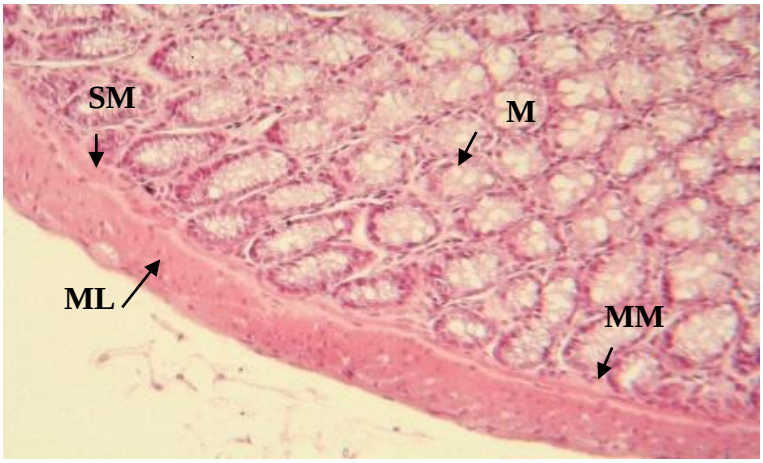

Figure 33. Histological section of colon of mouse received *C. sinensis* fruit (200 mg/kg).

Hematoxylin - eosin staining (× 100).

Mucosa (M). Muscularis (ML). Submucosa (SM). Muscularis Mucosa (MM).

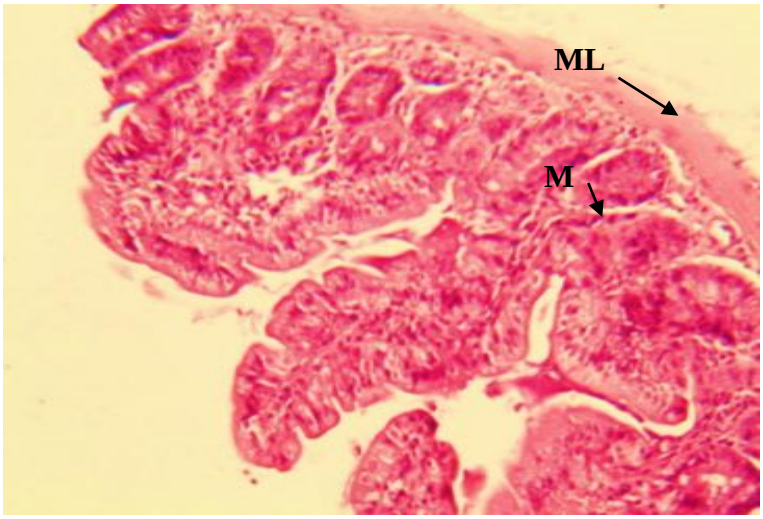

Figure 34. Histological section of colon of mouse administered with L- methionine (200 mg/kg) and treated with *R. canina* fruit (200 mg/kg). Hematoxylin - eosin staining (× 100).

Mucosa (M). Muscularis (ML).

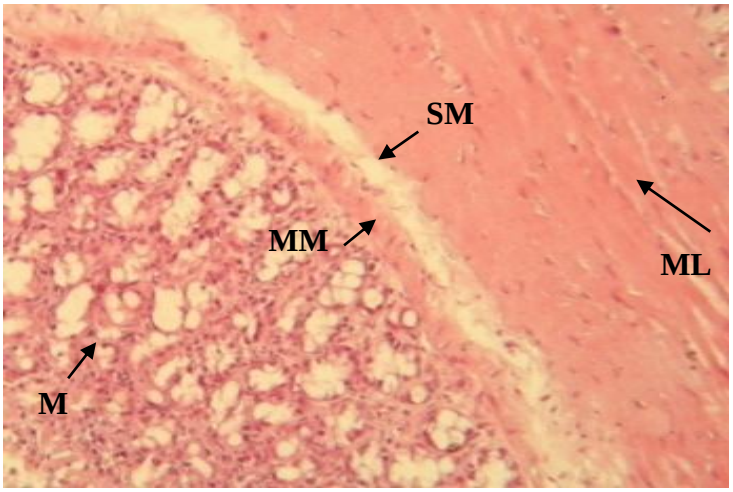

Figure 35. Histological section of colon of mouse received *R. canina* fruit (200 mg/kg).

Hematoxylin - eosin staining (× 100).

Mucosa (M). Muscularis (ML). Submucosa (SM). Muscularis Mucosa (MM).

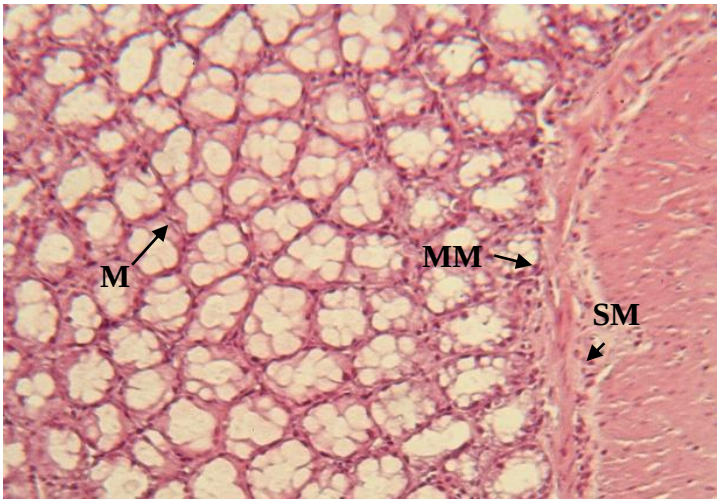

Figure 36. Histological section of colon of mouse administered with L- methionine (200 mg/kg) and treated with vitamin C (500 mg/kg). Hematoxylin - eosin staining (× 100).
Mucosa (M). Muscularis (ML). Submucosa (SM). Muscularis Mucosa (MM).

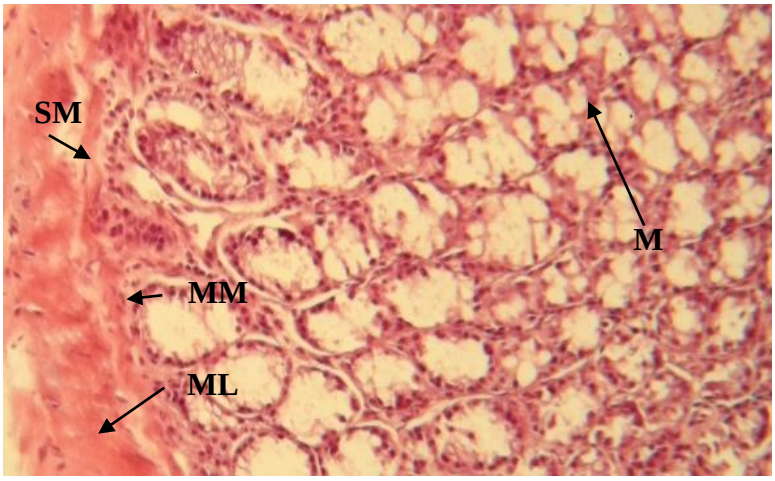

Figure 37. Histological section of colon of mouse received vitamin C (500 mg/kg).

Hematoxylin - eosin staining (× 100).

Mucosa (M). Muscularis (ML). Submucosa (SM). Muscularis Mucosa (MM).

In comparison with the group receiving L-methionine (200 mg/kg), microscopic examination of histological sections of mouse intestine showed that treatment with both fruits at a dose of 200 mg/kg or vitamin C at a dose of 500 mg/kg significantly reduced the histopathological changes caused by L-methionine, and also restored the integrity of the colonic epithelium.

The inflamed colon is exposed to oxidative stress produced by the infiltration of macrophages and neutrophils into the colonic tissue. An exaggerated intestinal immune response and the generation of reactive oxygen metabolites may play a key role in pathophysiology (Zerin et al., 2010).

Activation of the intestinal immune system and recruitment of inflammatory cells in the intestine help maintain inflammation and damage in the intestines (Wera et al., 2016).

The results of the present study are in agreement with the work of Dorghal and Houadek, (2014) which revealed necrosis of the sigmoid colon in mice treated with 400 mg/kg L-methionine for 12 days.

Histological examination revealed that the treatment with *Citrus sinensis* fruit restored the integrity of the intestine and the histopathological changes caused by the elevated homocysteine levels. Khan et al. (2016) reported that there is a marked reduction in

histopathological damage and a protective effect against inflammation in intestinal tissue in rat colitis treated with *Citrus sinensis* L.

The study of Gholap et al. (2012) also reported that *C. sinensis* fruit peel extract is effective in the treatment of UC in mice who showed less ulceration in histopathological observation. On the basis of these results, we can confirm that *C. sinensis* fruit exhibit protective role against intestinal inflammation.

On the other hand, our results showed that treatment with *Rosa canina* fruit corrected the intestinal histological damage caused by hyperhomocysteinemia, *via* restoring the integrity of the intestinal epithelium, inhibiting the over production of ROS.

Wanes et al. (2020) demonstrated that the *Rosa canina* fruit extract has a protective effect on dextran sulfate sodium-induced intestinal bowel disease in a mouse model. Over and obove, several studies have shown that rosehips are used worldwide, as an anti-inflammatory and gastroprotective agent (Lattanzio et al., 2011; Fetni and Bertella, 2020; Kubczak et al., 2020). On the basis of our results, we can confirm that *R.canina* fruit play a protective effect against inflammation.

We have also found that vitamin C treatment attenuates the histological and colonic damage caused by high dose of L- methionine. The clinical roles of vitamin C are of great interest, as there is evidence that oxidative damage is the cause of inflammation. Vitamin C nutrition may be more important for people with certain diseases (infectious and inflammatory). A high intake of vitamin C has a lower risk of developing a number of chronic inflammatory diseases such as gastrointestinal disorders (Jacob and Sotoudeh, 2002).

HHcy has been identified in previous studies as a risk factor for several chronic inflammatory disorders (Cordaro et al., 2021; Ji et al., 2022). Therefore, we planned to study the impact of L-methionine-induced hyperhomocysteinemia in causing intestinal inflammation and at the same time to estimate the protective capacity of the studied fruits.

According to the results obtained, the induced hyperhomocysteinemia showed significant intestinal alterations where the inflammatory process was well manifested. In addition, the fruits used in this research study also revealed a significant anti-hyperhomocysteinemic and anti-inflammatory effect.

Conclusion and Perspectives

Conclusion and perspectives

This work focuses on the valorization of Algerian fruits in the treatment of IBD based on two forms of studies: an *in vitro* study and an *in vivo* study. In this context, this investigation aims to explore the preventive and therapeutic effect of *Citrus sinensis*, *Rosa canina*, fruits and vitamin C on intestinal inflammation induced by the administration of a high dose of L-methionine during 21 days of experimentation.

Our findings showed the richness of these fruits in phenolic compounds, natural source of antioxidants against oxidative stress and inflammatory. These phenolic compounds contained in these two fruits have an important antioxidant and anti-inflammatory power, revealed by several *in vitro* and *in vivo* tests.

The evaluation of antioxidant activity by five different tests indicates that the extracts used have antioxidant activity.

The GC/MS performed on the extract of *R. canina* fruit allowed the identification of 65 different compounds, the electrochemical characterization also revealed that *R. canina* fruit is rich in ascorbic acid and has a good antioxidant capacity.

According to the results obtained, the *in vitro* study confirms that the *Rosa canina* fruit extract used has no toxic effect on normal human fibroblast cell lines and an antioxidant effect against H_2O_2-induced oxidative stress. In addition, *R. canina* fruit extract has a cytoprotective effect on human fibroblast cells which appears to protect cell viability.

In vitro anti-inflammatory activity has shown that both fruit extracts inhibit heat-induced protein denaturation and have powerful anti-inflammatory potential. This was proven and confirmed by the *in vivo* study.

The results of the *in vivo* study confirmed that hyperhomocysteinemia induces intestinal inflammation with a significant increase in the level of plasma markers (hs-CRP, homocysteine) and a decrease in antioxidant parameters (GSH and CAT) in the group of mice receiving 200 mg/Kg of L-methionine. This phenomenon was detected by the inflammatory process observed in the histological study.

According to the results obtained, the use of *Citrus sinensis*, *Rosa canina* fruits and vitamin C as a preventive treatment reduced the levels of plasma markers, confirming the anti-inflammatory and anti-hyperhomocysteinemic effect of these fruits. Moreover, due to the

significant decrease in liver antioxidant enzyme activity, this confirms that these fruits have potential antioxidant activities.

Histological analysis of the intestine of mice treated with these fruits shows a restoration of the integrity of the intestinal epithelium with a correction of intestinal damage.

In conclusion, the results of the activities carried out *in vitro* and *in vivo* studies indicate a significant effect of these fruits for antioxidant, anti-inflammatory, anti-hyperhomocysteinemic activities and for histological improvements elucidated by several tests.

For this reason, we recommend the use of these fruits for the prevention of inflammatory bowel diseases due to their significant antioxidant and anti-inflammatory power and should be proposed as possible candidates for the development of anti-inflammatory and anti-hyperhocysteinemic drugs.

However, these results remain preliminary and further experiments must be conducted in order to modify the future of IBD treatment, namely:

- the purification of the bioactive molecules of these fruits, in order to determine the fractions responsible for these effects;
- the realization of *in vivo* studies on IBD models with the bioactive molecules during different treatment periods;
- the determination of other plasma parameters;
- the elucidation of the molecular mechanism of our fruits at the level of enterocyte cells;
- the effect of these two fruits on the action of pro-inflammatory cytokines such as IL-1, IL-6, TNFα IL-23 and IL-17;
- characterization of the effect of these fruits on the regulation of certain pro-apoptotic, anti-apoptotic and other proteins involved in oxidative stress;
- the evaluation of the effect of *Rosa canina* fruits on other types of cancer lines.

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Annex

- **Trypsinization and cell division**

Normal human fibroblast cell line was used for in this part of the study.

- First, the medium was removed, and the flask was washed by 5 mL of DPBS.
- After removing the DPBS, 2 mL of trypsin (gibco® by Life Technologies, cascade biologics™) was added to the flask and ware incubated for 5 min in CO2 incubator (Thermo scientific "FORMA STERI-CYCLE").
- After that, 5 mL of medium was added to the trypsin and with a smooth pipetting to mix the solutions which then had been transferred in a conic tube
- Then the tube was centrifuged for 10 min/100 rpm.
- After taking out the supernatant, 1ml of the medium was added to the cells (by a smooth pipetting).
- At the end, 250 µL of the medium and cells solution was transferred to two T75 flasks filled with 14 mL of the medium each.

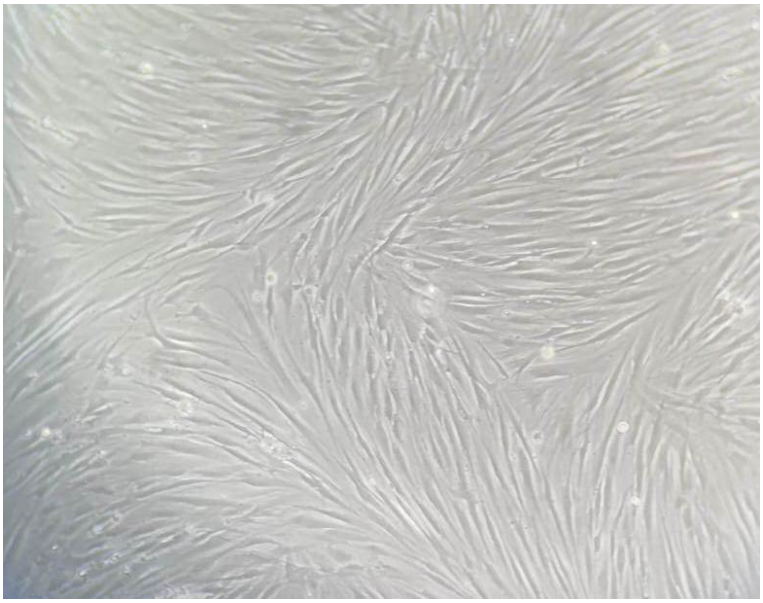

Figure. Human fibroblast cells (personel photo).

Calibration curve of gallic acid

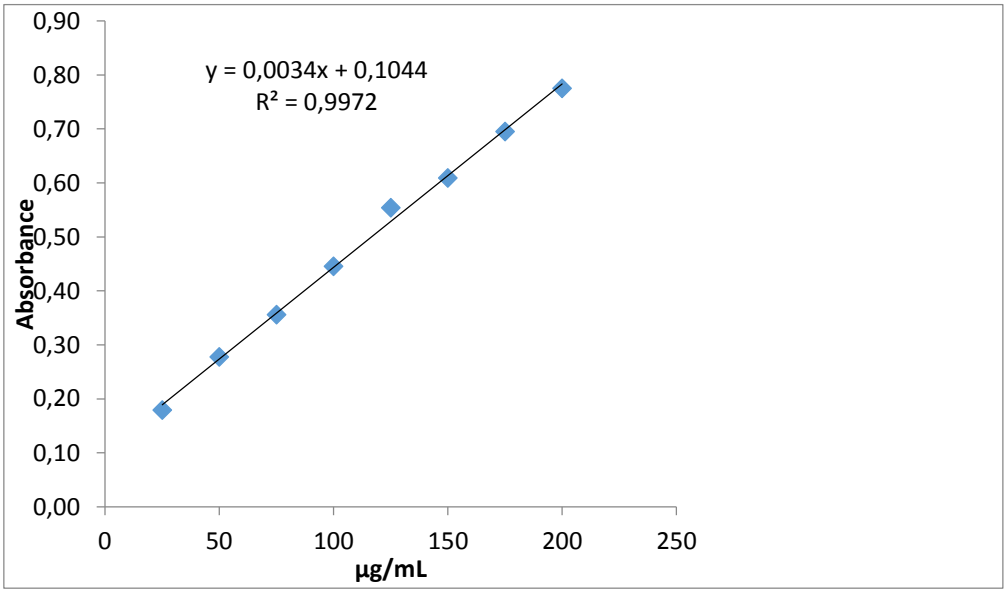

Figure. Calibration curve of gallic acid.

Calibration curve of quercetin

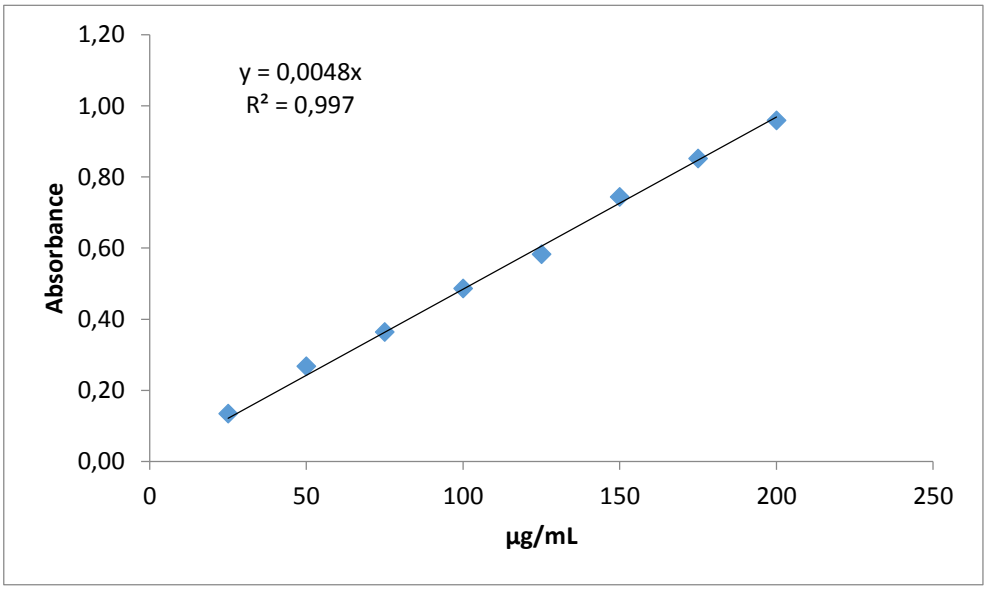

Figure. Calibration curve of quercetin.

Ascorbic acid calibration

The figure howed five points ascorbic acid calibration (5, 10, 25, 50 and 100 μM), linear regression equation and coefficient of regression (r^2). Data are given as baseline- subtracted currents (ΔnA).

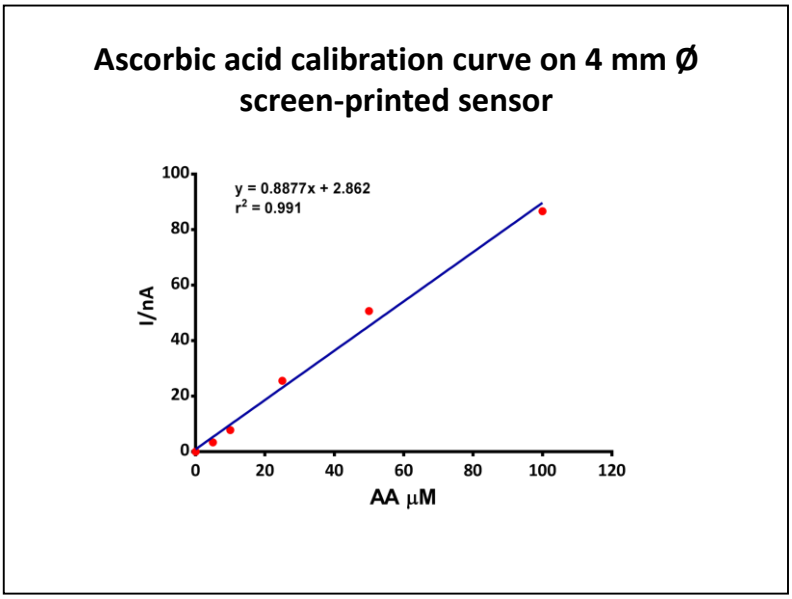

Figure. Ascorbic acid calibration.

Abstract

Abstract

Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, are chronic immune-mediated inflammatory diseases of the intestinal tract. Under low vitamin B conditions, homocysteine accumulates and leads to hyperhomocysteinemia and the association between hyperhomocysteinemia and the risk of IBD has been widely studied.

The objective of our work is to study the preventive effect of *Rosa canina* and *Citrus sinensis* fruits on intestinal inflammation induced by hyperhomocysteinemia in mice.

In the first part of this study we determinate two compounds, the polyphenol and flavonoid in fruit extracts of *Rosa canina* and *Citrus sinensis*, followed by an evaluation of the antioxidant activity of these fruit extracts by different methods: DPPH, ABTS, GOR, reducing power and CUPRAC. The extracts were then tested *in vitro* for their anti-inflammatory effect via the bovine serum albumin (BSA) denaturation test. The chemical composition, ascorbic acid content, electrochemical characterization and antioxidant capacity of *Rosa canina* fruit extract were determined. In addition, the *in vitro* antioxidant activity of *R. canina* fruit extract and its protective effect on hydrogen peroxide (H_2O_2)-stressed human fibroblast cells were evaluated using MTT assay.

In the second part, we studied the protective, anti-inflammatory and anti-homocysteinemic effect of the fruits: *Citrus sinensis*, *Rosa canina* and vitamin C on the intestinal inflammation induced by high doses of L-methionine during the 21 days *in vivo* experimental procedure. The levels of plasma inflammatory markers (hs-CRP, homocysteine), reduced glutathione (GSH) and catalase (CAT) in liver tissue were measured, and histological sections of intestinal tissue were examined.

The results show that both extracts are rich in polyphenols and have significant antioxidant power. Both extracts prevented the denaturation of BSA in a dose-dependent manner indicating that both extracts have anti-inflammatory activity, which was confirmed by the *in vivo* anti-inflammatory study. The extract of *Rosa canina* fruit indicates the presence of n-hexadecanoic acid, oleic acid and stearic acid, which were identified as the main compounds of this fruit. This fruit is rich in ascorbic acid and has an important antioxidant capacity. Moreover, the results show that *Rosa canina* fruit extract has a non-toxic effect on the viability of human fibroblasts with a protective effect against oxidative stress induced by hydrogen peroxide on fibroblasts. In addition, treatment with fruits and vitamin C led to a decrease in the level of plasma homocysteine, an increase in the levels of GSH and CAT. This was confirmed by the histological study of the restoration of the integrity of the intestinal epithelium.

This study revealed that the fruits of *Citrus sinensis* and *Rosa canina* have antioxidant and anti-inflammatory powers and can be considered as a natural source to prevent and treat IBD.

Key words: IBD, hyperhomocysteinemia, fruits, *Rosa canina*, *Citrus sinensis*, antioxidant and anti-inflammatory activity.

Résumé

Les maladies inflammatoires chroniques de l'intestin (MICI), regroupent deux pathologies : la maladie de Chron (MC) et la rectocolite hémorragique (RCH) appelée aussi la colite ulcéreuse. Les MICI correspondent à une inflammation chronique à médiation immunitaire du système digestif. Dans des conditions de faible teneur en vitamine B, l'homocystéine s'accumule entraînant une hyperhomocystéinémie et le lien entre l'hyperhomocystéinémie et le risque de MICI a bien etè établi. L'objectif de cette investigation est d'étudier l'effet préventif des fruits de *Rosa canina* et de *Citrus sinensis* sur l'inflammation du côlon induite par l'hyperhomocysteinemie chez les souris.

Dans la première partie de ce travail, la teneur en polyphénols et en flavonoïdes a été quantifiée dans les deux extraits de fruits, *Rosa canina* et de *Citrus sinensis*, suivie d'une évaluation de l'activité antioxydante par différentes méthodes : DPPH, ABTS, GOR, pouvoir réducteur et CUPRAC. Les extraits de fruits ont été testés *in vitro*, pour leur effet antiinflammatoire via le test de dénaturation de l'albumine sérique bovine (BSA). La composition chimique, la teneur en acide ascorbique, la caractérisation électrochimique et la capacité antioxydante de l'extrait de fruits de *Rosa canina* ont été déterminés. En outre, l'activité antioxydante *in vitro* de l'extrait de fruit de R*osa.canina* et son effet protecteur sur les cellules fibroblastes humaine stressées au peroxyde d'hydrogène (H_2O_2) ont été évalués en utilisant le test MTT.

Pour la deuxième partie, l'effet protecteur, anti inflammatoire et anti hyperhomocysteinémique des fruits : Citrus *sinensis*, *Rosa canina* et de la vitamine C sur l'inflammation du côlon induite par des doses élevées de L-methionine selon la procédure expérimentale *in vivo* de 21 jours a été étudié.

Les niveaux des marqueurs inflammatoires plasmatiques (hs-CRP, homocystéine), le glutathion réduit (GSH) et la catalase (CAT) dans le tissu hépatique ont été mesurés, et les coupes histologiques du tissu intestinal ont été examinées.

Les résultats montrent que les deux extraits de fruits, sont riches en polyphénols et ont un pouvoir antioxydant important. Les deux extraits empêchent la dénaturation de la BSA de manière dose dépendante, ce qui indique que ces deux extraits ont une activité anti-inflammatoire. Ceci a été confirmé par l'étude anti-inflammatoire *in vivo*.

L'étude *in vitro* de l'extrait de fruit *R.canina* note la présence d'acide n-hexadécanoïque, d'acide oléique et d'acide stéarique. Ces derniers ont été identifiés comme les principaux composés de ce fruit. En outre, l'extrait de fruit de *Rosa canina* a un effet non toxique sur la viabilité des fibroblastes humains pourvu d'un effet protecteur contre le stress oxydatif induit par le peroxyde d'hydrogène sur les fibroblastes. Pour l'étude *in vivo,* le traitement avec les fruits et la vitamine C a entraîné une diminution du niveau d'homocystéine plasmatique, une augmentation des niveaux de GSH et CAT. Cela a été confirmé par l'étude histologique de la restauration de l'intégrité de l'épithélium intestinal.

En conclusion, cette étude a révélé que les fruits de *Citrus sinensis* et *Rosa canina* sont pourvus des pouvoirs antioxydants et anti-inflammatoires et peuvent être considérés comme source naturelle pour prévenir et traiter les MICI.

Mots clés : MICI, hyperhomocystéinémie, fruits, *Citrus sinensis*, *Rosa canina,* activité antioxydante et anti-inflammatoire.

ملخص

يعتبر مرض التهاب الأمعاء (IBD)، والتي من بينها مرض كرون والتهاب القولون التقرحي، أمراضا التهابية مزمنة في الجهاز المعوي ومن أحد عوامل المتسببة في هدا المرض فهو زيادة تركيز الهوموسستيين في البلازما الدم الناتج من انخفاض في فيتامين ب. إن الهدف من بحثنا هو دراسة التأثير الوقائي لكل من فاكهة *Rosa canina* و *Citrus sinensis* على التهاب الأمعاء الناجم عن فرط الهوموسيستين في الدم عند الفئران.

في الجزء الأول من البحث، قمنا بتحديد كمية البوليفينولات والفلافونيدات في مستخلصي فاكهة *Rosa canina* و*Citrus sinensis*، ثم انتقلنا إلى تقييم النشاط المضاد للأكسدة في مستخلصات الفاكهتين المذكورتين بطرق مختلفة: DPPH، ABTS، GOR وCUPRAC. ثم اختبرنا للمستخلصات خارج العضوية لمعرفة تأثيرها المضاد للالتهابات عن طريق اختبار تمسخ زلال المصل البقري (BSA).

وكذلك تم تحديد التركيب الكيميائي لمحتوى حمض الأسكوربيك و التشخيص الكهروكيميائي والقدرة المضادة للأكسدة لمستخلص فاكهة *Rosa canina*. كما تم تقييم النشاط المضاد للأكسدة في المختبر لمستخلص فاكهة *R. canina* وتأثيره الوقائي على الخلايا الضامة الليفية البشرية المجهدة ببيروكسيد الهيدروجين (H_2O_2) باستخدام فحصMTT.

في الجزء الثاني من هدا البحث، درسنا التأثير الوقائي والتأثير المضاد للالتهابات والتأثير المضاد للهوموسستيين لكل من: *Citrus sinensis* و *Rosa canina* وفيتامين C على الالتهاب المعوي عند الفئران المتغذية على كمية عالية من المثيونين لمدة 21 يوم. تم قياس مستويات بروتين التفاعليc و الهوموسستيين في بلازما الدم، الجلوتاثيون المختزل' الكاتلاز في الكبد، مع تحضير قطاعات نسيجية على الأمعاء الغليظة.أظهرت النتائج أن كلا المستخلصين تحتوي على كميات من بوليفولينات ولهما قوة كبيرة في مضادات الأكسدة. تتميز كلا المستخلصين بنشاط مضاد للالتهاب وذلك من خلال عملية تمسخ BSA معتمدة على الجرعة المعطاة والتي اكدته الدراسة كمضاد للالتهابات في داخل العضوية.

بينت النتائج التي تم الحصول عليها خارج العضوية ان مستخلص فاكهة *R. canina* يشير إلى وجود بعض الاحماض الرئيسة من بينها: حمض الأوليك، حمض الستياريك وn-hexadecanoic. هذه الفاكهة غنية بحمض الأسكوربيك ولها قدرة كبيرة كمضاد للأكسدة. علاوة على ذلك، أظهرت النتائج أن مستخلص فاكهة *Rosa canina* لها تأثير غير سام على حيوية الخلايا الليفية البشرية مع تأثير وقائي ضد الإجهاد التأكسدي الناجم عن بيروكسيد الهيدروجين على الخلايا الضامة الليفية.

أظهرت الدراسة في الجسم الكائن أن العلاج بالفواكه وفيتامين C أدى إلى انخفاض في مستويات الهوموسيستتين في البلازما، وزيادة في مستويات الجلوثاثيون المختزل والكاتالاز مع تصحيح كل التغيرات التي حدثت على التركيب النسيجي للأمعاء خلال معاملته بالمثيونين.

في الخاتمة' إن النتائج المتحصل عليها في هده الدراسة تبين ان مستخلص كل من البرتقال *Citrus sinensis* ومستخلص بدور *Rosa canina* تتمتع بخصائص مضادة للأكسدة وللالتهابات ويمكن اعتبارها مصادر طبيعية للوقاية من مرض التهاب الأمعاء وعلاجه.

الكلمات المفتاحية: مرض التهاب الأمعاء , فرط الهوموسستيين , فاكهة , *Rosa canina* , *Citrus sinensis* , النشاط المضاد للاكسدة - النشاط المضاد للالتهاب.

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Titled: Preventive effects of pure vitamin C and Algerian oranges on bowel diseases induced by hyperhomocysteinemia and on cancer cell lines

Thesis submitted for the degree of Doctorate 3rd cycle

Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, are chronic immune-mediated inflammatory diseases of the intestinal tract. Under low vitamin B conditions, homocysteine accumulates and leads to hyperhomocysteinemia and the association between hyperhomocysteinemia and the risk of IBD has been widely studied.

The objective of our work is to study the preventive effect of *Rosa canina* and *Citrus sinensis* fruits on intestinal inflammation induced by hyperhomocysteinemia in mice.

In the first part of this study we determinate two compounds, the polyphenol and flavonoid in fruit extracts of *Rosa canina* and *Citrus sinensis*, followed by an evaluation of the antioxidant activity of these fruit extracts by different methods: DPPH, ABTS, GOR, reducing power and CUPRAC. The extracts were then tested *in vitro* for their anti-inflammatory effect via the bovine serum albumin (BSA) denaturation test. The chemical composition, ascorbic acid content, electrochemical characterization and antioxidant capacity of *Rosa canina* fruit extract were determined. In addition, the *in vitro* antioxidant activity of *R. canina* fruit extract and its protective effect on hydrogen peroxide (H_2O_2)-stressed human fibroblast cells were evaluated using MTT assay.

In the second part, we studied the protective, anti-inflammatory and anti-homocysteinemic effect of the fruits: *Citrus sinensis*, *Rosa canina* and vitamin C on the intestinal inflammation induced by high doses of L-methionine during the 21 days *in vivo* experimental procedure. The levels of plasma inflammatory markers (hs-CRP, homocysteine), reduced glutathione (GSH) and catalase (CAT) in liver tissue were measured, and histological sections of intestinal tissue were examined.

The results show that both extracts are rich in polyphenols and have significant antioxidant power. Both extracts prevented the denaturation of BSA in a dose-dependent manner indicating that both extracts have anti-inflammatory activity, which was confirmed by the *in vivo* anti-inflammatory study. The extract of *Rosa.canina* fruit indicates the presence of n-hexadecanoic acid, oleic acid and stearic acid, which were identified as the main compounds of this fruit. This fruit is rich in ascorbic acid and has an important antioxidant capacity. Moreover, the results show that *Rosa canina* fruit extract has a non-toxic effect on the viability of human fibroblasts with a protective effect against oxidative stress induced by hydrogen peroxide on fibroblasts. In addition, treatment with fruits and vitamin C led to a decrease in the level of plasma homocysteine, an increase in the levels of GSH and CAT. This was confirmed by the histological study of the restoration of the integrity of the intestinal epithelium.

This study revealed that the fruits of *Citrus sinensis* and *Rosa canina* have antioxidant and anti-inflammatory powers and can be considered as a natural source to prevent and treat IBD.

Keywords: IBD, hyperhomocysteinemia, fruits, *Rosa canina*, *Citrus sinensis*, antioxidant and anti-inflammatory activity.

Research laboratory: Laboratory for obtaining therapeutic substances, Constantine (Algeria).