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Evaluation of the antihyperlipidemic, anti-inflammatory and hepatoprotective effects of *Punica granatum* in mice

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Dedication

I dedicate this work...

To my beloved parents who have always been my source of inspiration and strength, for their endless love, continuous support and care, Thank you for everything.

To my brother and partner, Youcef Ayoub.
Together always to the top.

To my dear sister Asma, my brother Younes and my little princess Rania. I'm forever grateful to have you by my side.

To Haithem, Abdelhafid, Abdennour, Mehdi, Oussama and Abdallah, your friendship is always a source of comfort and joy, thank you for always being there.

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To my professors in med school, my mentors and colleges in Constantine University Hospital and Constantine Regional Military Hospital.

"Last but not least… I wanna thank me. I wanna thank me for believing in me. I wanna thank me for doing all this hard work. I wanna thank me for having no days off."

The search for knowledge never ends.

Zakarya Djama

Dedication

To my dear family,

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Youcef Ayoub Djama

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Contents

Abbreviations

- **Acetyl-CoA:** Acetyl coenzyme A
- **ALAT:** Alanine aminotransferase
- **ASAT:** Aspartate aminotransferase
- **CEH:** Cholesterol ester hydrolase
- **COX-2:** Cyclooxygenase-2
- **CRP:** C-reactive protein
- **DMAPP:** Dimethylallyl pyrophosphate
- **FPP:** Farnesyl pyrophosphate
- **HDL:** High-density lipoprotein
- **HMG-CoA:** 3-Hydroxy-3-methylglutaryl coenzyme A reductase
- **IDL:** Intermediate-density lipoprotein
- **IL-8:** Interleukin-8
- **LDL:** Low-density lipoprotein
- **LPL:** lipoprotein lipase enzyme
- **MCP-1:** Monocyte chemotactic protein-1
- **NSAIDs:** Nonsteroidal anti-inflammatory drugs
- **ONAB:** Office national des aliments du bétail
- **PAF:** Platelet activating factor
- **PG:** *Punica granatum*
- **RNS:** Reactive nitrogen species
- **ROS:** Reactive oxygen species
- **RPM:** Revolutions per minute
- **SQS:** Squalene synthase
- **SR-B1:** Scavenger receptor B1
- **TC:** Total cholesterol
- **TFA:** Trans-fatty acids
- **TG:** Triglycerides
- **TNF-α:** Tumor necrosis factor-alpha
- **VLDL:** Very-low-density lipoprotein

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Introduction

Introduction

Cardiovascular diseases (CVDs) represent a significant public health concern, accounting for over 17 million deaths annually, which equates to approximately 30% of global mortality. CVDs are linked to various cardio-metabolic risk factors, including dyslipidemia, diabetes, hypertension, obesity, physical inactivity, and smoking (**Capewell et al., 2010).**

In Algeria, as in all other countries, cardiovascular conditions occupy a preponderant position in both morbidity and mortality **(Ngwasiri et al., 2023).**

Dyslipidemia is a very common metabolic disorder characterized by elevated levels of triglycerides (TG), total cholesterol, low-density lipoprotein cholesterol (LDL-c), and reduced levels of high-density lipoprotein cholesterol (HDL-c). Currently, it is well established that hypercholesterolemia contributes to the development of atherosclerosis. The higher the cholesterol level, the greater the risk of cardiovascular complications **(Pan et al., 2016)**.

Clinical and experimental studies have revealed that high levels of LDL-C are associated with atherosclerosis and an increased risk of cardiovascular events. Furthermore, several hypotheses suggest that HDL-C concentrations exert an antiatherogenic effect, by reducing LDL oxidation **(Ferretti et al., 2009).**

Numerous studies have shown that atherosclerosis can be experimentally induced in animals by administering hypercholesterolemic diets. Indeed, in both animal models (mice, rats, rabbits) and humans, a cholesterol-rich diet leads to significant metabolic changes, including severe hypercholesterolemia, inflammatory responses, and oxidative stress.

This stress is characterized by an imbalance caused essentially by an exaggerated production of reactive oxygen species (ROS), which are responsible for biological alterations, such as lipid peroxidation, protein oxidation and a deficit in antioxidant defenses.

Medicinal plants have always been used to prevent or treat various diseases. They are used throughout the world, in traditional medicine, for their hypoglycemic, hypolipidemic and antioxidant activities **(Nasar et al., 2009).**

This study focuses on one such medicinal plant, *Punica granatum* (Pomegranate), which is found in several regions of Algeria. All parts of the plant are extensively used in traditional medicine for their antioxidant, antimicrobial, anti-cancer, and anti-diabetic effects **(Ahmed et**

al., 2005). Interest in natural antioxidants and their therapeutic properties has increased considerably, prompting scientific research to extract, identify, and quantify these compounds from various natural substances **(Huang et al., 2005).**

The present study aims to investigate the antihperlypedimic effect of *Punica granatum* peel powder on experimental hypercholesterolemia induced by a hyperlipidic and hypercaloric diet in *Mus musculus* mice. The goal is to prevent hypercholesterolemia and its associated disorders.

For this, various parameters will be measured, including total cholesterol, HDL-c, LDL-c triglycerides, hepatic transaminases (ASAT / ALAT), and CRP.

Additionally, the study will evaluate the impact of different treatments on the weight and food consumption of the animals.

CHAPTER 01: Hypercholesterolemia and inflammation

CHAPTER 1: Hypercholesterolemia and inflammation

I. General information about cholesterol

I.1. Definition

The name cholesterol originates from the Greek words: "*chole*-" (meaning bile) and "*stereos*" (meaning solid), and the chemical suffix "*-ol*" indicating an alcohol **(Mathew et al., 2008)**

Cholesterol is the main mammalian sterol. It is a waxy, fat-like substance present in all animals including humans and is vital for the functioning of every cell in the body.

Cholesterol serves as the precursor for steroidal hormones such as estrogen and testosterone. Additionally, it is a component of bile, which aids in the digestion of fats. Skin contains a unique type of cholesterol that, in the presence of sunlight, can convert into vitamin D. It also serves as a structural component of plasma membranes, where it regulates membrane fluidity, acts as a solubilizer of other lipids, and serves as a signaling mediator **(Kawakami et al., 2017).**

I.2. Structure

The molecular formula of cholesterol is $C_{27}H_{46}OH$, it consists of four carbon rings named: A, B, C and D. It has a hydroxyl group (-OH) on carbon 3 (C3), this group constitutes the polar head and therefore the hydrophilic part of cholesterol. Cholesterol molecular weight is: 386.65354 g/mol **(Figure 01) (Li et al., 2019).**

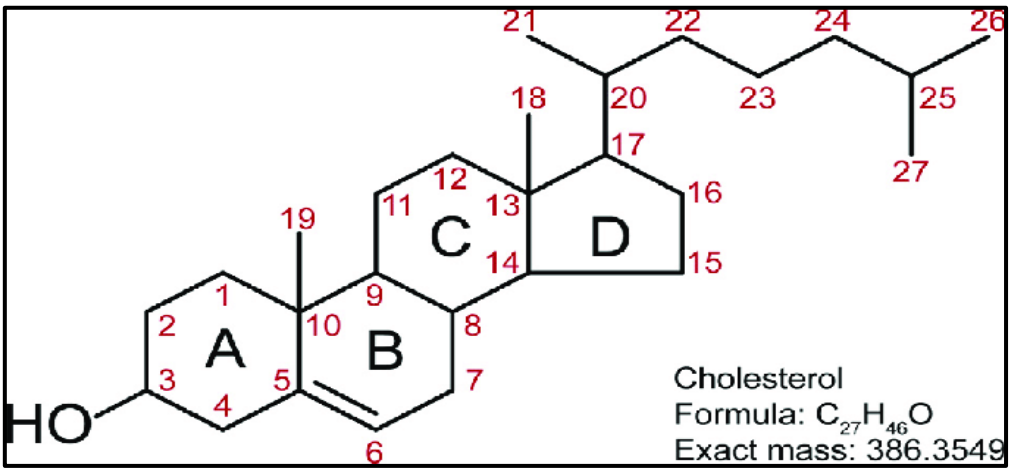

Figure 01: Structure of cholesterol with the numbering of the carbon atoms **(Li et al., 2019).**

I.3. Biological role of cholesterol

Contrary to its commonly perceived role as a significant health threat, cholesterol is actually a vital substance that serves many important purposes in the body. It provides multiple biological functions and is required for proper cellular homeostasis **(Luo et al., 2020).**

All steroid hormones, including glucocorticoids, mineralocorticoids, and sex hormones, are derived from cholesterol. Synthesis takes place in the placenta and ovaries for the production of estrogens, in the testicles for the production of testosterone, and in the adrenal cortex for the production of cortisol, aldosterone, and androgens **(Schade et al., 2020).**

It helps to maintain the stability of membrane fluidity and plays a role in membrane trafficking processes, which are necessary for the regulation of many trans-membrane signaling pathways.

Cholesterol is also involved in bile acid synthesis, which is essential for the absorption of fats, and it serves as a precursor of vitamin D synthesis in the skin.

Furthermore, it is vital for sperm development, immune system defense and the development and functioning of the central nervous system **(Mayengbam et al., 2021) (Figure 02).**

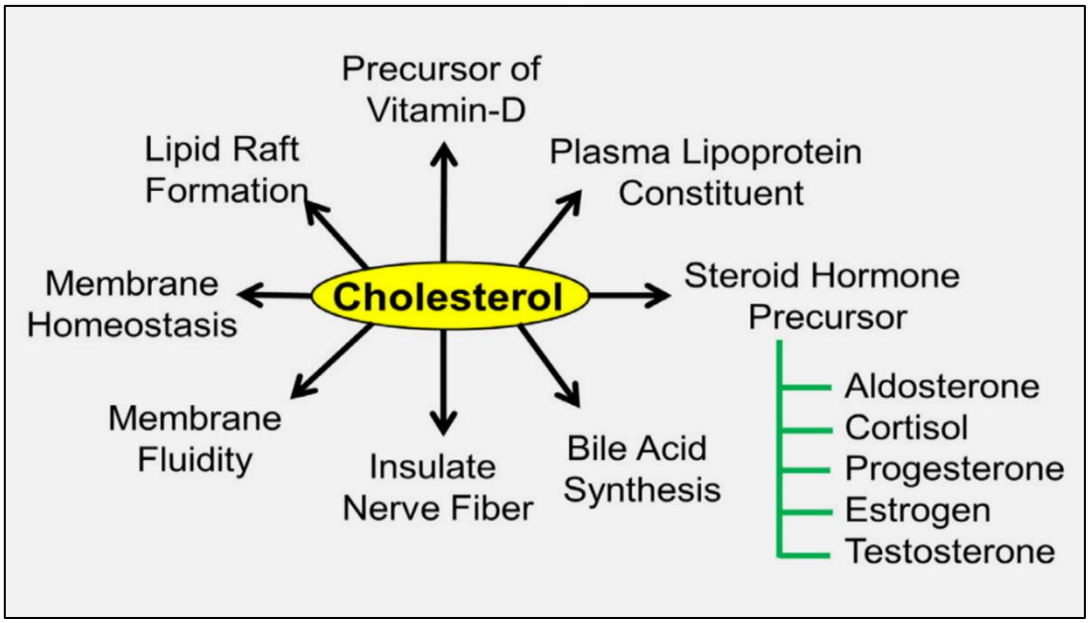

Figure 02: Essential biological functions of cholesterol **(Mayengbam et al., 2021).**

I.4. Transport of cholesterol in the blood

Since cholesterol is a water-insoluble molecule, it needs to be packed in order to be transported throughout the plasma. Transport in the bloodstream is facilitated by lipoproteins, which are spheroidal macromolecules containing proteins (apolipoproteins) and lipids (cholesterol or triglycerides), which allow the lipid component to be soluble in plasma **(Hegele, 2009) (Figure 03).**

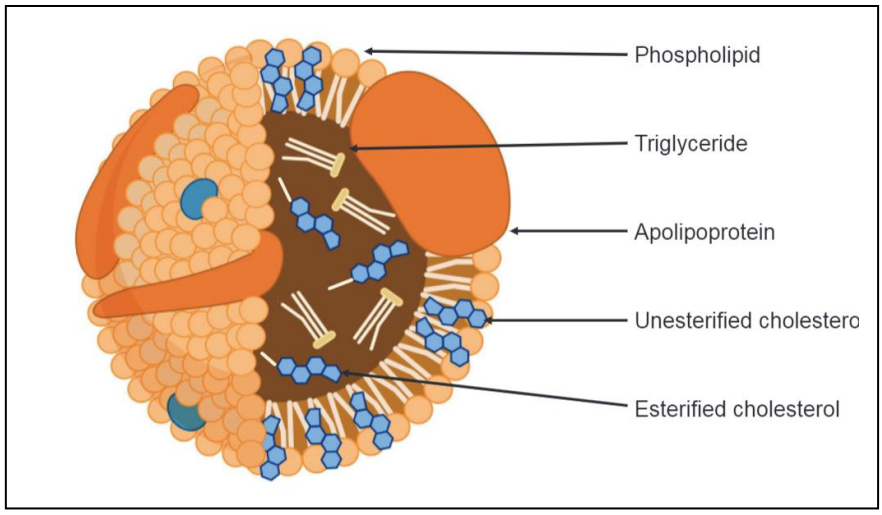

Figure 03: Structure of a lipoprotein particle **(Doi et al., 2023).**

There are five main classifications of lipoproteins in ascending order of density: Chylomicrons, Very Low-Density Lipoproteins (VLDL), Intermediate-Density Lipoproteins (IDL), Low-Density Lipoproteins (LDL), and High-Density Lipoproteins (HDL) **(Doi et al., 2023).**

Table 01: Lipoprotein classes and their density, size, composition and major lipid components **(Feingold, 2021).**

Lipoprotein	Density (g/ml)	Size (nm)	Composition		Major Lipids
			Protein	Lipid (%)	
Chylomicrons	<0.930	75-1200	1-2	98-99	Triglycerides
VLDL	0.930- 1.006	30-80	7-10	90-93	Triglycerides
IDL	1.006- 1.019	25-35	11	89	Triglycerides Cholesterol
LDL	1.019- 1.063	18- 25	21	79	Cholesterol
HDL	1.063- 1.210	5- 12	40-55	45-60	Cholesterol Phospholipids

Dietary cholesterol is absorbed into the lumen of the small intestine. It is solubilized in micelles by bile acids. Then, inside the enterocyte, cholesterol is esterified, packaged into chylomicrons, and transported to the liver. Hepatic cholesterol is incorporated into VLDL particles, which are secreted into the blood and hydrolyzed by plasma lipases to form IDL.

IDL particles are then converted into LDL, which are taken up by tissues expressing LDL receptors: the liver and other extra-hepatic tissues **(Giacomini et al., 2021).**

The elimination of cholesterol from extrahepatic cells is carried out by HDL particles, which accumulate and transport cholesterol esters to the liver, adrenal glands and gonads. Cholesterol esters are then transformed into free cholesterol by cholesterol ester hydrolase (CEH) for the synthesis of steroid hormones, bile acids in the liver and finally cholesterol excretion **(Röhrl et al., 2013).**

1.5. Low- and high-density lipoproteins

1.5.1. Low-density lipoprotein

Low-density lipoprotein (LDL) particles are formed when triglycerides are removed from VLDL by the lipoprotein lipase enzyme (LPL). LDLs transport most of the circulating cholesterol and constitute approximately 50% of the total lipoprotein mass in plasma. They transport cholesterol around the body to where it is needed **(Heidelbaugh, 2008)**

Each LDL particle contains one single apolipoprotein, which is Apo B-100, and lipids (21% and 79% of mass, respectively) **(Feingold, 2021)**. The lipid part consists of 6-8% free cholesterol, 45-50% cholesteryl ester, 18-24% phospholipid, and 4-8% triacylglycerols **(Hegele, 2009).**

Often referred to as "bad cholesterol" due to its association with the development of atherosclerosis, which is the cause of many cardiovascular diseases **(Katzmann et al., 2024).**

1.5.2. High-density lipoprotein

High-density lipoprotein (HDL) are small particles secreted from the liver and the intestine and consist of 50% protein (mostly apolipoprotein A-I and A-II), 20% cholesterol, 30% phospholipids, and only traces of triglycerides **(Heidelbaugh, 2008).**

These particles have a crucial function in the process of reverse cholesterol transport, which involves moving cholesterol from peripheral tissues to the liver to be catabolized, which is the mechanism by which HDL is considered as anti-atherogenic. In addition, HDL particles

have anti-oxidant, anti-inflammatory and anti-thrombotic properties, which contributes to their ability to reduce the risk of atherosclerosis and cardiovascular diseases **(Feingold, 2021)**. Therefore, often referred to as "good cholesterol" **(Wang et al., 2022).**

I.6. Sources of cholesterol

Cholesterol comes from two sources; endogenous source by biosynthesis "*de novo* synthesis" from two acetyl-CoA molecules and an exogenous source from the diet, with a ratio of around 70:30. This ratio varies between individuals depending on their genetic predisposition and dietary cholesterol intake **(Ikonen, 2008).**

I.7. Cholesterol biosynthesis and its regulation

I.7.1. Cholesterol biosynthesis

Cholesterol biosynthesis is a complex process, which occurs virtually in every mammalian cell, with liver and intestine being the anatomical sites responsible for more than 50% of total cholesterol biosynthesis. It involves a series of more than twenty enzymatic reactions taking place in several sub-cellular compartments **(Cerqueira et al., 2016 ; Giacomini et al., 2021) (Figure 04).**

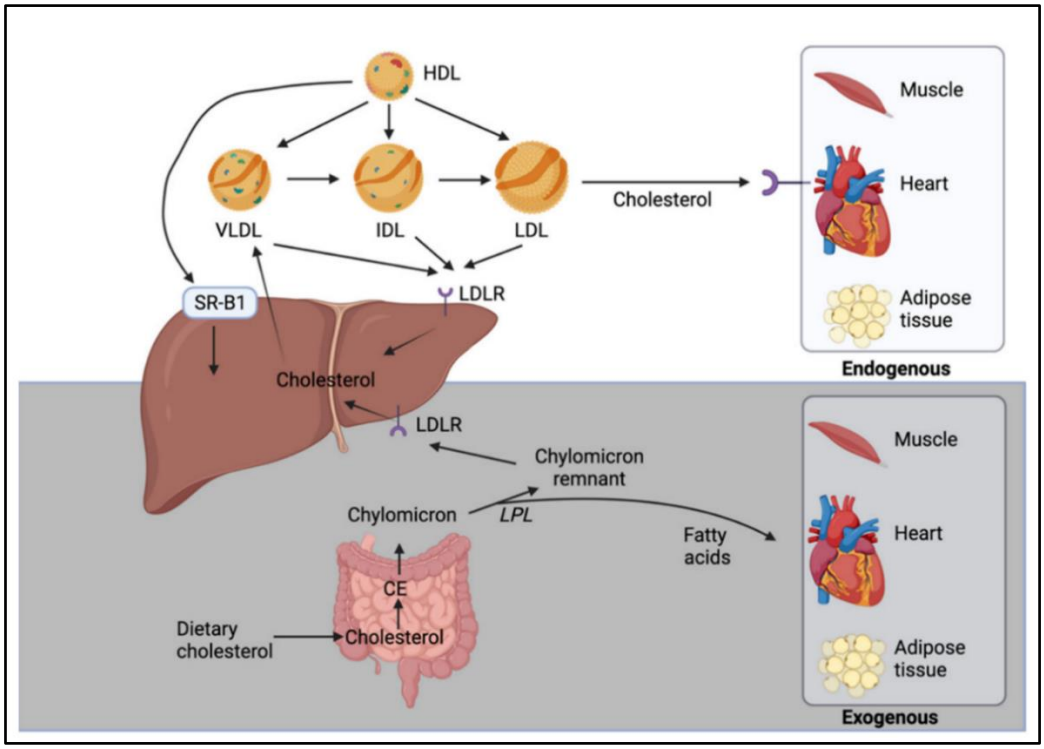

Figure 04: The endogenous (white) and exogenous (gray) pathways of cholesterol biosynthesis **(Vourakis et al., 2021).**

The levels of cholesterol in the plasma are maintained by biosynthesis through the endogenous pathway and absorption of dietary and biliary cholesterol through the exogenous pathway. In the endogenous pathway, cholesterol is synthesized by the liver and extrahepatic tissues and secreted into plasma, whereas the intestine is the primary site of the exogenous pathway of dietary cholesterol uptake. Alteration of either pathway will affect the concentration of plasma cholesterol **(Shepherd, 2001).**

I.7.1.1. Exogenous pathway

The exogenous pathway begins with the absorption from the diet in the intestines. The ultimate outcome is the transportation of fatty acids to adipose and muscular tissue, as well as the transportation of cholesterol to the liver.

After absorption, cholesterol undergoes re-esterification within the cells of the intestinal mucosa. It is then combined with different apoproteins and phospholipids to form lipoprotein particles known as chylomicrons. The chylomicrons are released into the intestinal lymph, then they enter the bloodstream through the thoracic duct, and finally attach to the walls of capillaries in adipose and skeletal muscle tissue. At these specific sites where the chylomicrons attach, they come into contact with the enzyme lipoprotein lipase. This enzyme then hydrolyses the triglyceride core and releases free fatty acids **(Cox et al., 1990).**

Following the elimination of the triglyceride core, remnant chylomicron particles are eliminated from the bloodstream and transported into the interior of hepatic cells. After a sequence of reactions, these substances are either transformed into bile acids, eliminated in bile, or integrated into lipoproteins that are originated in the liver (VLDL).

After a meal, chylomicrons are present in plasma for one to five hours under normal physiological conditions, which give it a milky appearance. Typically, they are eliminated from the bloodstream following a 12-hour fast **(Cox et al., 1990).**

I.7.1.2. Endogenous pathway

The endogenous pathway of cholesterol is a series of biochemical reactions that occur within cells to synthesize cholesterol. It’s a complex process that can be summed into 3 steps:

1. three molecules of acetyl-CoA condense to form HMG-CoA, which is then reduced to mevalonate by the first step-limiting enzyme HMG-CoA reductase (HMGCR).
2. subsequent reactions allow the transformation of mevalonate into FPP, an isoprenoid that generates squalene in a reaction catalyzed by squalene synthase (SQS).

3. squalene is then converted by the second rate-limiting enzyme squalene epoxidase (SQLE) into its epoxydic form, which is eventually cyclized to lanosterol by the enzyme lanosterol synthase. Additional reactions that are based on oxygen ultimately result in the synthesis of cholesterol. **(Giacomini et al., 2021) (Figure 05).**

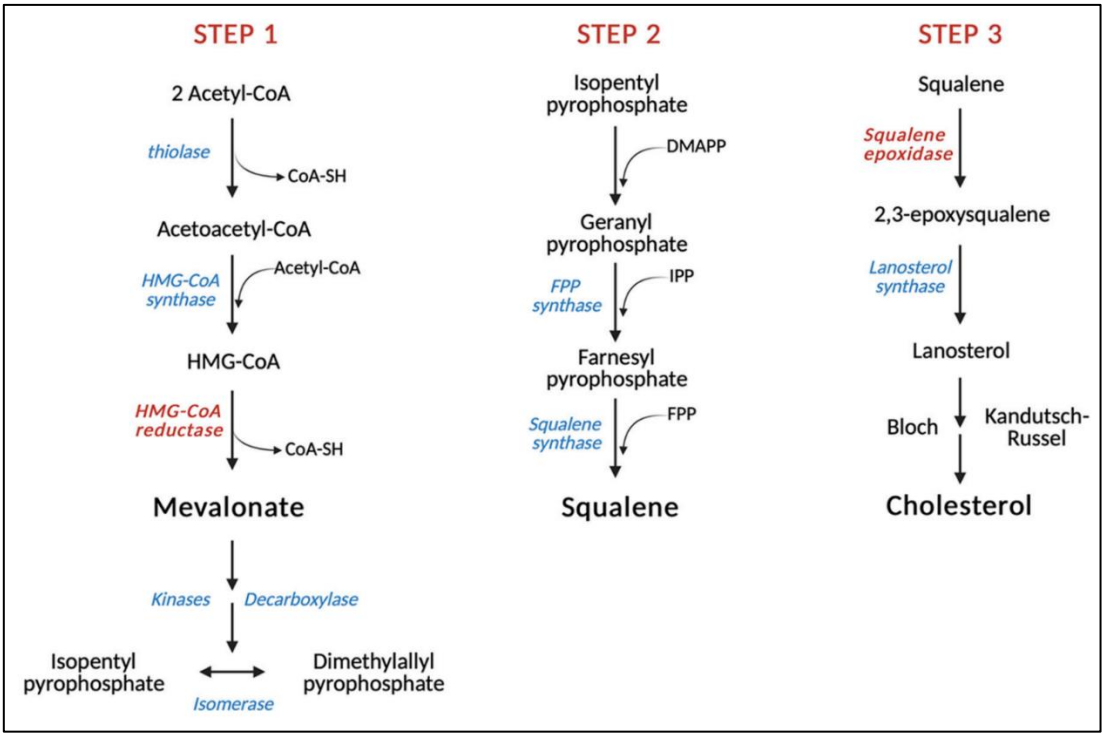

Figure 05: Schematic representation of the endogenous pathway of cholesterol biosynthesis **(Giacomini et al., 2021).**

I.7.2. Regulation of cholesterol biosynthesis

Cholesterol biosynthesis is regulated at multiple levels, including transcriptional, translational, and post-translational mechanisms. One key enzyme in cholesterol biosynthesis is HMG-CoA reductase, which is regulated by feedback inhibition by cholesterol and its metabolites **(Bhattarai et al., 2021).**

The key regulation step involves the conversion of HMG-CoA to mevalonate in the presence of HMG-CoA reductase. The enzyme HMG-CoA reductase acts as the rate-limiting enzyme and regulates the excessive production of cholesterol through a feedback mechanism. When the amount of cholesterol inside a cell increases, the levels of HMG-CoA reductase decrease by lowering the gene transcription of this enzyme.

The enzyme HMG-CoA reductase is also controlled by certain hormones like glucagon and insulin. The dephosphorylated form of HMG-CoA reductase which is the activated form is promoted by insulin thereby activating the enzyme and in turn increasing cholesterol synthesis,

whereas phosphorylation which is the inactivated form is triggered by glucagon thereby deactivating the enzyme and in turn reducing cholesterol synthesis. Insulin therefore stimulates cholesterol synthesis and, on the contrary, glucagon inhibits it **(Ness, 2015).**

II. Hypercholesterolemia

II.1. Blood cholesterol levels

Blood cholesterol levels are measured after a 12-hour fast except for water, using a lipid panel or lipid profile, which typically reports: Total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), and triglycerides (TG) **(Barter et al., 2007).**

Total and HDL cholesterol are measured directly from serum, while the value of LDL cholesterol is obtained by the Friedewald equation which is:

LDL cholesterol = (total cholesterol) - (high-density lipoprotein cholesterol [HDL-C]) - (triglycerides/5) in mg/dL or (triglycerides/2.2) in mmol/l

The measured LDL-C level is not totally accurate in patients with hypertriglyceridemia (up to 400 mg/dl or 4.5 mmol/l). In this case, a dosage of Apo-B proteins is therefore indicated, which can give an estimate of the LDL-C present in the blood **(Lee et al., 2020).**

Table 02: Acceptable, borderline, and high blood cholesterol levels for adults **(Grundy et al., 2019).**

Evaluation	Total cholesterol	HDL cholesterol	LDL cholesterol	Triglycerides
Good	Less than 200 (the lower, the better)	Ideal is 60 or higher; but, 40+ for males or 50+ for females is acceptable	Less than 100; below 70 if coronary artery disease is present	Less than 149; ideal is under 100
Borderline to moderately elevated	200-239	n/a	130-159	150-199
High	240 or higher	60 or higher	160 or higher; 190 is considered very high	200 or higher; 500 is considered very high
Low	n/a	Less than 40 for men and less than 50 for women	n/a	n/a

All values are in mg/dl (milligrams per deciliter) and are based on fasting measurements.

II.2. Normal cholesterol level variations

II.2.1 Within-day variation

An individual's blood cholesterol values can vary about 2-3% within the same day. Cholesterol levels are also reduced temporarily as a response to extreme pain, surgical procedures, and short-term physical effort **(Bookstein et al., 1990).**

II.2.2 Age and gender

Age and sex have an impact on cholesterol levels. Females under the age of 20 have greater amounts of cholesterol compared to males. Typically, adult males aged 20 to 45 have higher levels compared to females of the same age. Total cholesterol, LDL, and triglyceride levels rise with age, regardless of gender. The highest levels in men often occur between the ages of 40 and 60, whereas in women, they tend to occur between the ages of 60 and 80 **(Greenland et al., 1990).**

II.2.3 Seasonal variation

Cholesterol levels tend to be higher in the winter and lower in the summer independent of the country of origin, ethnicity, age, sex, and baseline lipids. The seasonal variation can reach 12% **(Kelly, 2005).**

Although the mechanism for this phenomenon is not fully clear, it is supposed to be linked to increases in temperature and/or physical activity and such variation could result in larger numbers of people being diagnosed as having hypercholesterolemia during the winter **(Ockene et al., 2004).**

II.2.4 Capillary vs. Venous sampling

Total cholesterol measured from capillary plasma (obtained via a finger stick) tends to run 2 to 4% higher than venous plasma cholesterol **(Sblendorio et al., 2008).**

II.3. Pathological variations "Hypercholesterolemia"

II.3.1 Definition of hypercholesterolemia

Hypercholesterolemia is a specific type of hyperlipidemia which refers to high blood cholesterol levels, by increase of low-density lipoprotein (LDL) **(Sharifi et al., 2019).**

It can be defined as a LDL-cholesterol level over 190 mg/dl, over 160 mg/dl with one major risk factor, or over 130 mg/dl with two cardiovascular risk factors **(Ibrahim et al., 2024).**

The major risk factors include:

- Age; males aged 45 years or older, females aged 55 years or older;
- A familial predisposition to early-onset atherosclerotic cardiovascular disease (occurring before the age of 55 in males and before the age of 65 in females);
- Diabetes;
- Hypertension;
- Smoking;
- Low levels of HDL-cholesterol (less than 40 mg/dl in males and less than 55 mg/dl in females) **(Ibrahim et al., 2024).**

Hypercholesterolemia is an issue faced in many communities and a source of concern for health experts, since it is one of the leading risk factors for the development of cardiovascular disorders such as atherosclerosis, acute myocardial infarction, and hypertension **(Matos et al., 2005).**

II.3.2. Causes of hypercholesterolemia

Hypercholesterolemia can be either due to primary (genetic or familial), or secondary (acquired) causes **(Al-Zahrani et al., 2021).**

II.3.2.1 Primary causes “Genetics”

Genetic contributions usually result from the combined impacts of numerous genes, referred to as "polygenic. However, in specific cases, they might be attributed to a single gene deficiency, as observed in familial hypercholesterolemia **(Bhatnagar et al., 2008).**

Familial hypercholesterolemia is a classical genetic disorder caused by mutations in the LDL-receptor gene. This leads to LDL-C levels higher than 190 mg/dl in heterozygotes and higher than 450 mg/dl in homozygotes. It is a result of loss-of-function mutations in the gene that encodes for the LDL receptor. The decrease in LDL receptor activity in the liver leads to a reduced rate of LDL clearance from the bloodstream. Therefore, he increased concentrations of LDL-C in familial hypercholesterolemia mostly result from a slower removal of LDL from the bloodstream **(Ibrahim et al., 2024).**

Other genetic factors include defective apolipoprotein B, proprotein convertase subtilisin/kexin type 9 gene gain-of-function mutation, LDL receptor adaptor protein mutation and polygenic hypercholesterolemia **(Al-Zahrani et al., 2021).**

II.3.2.2 secondary causes

Secondary (acquired) causes include excessive intake of dietary cholesterol. A diet high in saturated fats and sugar increases total cholesterol and LDL **(DiNicolantonio et al., 2016).** Trans fats or trans-fatty acids (TFA) are the result of partial hydrogenation of unsaturated fat. They have been shown to reduce levels of HDL while increasing levels of LDL. (Marchand et al., 2010) Industrially produced TFAs can be found in margarine, fried foods and baked goods such as biscuits and pies **(Dhaka et al., 2011).**

Other secondary causes are linked to medical conditions like hypothyroidism, diabetes mellitus, nephrotic syndrome, and cholestasis in addition to some medications such as cyclosporine and thiazide diuretics **(Bhatnagar et al., 2008).**

II.3.3. Consequences of hypercholesterolemia

The consequences of hypercholesterolemia are significant and can lead to various health issues **(Ibrahim et al., 2024).**

a- Hypercholesterolemia is associated with arteriosclerosis development. Cardiovascular diseases predominantly myocardial infarction and stroke remain the main cause of death worldwide **(Collado et al., 2021) (Figure 06).**

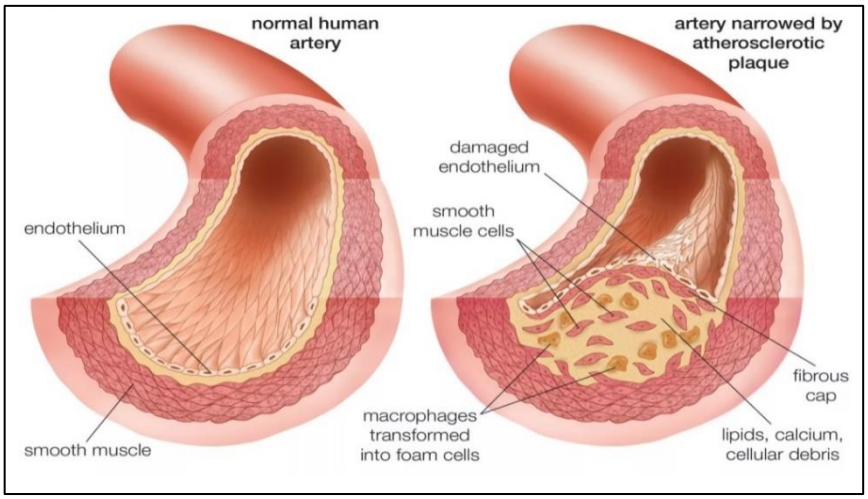

Figure 06: Normal artery vs. Artery narrowed by atherosclerotic plaque **(Collado et al., 2021).**

b- Hypercholesterolemia leads to an inflammatory response within the microvascular system, reflected by endothelial cell activation, leukocyte recruitment, rolling and adherence, as well as platelet activation and adhesion **(Stokes et al., 2007).**

c- Excess cholesterol in the brain can lead to brain strokes and has been linked to memory loss and mental function decline. High cholesterol may also accelerate the

formation of beta-amyloid plaques in people with Alzheimer's disease **(Xu et al., 2020).**

II.3.4. Treatment of hypercholesterolemia

The foundation for treating hypercholesterolemia involves maintaining a healthy lifestyle, achieving an optimal weight, avoiding smoking, engaging in 150 minutes of exercise weekly, and following a diet that is low in saturated and trans fats but high in fiber, fruits, vegetables, and fatty fish.

The drug class of choice is the Statin which is capable of reducing LDL-C by 22% to 50%. They have also been proven to lower the risk of cardiovascular events as both primary and secondary prevention **(Ibrahim et al., 2024).**

III. Inflammation

III.1. Definition

Inflammation is the immune system's response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation **(Medzhitov, 2010)**, and acts by removing injurious stimuli and initiating the healing process. It’s an innate defense mechanism and a protective response involving immune cells, blood vessels, and molecular mediators. **(Nathan et al., 2010)**. The cardinal signs of inflammation are explained by increased blood circulation, secretion of soluble mediators, vasodilatation, increased cellular metabolism, extravasation of fluids and cellular influx **(Ferrero-Miliani et al., 2007).**

Although inflammation is a natural defense mechanism, most human disorders such as cancer, atherosclerosis and autoimmune diseases are caused by acute and chronic inflammatory responses **(Ratan et al., 2022).**

III.2. Types of inflammation

Inflammation can divide into three types based on the time of the process that responds to the injurious cause; acute which occurs immediately after injury and lasts for few days, chronic inflammation that may last for months or even years when acute inflammation fails to settle, and subacute which is a transformational period from acute to chronic which lasts from 2 to 6 weeks **(Chen et al., 2018).**

III.2.1 Acute inflammation

Acute inflammation is an immediate, adaptive response with limited specificity caused by several noxious stimuli. Typically, as its name indicates (Latin word “*acutus”*, meaning

"sharp"), it has a sudden start and lasts for a brief period of time. It persists for few hours or days **(Damjanov, 2008).**

Acute inflammation starts after a specific injury that causes soluble mediators like cytokines, acute phase proteins, and chemokines to promote the migration of neutrophils which are the predominant cell type in almost all acute inflammatory reactions, and macrophages to the area of inflammation, , which results in local vasodilatation, with increased blood flow to the affected area resulting in redness and heat, increased vascular permeability, resulting in exudation of fluid into the interstitial tissue and swelling, and release of numerous mediators which recruit further inflammatory cells to the site and cause pain and loss of function **(Chen et al., 2018).**

The controlled acute inflammatory response is generally beneficial, and this can be seen clearly in providing protection against infectious organisms. However, it can become detrimental if not regulated, such as seen in septic shock **(Chen et al., 2018).** Or proceeds to a chronic inflammatory response when the initial infiltration of neutrophils is unable to eliminate the harmful initiating agent **(Fleit, 2014) (Figure 07).**

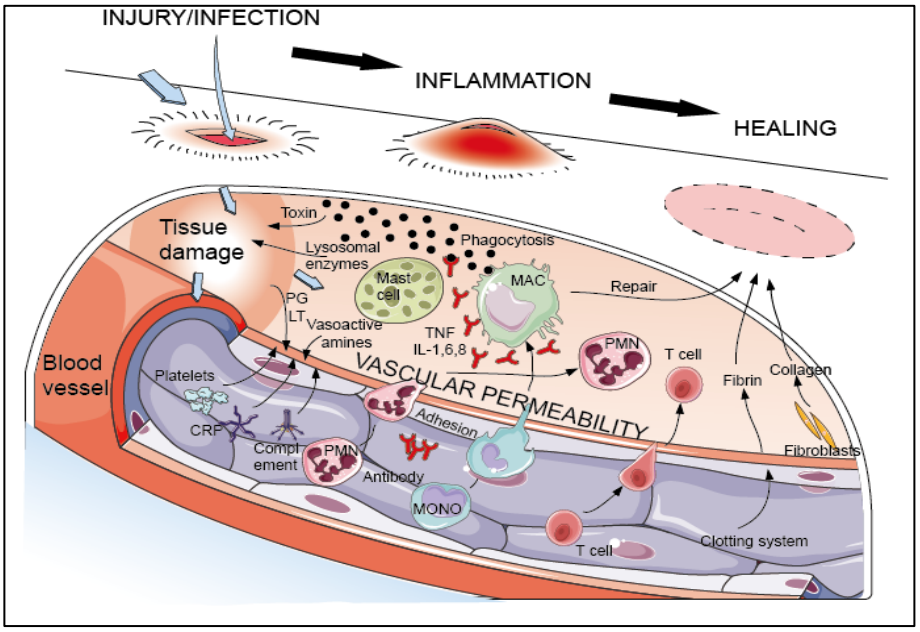

Figure 07: Cells and molecular mediators involved in acute inflammation **(Damjanov, 2008).**

III.2.2 Chronic inflammation

Chronic inflammation represents a long-term reaction (Greek chronos, ''time'') to an inflammatory stimulus characterized by continued recruitment of mononuclear leukocytes (monocytes and lymphocytes). Chronic inflammatory responses last for long periods of time,

varying from months, to years, to a lifetime in the case of certain chronic inflammatory diseases such as atherosclerosis, autoimmune diseases, cancers and some neurodegenerative diseases **(Damjanov, 2008)**

In contrast to acute inflammatory responses which mainly involve the recruitment of neutrophils from the blood, chronic inflammation is characterized by the continuous recruitment of circulating mononuclear leukocytes, including monocytes and populations of T lymphocytes. After leaving the bloodstream, monocytes differentiate into macrophages. In addition to the accumulation of macrophages and lymphocytes, chronic inflammatory can lead to the proliferation of fibroblasts; These fibrotic alterations can gradually cause organ dysfunction **(Fleit, 2014).**

Table 03: Acute vs. Chronic inflammation **(Šoltés et al., 2010).**

Inflammation	Acute	Chronic
Causative agent	Pathogens, injured tissues	Persistent acute inflammation due to non-degradable pathogens, persistent foreign bodies, or autoimmune reactions
Major cells involved	Neutrophils, mononuclear cells (monocytes, macrophages)	Mononuclear cells (monocytes, macrophages, lymphocytes, plasma cells, fibroblasts)
Primary mediators	Vasoactive amines, eicosanoids	INF- γ and other cytokines, growth factors, reactive oxygen species, hydrolytic enzymes
Onset	Immediate	Delayed
Duration	Few days	Up to many months or years
Outcomes	Resolution, abscess formation, chronic inflammation	Tissue destruction, fibrosis

III.3. Mediators of inflammation

Various chemical mediators from circulation system, inflammatory cells, and damaged tissue actively contribute to and regulate the inflammatory response.

A mediator may be defined as an endogenous chemical agent, which takes an active part in the development of the inflammatory response **(Abdulkhaleq et al., 2018).**

III.3.1. Cellular component

The cellular component consists of leukocytes, which generally reside in the bloodstream and must reach the inflamed tissue via extravasation to aid in inflammation. Some act as phagocytes, ingesting bacteria, viruses, and cell debris. Others secrete enzymatic granules that damage pathogenic invaders. Leukocytes also secrete inflammatory mediators that initiate and sustain the inflammatory response.

In general, acute inflammation is mediated by granulocytes, while chronic inflammation is mediated by mononuclear cells like lymphocytes and monocytes.

These cellular components interact dynamically with soluble mediators of inflammation to mount an effective immune response, eliminate pathogens, and promote tissue repair **(Serhan et al., 2005).**

III.3.2. Soluble mediators of inflammation

Soluble mediators are proteins that play crucial roles in orchestrating the inflammatory response. They are soluble in bodily fluids such as blood or interstitial fluid and act as signaling molecules to regulate various aspects of inflammation. Soluble mediators include cytokines, chemokines, lipid mediators (such as prostaglandins and leukotrienes), histamine, and other signaling molecules that can either promote or suppress inflammation **(Serhan et al., 2005).**

The type of immune response (either pro- or anti-inflammatory, cell mediated or humoral) induced by these molecules primarily depends on the early cytokines that are generated in response to bodily injury or invasion by pathogens **(Shachar et al., 2013).**

Even though soluble mediators are often successful at resolving inflammation, abnormal signaling of cytokines and chemokines signaling have been shown to play a role in numerous autoimmune disorders **(Alvine et al., 2015).**

III.4. Treatment of inflammation

There are several treatments for inflammation, depending on the cause and severity.

For acute inflammation caused by injury or infection, nonsteroidal anti-inflammatory drugs (NSAIDs) like ibuprofen and aspirin can help reduce pain and swelling.

For chronic inflammation associated with autoimmune disorders and other conditions, prescription medications are often needed. These can include disease-modifying anti-rheumatic

drugs (DMARDs) like methotrexate, and Corticosteroids in pill form or injections, but only for short periods due to side effects.

Lifestyle changes can also help reduce chronic inflammation such as:

- Getting regular moderate exercise for at least 20 minutes per day and maintaining a healthy weight.
- Using stress-reduction techniques like mindfulness meditation.
- Eating an anti-inflammatory diet rich in olive oil, nuts, fatty fish, fruits and vegetables **(Serhan, 2017).**

III.5. Link between hypercholesterolemia and inflammation

Inflammation and hypercholesterolemia are two conditions that are increasingly being recognized as interconnected. They can interact in a vicious cycle, with each condition exacerbating the other **(Collado et al., 2019).**

High levels of cholesterol can cause inflammation in the blood vessels, which can lead to the formation of plaques that narrow and stiffen the arteries. These plaques can rupture, leading to the formation of blood clots that can cause cardiovascular diseases. The progression of hypercholesterolemia is linked to endothelial cell dysfunction and increased oxidant stress. Cholesterol has been proven to disrupt and modify vascular structure as it accumulates within the lining of the vascular wall, and can interfere with endothelial function resulting to lesions, plaques, and emboli, as well as the formation of a highly pro-inflammatory condition **(Stapleton et al., 2010).**

In the other hand, inflammation can contribute to the development of hypercholesterolemia. Inflammatory cytokines can increase the production of cholesterol in the liver. This leads to higher levels of cholesterol in the blood, which can further contribute to the development of plaques in the arteries **(Simionescu, 2007)**. Monocyte chemotactic protein-1 (MCP-1) and interleukin-8 (IL-8) have been shown to be essential in hypercholesterolemic individuals by promoting the recruitment and attachment of monocytes, which leads to remodeling of the arterial wall **(Choudhary et al., 2004).**

Elevated cholesterol has also been shown to trigger the release of the inflammatory mediator C-reactive protein (CRP) which via IL-6, can worsen vascular dysfunction by promoting the generation of reactive oxygen species and increasing vascular permeability **(Pasceri et al., 2001).**

CHAPTER 02 : Pomegranate (*Punica granatum*)

CHAPTER 02 : Pomegranate (*Punica granatum*)

1. History

Punica granatum, commonly known as pomegranate, has a long history of use in various cultural contexts. The pomegranate tree is native to the Middle East and has been cultivated for over 5,000 years. It is associated with long-term fertility, abundance, and is considered a symbol of love, passion, and eternity in various cultures and mythologies **(Ainur et al., 2022) (Figure 08).**

In the religion of Islam, it is considered a sacred fruit, often symbolizing righteousness, good deeds, and the rewards of paradise. Pomegranates are among the fruits mentioned in the Quran as fruits of paradise **(Farhangi et al., 2014).**

The pomegranate, ar-Rummān in Arabic, has been mentioned three times in the verses of the Quranin verses 99 and 141 in chapter of Cattle and verse 68 in chapter of Merciful.

"And [We produce] gardens of grapevines and olives and pomegranates, similar yet varied. Look at [each of] its fruit when it yields and [at] its ripening. Indeed in that are signs for a people who believe." Verse (6:99) of chapter (6) sūrat l-anʿām (The Cattle).

Figure 08: *Punica granatum* Fruit **(Taoerdahong al., 2023).**

2. Geographic origin

Pomegranate is a fruit-bearing tree that is native to Central Asia and has a wide geographical distribution. It is one of the oldest fruit crops cultivated and is now grown in various regions around the world, including Mediterranean countries such as Algeria and Spain,

in addition to some areas of Asia, former Soviet countries, Argentina, Chile, and the United States of America **(Venat et al., 2021) (Figure 09).**

In Algeria, several kinds of pomegranate are reported, especially in northern Algeria, which is characterized by a subhumid climate. They are found in small gardens in Kabylia, the Mitidja plain (Chlef, Tipaza, Blida, Ain Defla) and it is also found in the south of the country (Ouargla, Adrar, etc.). Fourteen varieties are currently authorized for production and marketing by the State. Algeria therefore occupies a preponderant place among pomegranate producing countries despite technical and economic difficulties **(Meziane et al., 2016).**

The genetic diversity of *Punica granatum* totals more than 500 varieties, but only 50 cultivars are commercially grown, reducing the cultivated germplasm. The species are grown in temperate and subtropical regions. The geographical distribution of *Punica granatum* is influenced by various factors, including climate, soil conditions, and cultural practices **(Fahmy et al., 2022).**

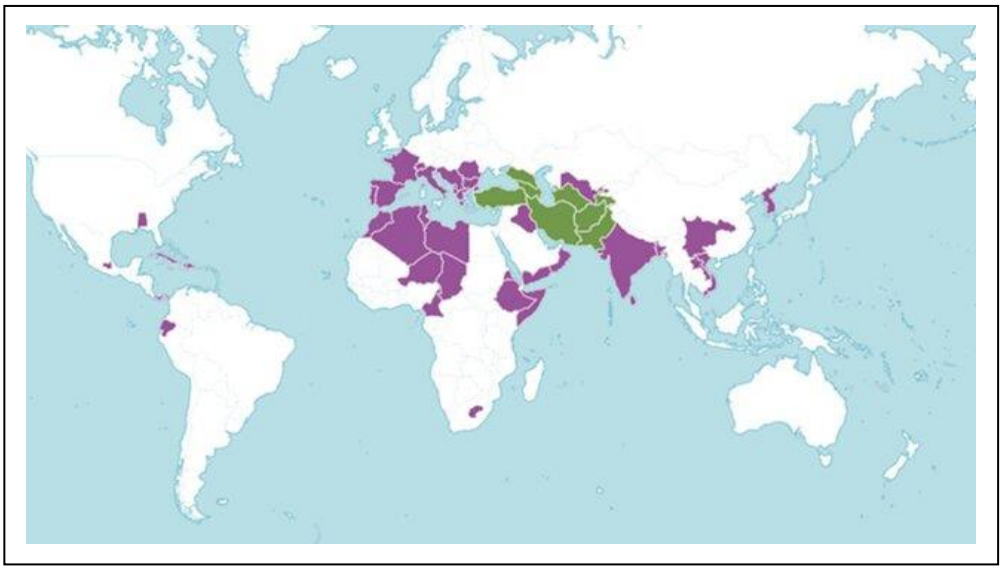

Figure 09: *Punica granatum* geographical distribution **(Fahmy et al., 2022).**

3. Nomenclature

The name of pomegranate varies from one place to another depending on the languages spoken in each country. According to **Hmid (2013)**, pomegranate nomenclature is:

- **Scientific name:** *Punica granatum*
- **English name:** Pomegranate
- **Arabic name:** Roman
- **French name:** Grenadier

- **Spanish name:** Granado

4. Classification

Table 04: *Punica granatum* classification

Kingdom	**Plantae**
Clade	Tracheophytes
Order	Asterids
Family	Lythraceae
Genus	*Punica*
Species	*Punica granatum* **(Panth et al., 2017)**

5. Botanical description of the pomegranate tree

Punica granatum, The plant has a bushy shape with multiple stems, and the bushiness is due to suckers that regularly arise from the base. The plant has an average height of 5-8 meters and is normally deciduous in nature. The leaves are dark green with a shiny appearance and are small with an alternate arrangement **(Rathore et al., 2020) (Figure 10).**

The pomegranate contains different parts as outlined below:

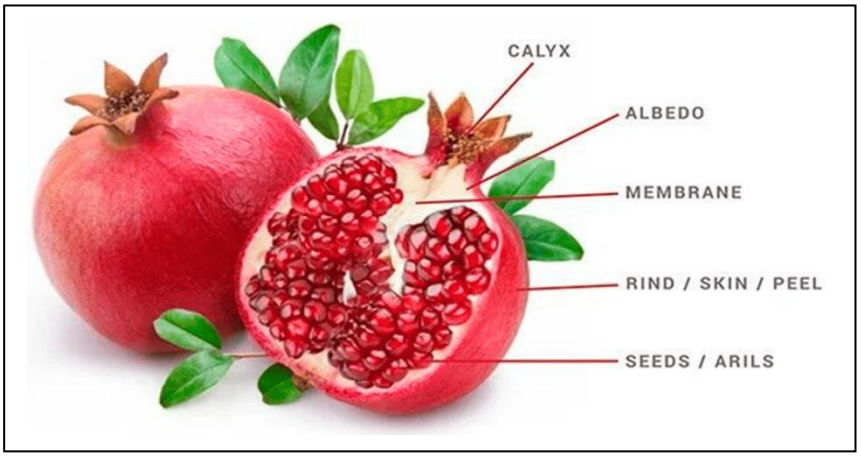

Figure 10: *Punica granatum* structure **(Alkhatib et al., 2022).**

5.1. Leaves

Leaves typically include characteristics such as being opposite or sub-opposite, glossy, and narrow. The leaves are usually lanceolate to oblong in shape, with a leathery texture and a

prominent midrib. They are often dark green and can vary in size, ranging from 3 to 7 cm in length **(Arra et al., 2024 ; Marcelino et al., 2023).**

5.2. Flowers

The flowers of pomegranate have a unique structure and formula. Pomegranate flowers comprise sepals, petals, stamens, and a pistil. The sepals are green and often hairy, while the petals are typically pink to red and have a distinctive star-shaped appearance. The stamens are arranged in whorls around the pistil, which consists of a stigma, style, and ovary **(Kshirsagar et al., 2023).**

5.3. Fruits

The fruit is round to oval-shaped and can range from 5 to 10 cm in diameter. It has a thick, leathery peel, inside the peel, there are numerous small juicy and sweet-tart arils **(Figure 11).**

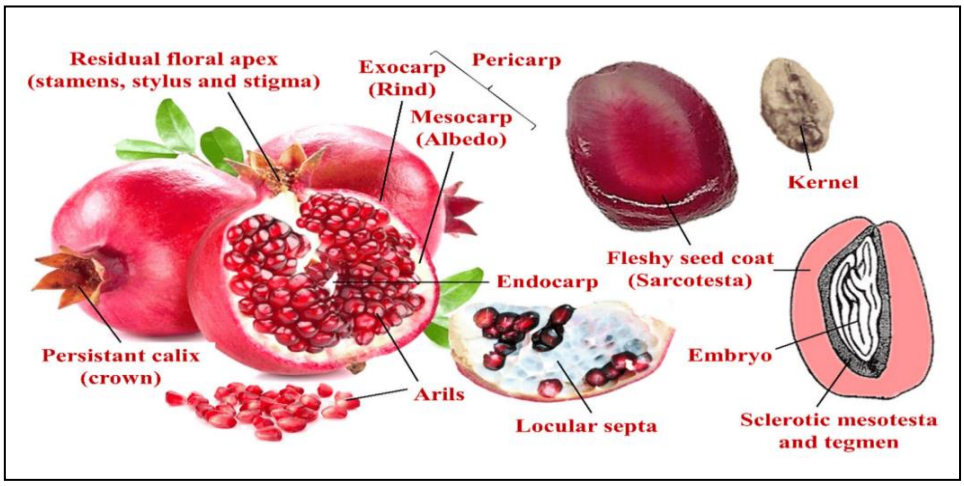

Figure 11: Anatomy of a typical pomegranate fruit and arils **(Montefusco et al., 2021).**

The botanical description of *Punica granatum* includes the following features:

1. **The peel**: The outermost layer, also known as the exocarp or the rind. It is thick, leathery, and tough, protecting to the arils inside. Typically, colored in vibrant red, though it can also be shades of yellow, pink, or purple depending on the variety.

2. **Mesocarp:** The spongy, white, and bitter layer beneath the exocarp. It forms the bulk of the interior structure of the fruit and is not typically eaten. It acts as a cushioning layer, protecting the arils from damage.

3. **Endocarp (Seed Covering):** It refers to the membrane that encloses the seeds. In a pomegranate, this is a delicate, transparent layer that separates the arils into locules

(compartments). Its structure is thin and paper-like, often appearing as a whitish, slightly translucent film.

4. **Arils**: The edible part of the pomegranate fruit is the aril, which surrounds the seeds. Known for their distinctive tart flavor, arils are consumed fresh, used in cooking, or processed for juice.

5. **Seeds (Kernels)**: Each aril contains a small, hard seed at its core. The seeds are edible, though their hardness can vary **(Fawole et al., 2013).**

6. Chemical constituents of permanganate

6.1. Primary metabolites

The primary metabolites found in *P.granatum* include organic acids, sugars, proteins, lipids, and amino acids. These compounds play essential roles in photosynthesis, respiration, and growth and development in plants. Some primary metabolites, such as sugars and fatty acids, accumulate differently depending on the cultivar, which can be useful in food processing and potentially provide health benefits. Additionally, the pomegranate genome contains UDP glycosyltransferase genes (PgUGTs), which play a crucial role in regulating signaling pathways and intracellular homeostasis by catalyzing the glycosylation of metabolites **(Li et al., 2023).**

6.2. Secondary metabolites

The secondary metabolites found in *Punica granatum* (pomegranate) include phenolic compounds, such as ellagic acid, flavonoids, and anthocyanins **(Table 02).** These compounds play crucial roles in plant defense, stress responses, and the development of various health-promoting properties in pomegranate **(Guo et al., 2022).**

Table 05: Phenolic compounds present in *Punica granatum* **(Singh et al., 2018).**

Family of phenolic compounds	Main identified compounds
Phenolic acids	Chlorogenic, caffeic, syringic, sinapic, pcoumaric, ferulic, vanillic, ellagic, gallic and cinnamic acids
Flavonoids	Catechin, epicatechin, quercetin, anthocyanins, procyanidins, flavonols and flavones
Tannins	Granatin A, granatin B, punicalin, punicalagin, corilagin, gallagyidilactone, pedunculagin and tellimagrandine

7. Uses of the pomegranate

The different parts of *P.granatum* are widely used in multiple fields:

7.1. Agri-food use

Pomegranates are a highly nutritious fruit with many potential uses in the agri-food industry. The fruit's peel and seeds, which account for about 54% of the pomegranate, can be converted into value-added products like food additives, bioactive compounds for food packaging, and antimicrobial agents to prevent food oxidation and pathogenic microbial growth. The arils are a delicious snack and can be used to produce pomegranate juice.

With proper processing and utilization of the whole fruit, pomegranate waste can be minimized and the nutritional and bioactive components can be optimized for various food applications such as jams and sauces **(Ko et al., 2021).**

7.2. Industrial use

Pomegranates have diverse industrial applications, particularly in the food, beverage, pharmaceutical, and cosmetic sectors. Pomegranate extracts, containing punicalagins and ellagic acid, are utilized in dietary supplements and functional foods due to their anti-inflammatory and anti-carcinogenic properties. The seeds are pressed to produce oil, which is incorporated into skin care products for its moisturizing and regenerative qualities.

Additionally, pomegranate peel, often considered a waste product, is repurposed for its high polyphenol content in natural dyes, animal feed, and bioactive compounds for medical applications. These varied uses underscore the fruit's economic and health-related significance in industrial applications **(Ain et al., 2023).**

7.3. Use in cosmetic products

Pomegranate has been recognized for its numerous benefits in cosmetic products, particularly in skincare. Its antioxidant properties, including vitamin C, tannins, anthocyanins, and ellagic acid, work to reverse free radical damage and protect the skin from further damage, making it an effective anti-aging ingredient **(Ko et al., 2021).**

It can be used in various forms, such as juice for toning, seeds for exfoliation, and as a face pack when mixed with bentonite clay. Pomegranate extract has also been shown to have moisturizing properties, help in wrinkle reduction, and provide anti-inflammatory effects, making it a valuable ingredient in cosmetic formulations. Furthermore, the high tannin content of pomegranate bark makes it an excellent cosmetic ingredient for fixing hair color, and its

polyphenols protect the intensity and radiance of the color from external aggressions (**Chakkalakal et al., 2022).**

7.4. Medicinal use

Pomegranate has a rich history of medicinal uses. It has been traditionally used to treat ulcers, diarrhea, and male infertility. Recent research has unveiled a plethora of pharmacological activities associated with pomegranate, including anti-diabetic, anti-tumor, anti-inflammatory, anti-malaria, and anti-fibrotic effects. Moreover, pomegranate has shown promise in improving gut microbiota, preventing obesity and diabetes. The fruit peel, in particular, is a valuable source of bioactive compounds like polyphenols, phenolic acids, tannins, and flavonoids, offering benefits such as lowering blood pressure, reducing oxidative stress, and improving heart health. Pomegranate's antioxidant properties have been linked to positive impacts on oxidative stress factors related to chronic diseases like diabetes and heart diseases **(Maphetu et al., 2022).**

Pomegranate juice and extracts have been shown to improve cardiovascular health by reducing blood pressure, cholesterol levels, and arterial plaque. Additionally, its antimicrobial properties help combat infections, and its anti-inflammatory effects benefit conditions like arthritis **(Ain et al., 2023).**

Material and methods

Material and methods

1. Material

1.1. Vegetal material

Pomegranate peel (*Punica granatum*) is the plant material used in this work. They were purchased at a local market in Constantine in November 2024. The peels were cleaned, air-dried and then crushed into a fine powder **(Figure 12).**

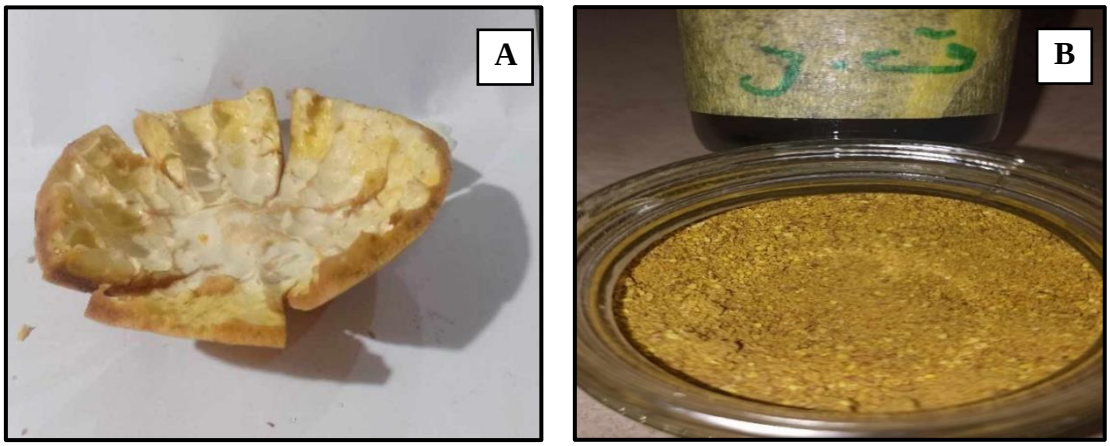

Figure 12: (A) Pomegranate peel **(B)** Pomegranate peel powder.

1.2. Animals selection

The experiment was carried out on male mice (20 mice), of the genus (*Mus*), species (*Mus musculus*), aged 2.5 to 3 months and weighing between 14g and 18g **(Figure 13)**

Figure 13: Animals selection.

The animals were maintained in favorable breeding conditions at the animal facility of the Department of Animal Biology, Faculty of Natural and Life Sciences, University of Frères Mentouri Constantine 1, at a temperature of 25 to 30°C, a humidity level between 45

and 60% and a photoperiod of 12 hours day and 12 hours night. In order to avoid inter-sex variability, the study was carried out on male mice.

During the experimental period, the mice were fed ONAB (Office national des aliments du bétail) food in the form of pellets **(Appendix 01)** and tap water ad libitum.

The animals were separated and divided into 4 groups according to the administered diet.

- The weight of the mice was taken every day at the same time (9:30 a.m.) during the 15-day treatment period.
- The amount of ingesta was recorded daily.

2. Methods

2.1. Treatment of mice

This study includes a group of 20 male mice, divided into 4 groups **(Figure 14)**.

The doses of cholesterol and the plant were calculated in relation to the weight of the mice in each group, i.e.: **(17g (egg yolk) × 3 times/day (3 meals) × 2 (hypercholesterolemia) = 450 mg/kg/day)** for cholesterol and **(150 mg/kg/day)** for *Punica granatum* peel powder. Cholesterol incorporated into the flour was administered in the form of balls (the weight of each ball = **0.1 g** for each dose).

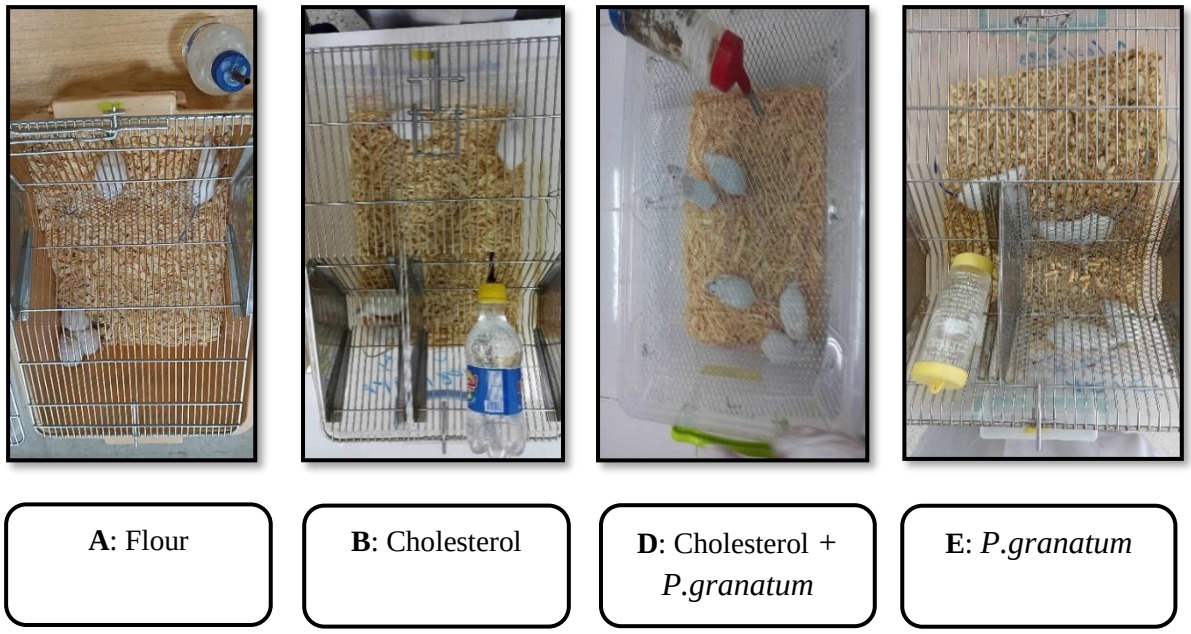

Figure 14: Distribution of mice in the different batches

Table 06**:** Treatment of mice

Experimental group	Treatment	Number of animals	Experience duration	Daily dose
01: Negative control	Flour	05	15 days	0.1 g/mouse
02: Positive control	Flour+ Cholesterol	05	15 days	450 mg/kg/day/mouse
03	Flour + Cholesterol + *Punica granatum* powder	05	15 days	150 mg/kg/day/*Punica granatum* powder/mouse. And 450mg/kg/day/Cholesterol/mouse
04	Flour + *Punica granatum* powder	05	15 days	150 mg/kg/day/*Punica granatum* powder

2.2. Blood sampling

After 15 days, each treatment step was followed by a blood sampling for the evaluation of certain biological parameters. Blood was collected from the retro-orbital sinuses of mice and was placed directly into heparin tubes.

Then, the blood was centrifuged at 2500 rpm (Revolutions per minute) for 5 minutes, then the serum was collected in Eppendorf tubes to carry out the biochemical assays at **Ibn Sina** medical analysis laboratory in Constantine (**Figure 15**).

Figure 15: Blood sampling from the retro-orbital sinuses and serum collection after centrifugation at 2500 rpm for 5 minutes.

2.3. Method of measurement of different biological parameters

All biological parameters were determined by the usual techniques used in the laboratory using an automated analyzer (**COBAS Pro**) and the spectrophotometer.

2.3.1. Total cholesterol measurement

Total cholesterol was measured by an enzymatic colorimetric method (Quimica Clinica Aplicada S.A kit) on serum. In this test the Cholesterol esters are hydrolyzed by cholesterol ester hydrolase to free cholesterol and fatty acids.

The free cholesterol produced and the pre-existing is oxidized by the enzyme cholesterol oxidase to Δ4 cholestenone and H_2O_2. The latter, in the presence of peroxidase, oxidizes the chromogen into a compound which is colored in red. The concentration of colored quinoneimine measured at 510 nm is directly proportional to the quantity of cholesterol contained in the sample.

2.3.2. Triglyceride measurement

Measurement of serum and tissue TG was carried out entirely through an enzymatic pathway, under the action of a lipase using the enzymatic colorimetric method (Quimica Clinica Aplicada S.A kit).

TG are hydrolyzed into glycerol and fatty acids using lipases. A series of reactions results in the formation of H_2O_2 which, in the presence of peroxidase and a chromogen, gives a colored compound, quinoneimine. The quinoneimine concentration is proportional to the total concentration of TG present in the sample. The TG concentration is determined at a wavelength λ=500 nm.

2.3.3. HDL-LDL measurement

For HDL-cholesterol, the used technique includes magnesium chloride which will precipitate LDL and VLDL; HDL cholesterol is then measured in the supernatant using the same enzymatic technique as total cholesterol. The normal value is >0.45 g/l.

For LDL cholesterol, the formula is as follows:

$$\textbf{LDL c = CT - [(TG ÷5) +HDL c]}$$

2.3.4. Transaminases measurement

Transaminases were determined by an enzymatic colorimetric method (Kit, Chronolab, System).

ALAT catalyzes the transfer of the amino group of L-alanine to α-ketoglutarate to produce L-glutamate. Reading is done by spectrophotometry at a wavelength λ=340nm.

ASAT catalyzes the transfer of the amino group of L-aspartate to α-ketoglutarate to produce L-glutamate. Reading is done by spectrophotometry at a wavelength λ=340 nm.

2.3.5. CRP measurement

The C-reactive protein (CRP) was measured using the Immunoturbidimetric test on the COBAS Pro auto analyzer. This test measures very low concentrations of CRP in serum samples.

2.4. Statistical Analysis

Results are presented in the form of mean ± standard deviation (n=5). The comparison of means between the four groups was carried out by the one-way ANOVA test and completed by the Tukey test. Statistical analysis was realized using SPSS software, version 23.0.

The comparison or correlation was considered, according to the probability (p), as follows:

- Not significant if $p > 0.05$
- Significant if $p < 0.05$

Significant difference was expressed by different letters (a, b and c).

Results and discussion

Results and discussion

1. Results and discussion of weight and food consumption

1.1. Effect of *Punica granatum* peel powder on mice weight and food consumption

1.1.1. Weight variation

Figure 16 illustrates the weight progression curves of the different groups. In the **(Negative control)** group, the weight of the animals over two weeks ranged from **32.14** g to **34.7** g respectively, indicating a non-significant weight increase ($p > 0.05$).

Similarly, the **positive control (cholesterol)** group showed a weight range from **35.9**g to **37.4**g respectively, also reflecting a non-significant weight increase ($p > 0.05$).

Also, the **(cholesterol + *P.granatum)*** group exhibited a weight range from **30.12**g to **31**g, respectively, demonstrating a significant weight increase ($p < 0.05$).

Finally, in the **(*P.granatum)*** group, the weight ranged from **29.16** g to **29.8** g, indicating a non-significant weight increase ($p > 0.05$).

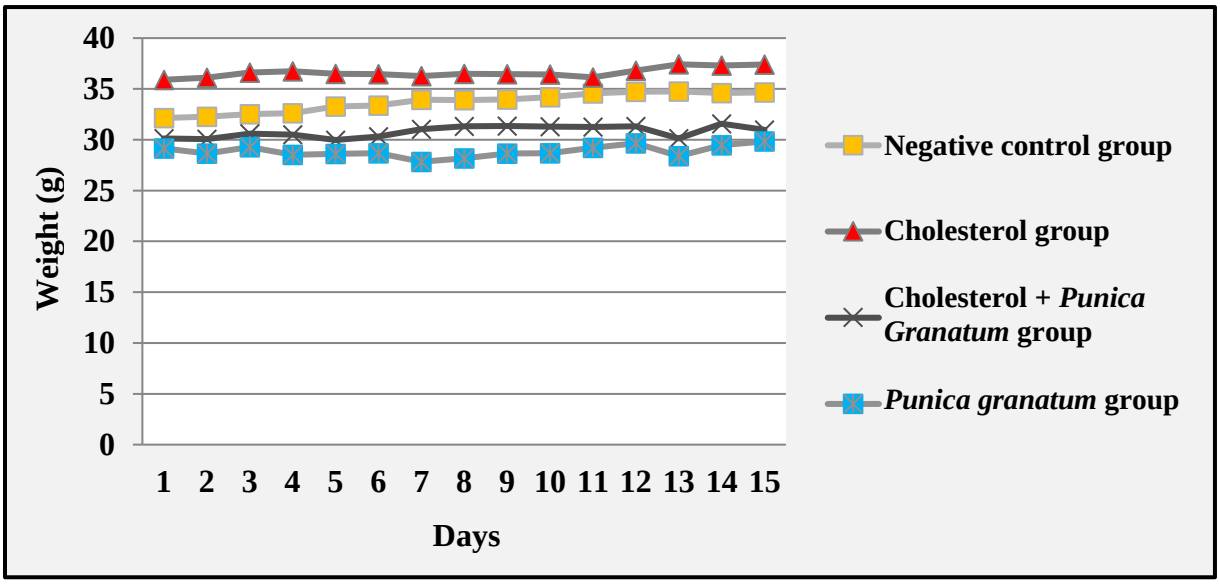

Figure 16. Effect of *Punica granatum* peel powder on the weight of mice.

1.1.2. Food consumption

During the treatment period, the **(Positive control)** group displayed higher food intake (ingesta) compared to the **(Negative control)** group. In contrast, the **(Cholesterol + *Punica granatum)*** group showed a significant reduction in food consumption from the first to the second week (70 g to 35.7 g, $p < 0.05$). Similarly, the ***(Punica granatum)*** group exhibited a significant decrease in food consumption compared to the **(Positive control)** group ($p < 0.05$) **(Figure 17).**

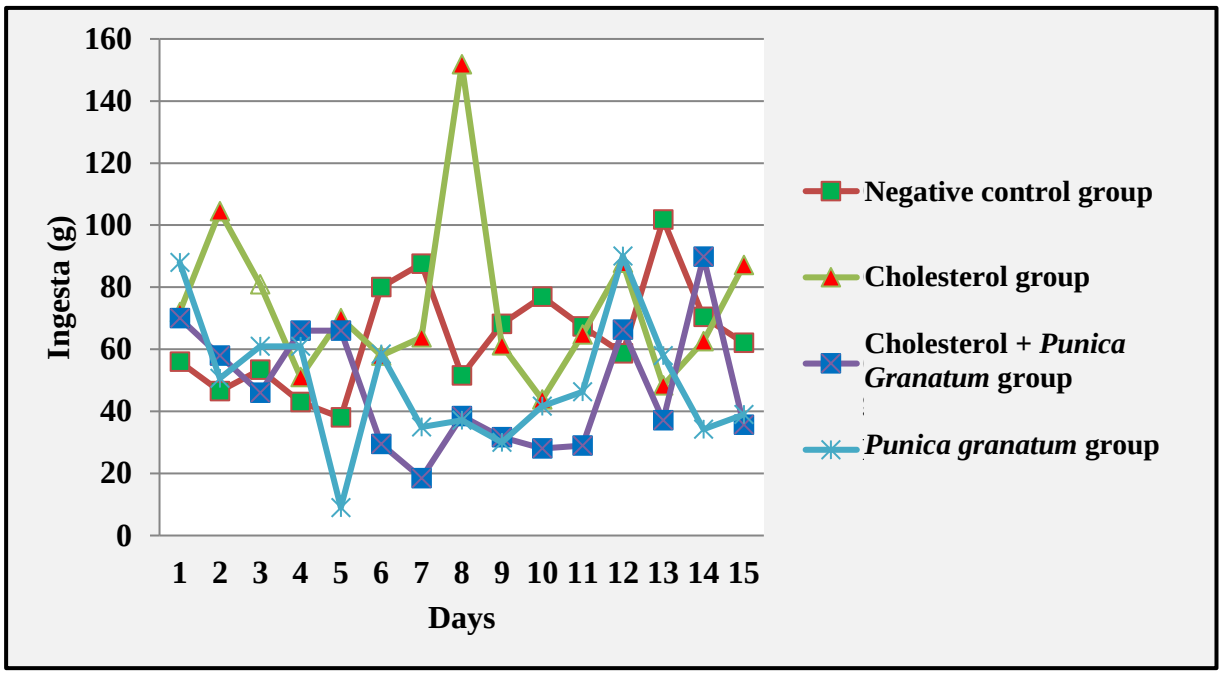

Figure 17: Effect of *Punica granatum* peel powder on food consumption.

1.2. General discussion of weight and food consumption

In our experiment, mice subjected to a hypercholesterolemic diet had significantly higher body weight and food intake compared to the negative control group. Additionally, treatment with *Punica granatum* peel powder decreased food intake in obese mice.

These findings align with existing literature, which reports that diets can induce obesity through hyperphagia in mice. Increased energy intake is a crucial factor in obesity development through adipose tissue accumulation. This type of obesity is associated with metabolic alterations in glucose, lipids, and proteins, akin to those seen in human obesity **(Kopelman, 2000)**

Our findings confirm that a hyperlipidemic diet induces obesity in mice, corroborating the results of **Armitage et al. (2005).**

The evaluation of the effects of different treatments on mice weight progression showed a weight increase in all groups, consistent with findings **by Zerizer et al. (2008),** who reported significant weight gain in mice treated for 18 days. These results suggest a clear relationship between treatment and mouse weight.

Negative control group exhibited normal weight gain associated with regular growth. In the ***Punica granatum*** treated groups, weight gain was lower than in the negative control group ($p > 0.05$), indicating that a dose of 150 mg/kg/day of *Punica granatum* peel powder might slightly reduce mouse growth. This observation is consistent with findings by **Fehri et al. (1995).**

The improvement in body weight in the group **(cholesterol + *Punica granatum)*** and ***Punica granatum*** group could be explained by the ability of *P.granatum* peel powder to restore triglyceride stock by enhancing insulin secretion and glucose levels **(Babu et al., 2007). Zerizer (2006)** also reported weight gain in mice treated for 18 days, supporting the relationship between hypercholesterolemia and mouse weight **(Messaoudi, 2021).**

The primary determinants of the energy density in food intake are lipids, which significantly affect satiety and weight gain. It is well established that high-energy diets rich in lipids, such as hypercholesterolemic diets, reduce satiety, increase hunger, and lead to weight gain. Intestinal lipid absorption is therefore enhanced in mice on a hypercholesterolemic diet. This diet clearly induces hyperphagia and an increased capacity for protein and lipid retention, promoting significant weight gain **(Bouanane et al., 2009).**

Regarding food consumption, no significant differences were observed among the groups, except for the ***Punica granatum*** and **cholesterol + *P.granatum*** groups, which showed a significant reduction. These results suggest a potential relationship between *Punica granatum* peel powder and mouse appetite where the peel powder may have appetite suppressing effects and enhance satiety.

2. Results and discussion of biochemical analysis

2.1. Results of biochemical analysis

2.1.1. Cholesterol

The results **(Figure 18)** show the effect of the different treatments on cholesterol.

We observed a significant increase in cholesterol levels in both the **Cholesterol group** and ***Punica granatum*** group **(1.24 ± 0.03 g/L** and **1.19 ± 0.09 g/L, respectively)** compared to the **Negative control** group. A non-significant decrease in cholesterol level was observed in the **(Cholesterol + *Punica granatum*)** group **(1.22 ± 0.05 g/L)** compared to the **(Cholesterol)** group.

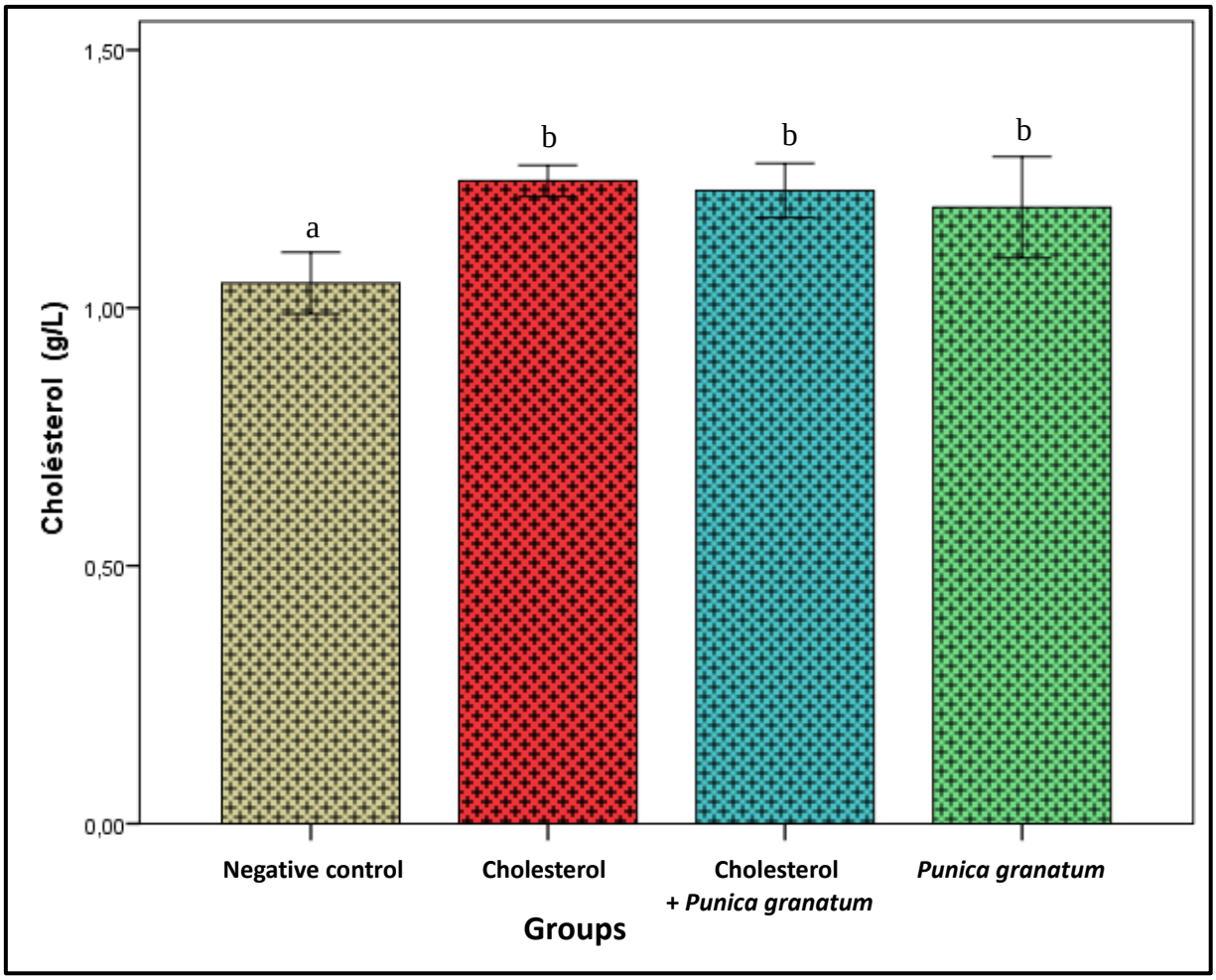

Figure 18: Effect of cholesterol and *Punica Granatum* on cholesterol levels in mice.

(Test ANOVA and Tukey ; a and b are significantly different ($p < 0.05$) ; n=5)

2.1.2. Triglycerides

The results **(Figure 19)** demonstrate the effect of the different treatments on triglycerides.

There was a non-significant increase in triglyceride levels in the **Cholesterol** group and ***Punica granatum*** groups (**1.19 ± 0.05 g/L and 1.24 ± 0.03 g/L, respectively)** compared to the **Negative control** group **(1.10 ± 0.09 g/L)**. A non-significant increase in triglyceride levels was observed in the **Cholesterol + *Punica granatum*** group **(1.32 ± 0.16 g/L)** compared to the **Cholesterol** group.

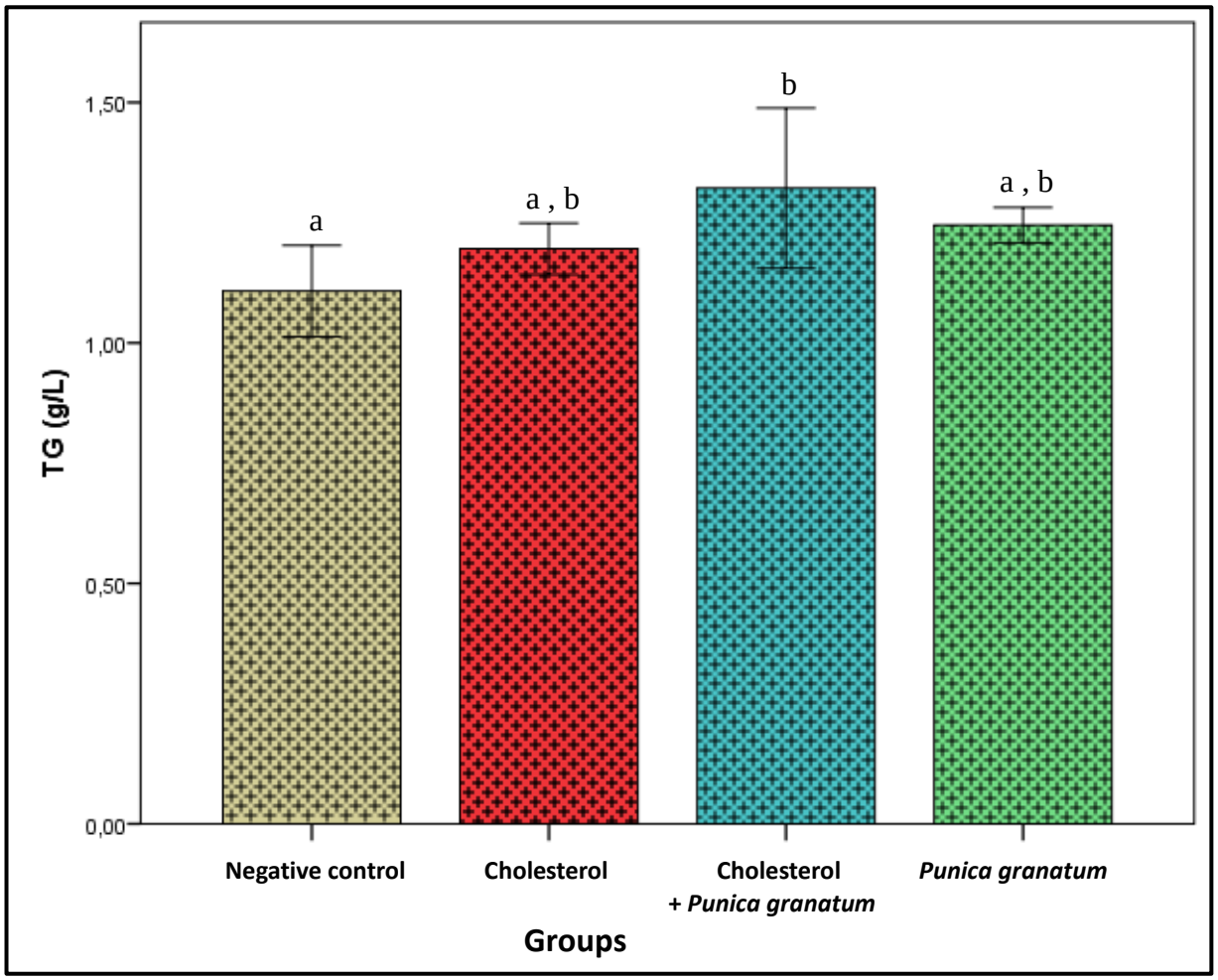

Figure 19: Effect of cholesterol and *Punica Granatum* on triglycerides levels in mice.

(Test ANOVA and Tukey ; a and b are significantly different ($p < 0.05$) ; n=5)

2.1.3. HDL

The results in **Figure 20** demonstrate the effect of the different treatments on HDL.

A non-significant decrease in the HDL level was noted in the **(Cholesterol)** group **(0.85 ± 0.02 g/L)** compared to the **(Negative control)** group **(0.92 ± 0.05 g/L)**. However, a non-significant increase in HDL level was reveled in the ***Punica granatum*** group **(0.94 ± 0.06 g/L)** compared to the **(Negative control)** group. Similarly, a non-significant increase in HDL level was observed in the **Cholesterol + *Punica granatum*** group **(0.90 ± 0.04 g/L)** compared to the **Cholesterol** group.

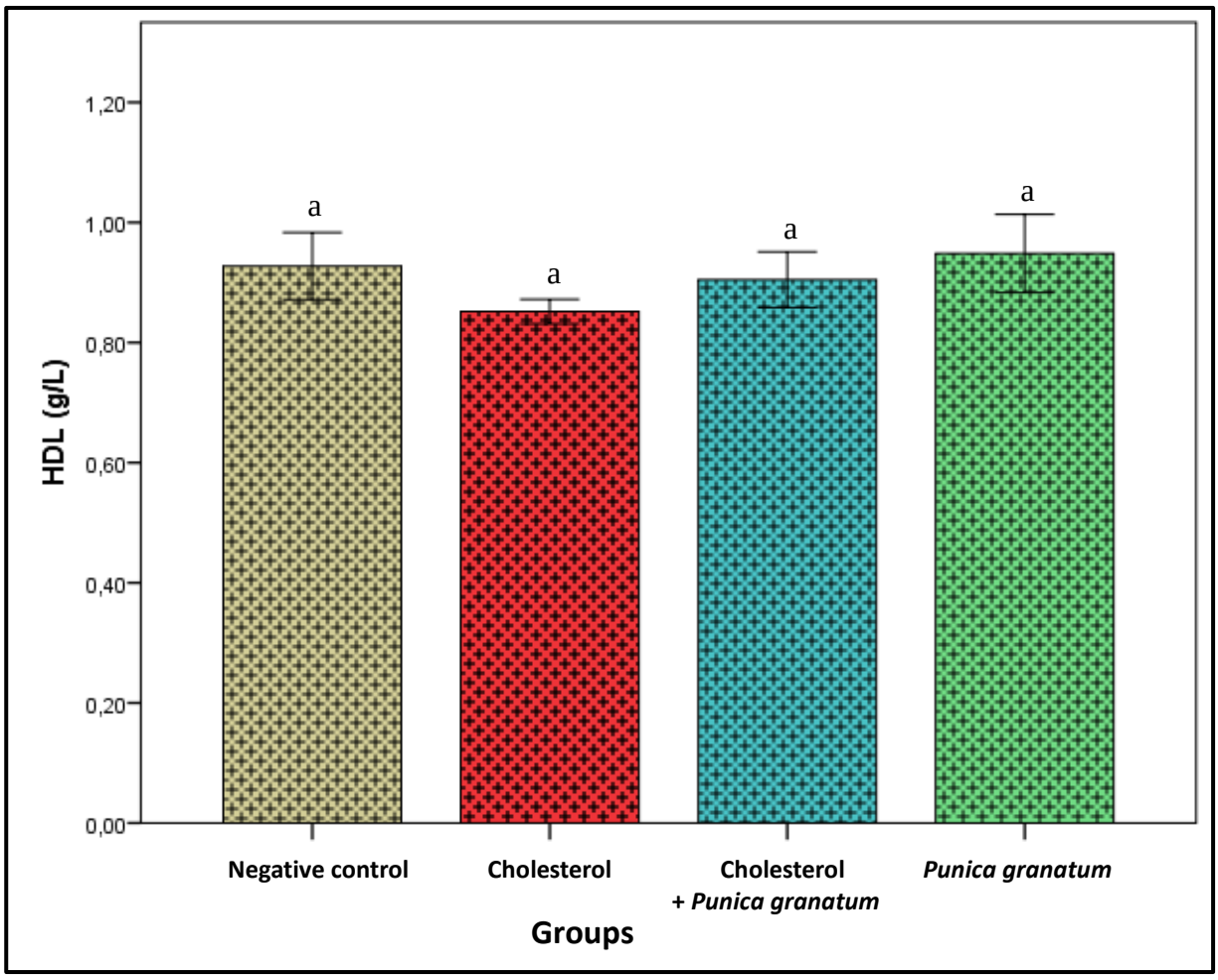

Figure 20: Effect of cholesterol and *Punica Granatum*on HDLlevels in mice.
(Test ANOVA and Tukey ;a are non-significantly different ($p > 0.05$) ; n=5)

2.1.4. LDL

The results in **Figure 21** demonstrate the effect of the different treatments on LDL.

We noted a significant increase in the LDL level in the **Cholesterol** group **(0.04 ± 0.00 g/L)** compared to the **Negative control** group **(0.02 ± 0.00 g/L).** In the other side, A non-significant decrease in LDL levels was observed in ***Punica granatum*** group **(0.01 ± 0.00 g/L)** compared to the Negative **control** group. A non-significant decrease in LDL level was reveled in **the (Cholesterol + *Punica granatum*)** group **(0.04 ± 0.00 g/L)** compared to the **(Cholesterol)** group.

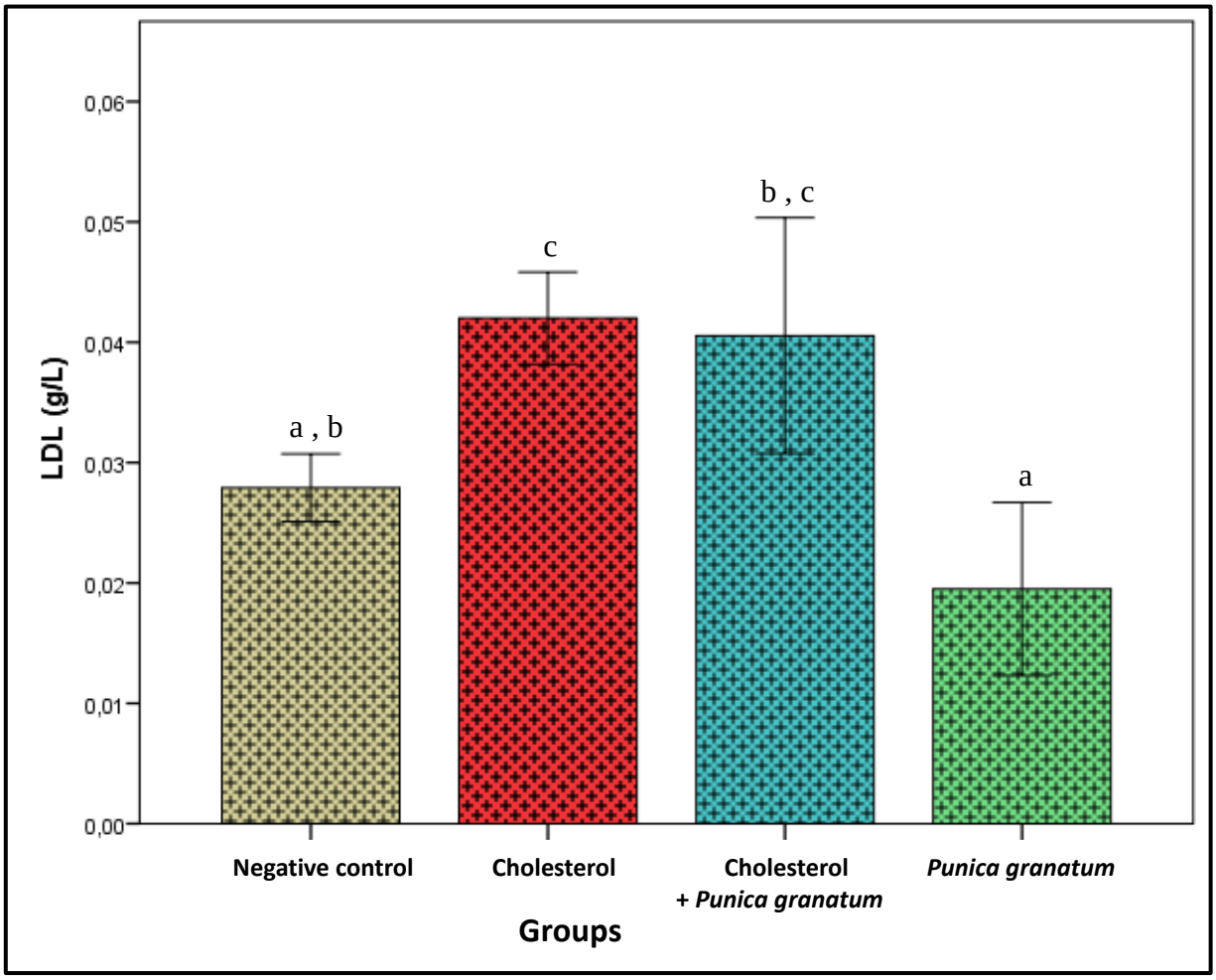

Figure 21: Effect of cholesterol and *Punica Granatum* on LDL levels in mice.
(Test ANOVA and Tukey ; a, b and c are significantly different ($p < 0.05$) ; n=5)

2.1.5. ASAT

The results **(Figure 22)** demonstrate the effect of the different treatments on ASAT.

We noted a significant increase in the ASAT level in the **Positive control (Cholesterol) group** (**290.19 ± 6.00 IU/L**) compared to the **Negative control** group **(243.86 ± 4.26 IU/L).** A significant decrease in the ASAT level was observed in the ***Punica granatum*** group **(209.75 ± 6.07 IU/L)** compared to the **Negative control** group. A significant decrease in the ASAT level was reveled in the (**Cholesterol + *Punica granatum*)** group **(237.50 ± 21.14 IU/L)** compared to the **Cholesterol** group.

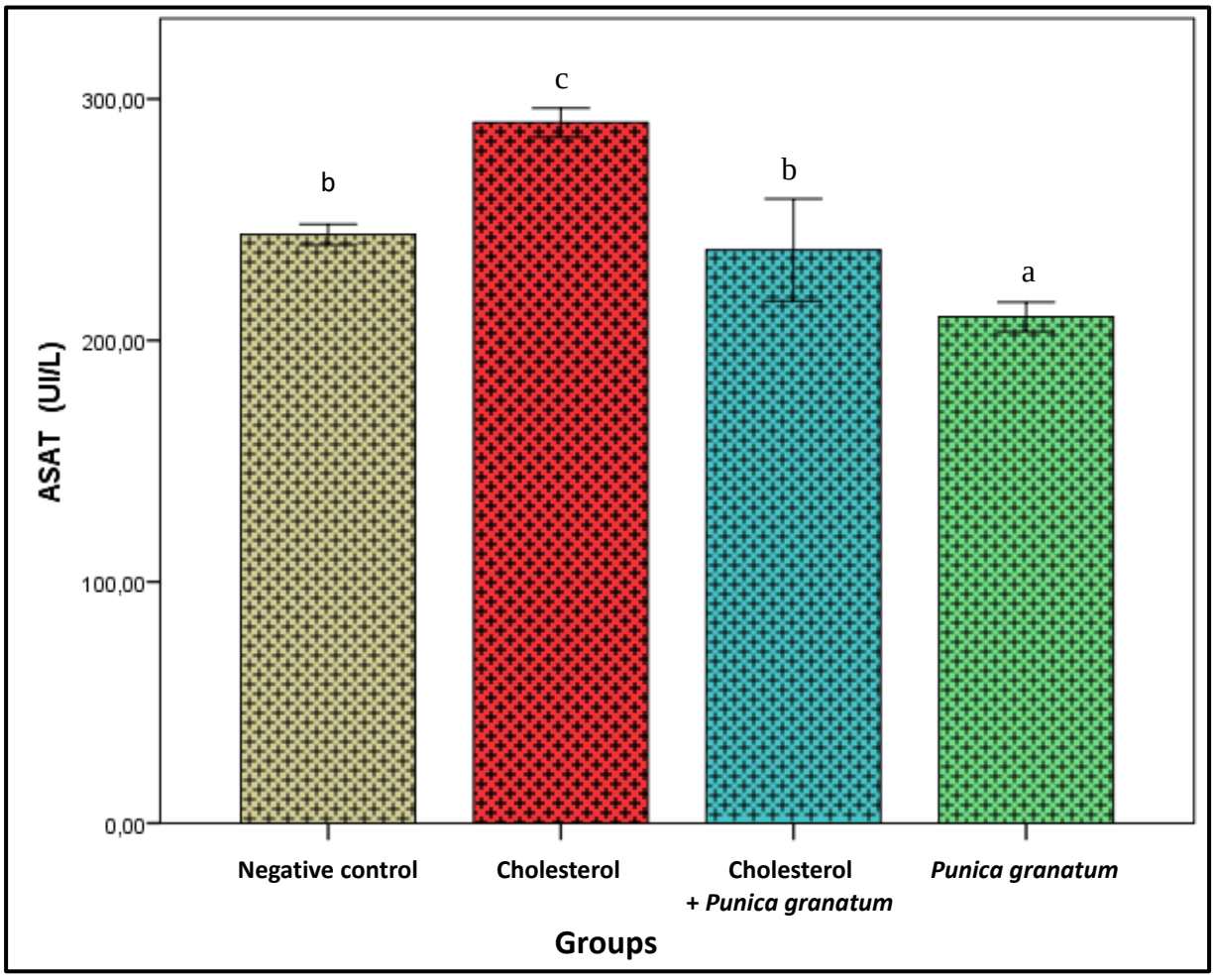

Figure 22: Effect of cholesterol and *Punica Granatum* on ASAT levels in mice.

(Test ANOVA and Tukey ; a, b and c are significantly different ($p < 0.05$) ; n=5)

2.1.6. ALAT

The results **(Figure 23)** demonstrate the effect of the different treatments on ALAT.

There was a significant increase in the ALAT level in the **Cholesterol** group **(43.10 ± 1.19 IU/L)** compared to the **Negative Control** group **(38.23 ± 0.96 IU/L).** A non-significant decrease in ALAT level was observed in the ***Punica granatum*** group **(36.30 ± 1.95 IU/L)** compared to the **Negative Control** group. Moreover, compared to the **Cholesterol** group a significant decrease in ALAT level was noted in the **Cholesterol + *Punica granatum*** group **(34.37 ± 2.49 IU/L)**.

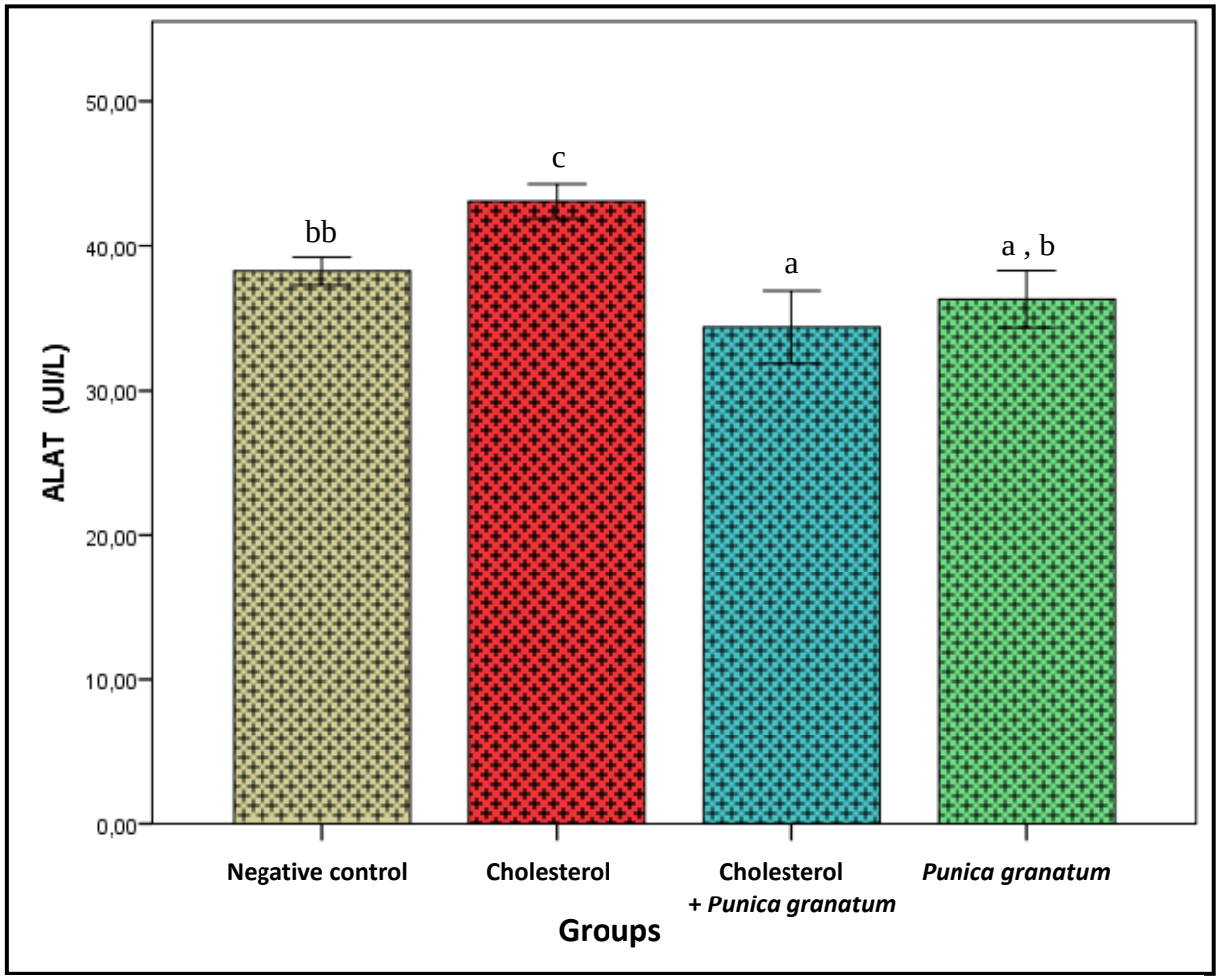

Figure 23: Effect of cholesterol and *Punica Granatum* on ALAT levels in mice.

(Test ANOVA and Tukey ; a, b and c are significantly different ($p < 0.05$) ; n=5)

2.1.7. CRP

The results **(Figure 24)** show the effect of the different treatments on CRP.

We observed a significant increase in the CRP level in **Cholesterol** group **(0.05 ± 0.1 mg/L)** compared to the **Negative control** group **(0.02 ± 0.00 mg/L).** A non-significant decrease in CRP level was observed in the **(*Punica granatum*)** group **(0.01 ± 0.00 mg/L)** compared to the **Negative control** group. Meanwhile, a significant decrease in CRP level was observed in **Cholesterol + *Punica granatum*** group **(0.02 ± 0.01 mg/L)** compared to the **(Cholesterol)** group.

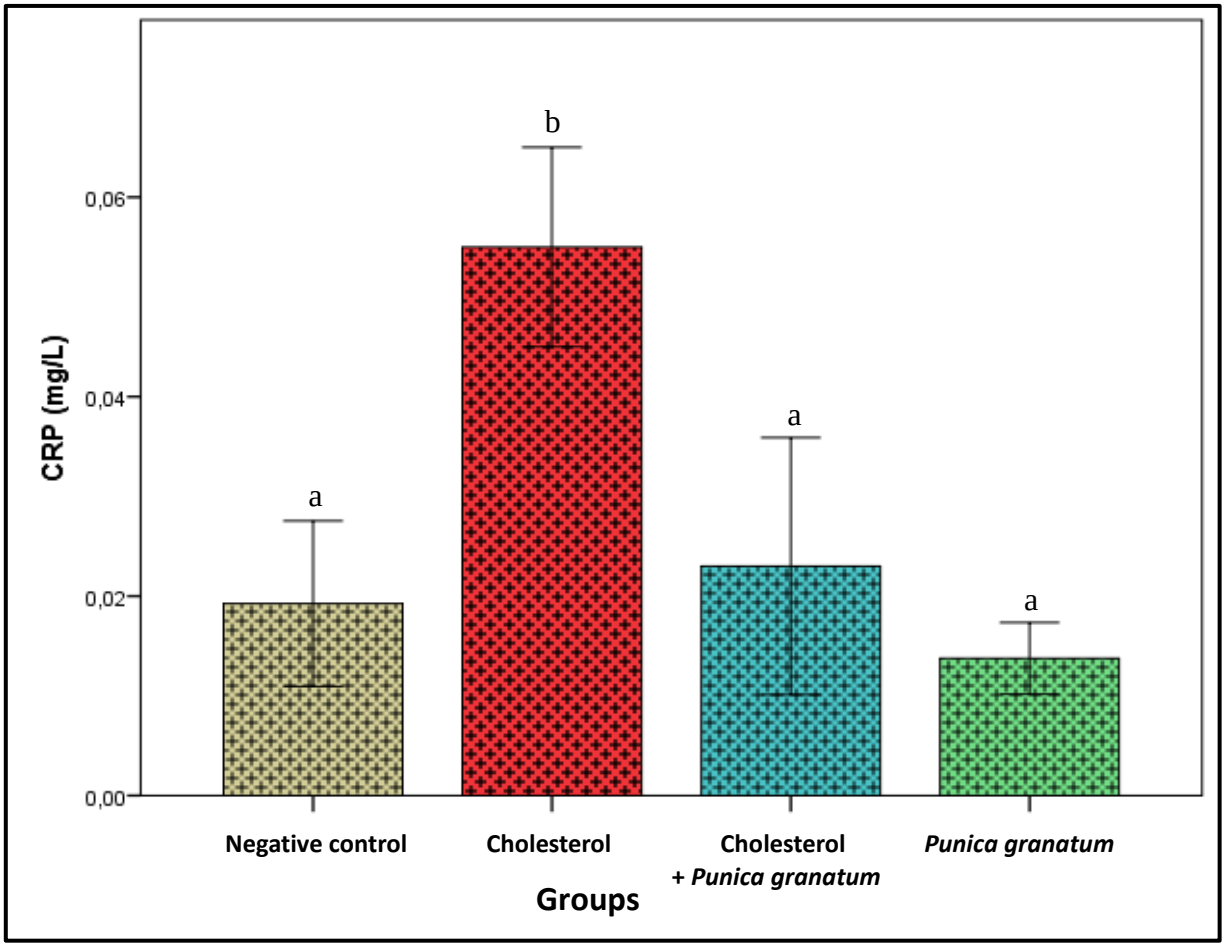

Figure 24: Effect of cholesterol and *Punica Granatum* on CRP levels in mice.
(Test ANOVA and Tukey ; a and b are significantly different ($p < 0.05$) ; n=5)

2.2 General discussion of biochemical analysis results

2.2.1. Cholesterol, TG, HDL and LDL

According to **figures 18, 19, 20 and 21**, treatment with cholesterol (450 mg/kg/day) revealed a significant elevation ($p<0.05$) in the level of total cholesterol and LDL-c, and a non-significant elevation ($p>0.05$) in serum TG levels. On the other hand, cholesterol caused a non-significant decrease ($p>0.05$) in serum HDL-c levels.

Cholesterol acts as a surfactant and suppresses the action of lipases to block the uptake of lipoproteins from blood circulation through the extrahepatic tissue resulting in increased blood lipid concentration **(Aarati et al., 2015).**

The induction of hyperlipidemia caused a non-significant increase in TG levels in mice of the **(Cholesterol)** group. TG represents the storage form of fatty acids in the body. TG are integrated into lipoproteins to ensure their transport in aqueous environments. Indeed, the diet enriched in cholesterol is responsible for an increase in serum and hepatic TG **(Yang et al., 2006).**

In the blood circulation, pre-HDLs can undergo the action of LCAT (Lecithin-Cholesterol Acyl Transferase) which, by esterifying the captured cholesterol, allows the formation of the hydrophobic core and the generation of mature HDL. The SR-B1 receptor (Scavenger Receptor class B type1) located in the caveolae is also capable of promoting cholesterol efflux **(Gautier et al., 2011).**

HDL is also called the “good cholesterol” because a high concentration of HDL in the blood constitutes protection against cardiovascular risks **(Dilmi-Bouras and Sadoun, 2002).** According to the study of **Obeid and Wolfgang (2009)**, the increase in plasma cholesterol is associated on one hand, with the reduction in HDL metabolism and on the other hand, with the distribution of plasma and tissue lipids.

The induction of hyperlipidemia by cholesterol also caused a significant increase in the LDL level in the **(Cholesterol)** group. Excess of this parameter is the cause of atherosclerosis **(Vergès, 2007).** As has been well accepted, oxidation of low-density lipoprotein cholesterol (LDL-c) plays an important role in atherosclerosis.

This accumulation causes a cascade of inflammatory processes, resulting in an unstable atherosclerotic plaque that ultimately bursts, causing myocardial infarction **(Wang et al., 2013).**

LDLs represents the final product of the VLDL-LDL-IDL metabolic cascade which is responsible for the transport of cholesterol from the liver to peripheral tissues **(Vergès, 2007).**

The oxidation of LDL contributed to the formation of foam cells and therefore inhibited the regression of macrophages from the plaques. The increase in LDL seems to be linked to their peroxidation by free radicals generated by cholesterol because this peroxidation inhibits the recognition of oxidized LDL by their native receptors and consequently, their increase in the plasma **(Laporte, 2000).** Our results are in agreement with the study of **Tselmin et al. (2013)** and differ from the study of **Amrane et al. (2012).**

- Our results show that serum cholesterol levels increased significantly ($p<0.05$) in the **(*Punica granatum*)** group **and the TG levels was increased non significantly** ($p>0.05$) **by *P.granatum* powder.** *Punica granatum* peel powder lowered LDL levels in a non-significant manner ($p>0.05$) **(Figure 21).** On the other hand, *Punica granatum* peel powder considerably increased HDL levels in a non-significant manner ($p>0.05$) **(Figure 20).**

Punica granatum is often considered to have cholesterol and TG lowering properties due to its antioxidant content. **Sadeghipour et al. (2014)** found that pomegranate extract reduced serum cholesterol, triglycerides, LDL, ALT, AST levels while increasing serum HDL levels in rats fed a high-lipid diet, compared to those treated with saline. This effect was attributed to the abundance of bioactive molecules present in the extract.

However, the results suggest that, under the conditions of this study, it may have a cholesterol/TG elevating effect. This could be due to a variety of factors, including the specific composition of the extract used, the dosage, or interactions with other dietary components or metabolic pathways in the test subjects.

Our findings indicated that the administration of *Punica granatum* peel powder led to a non-significant elevation in HDL cholesterol levels. This outcome may be attributed to the dosage of *P. granatum* employed. Nevertheless, the results imply that *Punica granatum* works in a dose-dependent manner. Certain fatty acids in *Punica granatum* peel powder have been shown to increase HDL-c, which is beneficial for human blood circulation, while oleic and linoleic acids are known to reduce LDL-c, which is bad cholesterol **(Njuguna et al., 2014).** Apolipoprotein A-1 is a core component of HDL that led to HDL formation in vivo. Transgenic expression of apolipoprotein A-1 was observed to result in reduced lesion formation in mice **(Lahiji al., 2003).**

However, our results revealed that administration of *Punica granatum* peel powder caused a non-significant decrease in LDL-c levels. This may indicate the probable fact that high doses of *Punica granatum* peel powder may be beneficial, regarding LDL-c lowering capacity.

Consumption of *Punica granatum* peel is linked to the reduction of cardiovascular risk factors, including glucose, free fatty acids, homocysteine and high blood pressure and to the inhibition of the formation of lipid peroxides, as well as the prevention of progression of atherosclerosis **(Figueroa et al., 2012; Poduri et al., 2012).**

Our results corroborate the study of **Rosenblat and Hayek., (2006)** who found that the concentrated juice of *Punica granatum* peel reduced significantly the LDL cholesterol by improving the total/HDL and LDL/HDL cholesterol ratios.

- The results show that serum cholesterol and LDL-C levels decreased non-significantly ($p>0.05$) in the **(Cholesterol + *Punica granatum*)** group. Meanwhile, a non-significant increase in triglyceride levels was observed. Meanwhile, a non-significant increase in HDL level was observed in the **(Cholesterol + *Punica granatum)*** group.

These findings imply that increasing the dosage of *Punica granatum* may give better results. This suggests that higher doses contain elevated concentrations of bioactive molecules such as polyphenols, which likely contribute to the observed effects.

The protective activity of polyphenols provided by diet against cardiovascular diseases is due to their ability to inhibit LDL oxidation, macrophage formation and atherosclerosis (**Aviram *et al.*, 2002**). The polyphenols of *Punica granatum* protect LDL against cell-mediated oxidation via two mechanisms which involve a direct interaction of the polyphenols with the lipoprotein and/or an indirect action linked to the accumulation of polyphenols in arterial macrophages. Pomegranate polyphenols have been shown to inhibit LDL oxidation by destroying reactive species of oxygen and nitrogen.

Furthermore, *Punica granatum* polyphenols increase serum paraoxonase activity, which leads to the hydrolysis of lipid peroxides in oxidized lipoproteins and in atherosclerotic lesions. These antioxidant and antiatherogenic properties of pomegranate polyphenols have been demonstrated *in vitro* as well as *in vivo* in humans and in atherosclerotic mice which are deficient in apolipoprotein E **(Aviram et al., 2002).**

The antihyperlipidemic efficacy of *Punica granatum* peel powder can be ascribed to its diverse array of bioactive constituents, notably polyphenolic compounds like ellagic acid, gallic

acid, punicalagins, and flavonoids such as quercetin and catechins. These polyphenols play a pivotal role by modulating key enzymes implicated in lipid metabolism, including HMG-CoA reductase responsible for cholesterol biosynthesis and lipoprotein lipase involved in triglyceride hydrolysis. Through the regulation of these enzymatic pathways, *Punica granatum* peel polyphenols effectively ameliorate serum lipid levels and enhance the lipid profile, thereby manifesting potent antihyperlipidemic effects **(Siddiqui et al., 2024).**

2.2.2. ASAT and ALAT

It is well known that ASAT is found in high concentrations in liver cells. ALAT is localized only in the cytosol, its concentration in non-hepatic tissues is very low and it is, in these cases, confined to the cytoplasm. The strong elevation in its serum concentration is relatively specific for damage to the hepatocyte. ALAT and ASAT are released in case of cell damage related to an increase in the permeability of the cell membrane or cell necrosis. ALAT tends to increase or decrease concomitantly with ASAT, but release of mitochondrial ASAT from the hepatocyte is believed to be involved in more severe cell damage than ALAT release **(Wang et al., 2011).**

The obtained results indicate a significant increase ($p<0.05$) in serum activity of ASAT and ALAT in the **(Cholesterol)** group compared to the other groups **(Figure 22)** and **(Figure 23).**

The hyperlipidemic diet increases the level of hepatic lipids. This excessive accumulation of lipids has exceeded the capacity of the mitochondria to oxidize them, and the microsomal oxidation pathway of long-chain fatty acids (LCFA), normally minor, takes over to oxidize the excess lipids **(Robertson et al., 2001).**

Due to liver cells damage, the rate of protein synthesis is reduced, resulting in low plasma protein levels in the **(Cholesterol)** group **(Chaturvedi et al., 2014).**

Microsomal oxidation of LCFAs generates large quantities of ROS, altering the respiratory chain. These modifications will in turn induce an increase in ROS production, thus forming a vicious circle between lipid peroxidation and ROS production. These lead to an increase in lipid peroxides (Thiols Barbituric Acid Reactive Species; TBARS) contributing to hepatocyte cytolysis (ALAT/ASAT) and inflammation **(Videla et al., 2004).** In addition, lipid peroxidation leads to protein oxidation, which is known to be deleterious to the cell. This results in an increase in the level of TG, hepatocyte cytolysis (increase in the ALAT/ASAT ratio) and

the presence of oxidative stress (increase in TBARS). This increase in aminotransferase activity is explained by the increase in the permeability of the hepatocyte membrane **(Van Herpen et al., 2008).** The numerous studies carried out on mice receiving a high-lipid diet have demonstrated a clear improvement in the serum concentration of plasma enzymes **(Bernal et al., 2013) (Messaoudi S., 2021).**

- According to the obtained results, the administration of *Punica granatum* peel powder significantly reduced ($p<0.05$) the ASAT rate and non-significantly ($p>0.05$) the ALAT rate in the **(*Punica granatum*)** group compared to the **(Negative control)** group.

The significant reduction in ASAT levels suggests that the administration of *Punica granatum* peel powder may have a protective effect on the liver, potentially reducing liver damage or inflammation. However, the non-significant reduction in ALAT levels indicates that the effect on this enzyme was not as pronounced.

- In the other side, the (**Cholesterol + *P.granatum)*** group results show that the treatment with the *P.granatum* peel powder induced a significant decrease of ASAT and ALAT. These results confirm the hepatoprotective effect of *P.granatum.*

In this study, the decrease in transaminases in groups treated with *P.granatum* may be due to a reduction in the supply of amino acids as a result of improvement in the defense system against proteolysis, or by a decrease in the coenzyme of these enzymes. This can also be explained by the reduction of gluconeogenesis process, which requires the intervention of ASAT and ALAT **(Derouiche and Kechrid, 2013).**

The observed low activities of ALAT and ASAT in mice fed *Punica granatum* peel powder could result from the possible role of phenolic compounds in maintaining cellular integrity **(Oluba et al., 2008).** The ability of hepatoprotective substances to reduce the damaging effects or to preserve the mechanisms of liver functioning against the disturbances of a hepatotoxin is an index of their protective effect **(Sakr et al., 2011).**

These results confirmed that *Punica granatum* peel powder has the ability to stabilize liver cell membranes, prevent enzymes leakage and prevent the production of free radicals and neutralize them. The reduction in serum levels of liver enzymes by the consumption of *Punica granatum* peel powder is a good indicator of the hepatoprotective activity of pomegranate.

Our results are consistent with the work of **Wei et al., (2015)** who confirmed that pomegranate peel extract has protective properties against hepatic fibrosis induced by CCl_4, whose mechanisms could be associated with their antioxidant activity.

These results are in agreement with those of **Sadeghipour et al. (2014)** and **Hasona et et al. (2016)**, who observed that *Punica granatum* peel extract decreased serum LDL, ASAT, and ALAT in obese rats and protected liver damage, including changes in fat in the hepatocyte.

2.2.3. CRP

Data analysis reveals a significant increase ($p<0.05$) in serum CRP levels in the **(Cholesterol)** group compared to the other groups.

Elevated cholesterol levels are linked to heightened inflammation within the body, often indicated by increased levels of CRP (C-reactive protein). The presence of excess cholesterol in arteries initiates inflammation, prompting the liver to produce CRP as part of the body's immune reaction. Our results confirm those of **Zerbato (2009),** who indicate that CRP is characterized by a rapid increase in its concentration in serum during the inflammatory phase.

- According to the obtained results, the administration of ***Punica granatum*** peel powder reduced non-significantly ($p>0.05$) the CRP level. On the other hand, the **(Cholesterol + *P.granatum)*** group reduced significantly the CRP level**.**

Inflammatory disorders are caused by the excessive production of pro-inflammatory mediators such as TNFa, GM-CSF, IL-1, IL-6, IL-8, leukotriene B4 and PAF, the activity of inflammatory cells such as neutrophils, monocytes and macrophages, and excessive production of reactive oxygen species (ROS).

Pomegranate peels are widely used to treat inflammatory disorders, ulcers and infections **(Bachoual et al., 2011).** *Punica granatum* peel powder is rich in phenolic compounds including flavonoids, carotenoids and triterpenoids, these compounds have an anti-inflammatory and antioxidant properties that make the fruit beneficial for health **(Tlili et al., 2011).** These anti-inflammatory agents do not neutralize the activity of ROS but neutralize the activity of reactive nitrogen species (RNS) **(Abdelwahab et al., 2011).** Some studies report that the peel, roots, leaves, flowers have beneficial medicinal effects on certain pathologies, mainly in the prevention and treatment of cancer through the inhibition of pro-inflammatory factors and tumor initiators and also reduce cell proliferation, cardiovascular diseases (anti-atherogenic effect) **(Akpinar-Bayizit et al., 2012).**

Our results confirm the study of **Achraf et al. (2018)** where 12 healthy males were administered a daily intake of 500 milliliters (ml) of pomegranate juice over a 15-day period demonstrated notable reductions in the inflammatory biomarker C-reactive protein (CRP) levels and indices of muscle damage relative to those subjected to a placebo intervention.

Punica granatum can effectively prohibit the production of prostaglandin or leukotriene by inhibition of eicosanoid enzymes of cycloxygenase and lipoxygenase so that it can increase the application of the oil or its derivatives as internal or external anti-inflammatory substances **(Schubert et al., 1999).** Analysis of the production of prostaglandin E2, nitric oxide, and TNFα in RAW 264.7 macrophages, as well as TNFα in human lymphocytes, indicated a reduction of all mediators **(Escandell et al., 2007).**

The *in vitro* study by **Bachoual et al. (2011)** proved that the aqueous extract of pomegranate peels had no effect on the concentrations of superoxide superoxyde ($O2^{\bullet-}$) or hydrogen peroxide (H_2O_2), but it strongly inhibited the activity of the myeloperoxidase.

Another study showed that the administration of *Punica granatum* extract (6 mg/day/mouse) for 4 weeks reduced cholesterol levels (total and LDL cholesterol), and reduced the expression of inflammatory genes (COX-2 and interleukins 1b and 6) in the colon and visceral adipose tissue. These results ensure the importance of the consumption of pomegranate extract rich in polyphenols in the control of metabolic and inflammatory disorders associated with obesity **(Neyrinck et al., 2013).**

Conclusion

Conclusion

The aim of this study was to investigate the antihyperlipidemic effect of *Punica granatum* (pomegranate) peel. Hyperlipidemia was induced in mice through a hypercholesterolemic diet, while the peel powder of *Punica granatum* was used as a treatment (150 mg/kg/day). Four groups were used in this study: negative control, positive control (Cholesterol), cholesterol + *P. granatum*, and *P. granatum* group.

Multiple biochemical parameters were measured, including the lipid profile as the main indicator of hypercholesterolemia (Total Cholesterol, Triglycerides (TG), HDL-c, LDL-c), transaminases (ASAT and ALAT) as markers for heart and liver diseases, and CRP as a marker of inflammation. The weight of the animals and their food intake were also monitored to assess the physiological state of the animals and the effect of the different treatments on their weight.

The results showed that the hypercholesterolemic diet induced hyperphagia in the Cholesterol group, leading to a significant increase in weight due to an accumulation of lipids in adipose tissue, compared to the other groups. This change was associated with a decrease in HDL-c and an increase in plasma levels of Cholesterol, Triglycerides, LDL-c, as well as hepatic secretions of transaminases (ASAT and ALAT) and the cardiovascular inflammation biomarker CRP.

Treatment with *Punica granatum* peel powder demonstrated a hypolipidemic effect, as evidenced by improvements in various biochemical parameters. However, the lipid profile exhibited a non-significant improvement, potentially due to the inadequate dose administered to the animals. Conversely, significant reductions were observed in other parameters, suggesting that the ingestion of this plant possesses anti-inflammatory, hepatoprotective, and preventive properties against cardiovascular diseases.

In light of these promising results, future research should focus on:

1. Conducting more *in vivo* and *in vitro* studies, particularly phytochemical studies and assessments of antioxidant and other biological activities (such as cell culture assays) to compare results and develop more effective therapeutic substances.
2. Determining the effective and precise quantity of *Punica granatum* peel powder, potentially in combination with different types of antioxidants of plant origin (fruits and vegetables), to combat the complications associated with obesity.

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Appendix

Appendix : Components of mouse food (ONAB) (National Livestock Office).

Proteins	15%
Lipids	2,5%
Cellulose	8%
Humidity	13%
Vitamin A	150.000 IU
Vitamin D3	200.000 IU
Vitamin E	3 mg
Iron	6 mg
Cu	1,2 mg
Zn	14,400 mg
Cobalt	60 mg
Mn	10,800 mg
Iodine	150 mg
Selenium	300 mg
Ca^{+2}	1%
Phosphorus	0,8%

Abstract

Abstract

The aim of our work is to evaluate the effect of *Punica granatum* peel powder on hyperlipidemia caused by a hypercholesterolemic diet. The study aimed to evaluate the effect of *P.granatum* on body weight and on some biochemical parameters including lipid profile (total cholesterol, triglycerides, HDL-c, and LDL-c), hepatic parameters (ASAT and ALAT) and markers of inflammation such as CRP.

For this purpose, *Mus musculus* mice received the standard diet, the hypercholesterolemic diet, the hypercholesterolemic diet with *P.granatum* peel powder, or only *P.granatum* peel powder for 15 days.

Our results showed that the hypercholesterolemic diet induced hyperphagia and obesity in mice on the hypercholesterolemic diet group compared to negative control group. The increase in fat mass is accompanied by notable changes with an increase in plasma levels of cholesterol, TG, LDL-C, ASAT, ALAT and CRP and a decrease in HDL-C. The beneficial effect of *Punica granatum* peel powder is marked by a reduction in cholesterol, LDL-c, ASAT, ALAT and CRP levels.

Based on the diverse findings, *Punica granatum* peel exhibits hypolipidemic, hepatoprotective, and anti-inflammatory properties, making it a promising candidate for pharmaceutical treatment against hyperlipidemia.

Keywords: Hypercholesterolemic diet, *Mus musculus* mice, *Punica granatum*, biochemical parameters, antihyperlipidemic effect.

Résumé

L'objectif de notre travail est d'évaluer l'effet de la poudre d'écorce de *Punica granatum* sur l'hyperlipidémie causée par un régime hypercholestérolémique. L'étude visait à évaluer l'effet de *P.granatum* sur le poids corporel et sur certains paramètres biochimiques dont le profil lipidique (cholestérol total, triglycérides, HDL-c, LDL-c), le bilan hépatique (ASAT et ALAT) ainsi que sur des marqueurs d'inflammation tels que la CRP.

Dans ce but, des souris *Mus musculus* ont reçu le régime standard, le régime hypercholestérolémique, le régime hypercholestérolémique avec de la poudre d'écorce de *P. granatum*, ou seulement de la poudre d'écorce de *P.granatum* pendant 15 jours.

Nos résultats ont montré que le régime hypercholestérolémique induisait une hyperphagie et l'obésité chez les souris du groupe sous régime hypercholestérolémique par rapport au groupe témoin négatif. L'augmentation de la masse grasse est accompagnée de changements notables avec une augmentation des niveaux plasmatiques de cholestérol, de TG, de LDL-C, d'ASAT, d'ALAT et de CRP et une diminution du HDL-C. L'effet bénéfique de la poudre d'écorce de *P.granatum* est marqué par une réduction des niveaux de cholestérol, de LDL-c, d'ASAT, d'ALAT et de CRP.

Sur la base des résultats diversifiés, la poudre d'écorce de *P.granatum* présente des propriétés hypolipidémiantes, hépatoprotectrices et anti-inflammatoires, ce qui en fait un candidat prometteur pour le traitement pharmaceutique contre l'hyperlipidémie.

Mots-clés : Régime hypercholestérolémique, souris *Mus musculus*, *P.granatum*, paramètres biochimiques, effet antihyperlipidémique.

ملخص

الهدف من عملنا هو تقييم تأثير مسحوق قشر *Punica granatum* على فرط شحميات الدم الناتج عن اتباع نظام غذائي يؤدي إلي فرط الكوليستيرول في الدم. هدفت الدراسة إلى تقييم تأثير *P.granatum* على وزن الجسم وعلى بعض المعايير البيوكيميائية التي تتضمن فحص الدهنيات (الكوليسترول الكلي، ثلاثي الغليسريد، HDL-c، LDL-c)، موشرات الكبد (ASAT وALAT) ومؤشرات الالتهاب مثل CRP.

لهذا الغرض، تلقت فئران *Mus musculus* النظام الغذائي النموذجي، النظام الغذائي لفرط الكولسترول، النظام الغذائي لفرط الكولسترول مع مسحوق قشر *P.granatum* ، أو مسحوق قشر *P.granatum فقط* وحده لمدة 15 يومًا.

أظهرت نتائجنا أن النظام الغذائي الخاص بفرط كوليسترول الدم يسبب فرط الأكل والسمنة لدى الفئران في مجموعة النظام الغذائي لفرط كوليستيرول الدم مقارنة بالمجموعة المرجعية السلبية. تترافق الزيادة في كتلة الدهون مع تغيرات ملحوظة من خلال زيادة في مستويات البلازما لكل من الكوليسترول، ثلاثي الغليسريد، LDL-C، ASAT، ALATوCRP مع انخفاض في HDL-C. يتمثل التأثير النافع لمسحوق قشر *Punica granatum* في خفض مستويات الكوليسترول، LDL-c، ASAT، ALAT، وCRP.

بناءً على النتائج المتنوعة، يُظهر قشر *Punica granatum* خصائص خافضة لشحوم الدم، واقية للكبد ومضادة للالتهابات، مما يجعله مرشحًا واعدًا للعلاج الدوائي ضد فرط شحميات الدم.

الكلمات المفتاحية: نظام الغذائي لفرط كوليستيرول الدم، فئران *Mus musculus* ، *Punica granatum*، معايير بيوكيميائية، تأثير مضاد لفرط شحميات الدم.

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Evaluation of the antihyperlipidemic, anti-inflammatory and hepatoprotective effects of *Punica granatum* in mice

Thesis submitted for obtaining the **Master's Degree in Molecular and Cellular Immunology**

Abstract

The aim of our work is to evaluate the effect of *Punica granatum* peel powder on hyperlipidemia caused by a hypercholesterolemic diet. The study aimed to evaluate the effect of *P.granatum* on body weight and on some biochemical parameters including lipid profile (total cholesterol, triglycerides, HDL-c, and LDL-c), hepatic parameters (ASAT and ALAT) and markers of inflammation such as CRP.

For this purpose, Mus musculus mice received the standard diet, the hypercholesterolemic diet, the hypercholesterolemic diet with *P.granatum* peel powder, or only *P.granatum* peel powder for 15 days.

Our results showed that the hypercholesterolemic diet induced hyperphagia and obesity in mice on the hypercholesterolemic diet group compared to negative control group. The increase in fat mass is accompanied by notable changes with an increase in plasma levels of cholesterol, TG, LDL-C, ASAT, ALAT and CRP and a decrease in HDL-C. The beneficial effect of *Punica granatum* peel powder is marked by a reduction in cholesterol, LDL-c, ASAT, ALAT and CRP levels.

Based on the diverse findings, *Punica granatum* peel exhibits hypolipidemic, hepatoprotective, and anti-inflammatory properties, making it a promising candidate for pharmaceutical treatment against hyperlipidemia.

Keywords: Hypercholesterolemic diet, Mus musculus mice, *Punica granatum*, biochemical parameters, antihyperlipidemic effect.

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