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Effect of vitamin C on cardiovascular diseases induced by hypercholesterolemia in rats

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Dedication

Thanks to ALLAH who have lightened my path and who granted me the ability to accomplish this work.

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Abbreviations

ALAT : Alanine Amino Transferase

ASAT: Aspartate Amino Transferase

C1q : Complement Component

DHA : Dehydroascorbic Acid

DNA : Deoxyribonucleic Acid

ER : Endoplasmic Reticulum

FCH : Familial Combined Hyperlipidemia

GLO : Gulonolactone Oxidase

GLUT : Glucose Transporters

HCHF: High levels of Cholesterol and High levels of Fat

HFH: Homozygous Familial Hypercholesterolemia

IPP :Iso Pentenyl Pyrophosphate

IV: Intra-Veineuse

LDLR : Low-Density Lipoprotein Receptor

LV : Left Ventricular

NO: Nitric Oxide

PIT : Pathologic Intimal Thickening

PRR : Pattern Recognition Receptor

SAP: Serum Amyloid Pcomponent

SVCT : Sodium Vitamin C Transporters

TCFA : Thin Cap Fibro Atheroma

UL : Upper Tolerable Limit

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Introduction

The dietary cholesterol is responsible for both the development of hypercholesterolemia and atherosclerosis has been the focus of many investigators. Many studies in rabbits (and other animal models) and in human diet and epidemiologic investigations indicated the importance of dietary cholesterol on serum cholesterol levels and its associated effects (**Sereday et al.,2004**). Lipids are considered one of the most elemental nutrients for humans. It may lead to disruption of signaling network and could be associated with some pathological stats, such as cancer, cardiovascular and similarly with inflammatory complication **(Jana et al., 2015).** Studies have shown that the increase in morbidity and mortality in low- and middle-income countries will increase the number of worldwide deaths from cardiovascular disease to 2330 million by 2030 **(Mathers and Loncar ,2006).** Therefore, the prevention of cardiovascular disease is an important topic of research. Atherosclerosis (AS) is the most common cause of cardiovascular disease, resulting in insufficient blood supply to the coronary arteries and subsequent myocardial ischemia and hypoxia, which are fundamental pathological processes of coronary heart disease (CHD). The under- lying mechanisms are very complex. Studies have been trying to interpret this disease by investigating various factors, including "endothelial injury" **(Ross and Glomset ,1976),** "lipid infiltration,""inflammatory response" **(Ross,1999),** and "thrombosis." However, it is clear that disruption of the integrity of the vascular endothelium and its dysfunction are part of the initial factors causing AS **(Stasch et al .,2002) and (Yanagisawa et al.,1988).** Therefore, the protection of endothelial function is significant for the prevenion and treatment of AS. Vascular **(Goligorsky et al., 2001).** Vitamin C or ascorbic acid, also known as antiscorbutic vitamin is an "enediolactone" of an acid similar to L-glucose. Plants and almost all animals except primates and guinea pigs synthesize this vitamin. It is watersoluble and widely distributed in plants and animal tissues. prolonged deficiency in man results to a condition known as scurvy. essentially, there are no storage forms of this vitamin in animal tissues but there is high concentrations in "metabolically highly active" organs such as adrenal cortex, liver, corpus luteum **(Chatterjea and Shinde, 2002).** Dietary sources consist chiefly of vegetables and fruits **(Annette and John, 1985).** This vitamin is concerned with synthesis of mucopolysaccharides of basement membranes of epithelial tissues, collagen and also in wound healing as well as antibody synthesis and healthy dentition. The activity

of this vitamin is also significant in vital metabolic activities including tryptophan metabolism, formation of active tetra hydrofolate, formation of ferritin as cellular antioxidant, iron absorption, **(Chartterjea and Shinde, 2002).** An association between vitamin C and atherosclerosis has been suggested in studies that evaluated the relationship between vitamin C and cholesterol levels **(Spittle, 1972)** and **(Dubic and Hunter, 1987).** Ascorbic acid levels were also found to be lower in patients with diagnosis of cardiac infarction and diabetes mellitus **(Chatterjea and Shinde, 2002).**

Objectives

In the present study, our objectives were to :

1-Establish the effect of hypercholesterolemia and vitamin C on the weight and diet of rats.
2-Evaluate the effect of high levels of vitamin C (500mg/kg) intake on hypercholesterolemia induced by diet rich in fats by measuring the levels of T-Ch, HDL-c, LDL-c and TG.
3-Evaluate the effect of vitamin C on the inflammation by measuring C-reactive protein induced by hypercholesterolemia.
4-Evaluate the effect of vitamin C on the enzymes Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT).
5-Evaluate the effect of vitamin C on the blood glucose .

Chapter 01

Cholesterol and Hypercholestrolemia

I.1. Cholesterol

I.1.1.Definition of cholesterol

Cholesterol is a waxy, fat- like substance that has many critical functions in the body **(Michael and Murayn, 2013).** It is an important component in cell membrane that maintains the structure and function of the cells. Moreover, cholesterol is a precursor of sex hormones, corticosteroid, and vitamin D. This vitamin is involved in bone formation, modulates immune system, and regulates gene expression **(Widmaier et al.,2013).** Cholesterol can be catabolized in to bile acids that play an important role in digestion and absorption of fat diets and fat-soluble vitamins **(Ikonen , 2008).**

I.1.2.Structure of cholesterol

Cholesterol molecular weight is: 386.65354 g/mol **(Kathleen, 2017).** It is not a simple molecule. At 27-carbons, it comprises four fused rings, an aliphatic side-chain, and a hydroxyl group **(Brown and Sharp , 2015). (Figure 01 and 02).**

I.1.3.Transport of cholesterol

Cholesterol is transported throughout the bloodstream by joining to specific proteins and lipids forming lipoproteins. There are four main types of lipoprotein acting as cholesterol carriers in circulation: chylomicrons, very low-density lipoproteins (VLDL), low-density lipoprotein (LDL) “bad cholesterol”, and high- density lipoprotein (HDL) “good cholesterol” **(Widmaier et al.,2013). (Figure 03).**

I.1.4. High and low density lipoprotein

I.1.4.1.Low density lipoprotein LDL

Low-density lipoprotein (LDL) ‘bad cholesterol’ (**Widmaier et al, 2013),** It is a major carrier of cholesterol to peripheral cells, accounting for over 60% of the total cholesterol in plasma. **(Mozaffarian et al ., 2015).** LDL constitutes ~50% of the total lipoprotein mass in the plasma. It contains a single protein, ApoB100 , and lipids (~25 and ~75% of mass, respectively), the latter consisting of ~6–8% free cholesterol, ~45–50% cholesteryl ester, ~18–24% phospholipid, and ~4–8% triacylglycerols **(Hegele, 2009)**.

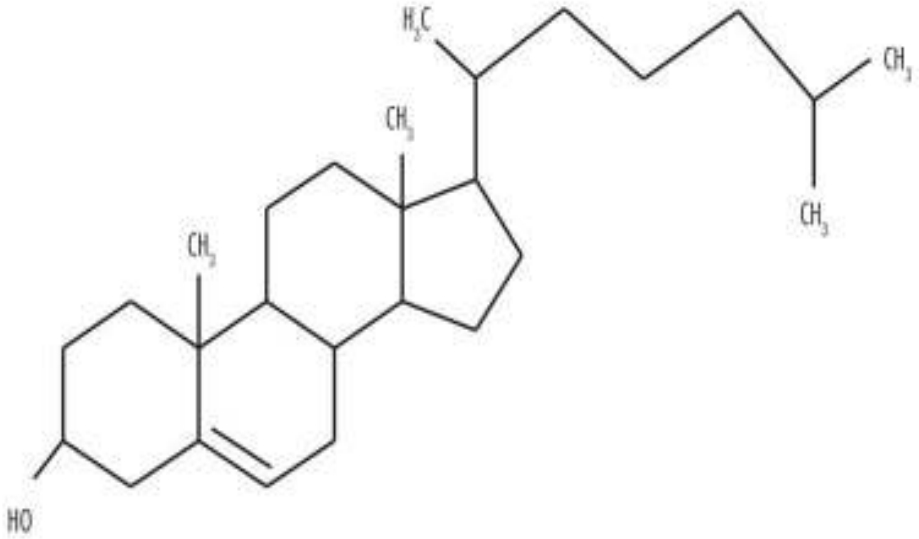

Figure 01: Chemical structure of cholesterol **(Watson and Meester, 2017).**

Figure 02: A sphere model of cholesterol molecule (carbon in blue, oxygen in red, hydrogen in white) **(Di Scala and Fantini, 2017).**

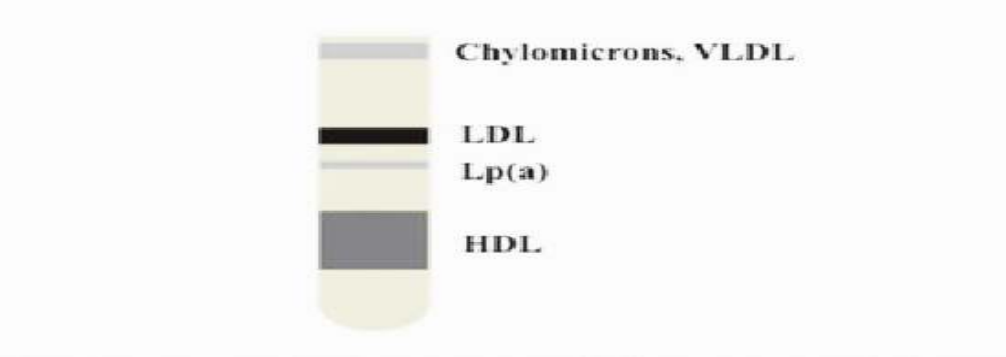

Figure 03: Schematic digram of lipoprotein-subclass separation after gradient density centrifugation**(Loregger et al ., 2017).**

I.1.4.2. High density lipoprotein HDL

High density lipoprotein (HDL) 'good cholesterol' (**Widmaier, 2013).** It elicits cardioprotective function by reverse cholesterol transport to the liver to be catabolized, moreover, HDL has antioxidant and anti-inflammatory effects as well as involved in nitric oxide (NO) homeostasis **(Xu ,2013).**

I.1.5. Biosynthesis of Cholesterol

The cells get its cholesterol through two pathways, endogenous source by means of biosynthesis in liver (80%) and exogenous source from the diet (20%) **(Ikonen, 2008).** Each cells synthesizes cholesterol from simpler molecules. The body compensates for any absorption of additional cholesterol by reducing cholesterol synthesis **(Lecerf and De Lorgeril, 2011).** Usually found as free cholesterol in the cells of the liver (hepatic cells) following synthesis; and in the bloodstream, where it is transported as inactive esters, the role of cholesterol cannot be overemphasized. The quantity of cholesterol that is required everyday by an average human body is about 900 mg and of this amount, a range of between 200 to 500 mg is provided by the diet while the rest is produced by the hepatic cells and the central nervous system **(Levy et al., 2007).(figure 04).**

I.1.5.1.Exogenous Pathway

Cholesterol absorption occurs primarily in the duodenum and proximal jejunum at levels of efficiency that vary greatly among different individuals **(Kesäniemi et al., 1987)** and **(Bosner et al ., 1999).** There are two main phases of cholesterol absorption **(Turley, 1999).**

a) The first takes place in the lumen and involves digestion and hydrolysis of dietary lipids followed by solubilization of cholesterol in mixed micelles containing bile acid and phospholipids. This solubilization facilitates the movement of cholesterol from the bulk phase of the lumen to the surface of the enterocyte.

b) In the second phase, cholesterol crosses the mucosal cell membrane by simple diffusion, and probably by facilitated diffusion as well. Thus far, a specific cholesterol transporter in the microvillus membrane of the enterocyte has not been identified **(Turley, 1999)(Kramer et al., 2000).** Within the cell the cholesterol is re-esterified

and incorporated in to apolipoprotein (B) containing nascent lipoproteins that are secreted into the lymph **(Turley, 1999).** (**Figure 05**)

c) The chylomicrons enter the bloodstream through the thoracic duct, and bind to the wall of capillaries in adipose and skeletal muscle tissue. Then cholesterol after a series of reaction either converted into bile acids, excreted in bile, or incorporated into lipoproteins originated in the liver **(Rafael et al.,1990).**

I.1.5.2.Endogenous Pathway

Cholesterol synthesis occurs in the cytoplasm and microsomes from the two-carbon acetate group of acetyl-CoA **(Clayton,1998).** The process has five major steps:

1. Acetyl-CoAs are converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) by HMG-CoA synthase.

2.3-hydroxy-3-methylglutaryl-CoA is converted to mevalonate by HMG-CoA reductase (HMG-CoA-R).

3. Mevalonate is converted to the isoprene based molecule, isopentenyl pyrophosphate (IPP), with the concomitant loss of CO2

4. Isopentenyl pyrophosphate is converted to squalene

5. Squalene is converted to cholesterol in the endoplasmic reticulum (ER).

From its site of synthesis, cholesterol is transported to other cellular destinations, prevalently plasma membranes, where 70-90 % of cellular cholesterol resides. It is reported that cholesterol reaches the cell surface within 10-20 min after synthesis in the ER. **(Lange and Matthies, 1984)** and **(Urbani and Simoni, 1990).** Recent evidences indicate caveolae as the initial site on the cell surface where new cholesterol appears **(Fielding, P and Fielding, C, 1995)** and **(Fielding, P and Fielding, C, 1996)** .Caveolae are distinctive, flask-shaped invaginations of the plasma membrane found in many cells. In contrast to coated pits, which are constitutively endocytosed, caveolae remain attached to the plasma membrane with their release being affected by unknown signal. They have a characteristic lipid composition, rich in cholesterol and glycosphingolipids, and are associated with the presence of a 22 kD protein called caveolin-1 **(Anderson,1998)** and **(Kurzchalia and Partan, 1999).** It is also reported that caveolin-1 is required for the translocation of newly synthesized cholesterol from the ER directly to caveolae. The arrival of new cholesterol in caveolae is followed by the immediate movement of the sterol to noncaveolae membrane and possibly out of the cell **(Smart, 1996).** (**Figure 04 and 05**).

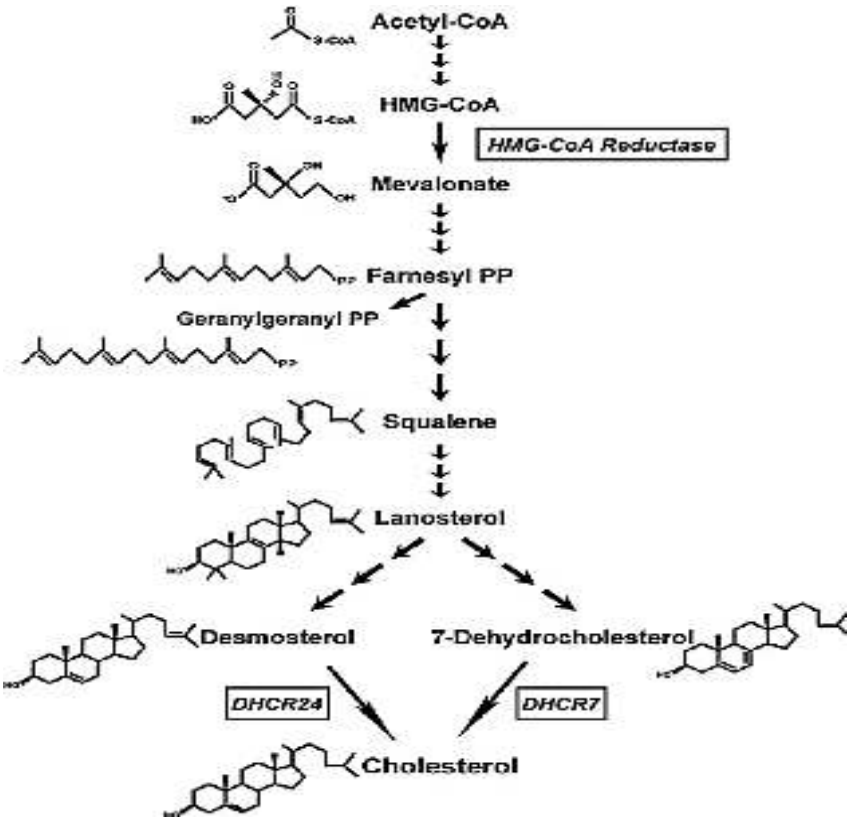

Figure 04: Mevalonate pathway endogenous pathway **(Cortes et al., 2014).**

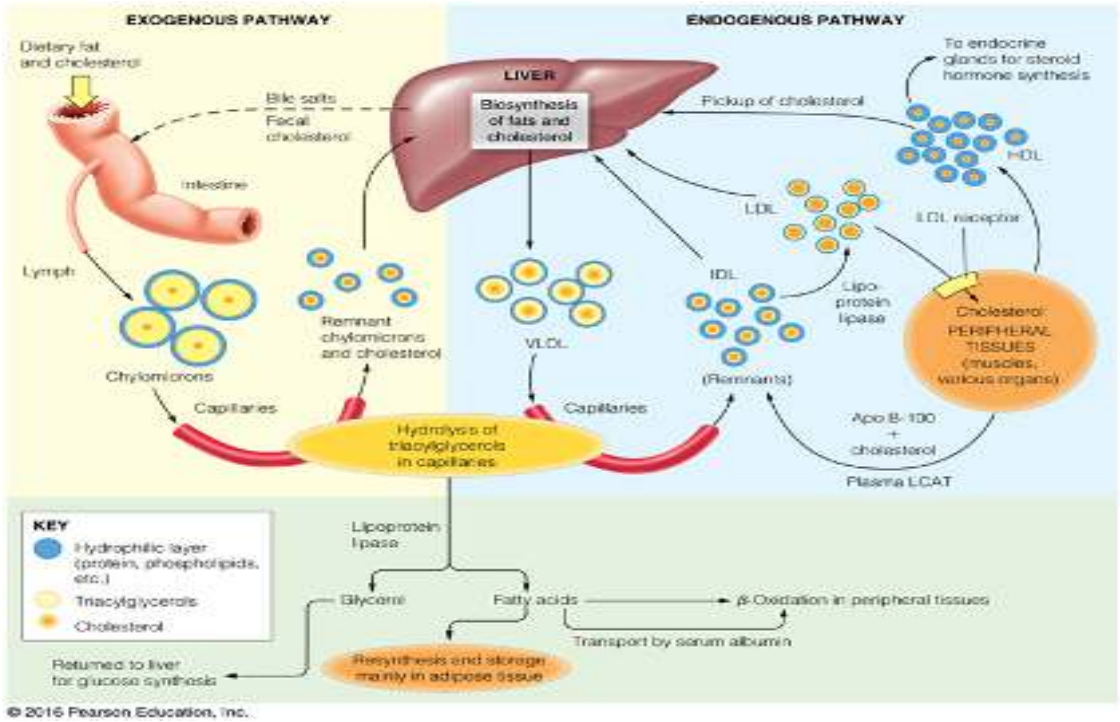

Figure 05 : Exogenous and the endogenous pathway synthesis of cholesterol **(pearson, 2016).**

I.1.6.Biological functions of cholesterol

1. Cholesterol is an important component of the cell membranes, including organelle membranes inside the cell.

2. The right proportion of phospholipids, fatty acids and cholesterol in cell membranes allows them to be flexible while still holding their shape.

3. Cholesterol is used by the body as raw material for the healing process. This is the reason the injured areas in the arteries (as in atherosclerosis) have cholesterol along with several other components (such as calcium and collagen) in the "scar" tissue that is formed to heal the "wound" **(Enig, 2000)**

4. Cholesterol is found in large amounts in brain tissue where it is needed for normal brain function. Research has shown that cholesterol in eggs is helpful to older people whose memory is declining **(Singer, 1995)**

5. Cholesterol often elevates as part of a protective immune system response to chronic infection.

6. Infants need plenty of cholesterol for proper brain development and cholesterol is normally found in large amounts in human breast milk.

7. Adrenal and gonadal hormones are made from cholesterol. These are the stress handling, energy producing and reproductive hormones. (This is why serum cholesterol normally elevates with excessive or prolonged stress.)

8. Cholesterol is vital for proper nerve function. Three quarters of the myelin membrane is made from fat and of that nearly one quarter is cholesterol.

9. Vitamin D is made from cholesterol in the skin.

10. Cholesterol is converted into bile salts in the liver which are needed to break down and emulsify fats.

11. Cholesterol is needed in large amounts in the skin where it is vital for skin health and strength.

12. Although lowering serum cholesterol does seem to decrease deaths from heart disease, it

"does not, in the least , improve overall mortality rates. People who achieved the lowest cholesterol levels 160 units or less had unexpectedly higher rates of death from other causes, such as liver cancer, stroke, lung disease, alcoholism and suicide**."(Donald et al., 1996)**

I.1.7. Cholesterol homeostasis

Whole-body cholesterol homeostasis is determined by cholesterol absorption, cholesterol synthesis and cholesterol excretion, and losing control of any of these processes leads to an increase in plasma cholesterol. Liver and intestine are the major sites that control cholesterol homeostasis. The liver synthesizes cholesterol for secretion in nascent lipoproteins when blood levels of cholesterol are low, and removes excess cholesterol from the blood by taking up chylomicron remnants, high density lipoprotein (HDL), very low density lipoprotein (VLDL) and low density lipoprotein (LDL) particles. It converts cholesterol into bile acids, and secretes cholesterol and bile acids into bile for elimination from the body. The intestine regulates influx of cholesterol from the lumen and efflux of cholesterol back into the lumen to control the amount of cholesterol that enters the body **(van der et al ., 2013).**

I.2.Hypercholesterolemia

I.2.1. Definition of hypercholesterolemia

Hypercholesterolemia is characterized by LDL cholesterol exceeding 159 mg/dl **(NCEP,2001).** Diets containing high levels of cholesterol and high levels of fat (HCHF) are frequently the culprit in causing hypercholesterolemia. In addition, genetic factors influence susceptibility to diet-induced hypercholesterolemia **(Goldstein and Brown, 1979).**

Hypercholesterolemia has been implicated as pathogenesis of pancreatitis, hepatitis, renal injury, stroke, atherosclerosis, and metabolic syndrome by oxidative damage-dependent mechanism **(Olorunnisola et al., 2012).**

I.2.2.Causes of hypercholesterolemia

I.2.2.1.Genetic

Hypercholesterolemia is a complex disorder often due to multiple genetic defects and rarely due to a single genetic defect **(Goldstein, 1979).** Much research has been devoted to

understanding the genetic variants and environmental factors that contribute to elevated blood LDL cholesterol **(Burnstock, 1998)** and **(Yanni, 2004).**

I.2.2.1.1. Polygenic hypercholesterolemia

Polygenic hypercholesterolemia is the most common form of hypercholesterolemia caused by a susceptible genotype, still unknown, aggravated by excessive saturated fat, trans fatty acid and cholesterol intake. The patients did not exhibit any LDL-receptor defect and manifested a moderate hypercholesterolemia (240–350 mg/dL) with serum triglyceride concentrations within the reference range **(Ruiu et al., 2009).**

I.2.2.1.2. Familial hypercholesterolemia

Familial hypercholesterolemia (FH) is an autosomal codominant genetic disorder of lipoprotein metabolism, usually caused by mutations in the low-density lipoprotein (LDL) receptor (LDLR) gene or other genes that affect LDLR function. Patients can be heterozygous (HeFH) with one mutated allele, homozygous (HoFH) with two identical mutations, or compound heterozygous with different mutations in each allele **(Sniderman, 2014).** Hypercholesterolemia is present from childhood, leading to early development of Coronary Heart Disease CHD **(Goldberg et al., 2011).** And characterized by a high concentration of serum LDL cholesterol **(Soutar and Naoumova, 2007).**

I.2.2.1.3.Familial combined hypercholesterolemia

Familial combined hyperlipidemia (FCH) is a common genetic lipid disorder. Affected subjects characteristically have elevated levels of plasma total cholesterol, triglycerides, and apolipoprotein (apo) B, and are more prone to develop premature cardiovascular disease (CVD) **(Genest et al., 1992)** and **(Goldstein et al., 1973).**The prevalence of this disease is 1–2% in the general population **(Hoffer et al., 1996).** And 10–20% among patients with premature coronary disease **(Goldstein et al., 1973)**.

I.2.2.2. Foods high in saturated fats and Cholesterol

There is substantial support suggesting that dietary SFA raise total and LDL cholesterol levels, thereby increasing the risk of atherosclerosis and CVD **(Sharrett et al., 2001).** Recent studies suggest that not all SFAs have the same impact on serum cholesterol levels. Unlike LDL, HDL is inversely associated with cardiovascular disease **(Santos-Gallego and**

Badimon, 2012). SFAs have varying effects on LDL and HDL levels suggesting the ratio of LDL:HDL cholesterol is a better measure of CVD risk **(Kris-Etherton and Yu, 1997)** and **(Mensink et al., 2003).**In contrast palmitic acid , and myristic acid both increase LDL cholesterol levels while lowering HDL to LDL ratios **(Zock et al., 1994).** Over 200 risk factors have been identified for cardiovascular disease **(Castelli, 1996).** In recent years, focus upon diet and circulating lipids has shifted from the assumption that a high fat diet raises blood cholesterol, which in turn is associated with conditions such as coronary heart disease, to include the role of omega -3 fatty acids in the modulation of cardiovascular functions **(De Meester, 2009)** and **(Dubnov et al., 2008)** and **(Simopoulos, 2009)**.

I.2.2.3.Other diseases

Some people suffering from diabetes may have high levels of cholesterol **(Kathleen, 2017).**

I.3. Complications of hypercholesterolemia

A – Atherosclerosis

Hyperlipidemia is the most important risk factor for atherosclerosis, which is the major cause of cardiovascular disease. Atherosclerosisis a pathologic process characterized by the accumulation of lipids, cholesterol and calcium and the development of fibrous plaques with-in the walls of large and medium arteries **(Wouters et al., 2005).**

B -Coronary Artery Disease (CAD)

Atherosclerosis, the major cause of coronary artery disease, characterized by the accumulation of lipid and the formation of fibrous plaques within the wall of the arteries resulting in narrowing of the arteries that supply blood to the myocardium, and results in limiting blood flow and insufficient amounts of oxygen to meet the needs of the heart. Elevated lipid profile has been connected to the development of coronary atherosclerosis **(Gao et al., 2012).**

C -Myocardial Infarction (MI)

MI is a condition which occurs when blood and oxygen supplies are partially or completely blocked from flowing in one or more cardiac arteries, resulting in damage or death of heart cells. The occlusion may be due to ruptured atherosclerotic plaque. The studies show that

about one-fourth of survivors of myocardial infarction were hyperlipidemic **(Nickolas, 2003).**

D -Ischemic stroke

stroke is the fourth leading cause of death. Usually strokes occur due to blockage of an artery by a blood clot or a piece of atherosclerotic plaque that breaks loose in a small vessel within the brain. Many clinical trials revealed that lowering of low-density lipoprotein and total cholesterol by 15% significantly reduced the risk of the first stroke **(Amarenco, 2009).**

Chapter02

Inflammation and

Cardiovascular diseases

II.Cardiovascular diseases

II. 1.Inflammation

II. 1.1.Definition

Inflammation is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritantsacts by removing injurious stimuli and initiating the healing process(**Ferrero et al .,2007**).It is therefore a defense mechanism that is vital to health**(Nathan,2010).**At the tissue level, inflammation is characterized by redness, swelling, heat, pain, and loss of tissue function, which result from local immune, vascular and inflammatory cell responses to infection or injury**(Takeuchi, 2010).**Important microcirculatory events that occur during the inflammatory process include vascular permeability changes, leukocyte recruitment and accumulation, and inflammatory mediatorrelease(**Ferreroet al .,2007**) and **(Chertov, 2000).**

II. 1.2. Types of inflammation

II.1.2.1. Acute inflammation

Acute inflammation is a short-term process, usually appearing within a few minutes or hours and begins to cease upon the removal of the injurious stimulus**(Kumar, 1998).**It involves a coordinated and systemic mobilization response locally of various immune, endocrine and neurologicalmediators. In a normal healthy response, it becomes activated, clears the pathogen and begins a repair process and then ceases**(Kumar, 2004).**

However, uncontrolled acute inflammation may become chronic, contributing to a variety of chronic inflammatory diseases**(Zhou, 2016).**

II.1.2.2. Chronic inflammation

Chronic inflammation occurs when acute inflammatory mechanisms fail to eliminate tissue injury **(Lintermans et al, 2014).**and may lead to a host of diseases, such as cardiovascular diseases, atherosclerosis, type 2 diabetes, rheumatoid arthritis, and cancers **(Sugimoto et al 2016).**

II.1.3. C-reactive protein

II 1.3.1.Definition

C-reactive protein (CRP) is an annular (ring-shaped), pentameric protein found in blood plasma, whose levels rise in response to inflammation. It is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion by macrophages and T cells. Its physiological role is to bind to lysophosphatidylcholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via C1q(**Thompson and pepys ,1999**). CRP is synthesized by the liver (**Pepys and Hirschfield,2003**). In response to factors released by macrophages and fat cells (adipocytes).(**Lau et al.,2005**). It is a member of the pentraxin family of proteins.**(Pepys and Hirschfield,2003).**It is not related to C-peptide (insulin) or protein C (blood coagulation). C-reactive protein was the first pattern recognition receptor (PRR) to be identified **(Mantovani et al.,2008).**

II. 1.3.2.Structure

CRP consists of five no covalently associated protomers arranged symmetrically around a central pore. The overall dimensions of the CRP pentamer are about 102 A outside diameter with a central pore diameter of 30 A and a protomer diameter of 36 A. The protomer consists of 206 amino acids folded into two antiparallel sheets with a flattened jellyroll topology**(Fig. 06)**. The structure is very similar to that of SAP, although certain unique features characterize each protein.**(Marnell et al., 1995).**On the opposite side of the protomer, two Ca ions are ligated to side chains and main chain carbonyls of the polypeptide chain at a distance of 4 A from each other. Both Ca2+ articipate in ligand-binding.Electronmicroscopic**(Roux et al., 1983).**

II.1.3.3.Function

C-reactive protein has ability to recognize pathogens and to mediate their eliminationby recruiting the complement system and phagocyticcells makes CRP an important constituent of the firstline of innate host defense. Furthermore, the proteinappears to play a role in the clearance of apoptotic andnecrotic host cells, thus contributing to restoration ofnormal structure and function of injured tissues. Likeother elements of immunity, CRP perhaps has not onlyprotective, but also potentially harmful effects. Thus,recently CRP has been

implicated in atherogenesis **(Torzewski et al., 2000)** and**(Zwaka et al., 2001)** and in themediation of tissue damage in acute myocardial infarction**(Griselli et al., 1999).**

II. 1.3.4.C- Reactive protein and inflammation

C-reactive protein (CRP) is a member of the phylogeneticallyancient and highly conserved 'pentraxin' familyof proteins, which also includes serum amyloid Pcomponent (SAP), a constituent of all amyloid deposits.In man and other animal species,
(VolanakisandKaplan, 1971).

II.1.3.5.C- Reactive protein level and clinical implication and indication

In healthy adults, the normal concentrations of CRP varies between 0.8 mg/l to 3.0 mg/l.The rate of CRP production increases with inflammation, infection, trauma, necrosis, malignancy, and allergic reaction. The CRP level also increases with age, possibly due to increasing subclinical condition. There is also no seasonal variations of CRP levels. (**Pepys,2003**). CRP between 100 to 500 mg/dL is considered as bacterial inflammation. CRP concentrations between 2 to 10 mg/dL are considered as metabolic inflammation (metabolic pathways that causes arteriosclerosis and Type II diabetes mellitus). Once inflammation subsides, CRP level falls quickly because of its short half-life (4 to 7 hours) (**Bray et al ., 2016**) .

III.Atherosclerosis

III.1.definition

Atherosclerotic lesions develop in the walls of large arteries and cause occlusion of blood vessels as a result of either arterial wall thickening or thrombus formation on the surface of unstable plaques. This latter condition is especially dangerous, since it can lead to a sudden and often fatal thromboembolia, which represents the first clinical manifestation of atherosclerosis in many patients. By contrast, early stages of the disease usually pass unnoticed. Recent studies have demonstrated that asymptomatic atherosclerosis is, in fact, a widespread condition among young adults **(Simmons et al ., 2012)** and**(Tuzcu et al ., 2001).** In this cohort of subjects, the incidence of atherosclerotic lesions reaches 100%, although no clinical manifestations can be observed **(Berenson et al ., 1998)** and **(Tuzcu et al ., 2001)** The development of atherosclerosis is a complex process, which, despite the significant

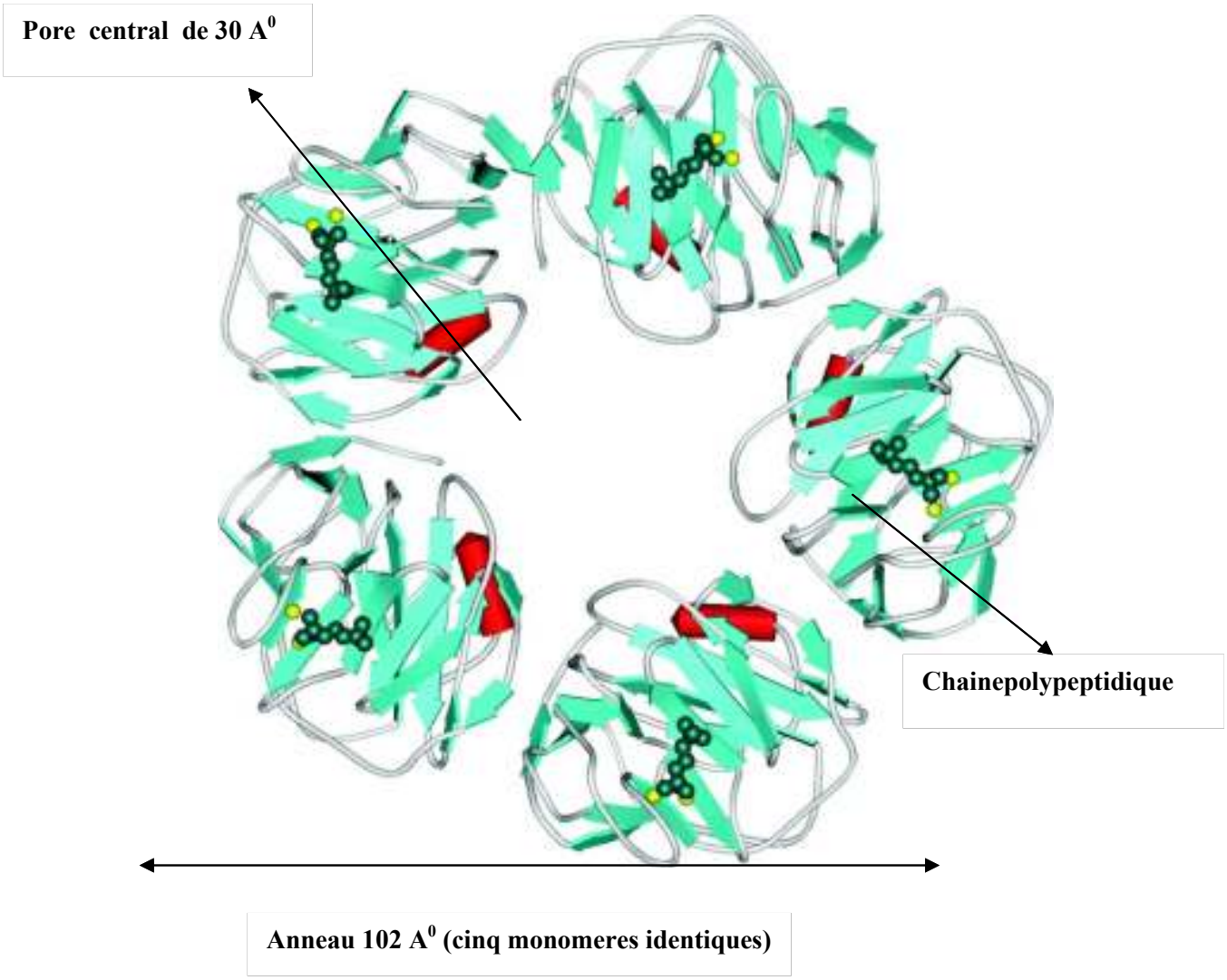

Figure 06: Crystal structure of C-reactive protein complexed with phosphocholine**(Thompson et al. 1999).**phosphocholine complex The calcium ions are yellow, and phosphocholine is green.

progress made during the last decade, still remains to be fully understood. Atherosclerosis and related cardiovascular disorders are associated with several known risk factors, including elevated plasma cholesterol level, diabetes, tobacco smoking and others **(Anderson et al ., 1991)** and **(Fowkes et al ., 2013).**

III.2.Anatomy of a normal artery

A large artery consists of three morphologically distinct layers. **(Figure 07)**

1-The intima, the innermost layer, is bounded by a monolayer of endothelial cells on theluminal side and a sheet of elastic fibres, the internal elastic lamina, on the peripheral side. The normal intima is a very thin region **(Figure08)** and consists of extracellular connective tissue matrix, primarily proteoglycans and collagen.

2- The media, the middle layer, consists of smooth muscle cells SMCs.

3-The adventitia, the outer layer, consists of connective tissues with interspersed fibroblasts and smooth muscle cells (SMCs) **(Aldons, 2000).**

III.3. Atherosclorosis development

The cells populating the intinial layer in the developement of atheroscletic lesion can be either resident mesenchymalcells , such as smooth muscle cells or inflammatory cells , such as monocytes macrophages **(Kruth,2002).**Along with macrophqges , smooth muscular cells also take part in lipid uptake and canbe transformed into foam cells. The LDL are recognised by macrophages as pathogens that have to be cleared by phagocytosis **(Kruth,2002).**Phago-cytosis-mediated lipid accumulation in atherosclerosis can therefore be regarded as a variation of innate immune response **(Andreeva,1997).**Lipid accumulation also triggers processes that are typical for the reparative phase of inflammation, such as proliferation and extracellular matrix synthesis leading to the fibrosis **.**Gradual development of such focal lesion areas leads to a diffuse intimal **(Orekhov et al., 2016).(Figure09 and 10).**

Atherosclerotic plaques can be protected from the bloodstream by formation of a fibrous cap, which serves as a barrier for lipoproteins and inflammatory cells **(Orekhov et al., 2016)**.

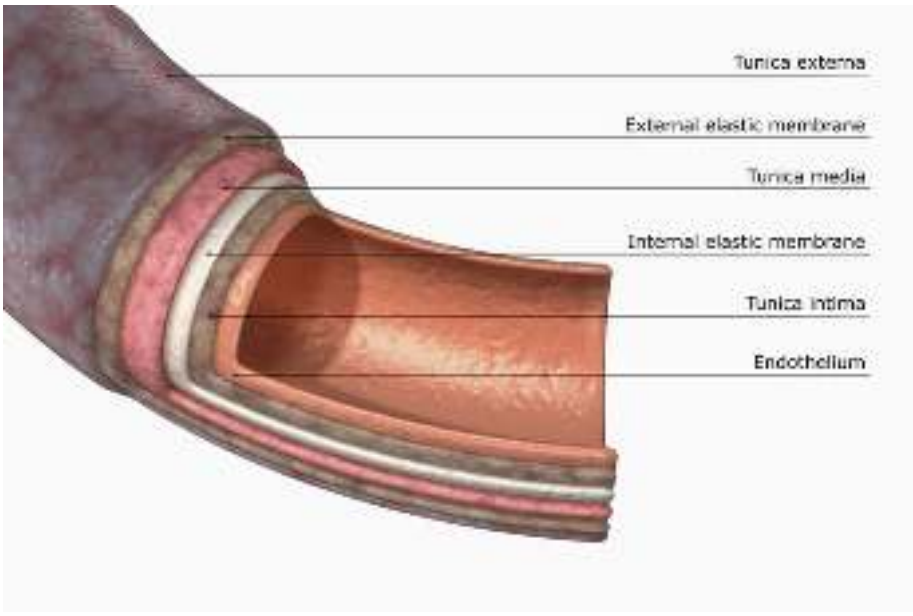

Figure 07: Artery Structure**(web site1)**

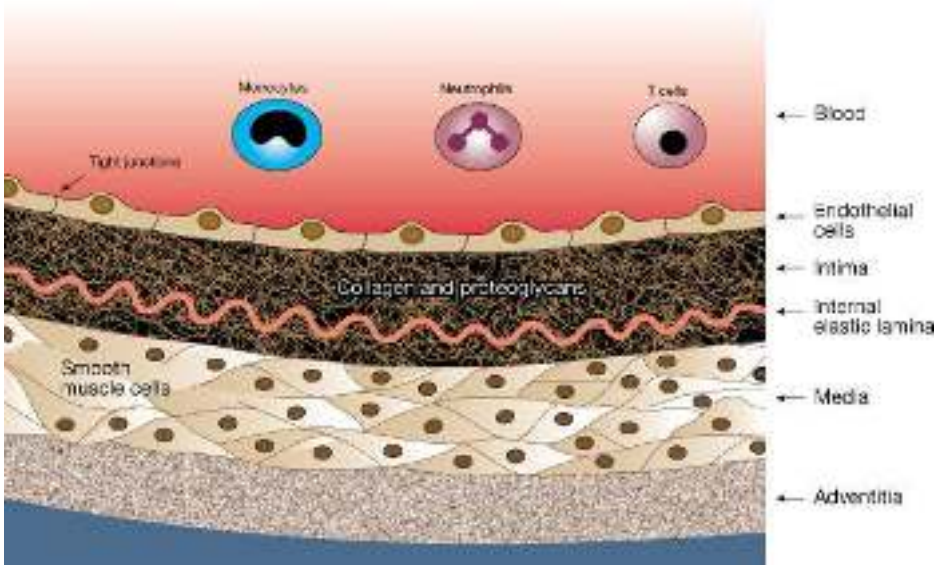

Figure 08: Anatomy of normal arterial and composition **(Aldons, 2000)**

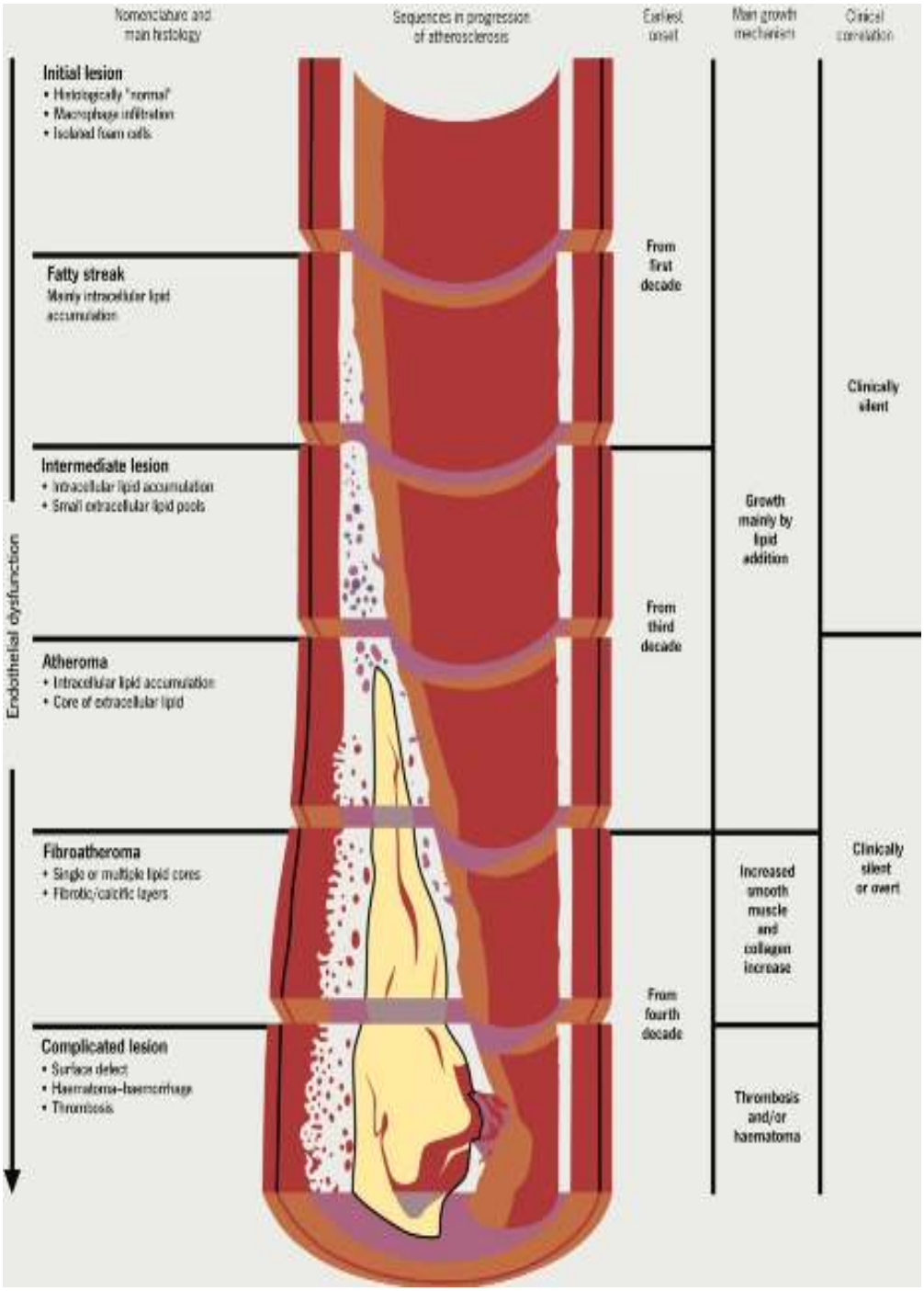

Figure09: the progression of atherosclerosis**(site2)**

III.4. Atherosclerosic plaque

Atherosclerotic plaque rupture with luminal thrombosis is the most common mechanism responsible for the majority of acute coronary syndromes and sudden coronary death. The precursor lesion of plaque rupture is thought to be a thin cap fibroatheroma (TCFA) or "vulnerable plaque". TCFA is characterised by a necrotic core with an overlying thin fibrous cap (≤65 μm) that is infiltrated by macrophages and T-lymphocytes. Intraplaquehaemorrhage is a major contributor to the enlargement of the necrotic core. Haemorrhage is thought to occur from leaky vasa vasorum that invades the intima from the adventitia as the intima enlarges. The early atherosclerotic plaque progression from pathologic intimal thickening (PIT) to a fibroatheroma is thought to be the result of macrophage infiltration (**Sakakura et al 2013).**

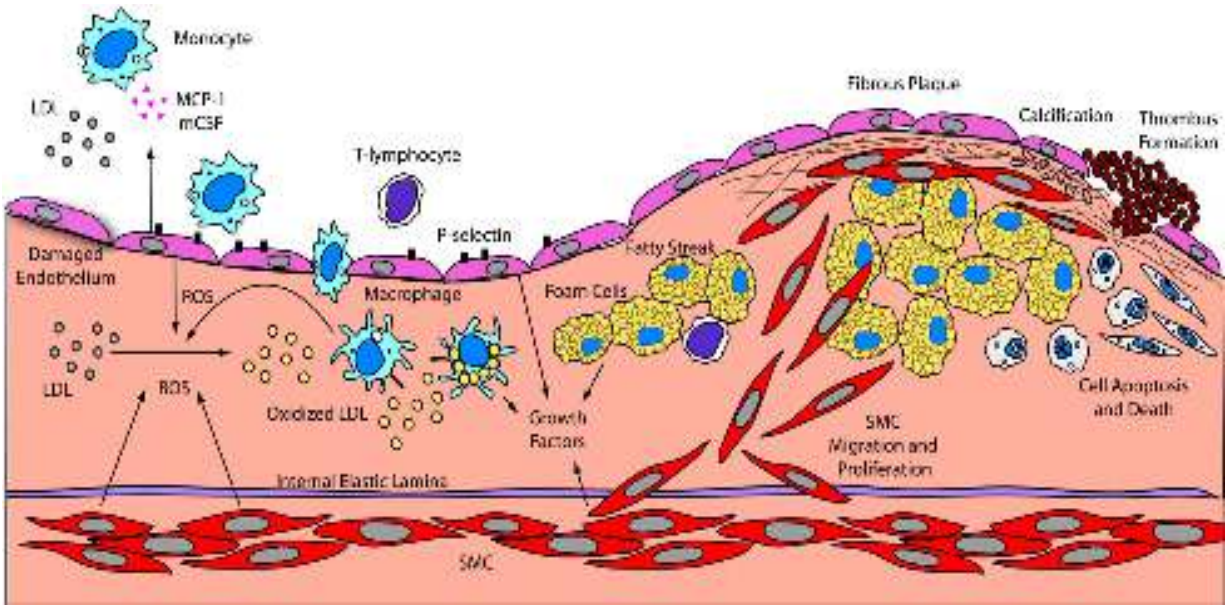

Figure 10: Endothelial injury dysfunction (cause of atherosclorosis) **(Madamanchi.,2005).**

Chapter03

Vitamin c

IV.Vitamin C

IV.1. Definition

Vitamin C was first identified in fruits (citrus), vegetables and adrenal glands as hexuronic acid in the 1920s by Albert Szent-Györgyi, a Hungarian biochemist. With the help of an American physician named Joseph Svibely, Szent-Györgyi was able to identify that hexuronic acid was vitamin C through scurvy research on guinea pigs. It was later named ascorbic acid (meaning "without scurvy" from Latin) by Szent-Györgyi and Norman Haworth, a scientist who also studied vitamin C **(Grzybowski and Pietrzak, 2013).**The particular mechanism that prevents synthesis is the absence of gulonolactone oxidase (GLO) (**Mandl, 2009**). Which is necessary to catalyze the enzyme L-gulono-1,4-lactone oxidase, the final step in the biosynthetic pathway of vitamin C **(Gallie, 2013).**There are numerous reasons why vitamin C is important to our health,but many involve its aspect as essential factor in the synthesis of collagen, carnitine and norepinephrine **(Chatterjee, 1973)**. The two main components of vitamin C are ascorbate and dehydroascorbic acid (DHA) (**Doll and Ricou, 2013)**

IV.2. Sources of vitamin C

IV.2. 1.Distribution in foods

Vitamin C is widely distributed in both plants and animals, occurring mostly (80–90%) as ascorbic acid but also as dehydro ascorbic acid. The proportion of both species tends to vary with food storage time, due to the time-dependent oxidation of ascorbic acid. Fruits, vegetables, and organ meats (e.g., liver and kidney) are generally the best sources; only small amounts are found in muscle meats **(Table 01).** Plants synthesize l-ascorbic acid from carbohydrates; most seeds do not contain ascorbic acid but start to synthesize it on sprouting. Some plants accumulate high levels of the vitamin C (e.g., fresh tea leaves, some berries, guava, rose hips). **(Gerald et al., 2017).**

Table 01: Vitamin C Contents of Foods **(USDA.2017).**

Food	**VitaminC,mg/100g**
Fruits	
Apple	5
Banana	9
Cherry	7–10
Grapefruit	34
Guava	228
Lemon	53
Melons	8–37
Orange	59
Peach	7
Rasp berry	26
Rose hips	426
Strawberry	59
Tangerine	27
Vegetables	
Asparagus	6
Broccoli	89
Cabbage	37
Carrot	3
Cauliflower	48
Celery	3
Collards	35
Corn	7
Kale	120
Leek	12
Potato	11
Onion	7
Pea	40
Parsley	133
Pepper	80–128
Cereals	(none)
Animal Products	
Beef	0
Milk cow	0–1
Milk, human	5

IV.2. 2.Biosynthesis of ascorbic acid

Most higher animals (and probably all green plants) can synthesize vitamin C. They make it from glucose via the glucuronic acid pathway. **(Figure 11 ,Table 02).** The enzymes of this pathway are localized in the kidneys of amphibians, reptiles, egg-laying mammals, and the more primitive orders of birds. Evolutionary loss of ascorbic acid biosynthetic capacity appears to have occurred in invertebrates, teleost fishes, several species of birds (e.g., red-vented bulbul), and some mammals (humans, other primates, guinea pigs, most bats, and a few mutant strains of rats). These species do not express the last enzyme in the biosynthetic pathway, l-gulonolactone oxidase. While all species studied have the gene, in some it is so highly mutated that it yields no gene product. The loss of this single enzyme renders ascorbic acid, an otherwise normal metabolite, a vitamin. Therefore, scurvy can correctly be considered a congenital metabolic disease, hypoascorbemia. **(Gerald and James, 2017).**

IV.3. Transport of vitamin C

The two main components of vitamin C are ascorbate and dehydro ascorbic acid (DHA) **(Mandl, 2009).** The transport of ascorbate through the human body involves two sodium-dependent vitamin C transporters (SVCT): SVCT1 and SVCT2 **(Li and Schellhorn, 2007).** The majority of ascorbate is transported by SVCT1 in epithelial cells (e.g. intestine, kidney and liver), and the remaining is transported by SVCT2 in specialized cells (e.g. brain and eye) **(Tsukaguchi et al., 1999).** DHA (the oxidized form of ascorbate) is transported in the human body through 2 glucose transporters (GLUT): GLUT1 and GLUT3 **(Li and Schellhorn, 2007).** (a third transporter, GLUT 4, is used only for insulin-sensitive tissues **(Padayatty and Levine, 2001).**). Once DHA has been transported inside the cell by a GLUT, it is reduced back to ascorbate **(Li and Schellhorn, 2007).** The distribution and homeostasis of vitamin C in the human body is regulated by the SVCTs, GLUTs, facilitated diffusion through channels and exocytosis in secretory vesicles **(Wilson, 2005).** The main concentrations of vitamin C are located in brain and adrenal cells. **(Figure 12) .**

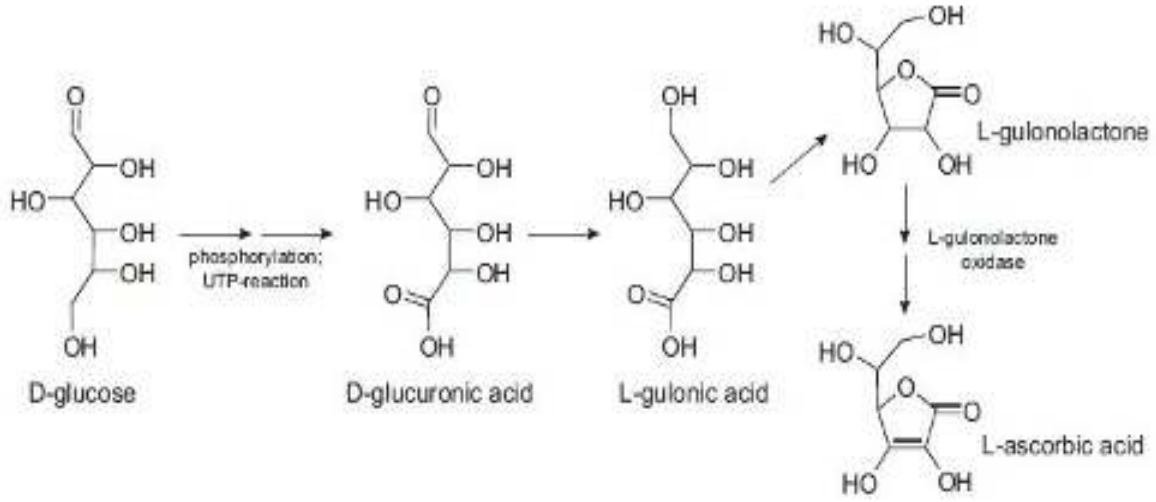

Figure 11 : Biosynthesis of ascorbic acid. **(Gerald and James, 2017).**

Table 02: Estimated rates of ascorbic acid biosynthesis in several species **(Gerald and James ., 2017).**

Species	Synthetic Rate, mg/kg BW	$T_{1/2}$,[a] days	Turnover, %/day
Mouse	125	1.4	50
Golden hamster	20	2.7	26
Rat	25	2.6	26
Rabbit	5	3.9	18
Guinea pig	0	3.8	18
Human	0	10–20	3

[a]*half-life in the body.*

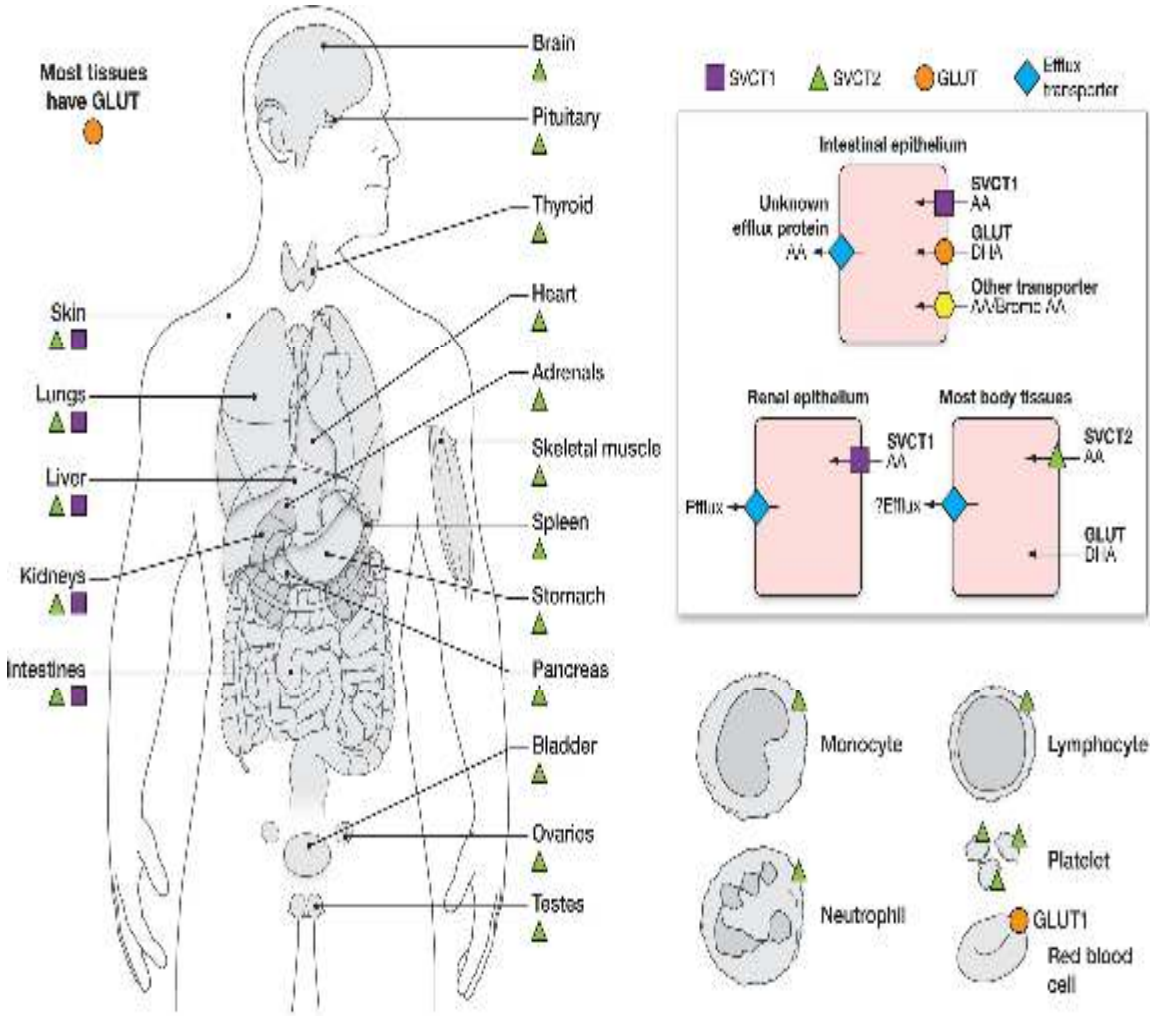

Figure 12 : Vitamin C transporters. Distribution of vitamin C transporters in human tissues. **(Padayatty and Levine, 2016)**

IV.4.Absorption and tissue distribution of vitamin C

Vitamin C is absorbed from the small intestine in humans, achieving peak plasma vitamin C concentrations approximately 120–180 min after ingestion. **(Corpe et al., 2013).** It should be noted that for many human tissues, accurate vitamin C concentrations in health and in disease states are not known. The major portion of vitamin C in humans is in the liver, brain, and skeletal muscle. Although skeletal muscle vitamin C concentrations are not high when compared to other cell types, skeletal muscle constitutes 31–38% of body mass **(Janssen et al., 2000).** For animal vitamin C concentrations, rodent data are most abundant. Reported tissue concentrations of vitamin C in the rat are much higher than those in humans This may be because fresh tissue is easily obtained under controlled conditions from rodents, thus avoiding oxidation and irreversible degradation of vitamin C. Note that plasma vitamin C was reported to be 90 μM in rat , although more recent studies with modern assays have shown plasma concentrations of 40–70 μM in rat **(Corpe et al., 2013) .** Red blood cells are the only body compartment other than saliva that have vitamin C concentrations that are similar to that of plasma or lower than **(Evans et al., 1982)** and **(Jacob et al., 1987)** and **(Li et al., 2012) (Figure13).**

IV.5.Chemistry and metabolism of vitamin C

Because electrons from vitamin C can reduce oxidized species, or oxidants, vitamin C is often termed an antioxidant, but this terminology is misleading. Electrons from ascorbate can reduce metals such as copper and iron, leading to formation of superoxide **(Parrow et al., 2013).** Ascorbate loses electrons sequentially. When one electron is lost, the first product is ascorbate radical. Most radical species have short lives less than 1 millisecond. Ascorbate radical is different, in that the half-life can be in many seconds, or even minutes, depending on absence of oxygen and electron acceptors, especially iron (**Buettner, 1993**). For example, under some conditions ascorbate radical can be measured in blood and extracellular fluid samples **(Chen et al., 2007)**. When a second electron is lost, a more stable species formed, in comparison with ascorbate free radical. The formed species is dehydroascorbic acid (DHA), which exists in hydrated and anhydrous forms. As discussed below, (DHA) has affinity for facilitated glucose transporters (GLUTs) and is transported by a number of them **(Rumsey et al.,1997)**

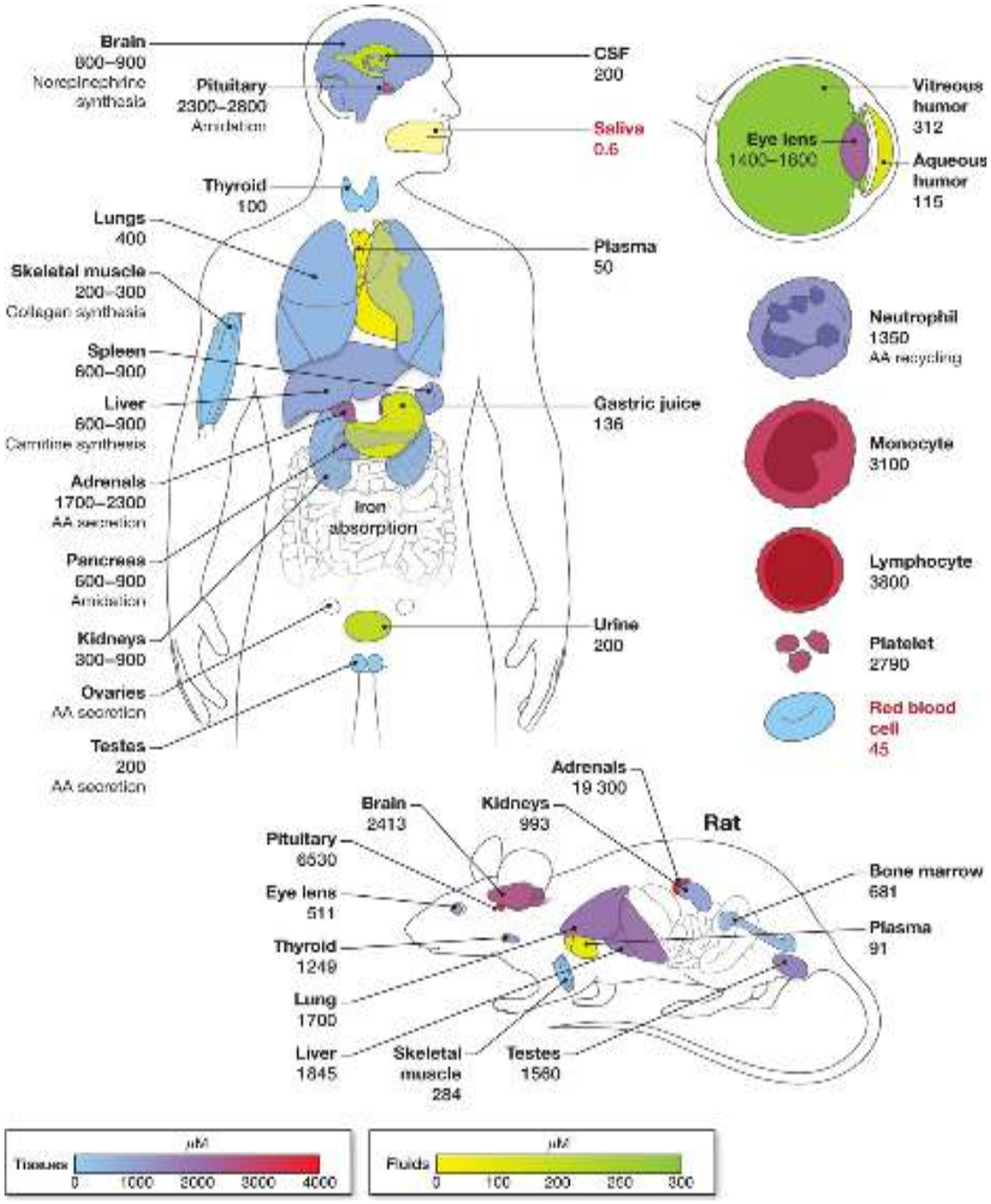

Figure 13 : Vitamin C concentrations in human and rat tissues and fluids. (Concentration of vitamin C in body tissues and body fluids are shown in M.) (S. J. Padayatty and Levine, 2016)**.**

and **(Rumsey et al.,2000)** and (**Corpe et al.,2013).** Both (DHA) and ascorbate radical are reversibly reduced to ascorbate. (DHA) half-life is only minutes, due to hydrolytic ring rupture. Once the ring structure is lost, the product 2, 3-diketogulonic acid cannot reform its precursors (DHA), ascorbate radical, and ascorbate. **(Figure 14) .**

IV.6.Vitamin C physiology

vitamin C acts as a cofactor and reduces certain enzymes by providing them with electrons, due to its chemical structure **(Padayatty and Levine, 2016).** Those enzymes can react with biomolecules known as lipids, proteins and DNA, and cause harm. In order to help prevent that, vitamin C reduces oxygen species when lipid peroxidation is formed, reduces radical inhibitors in protein oxidation, and prevents nitrosamine formation to reduce DNA damage **(Padayatty and Levine, 2001).**

Current knowledge of vitamin C physiology in humans is largely limited to vitamin C dose–concentration relationships, bioavailability and renal excretion in healthy young subjects **(Levine et al., 1996)** and **(Levine et al., 2001).** Plasma vitamin C concentrations depend on dietary intake; vitamin C absorption by the gastrointestinal tract; distribution in body fluids and uptake by tissues; irreversible metabolism of vitamin C (utilization); and vitamin C excretion by the kidneys. All of these factors may be altered in disease, and may also vary depending on body composition, genetics, and perhaps other factors such as physical activity. However, the most important variable identified so far that determines plasma vitamin C concentration is dietary intake. **(Padayatty et al., 2003)**

IV.7.Vitamin C in disease states

Vitamin C is crucial to our overall health and wellbeing. It should be considered a functional food ingredient, as it is an important bioactive compound with antioxidant properties **(Padayatty and Levine, 2016).**

IV.7.1. Role of recycling ability in the presence of microorganisms

The role of vitamin C in health could be related to its recycling ability in the presence of microorganisms **(Wang et al., 1997)**. When microorganisms are present, the amount of vitamin C in neutrophils is 30 times higher than in neutrophils that do not have microorganisms **(Wang et al., 1997)**

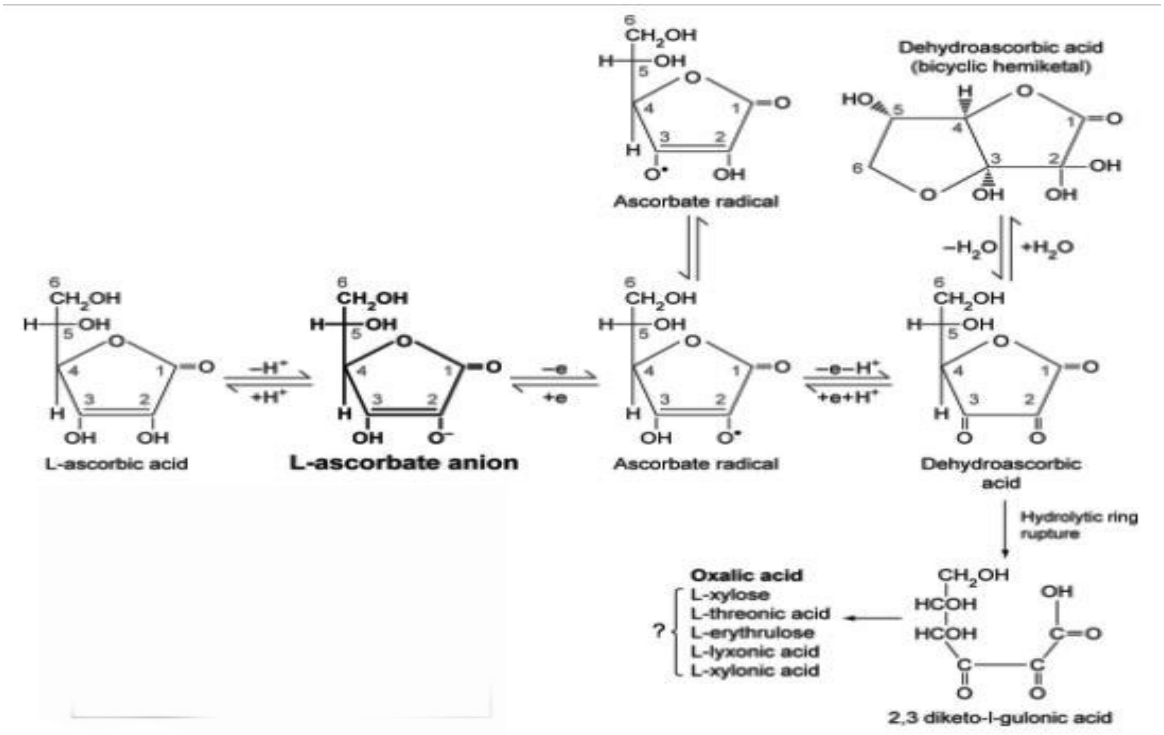

Figure 14 : Vitamin C chemistry (Padayatty and Levine, 2016)

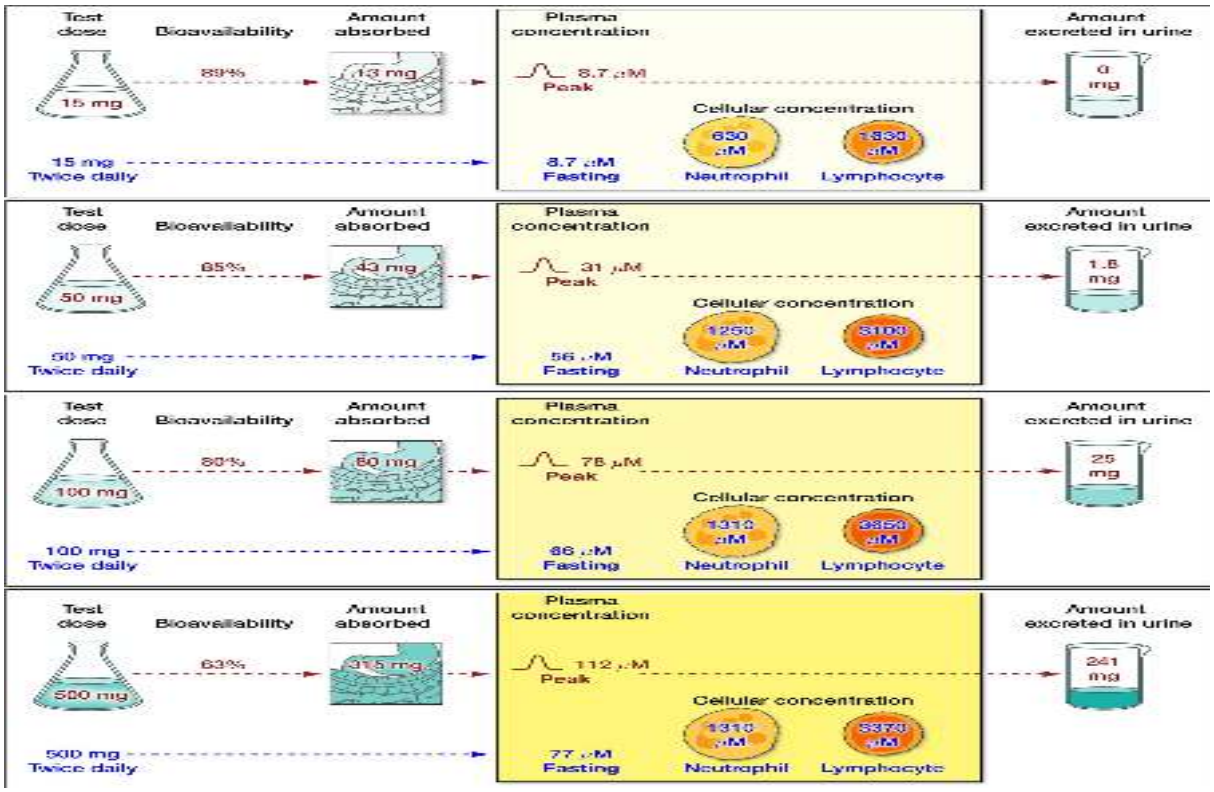

Figure 15 : Ascorbate flux in humans. The relationship between the daily intake of known doses of vitamin C and its absorption,**(Padayatty and Levine, 2016)**

This is important due to the ability of vitamin C to provide oxidant protection by scavenging excessive reactive oxygen species (ROS), which cause oxidant damage**(Wang et al., 97).**When more vitamin C is produced due to recycling, the ability to protect the body against damage is increased. **(Cook and Reddy, 2001).**

IV.7.2. Role of facilitate proper iron levels

vitamin C is important in its ability to facilitate proper iron levels. In one comparison study, individuals who consumed 247 mg of vitamin C daily had 35% higher iron absorption levels than those who consumed 51 mg of vitamin C daily **(Cook and Reddy, 2001).**

IV.7.3. Role of vitamin C as an antioxidant

The importance of vitamin C stems from its powerful antioxidant capacity. The term antioxidant has been defined as, "any substance that, when present at low concentrations compared to those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate **(Halliwell and Gutteridg, 1995).** Out of the three different antioxidant defense systems, vitamin C is classified as a chain breaking antioxidant; specifically, an aqueous phase chain breaking antioxidant **(Young and Woodside, 2001).** As an antioxidant, vitamin C protects low-density lipoproteins (LDLs) from being oxidized, decreases damaging oxidation in the stomach, and facilitates the absorption of iron **(Padayatty and Levine, 2001).** In one study examining the chemical composition of broccoli and cauliflower, vitamin C was shown to have the highest positive correlation of phytochemicals (phenol, flavonoid and glucosinolate), and the second highest antioxidant activity (only 9.5% lower than total phenol) **(Bhandari and Kwak, 2015).** In another study highlighting the antioxidant capacity of jujube fruits, vitamin C showed the highest correlation coefficient in the 2,2′-azinobis (3-ethylbenzothiazoline-6) sulfonic acid (ABTS) scavenging method (which establishes antioxidant activity) **(Kou et al., 2015).**

IV.7.4. Role in cognitive functions (high concentration in the brain)

The importance of an adequate amount of daily vitamin C over a lifetime could help prevent certain degenerative diseases. For example, vitamin C is a main defense against dopamine auto toxicity, a major component of Parkinson's disease **(Traber and Stevens, 2011).**

IV.8.Vitamin C deficiency – symptoms

A person who has a normal intake of vitamin C will have plasma levels of >28 μmol/L or 0.4-0.99 mg/dl **(Smythies, 1996)** and **(Jacob, 1990) .** Another with depleted vitamin C will have plasma levels between 11–28 μmol/L or 0.2-0.39 is an essential and water-soluble nutrient that is quickly excreted. Vitamin C has a half-life of 16 days in our bloodstream and, if ingestion of it stops completely, it will be eliminated within 35- 40 days **(Noble et al ., 2013) .** Blood plasma levels drop fairly quickly; however, the symptoms of deficiency take much longer to develop **(Delanghe et al., 2011).** In order to prevent deficiency, humans must ingest 10 mg of vitamin C daily (this prevents deficiency; but, does not provide enough to reach normal plasma levels) **(Lindblad et al., 2013).** One of the earliest symptoms is, unfortunately, very non-specific: fatigue. This is due to the fact that vitamin C is involved in the biosynthesis of carnitine, a compound essential for producing energy by transporting long-chain fatty acids into the mitochondria **(Carr and Frei, 1999).** Vitamin C is required by proline hydroxylase and lysine hydroxylase (enzymes in procollagen biosynthesis), and a deficiency leads to unstable collagenous structures **(Carr and Frei, 1999)** and **(Phillips and Yeowell, 1997)** . This causes tooth loss, joint pain, bone and connective tissue disorders, poor wound healing and, more specifically: bleeding, bruising, edema, hemorrhage, gingivitis, and corkscrew hairs **(Phillips and Yeowell, 1997)** and **(Al-Dabagh , 2013).**

IV.9.Vitamin C overdose – effects

There have been some reports of negative effects in larger doses, and to help prevent that, the USDA set the upper tolerable limit (UL) for vitamin C at 2 g **(IOM and FNB, 2000).** Gastrointestinal distress and diarrhea are the most common side effects, which have been shown in single oral doses of 5-10 g or greater than 2 g daily, with symptoms disappearing within 1-2 weeks **(Fukushima and Yamazaki, 2010).**

There are documented cases of oxalate stone formation in subjects with renal issues; however, the incidence rate is low. A majority of the data surrounding oxalate formation provides statistically significant findings; but, not clinically significant ones **(Auer et al .,1998).** An additional concern deals with an overproduction of iron. Vitamin C increases iron absorption by helping to transport iron across the epithelium in the small intestine (**Lykkesfeldt J et al .,2014).** Though this is a beneficial effect in most cases, there is potential for an iron overload in some individuals with diseases such as hemochromatosis, sideroblastic anemia, beta-thalassemia major and sickle cell anemia **(Nienhuis, 1981)** and **(Gerster,1999).**

IV.10.Optimal daily intake:

As with most nutrients, there are always questions about optimal intake. This can vary dramatically when considering age, health, lifestyle and gender. In terms of determining a daily amount best suited for the general population, the most recent RDA has been calculated at 90 mg for men and 75 mg for women, daily **(Table 03) (Deruelle and Baron, 2008).**

V.Management and prevention of chronic diseases with vitamin C

Vitamin C has been shown to lower blood pressure. After an initial vitamin C dose of 2 g, and subsequent daily doses of 500 mg for one month, the participants' mean blood pressure dropped 9.1%, providing evidence that long-term vitamin C treatment can reduce blood pressure in patients with hypertension **(Duffy et al., 1999).** The use of vitamin C can also aid patients who are undergoing treatment. One study on breast cancer patients undergoing chemotherapy/ radiotherapy showed that a once weekly IV dose of 7.5 g vitamin C decreased their overall side-effects (such as nausea, loss of appetite, and fatigue) by 37.5%, showcasing its benefits in conjunction with standard therapies **(Vollbracht et al., 2011).** Another study on the use of supplemental oxygen showed that hyperoxia can cause negative side effects such as reduced coronary blood velocity (CBV) by 28%, increased relative coronary resistance by 34%, and decreased left ventricular (LV) systolic velocity by 11%, all of which were eliminated with an IV dose of 3 g vitamin C **(Gao et al., 2012).** Vitamin C plasma levels have also an inverse correlation with the incidence of heart disease, with every 20 μmol/L increase in plasma yielding a 17% decrease in risk for heart failure **(Pfister et al., 2011).**

Table 03: Comparison of optimal vitamin C intake **(Pacier and Martirosyan, 2015).**

Study/Organization	**Recommended Intake (Healthy Adults)**	**Reasoning**
USDA RDA **(IOM and FNB, 2000)**	90 mg oral (men), 75 mg oral (women)	Meets nutrient requirements for 97-98% of the population; calculated from an EAR
Vitamin C depletion-repletion pharmacokinetic studies in 7 healthy inpatient volunteers by using 7 doses from 30-2500mg. **(Levine et al., 1996)**	200 mg oral	Bioavailability was complete at 200 mg
The combined evidence from human metabolic, pharmacokinetic, and observational studies and Phase II RCTs. **(Frei et al 2012)**	200 mg oral	Maximizes the vitamin's potential health benefits with the least risk of inadequacy or adverse health effects.
15 healthy female inpatients received in succession daily vitamin C doses of 30, 60, 100, 200, 400, 1,000, and 2,500 mg. **(Levine et al., 2001)**	90 mg oral (women)	Using FNB guidelines and on the basis of determination of an EAR; produces a median of ≈80% vitamin C saturation of neutrophils; minimal urine excretion
Review of past findings, and analysis of recent findings, on optimal vitamin C dosage. **(Ordman, 2010)**	500 mg oral twice daily	500 mg of vitamin C taken every 12 hrs may reduce many major causes of chronic disease, aging decline, and colds

Material and methods

Material and methods

1-Material

1-1-Animals

Young female rats of the Wistar Albinos weighing between (110.5g-169g).were used for the experimental study. All animals were born in animal house of the university des frères Mentouri- Constantine1), and they were housed in plastic cages with free access to water and diet every day at room temperature . Composition of diet is shown at **(Table 04).**

1.2. Chemical products

Chemical Products used in our study are:

Acid ascorbique (Nutri Power vitamine C powder pure 100% chine) , chloroform, formalin 10%, different concentrations of ethanol (60%, 75%, 96% and), NaCl, butanol, xylene, paraffin and glycerin. methanol ,buthanol , acid, picric acid, eosine, hematohxyline.

2. Methods

2.1. Biochemical analysis

2.1. a. Treatment of rats

After acclimatization to the laboratory conditions for 10 days, the twenty four rats were divided into four groups and fed with control and experimental diets **(Table 05).**

The control group (C) was fed with normal diet , group (CH) was fed with diet rich in fats , group (CHVC) was fed with diet rich in fats and treated with vitamin C (500mg/kg) and the group (VC) was fed with normal diet and vitamin C (500mg/kg) only. The vitamin C is administered to the rats using orogastric tubes. The animals were kept under standard laboratory conditions of humidity and allowed free access to food and water.

The weight, diet and water consumed by rats were taken throughout the experiment at the same time. Good hygiene was maintained by constant cleaning and removal of excrement and spilled feed from cages daily .This lasted for a period of 30 days.

2.1. b.Blood samples

After 30 days of experiment, animals were fasted overnight and the blood was obtained from the retro orbital sinus and collected into EDTA tubes by using glass capillaries. The blood was centrifuged immediately at 3000/rpm during 15 minutes .The plasma hs-CRP values and lipids(Total cholesterol,HDL-c, LDL-c, and triglyceride) , glucose , ALT and AST were measured by immunoturbidimetric method on a Cobas Integra 400 plus analyzer (Roche) and auto analyzer INTEGRA 400 respectively. The analysis was performed in the medical laboratory EL AMINE.

2.2. Histological analysis

After the blood samples collection, the animals were sacrificed, and samples for light microscopic investigations were obtained from aorta, heart , liver stomach , spleen and kidney . The aorta was divided into 4 sections (arch, thoracic, abdominal, and iliac).

The samples were rincedfrom all adherent tissues in NaCL 0.9% thenthe samples were:

- Fixed in formol 10%.
- Placed the different parts of the aorta in the Bouin solution for 5 min for color.
- Dehydrated in ascending percentage of ethanol (50%, 70% and 95% for 1h and 30min for each concentration) then in butanol for three days.
- Cleared in xylene for 10min at two exchanges
- Embedded in paraffin at 60 °C for 1h and 30 min at three exchange.
- Embed tissue into paraffin blocks
- Tissues are microtomed at 5 µm thick
- The sections dried on a hot plate at 37°C.
- Deparaffinization in xylene for two hours.
- Staining sections with hematoxylin-eosin (annex).

2.3 Statistical analysis

Data were analyzed using the statistical Package for Social Sciences (SPSS) programme, version 20 . In each assay , the experimental data represent the mean ± Ecartype deviations . The results were analyzed for differences between the groups , by one –way ANOVA test , and Tukey's multiple comparison tests. P values less than 0.05 were considered statistically significant.

Table 04: Composition of diet taken by rats during 30days

Animal diet	Diet rich in fats material (7rats)
Corn	Chips(3.5g per day)
Soy	Fats (8.4g)
Barley	Margarine (16.8g per day)
Cellulose	Cake (35g per day)
Minerals	Mayonnaise
Vitamins	
Water guedila	

Table 05: Treatment of rats for 30 days

Experimental group	Treatment	Number of animals	Daily dose
(C)	Normal std Diet	5	200g
(CH)	Normal std diet+diet rich in fats	5	200g+45.35g/5rats
(CHVC)	Vitamin C+ Normal std diet + diet rich in fats	7	500mg /kg +200g + (63.7g/7rats)
(VC)	vitamin C+ Normal std Diet+	7	500mg/kg + 200g

Results and Discussion

1. Results

1.1. Weight and diet experiments

The objective of this experiment was to evaluate the effect of diet rich in fats and vitamin C on the weight and diet consumption of rats.

1.1.1. Effect of diet rich in fats on weight and diet

a. Weight variation

The weight taken from rats between the first and the fourth weeks was (169.99±2.19g)and (188.21g±1.97g) respectively in group (CH) is increased very highly and significantly between the two weeks p=0.000 **(Figure16)** and **(Table 07).** Also we have obtained that the weight of rats in the control group (C) in the first week (160.03±5.82g) and in the fourth week 192.45g±1.13g) is increased very highly and significantly. p=0.000 **(Figure16)** and **(Table 06).**

b. Diet variation

The diet consumed by rats between the first week (95.27±19.84g) and the fourth week (59.49±7.25g) in group (CH) is decreased significantly (P=0.02) **(Figure17)** and **(Table07 annex).** However in the control group (C) the diet consumed by rats during the first week **(**85.35g±4.26g) and the fourth week (95.05g±8.67g) is shows a non significant increase.
.**(Figure 17)** and **(Table 06).**

1.1.2 Effect of vitamin C intake on weight and diet

a. Weight variation

The weight taken from rats during the first second third and fourth weeks was (151.91±4.41g),(164.78±3.22g),(176.79±2.69g) and (187.28±2.68g) respectively. Our result indicated that there is an increase of weight between groups but not significantly p=0.065. However the Tukey test indicated an increase significantly in the weight of rats between the first and the fourth week p=0.054. However the weight of rats in group (VC) during the first and the fourth weeks was (138.46±5.59g) and (175.91±3.38g) respectively. The weight of rats is increased very highly significantly p=0.000 **(Figure16) and (Table 09 annex).**

b. Diet variation

The diet consumed by the rats in the first week (103.6±18.09g) and the fourth week (107.5±3.14g) in the group (CHVC) is increased not significantly p= 0.241 . Also we have obtained that the diet consumed by the rats in the first week (133.27±8.15g) and the fourth week (149.68±26.74g) in group (VC) is increased not significantly p=0.423.

.**(Figure 17)** and **(Table 08 and 09 annex).**

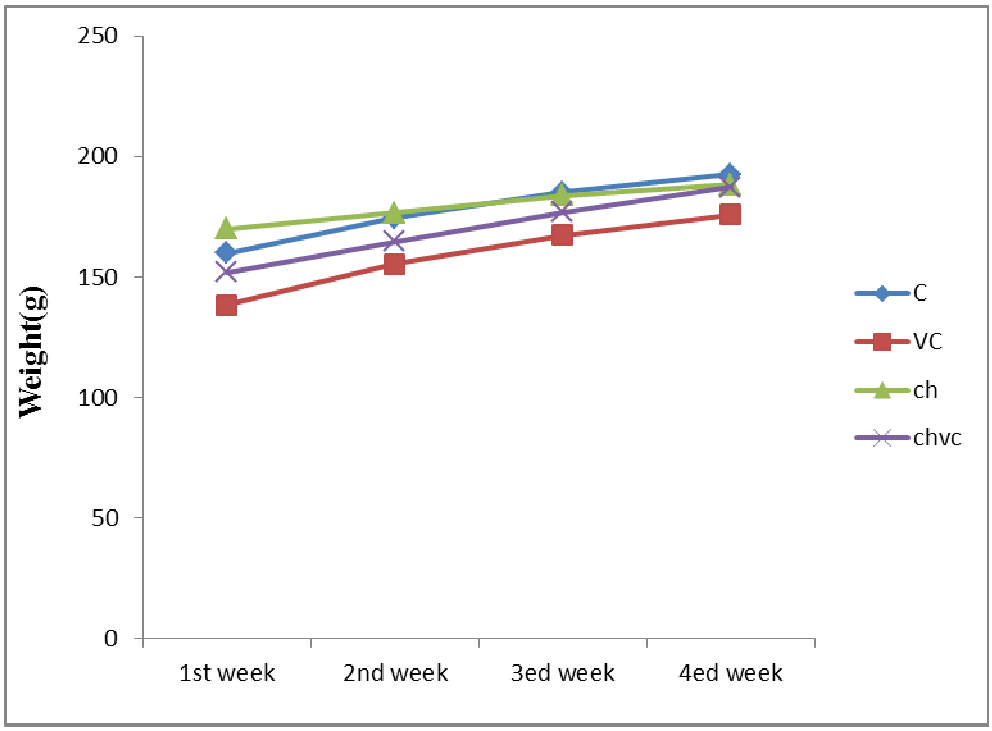

Figure16: Effect of diet rich in fats and vitamin C intake on the weight of rats during 30days.

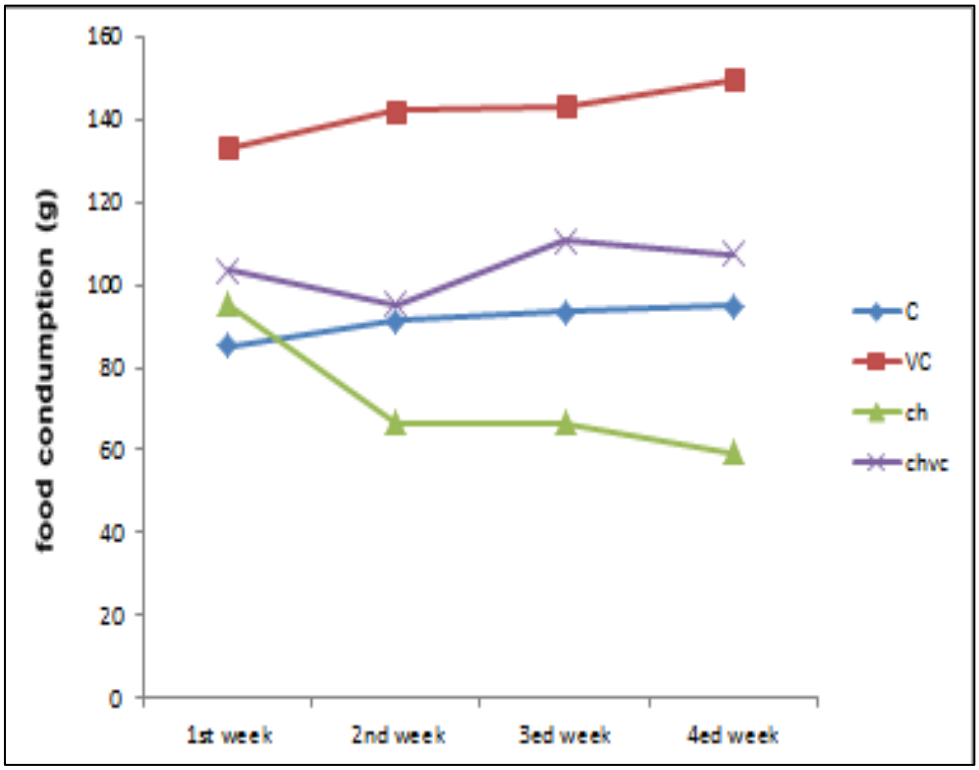

Figure17: Effect of diet rich in fats and vitamin C intake on the diet consumption of rats during 30days.

1.2. Biochemical results

1.2.1. hs- CRP measurement

The present data showed that there is a very highly significant difference in the means for the plasma hs-CRP concentrations between groups p=0.000. The Tukey test revealed that the concentration of hs-CRP in group (CHVC) (0.108± 0.117mg /l) is decreased very highly significantly when it is compared to the group (CH) (0.5g ± 0.05mg/l) p= p=0.000. Also, the concentration in the group (VC) (0.02 ± 0.0063mg / l),decreased highly significantly when it is compared to the group (C) (0.27 ± 0.17mg / l) (p=0.018) (**Figure 18**).

1.2.2 Total cholesterol

The result of determination of total cholesterol in the groups (C) (0.56 ±0.11g / l),(CH) (0.58 ± 0.07g/l),(CHVC) (0.52 ± 0.06g/l),(VC) (0.516 ± 0.06g/l). The present data indicated that there is a difference in the means for the concentration of total cholesterol but not significantly between groups p≥0.05. The Tukey test demonstrated that the concentration of total cholesterol in the groups (CHVC) and (VC) is decreased not significantly P ≥0.05.Also there is a decrease in the concentration of t-ch in group (VC) when it is compared to the control group (C) p≥0.05(**Figure 19).**

1.2.3 Triglyceride

The result of the determination of the triglyceride was in the groups (C) (0.383 ±0.16g / l), (CH) (0.402 ±0.05g/l) , (CHVC) (0.586 ±0.15g/l) and (VC) (0.366 ±0.06g/l). Our data indicated that there is a difference between groups not significantly p=0.084. The Tukey test demonstrated that the concentration of triglyceride in group (CHVC) is increased not significantly when it is compared to the group (CH) p=0.243 on the other hand the concentration of triglyceride is decreased not significantly in group VC when it is compared to the control goup (C) (**Figure20)**.

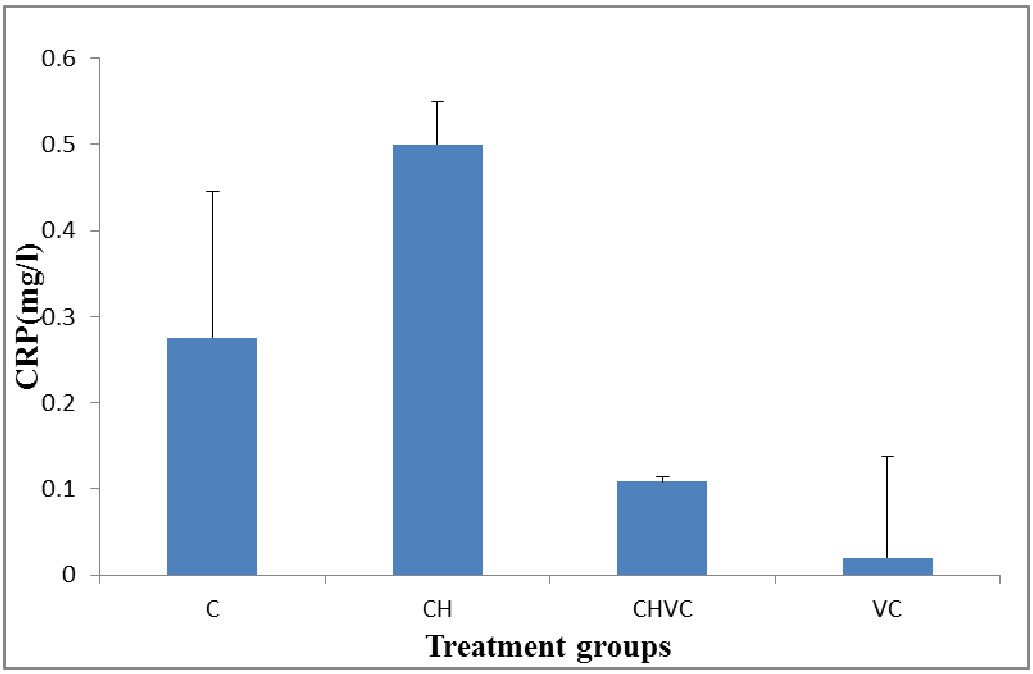

Figure 18: Interaction of diet rich in fats and vitamin C on the plasma ultra sensitive CRP in rats during 30days.

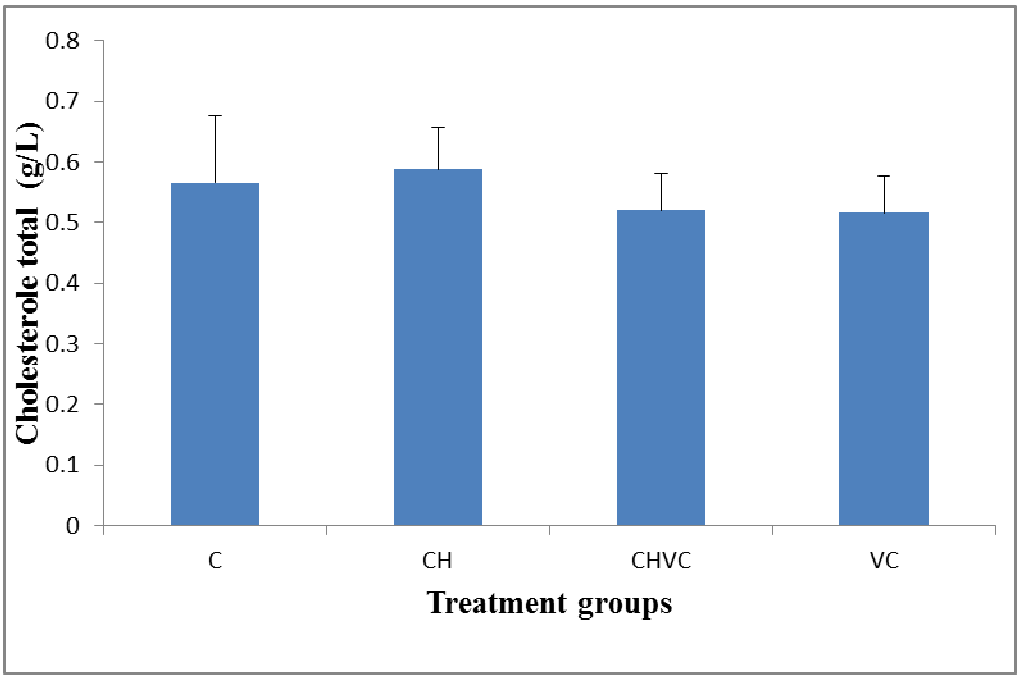

Figure 19: Interaction of diet rich in fats and vitamin C on the total cholesterol in rats during 30days.

1.2.4 LDL-cholesterol

The result of the determination of the LDL-cholesterol was in the groups (C) (0.08 ± 0.0081g / l), (CH) (0.096 ±0.02g / l) , (CHVC) (0.057 ±0.014g / l) and (VC) (0.014 ±0.016g /l).Our data indicated that there is a difference between groups significantly p=0.03.

The Tukey test revealed that the concentration of LDL-cholesterol in groups (CHVC) is decreased highly significantly when it is compared to the groups (CH) (p=0.019) and decreased not significantly in group (VC) when it is compared to the control group (c) respectively(p=0.986) (**Figure 21).**

1.2.5 HDL -cholesterol

The result of the concentration of HDL- cholesterol were in groups (C) (0.468±0.08g / l), (CH) (0.48±0.062g / l) , (CHVC) (0.5±0.05g / l) and (VC) (0.46 ±0.05g / l).Our data indicated that there is a difference but not significantly between groups p=0.886).

TheTukey test demonstrated that the concentration of HDL cholesterol in the group (CHVC) is increased not significantly when it is compared to the group (CH) p≥0.05.However the HDL- cholesterol levels were decreased not significantly in the group (VC) when it is compared to the group (C) p≥0.05. (**Figure 22).**

1.2.6 Glucose

The result of the concentration of the glucose was C (0.818 ± 0.84g/l), (CH) (1.02 ± 0.94g/l) , (CHVC) (1.708 ± 0.84g / l) (VC) (1.22 ± 0.74g / l). Our data indicated that there is a difference between groups not significantly p≥0.05. The Tukey test demonstrated that the concentration of the glucose in group (CHVC) is increased but not significantly (p=0.185) when it is compared to the group(CH). The glucose levels were increased but not significantly in group (VC) when it is compared to the control group (C) (p=0.636).**(Figure 23)**

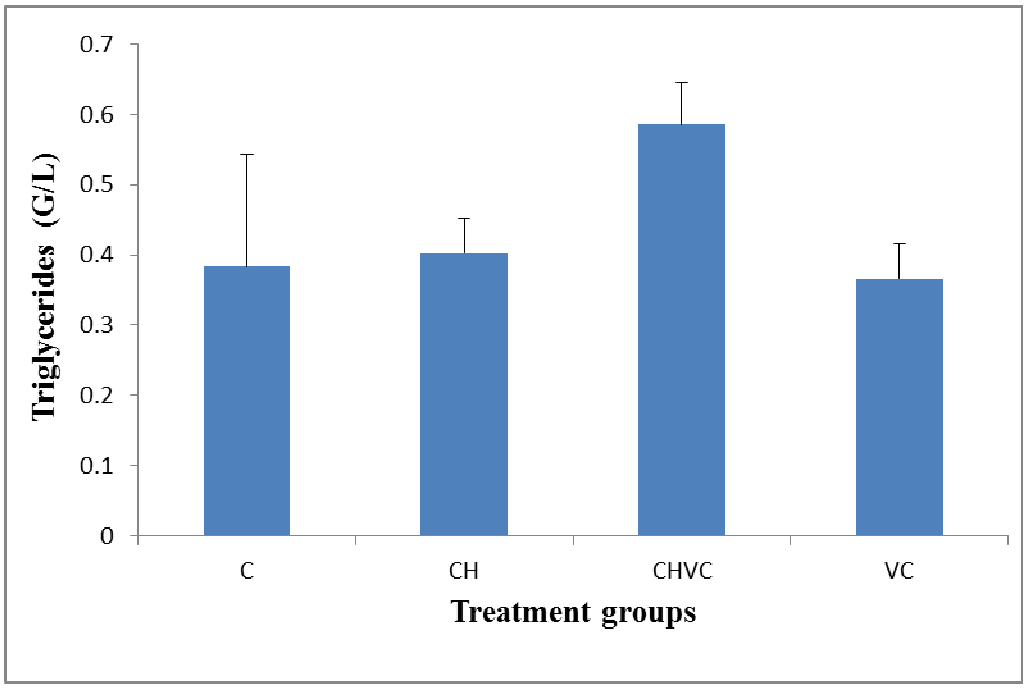

Figure 20: Interaction of diet rich in fats and vitamin C on the triglyceride in rats during 30days.

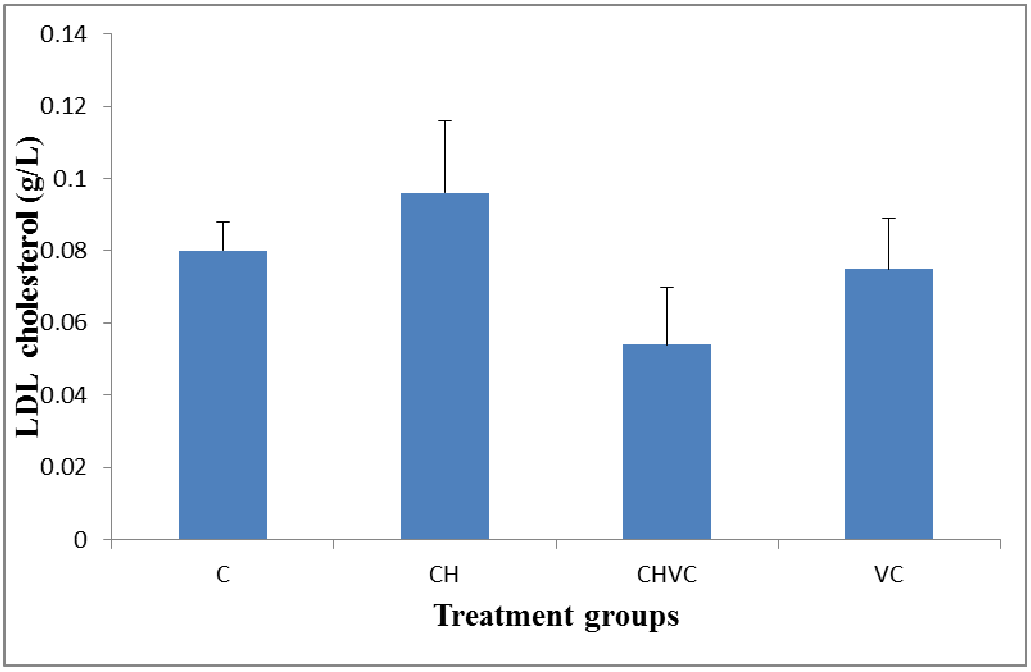

Figure 21: Interaction of diet rich in fats and vitamin C on the LDL- cholesterol in rats during 30days.

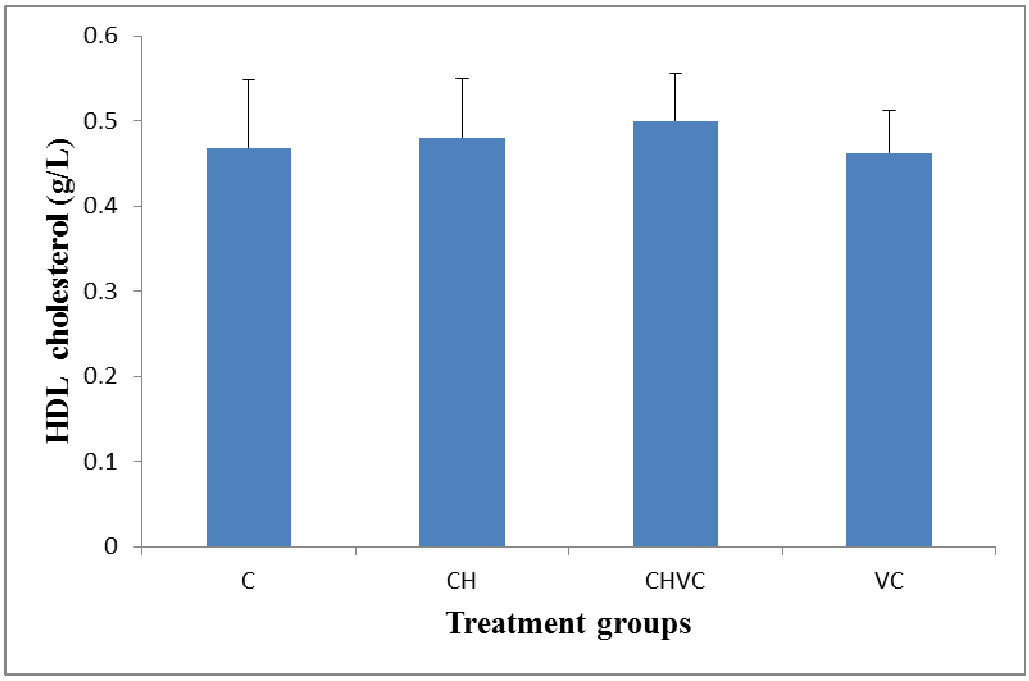

Figure 22: Interaction of diet rich in fats and vitamin C on the HDL- cholesterol in rats during 30days.

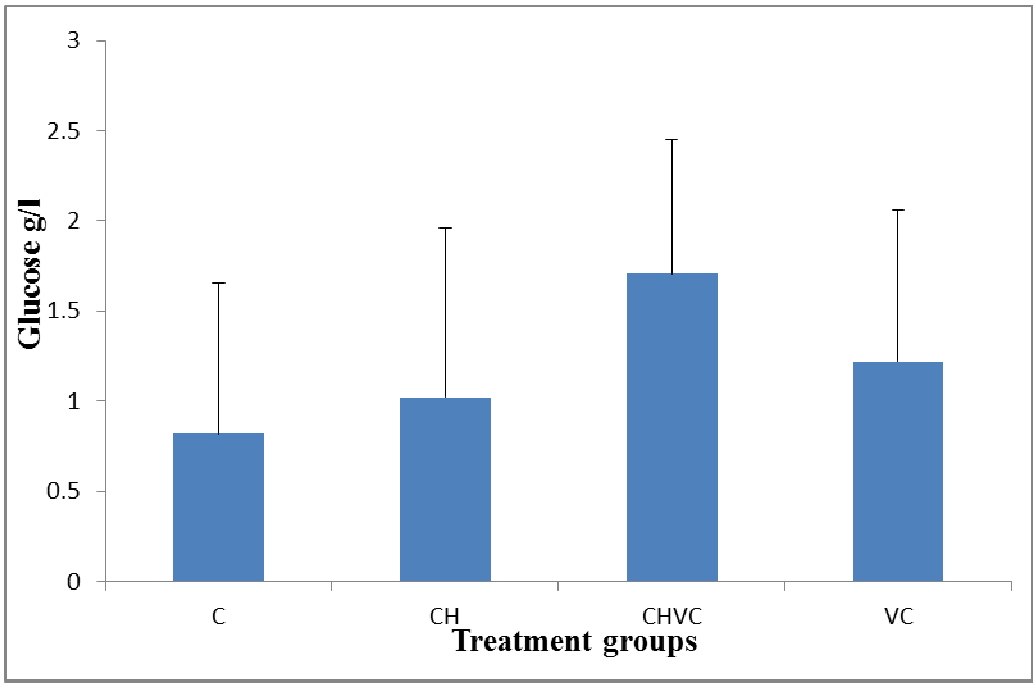

Figure 23: Interaction of diet rich in fats and vitamin C on the plasma glucose in rats during 30days.

1.2.7 Aspartate Amino Transferase (AST)

The results of the concentration of AST were in the group (C) (129.16 ±29.U/l), (CH) (100.2±6.06U̓/l) , (CHVC) (92.6±40.38U̓/l) VC(G4) (82.6±14.703uU̓/ l).Our data indicated that there is a difference but not significantly between groups (P=0.115).

The Tukey test demonstrated that the concentration of AST in group (CHVC) is decreased but not significantly (p=0.976) when it is compared to the (CH). The levels AST were decreased but not significantly in the group (VC) when it is compared with control group(C) (p=0.098).(**Figure 24)**.

1.2.8 Alanine AminoTransferase (ALT)

The results of the concentration of ALT were in the group (C) (39.4±8.68 U/l), (CH) (40.2±10.12 U/l), (CHVC) (49.8±41.07 U/l) and (VC) (24±3.4 U/l). Our data indicated that there is a difference significantly between groups (P=0.02).The Tukey test demonstrated that the concentration of ALT in group (CHVC) is increased but not significantely (p=0.299) when it is compared to the (CH). The level of ALT was decreased significantly in (VC) when it is compared to the control group C (p=0.059) (**Figure 25).**

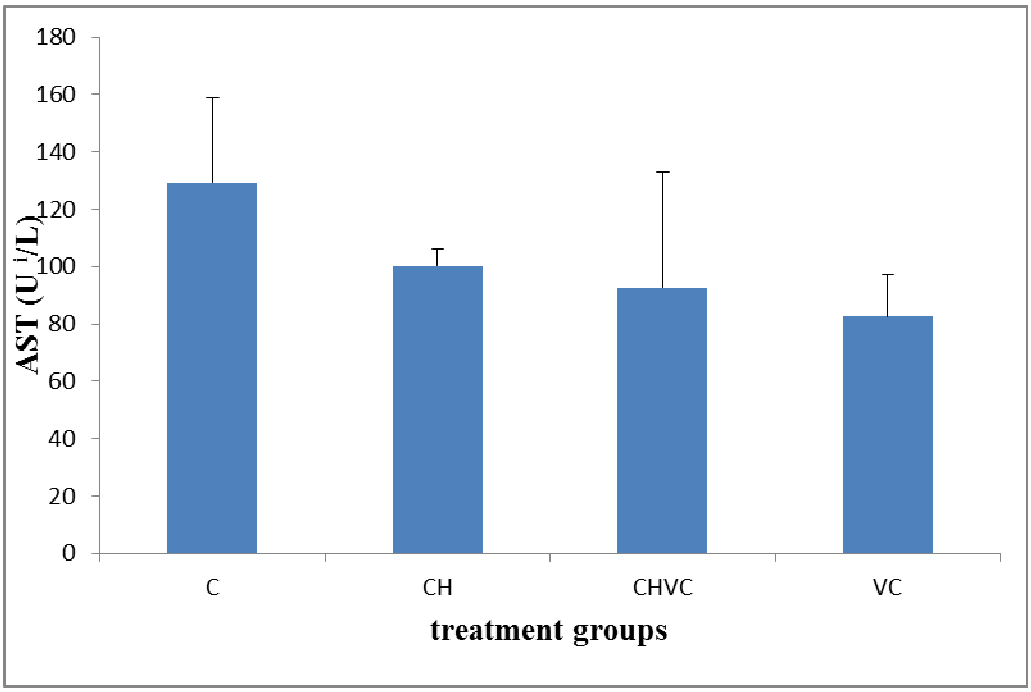

Figure 24: Interaction of diet rich in fats and vitamin C on the plasma enzyme Aspartate Amino Transferase (AST) in rats during 30days.

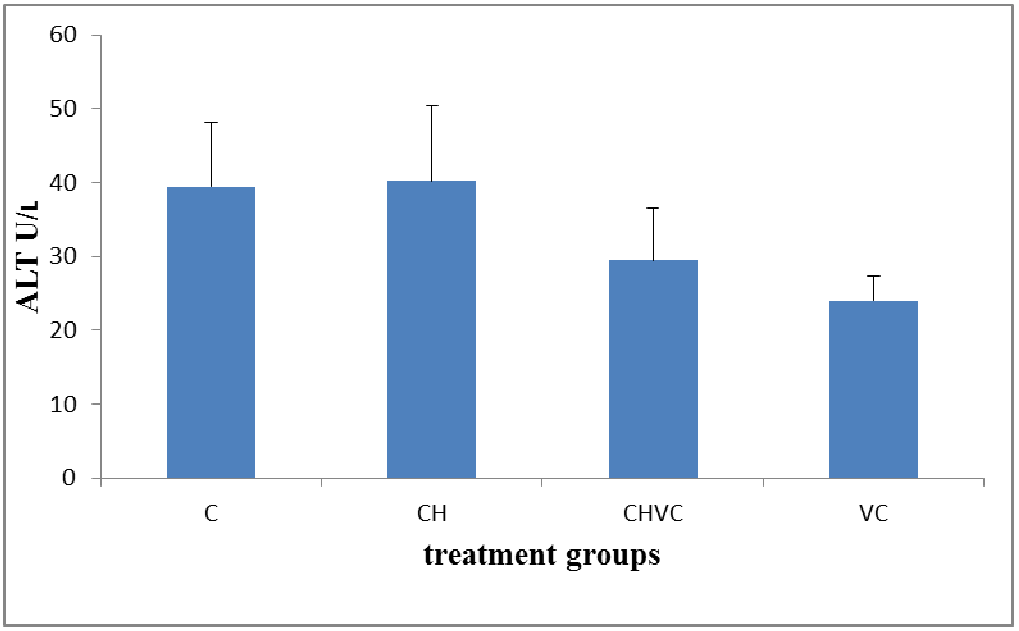

Figure 25: Interaction of diet rich in fats and vitamin C on the plasma enzyme Alanine Amino Transferase (ALT) in rats during 30days.

1.3. Behavior investigations

During our study we have noticed that the animals in the group (CHVC) and group (VC)are very active due to vitamin c intake. when it is compared to other groups. Also we have observed the normal excrement in three groups , but diarrhea in group (VC). **(photo 01** and **02)**

1.4. Morphological investigation

The weight of organs (heart, spleen, kidney, aorta, stomach and liver) in (C) group was (0.61 ±0.05) g (0.89 ±0.13 g) (1.11 ±0.07 g) (0.076 ±0.004 g) (1.22±0.06 g) (6.80 ±0.84 g) , in (ch) group was (0.61 ±0.05 g) (1.44 ±0.11 g) (1.28 ±0.062 g) (0.06 ±0.007 g) (1.21 ±0.15 g) (8.062 ±0.524 g) . in (VC) group was (0.55 ± 0.04 g) (0.95 ± 0.21 g) (1.37 ±0.179 g) (0.12 ± 0.02 g) (1.16±0.11 g) (8.75± 0.61 g) and in (CHVC) group was (0.65 ± 0.09g) (0.75± 0.12 g) (1.37 ± 0.14 g) (0.11 ± 0.049 g) (1.13 ±0.12 g) (9.20±0.63 g) respectively .

Our data showd that the weight of liver , kidney and spleen was increased in the group (ch) when it is compared to the controle group (c) . in the other side the weight of liver and heart was increased in the group (chvc) when it is compared to the (vc) group but the weight of spleen decreased (slithely). **(Figure 26) .**

1.5. Histological investigation

For the histological investigation we have obtained sections of all organs stained by hematoxyline eosin (heart, spleen, kidney, aorta, stomach and liver). The histological sections are observed by light microscopy and no photo was taken by orthoplan because the sections appear opaque.

photo 01 :VC group excrement

photo 02 :CHVC group excrement

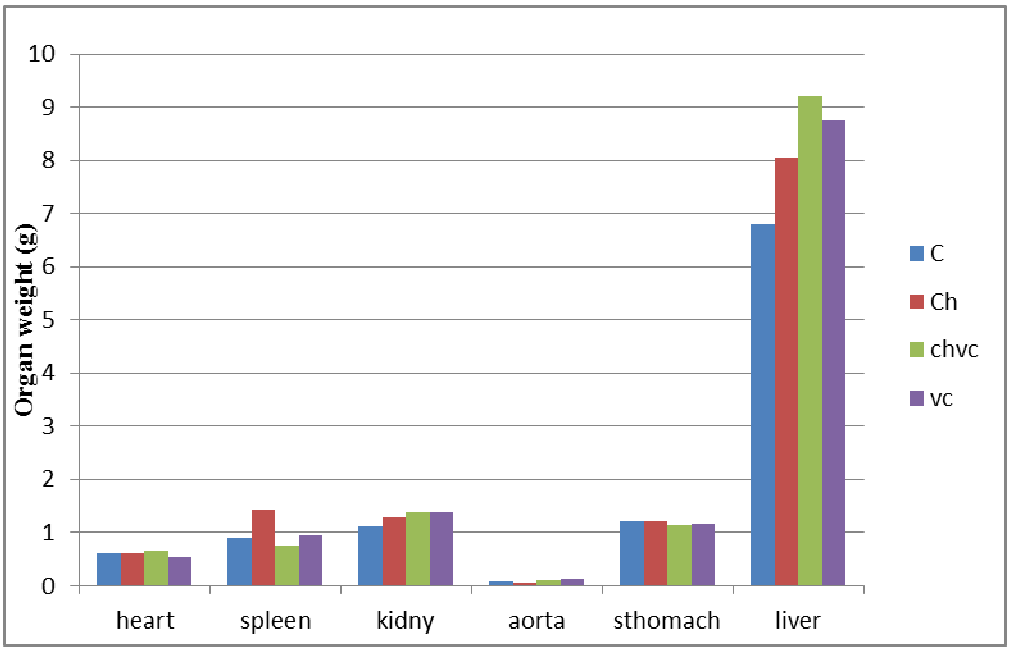

Figure26 : The weight of organs (heart, spleen, kidney, aorta, stomach and liver) changing in four groups

2.Discussion

This research investigated the relationship between dietary intake rich in fats and inflammation which induced some diseases such as cardiovascular diseases by an increase of some parameters such as hs-CRP , lipids ,ALT, AST and glucose .On the other hand we evaluates the protective effect of vitamin C on the reduction of these parameters.

The first experiment evaluates the effect of diet rich in fats on the weight and diet consumption of rats. The result showed an increase highly significantly in the weight followed by a decrease in the diet consumption in rats fed with diet rich in fats . Our result is agrees with those obtained by **(Guennoub et Redouane ali, 2017)** et **(Belili et Mekki , 2016)** who reported an increase in weight of rats fed with diet rich in fats during 26 days and wistar male rats fed with diet rich in lipids during two months respectively. However we have detected a decrease in the diet consumption in same group (CH). This results is in agreement with those of **(Guennoub et Redouane ali 2017 and Boufedeche et al., 2018)** who reported a decrease of diet consumption by rats male and female fed with diet rich in fats during 26 and 21 days respectively. The second experiments evaluates the effect of vitamin C on the weight and diet consumption in the group of rats fed with diet rich in fats and treated with vitamin C. The weight of rats and diet consumption are increased highly significantly respectively in both groups treated with vitamin C .Our results is not agrees with **(Nayanatara et al.,2005)** reports, normal rats feed with Vit C (20mg/kg/day) does not show any changes in body weight in comparison to control group.

The biochemical results showed an increase in the total cholesterol concentration in rats fed with diet rich in fats, followed by a decrease in the concentration of the total cholesterol in group fed with diet rich in fats and treated with vitamin C (500mg/kg) , this indicated that vitamin C could decrease the concentration, of total cholesterol in the plasma of rats. Our result is agrees with those of **(Chakravarti et al. ,1975)** and those of **(Sadava et al .,1982)** who reported that , the administration of cholesterol to the rabbits , rats and pigs could increase the cholesterol levels in the plasma and tissues, but the elevation is significantly smaller when vitamin C is supplemented to this animals. Also they showed that these animals could synthesize themselves this vitamin.

Without the atherogenic diet the supplementation with vitamin C decreases the cholesterol total so this work is in agreement with the work of **(Nambisan and Kurup, 1974)** and **(Chen and thacker,1985)** who reported that extra vitamin C in the absence of dietary cholesterol decreases the cholesterol level in the serum , liver, and aorta of rats.

The concentration of triglyceride is increased in rats fed with diet rich in fats and treated with vitamin C but is decreased in the group of rats treated only with vitamin C. Our results is agrees with the study of (**Ginter, 1978), (Sokoloff et al.,1966)** and **(Sadava et al.,1982)** who treated the hamsters , rats or rabbits respectively by extra vitamin C and they reported that the increase in triglyceride levels caused by an atherogenic diet is partially prevented by vitamin C . Also our result is agrees with those of **(Nambisan and Kurup, 1974)** who found that the level of triglyceride is decreased in rats fed with normal diet and supplemented with vitamin C. In our study we have obtained that the LDL- cholesterol is decreased in the groups treated with vitamin C. The present result agrees with previous reports as documented by **(Chattrjea and Shinde, 2002)** who observed a reduction in serum cholesterol in experimental animals administered with vitamin C.

Another study reported that LDL- cholesterol levels were about three times higher in the group of vitamin-dependent rats , receiving a vitamin deficient diet than in the control group **(Horio et al ., 1991).** We could explain that vitamin C increases the number of LDL-receptors in cultured arterial smooth muscle cells **(Aulinskas et al.,1983).** Also , it has been suggested that the vitamin C plays an important role in the metabolism of lipoprotein (a) , which has been linked with atherosclerosis **(Mbewu and Durrington,1990)** and **(Rath,1991).** The reduction in LDL-cholesterol points to the fact that adequate vitamin C intake can reduce the incidence of atherosclerosis .Mean while both (Anderson et al .,1999) and **(Bsoul and Terzhalmy ,2004)** , noted that animal fed on vitamin C had reduced risk of coronary heart disease.

The result in this study showed that , there is an increase in HDL- cholesterol in rats fed with diet rich in fats and treated with vitamin C . Our result is agrees with those of **(Hillstrom ,2003)**. who reported that the vitamin C could inhibit the oxidation of HDL even in human and the work of **(Horio et al .,1987)** and **(Uchida et al .,1990)** mentioned that , When vitamin-dependent rats were put on a vitamin C-deficient diet, their plasma HDL- cholesterol level became lower than in mutant rats that were given the vitamin C .

A plausible explanation for the observed effect on serum lipids may be due to the activation of the enzyme 7 alpha-hydroxylase by vitamin C which enhances the conversion of plasma cholesterol into bile acid hence resulting in a decrease in serum levels of cholesterol.

In fact **(Mayes, 1996)** observed that deficiency of vitamin C inhibits 7 alpha-hydroxylase leading to the block in bile acid synthesis and accumulation of cholesterol in serum with subsequent athereosclerosis in scorbutic Guinea pigs. Steroid hormones synthesis requires cholesterol as the precursors and vitamin C plays a role in hydroxylating the steroid hormone

in the adrenal glands. It also directly meditates through a rate limiting hydroxylation of side chains, the conversion of cholesterol into steroid hormones as documented by **(White et al., 1978).**The reduction in LDL-cholesterol points to the fact that adequate vitamin C intake can reduce the incidence of athereoslerosis. Both **(Anderson et al., 1999)** and **(Bsoul and Terezhalmy, 2004),** noted that animal fed on vitamin C had reduced risk of coronary Heart disease. The observed decrease in total cholesterol ,LDL and most significantly the ability to lower the levels of the atherogenic predisposing factor (serum – LDL cholesterol)yet desirably increasing the level of HDL implies that dietary vitamin C on account of its effect on lipid profile may have a protective effect against arteriosclerosis.

In our research we have obtained that the concentration of blood glucose is increased in the group of rats fed with diet rich in fats and treated with vitamin C and decreased in group of rats treated with vitamin C only. This results is agrees with the work of (**Shojaoddiny and Ardekani , 2007),** who indicated that daily consumption of 1000 mg supplementary vitamin C may be beneficial in decreasing blood glucose and lipids in patients with type 2 diabetes and thus reducing the risk of complications.

The reduction of AST and ALT by vitamin C is in agreement with **(Rekka et al.,1992)**, who found that serum transaminases returned to normal activities with the healing of tissue parenchyma and regeneration of hepatocytes and renal tissues. Vitamin C induced suppression of increased ALT and AST activities. **(Ayyoubi et al., 2015).**

Conclusion

Conclusion

We conclud that the treatment with vitamin C (500mg/Kg) in rats could ameliorate the concentration of lipids and hs-CRP and prevented from inflammation and cardiovascular diseases.

For future work we propose some experimental work :

-We need to modulate dose and evacuate the effect.

-Treatment of animals (rats and mice) with the same dietry during more than 30 days.

- Determination of pro-inflammatory cytokines.

-Determination of mechanisms of ascorbate secretion.

- Determination of antioxidant enzyme catalase-SOD.

-Compare the effect of this dose with the therapeutic uses of another dose intravenously administered vitamin C.

References

Reference

- Al-Dabagh, A., Milliron, B. J., Strowd, L., Feldman, S. R. (2013). A disease of the present: scurvy in "well-nourished" patients. Journal of the American Academy of Dermatology. 69(5) : 246-247.
- Afkhami, A. M., Shojaoddiny A. A.(2007). Effect of vitamin C on blood glucose, serum lipids & serum insulin in type 2 diabetes patients. Indian J Med Res.26(5):4714.
- Aldons, J .L. (2000). Atherosclerosis. Nature. 407(6801): 233–241.
- Amarenco, P., Labreuche, J. (2009). Lipid management in the prevention of stroke: review and updated meta-analysis of statins for stroke prevention. The Lancet Neurology, 8(5), 453-463
- Anderson, K. M., Wilson, P. W., Odell, P. M., & Kannel, W. B. (1991). An updated coronary risk profile. A statement for health professionals. Circulation. 83(1): 356-362.
- Anderson, R. G. (1998). The caveolae membrane system. 67:199-225
- Anderson, J. W., Gowri, M. S., Turner, J. (1999) Antioxidant supplementation effects low density lipoprotein oxidation for individuals with type 2 diabetes mellitus. J. Am. Coll. Nutr. 18: 457 – 461.
- Andreeva, E. R., Pugach, I. M., & Orekhov, A. N. (1997). Collagen-synthesizing cells in initial and advanced atherosclerotic lesions of human aorta. Atherosclerosis. 130(1-2): 133-142.
- Auer, B. L., Auer, D., Rodgers, A. L. (1998). The effect of ascorbic acid ingestion on the biochemical and physicochemical risk factors associated with calcium oxalate kidney stone formation. Clinical chemistry and laboratory medicine. 36(3):143-148.
- Barquera, S., Pedroza-Tobias, A., Medina C., Hernandez-Barrera L., Bibbins-Domingo, K., Lozano R., Moran AE. (2015). Global overview of the epidemiology of atherosclerotic cardio- vascular disease. Arch Med Res. 46: 328–38.
- Belili, A.,Mekki, H.(2016). Impact d'un régime hyperlipidique sur les modifications structurelles et de la muqueuse intestinale et sur les paramètres biochimiques chez les rats wistar. Mémoire pour l'obtention du diplôme de Master . Spécialité: Régulation endocrinienne et physiopathologie .Université Djilali Bounaama de Khemis Miliana : 27-28 .

- Berenson, G. S., Srinivasan, S. R., Bao, W., Newman, W. P., Tracy, R. E., Wattigney, W. A. (1998). Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. New England journal of medicine. 338(23) : 1650-1656.
- Bhandari, S. R., and Kwak, J.-H. (2015). Chemical composition and antioxidant activity in different tissues of Brassica vegetables. Molecules.20(1) : 1228–1243.
- Birney, E. C., Jenness, R., and Ayaz, K. M. (1976). Inability of bats to synthesise L-ascorbic acid. Nature. 260(5552): 626.
- Bosner, M. S., Lange, L. G., Stenson, W. F., and Ostlund, R. E. (1999). Percent cholesterol absorption in normal women and men quantified with dual stable isotopic tracers and negative ion mass spectrometry. Journal of Lipid Research. 40(2): 302–308.
- Bray, C., Bell, L. N., Liang, H., Haykal, R., Kaiksow, F., Mazza, J. J., Yale, S. H. (2016). Erythrocyte Sedimentation Rate and C-reactive Protein Measurements and Their Relevance in Clinical Medicine. WMJ. 115: 317–21.
- Brown, A.J., Sharpe, L.J .(2015). Cholesterol synthesis. In: Biochemistry of lipids, lipoproteins, and membranes . 6: 327-358.
- Buettner, G. R. (1993). The pecking order of free radicals and antioxidants: lipid peroxidation, α-tocopherol, and ascorbate. Archives of Biochemistry and Biophysics . 300(2): 535–543.
- Burnstock,G.,Aliev, G.(1998). Watanabe rabbits with heritableh ypercholesterolaemia : a model of atherosclerosis. Histology and histopathology.13(3): 797-817.
- Carr, A. C., Frei, B. (1999). Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans–. The American journal of clinical nutrition. 69(6) : 1086-1107.
- Castelli, W. P. (1996). Lipids, risk factors and ischaemic heart disease. Atherosclerosis. 124: 1-9.
- Chatterjee, I. B. (1973). Evolution and the biosynthesis of ascorbic acid. Science . 182(4118):1271–1272.
- Chatterjea, M. N.,Shinde, R. (2002). Textbook of Medical Biochemistry .5: 154 – 157.

- Chen, Q., Espey, M. G., Sun, A. Y., Lee, J.-H., Krishna, M. C., Shacter, E., Buettner, G. R. (2007). Ascorbate in pharmacologic concentrations selectively generates ascorbate radical and hydrogen peroxide in extracellular fluid in *vivo*. Proceedings of the National Academy of Sciences. 104(21): 8749–8754.
- Chertov, O., Yang, D., Howard, O. M., Oppenheim, J. J. (2000). Leukocyte granule proteins mobilize innate host defenses and adaptive immune responses. Immunological reviews.177(1): 68-78.
- Clayton, P.T. (1998) : Disorders of cholesterol biosynthesis. Arch Dis Child; 78:185-189.
- Combs, G. F., McClung , J. P. (2017). Fundamental Aspects in Nutrition and Health. 5 : 269.
- Cook, J. D., Reddy, M. B. (2001). Effect of ascorbic acid intake on nonheme-iron absorption from a complete diet. The American journal of clinical nutrition. 73(1) : 93-98.
- Corpe, C. P., Eck, P., Wang, J., Al-Hasani, H., and Levine, M. (2013). Intestinal dehydroascorbic acid (DHA) transport mediated by the facilitative sugar transporters, GLUT2 and GLUT8. Journal of Biological Chemistry. 288(13): 9092–9101.
- Cortes, V. A., Busso, D., Maiz, A., Arteaga, A., Nervi, F., Rigotti, A. (2014). Physiological and pathological implications of cholesterol. Frontiers in Bioscience (Landmark Edition). 19: 416–428.
- Delanghe, J. R., Langlois, M. R., Buyzere, M. L., Na, N., Ouyang, J., Speeckaert, M. M., Torck, M. A. (2011). Vitamin C deficiency: more than just a nutritional disorder. Genes & nutrition. 6(4): 341.
- De Meester, F., van Lenthe, F. J., Spittaels, H., Lien, N., De Bourdeaudhuij, I. (2009). Interventions for promoting physical activity among European teenagers: a systematic review. International Journal of behavioral nutrition and physical activity. 6(1): 82.
- Di Scala, C., Fantini, J. (2017). Hybrid in silico/*in vitro* approaches for the identification of functional cholesterol-binding domains in membrane proteins. Cholesterol Homeostasis: Methods and Protocols : 7–19.
- Doll, S., Ricou, B. (2013). Severe vitamin C deficiency in a critically ill adult: a case report. European Journal of Clinical Nutrition. 67(8): 881.

- Donald O., Rudin, M.D., Felix, Clar.(1996).Omerga-3 Oils: A Practical Guide, Avery Publishing Group. 18.
- Dubnov, G., Pella, D., Singh, R. B. (2008). The Effect of an Alpha-Linolenic-Acid-Rich Diet on the Circadian Rhythm of. Nutrition Research at the Leading Edge : 191.
- Duffy, S., Gokce, N., Holbrook, M., Huang, A., Frei, B., Keaney Jr, J. F., Vita, J. A. (1999). Treatment of hypertension with ascorbic acid. The Lancet. 354(9195) : 2048-2049.
- Durrington, P.N. (2007). Diagnosis and Management. London: Hodder Arnold; Hyperlipidaemia : 3.
- Enig, M. G. (2000). Know your fats: The complete primer for understanding the nutrition of fats, oils and cholesterol . Bethesda Press Silver Spring, MD: (120).
- Evans, R. M., Currie, L., and Campbell, A. (1982). The distribution of ascorbic acid between various cellular components of blood, in normal individuals, and its relation to the plasma concentration. British Journal of Nutrition. 47(3): 473–482.
- Fan, C., Pacier, C., and Martirosyan, D. M. (2014). Rose hip (Rosa canina L): A functional food perspective. Functional Foods in Health and Disease. 4(12) : 493–509.
- Felix-Redondo, F.J., Grau, M. and Fernandez-Berges, D., 2013. Cholesterol and cardiovascular disease in the elderly.Facts and gaps. Aging and Disease. (4): 154-169.
- Ferrero-Miliani, L., Nielsen, O. H., Andersen, P. S., Girardin, S. E. (2007). Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation. Clinical and Experimental Immunology, 147(2), 227-235.
- Fielding, P. E., Fielding, C. J. (1995). Plasma membrane caveolae mediate the efflux of cellular free cholesterol. Biochemistry, 34(44), 14288-14292.
- Fielding, P. E., Fielding, C. J. (1996). Intracellular transport of low density lipoprotein derived free cholesterol begins at clathrin-coated pits and terminates at cell surface caveolae. Biochemistry, 35(47), 14932-14938.
- Fowkes, F.G., Rudan D., Rudan, I., Aboyans V., Denenberg JO., McDermott MM., Norman PE., Sampson UK., Williams LJ., Mensah GA., Criqui MH.(2013).Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a system- atic review and analysis. Lancet. 382: 1329–40.

- Gallie, D. R. (2013). Increasing vitamin C content in plant foods to improve their nutritional value—Successes and challenges. Nutrients. 5(9): 3424–3446.
- Gao, W., He, H. W., Wang, Z. M., Zhao, H., Lian, X. Q., Wang, Y. S., Wang, L. S. (2012). Plasma levels of lipometabolism-related miR-122 and miR-370 are increased in patients with hyperlipidemia and associated with coronary artery disease. Lipids in health and disease. 11(1) : 55.
- Gao, Z., Spilk, S., Momen, A., Muller, M. D., Leuenberger, U. A., Sinoway, L. I. (2012). Vitamin C prevents hyperoxia-mediated coronary vasoconstriction and impairment of myocardial function in healthy subjects. European journal of applied physiology.112(2): 483-492.
- Genest, J. J., Martin-Munley, S. S., McNamara, J. R., Ordovas, J. M., Jenner, J., Myers, R. H., Schaefer, E. J. (1992). Familial lipoprotein disorders in patients with premature coronary artery disease. Circulation .85(6) : 2025-2033.
- Gerald, F. Combs, Jr., James P. McClung. (2017) . The vitamins: fundamental aspects in nutrition and health. Academic press. 5:269-270.
- Gerster. (1999). High-dose vitamin C: a risk for persons with high iron stores? . International journal for vitamin and nutrition research. 69(2) : 67-82.
- Goldberg, A. C., Hopkins, P. N., Toth, P. P., Ballantyne, C. M., Rader, D. J., Robinson, J. G. Ziajka, P. E. (2011). Familial hypercholesterolemia: Screening, diagnosis and management of pediatric and adult patients: Clinical guidance from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. *Journal of clinical lipidology*. *5*(3) : 1-8 .
- Goldstein, J. L., and Brown, M. S. (1979). The LDL receptor locus and the genetics of familial hypercholesterolemia. Annual Review of Genetics. 13(1): 259–289.
- Goldstein, J. L., Schrott, H. G., Hazzard, W. R., Bierman, E. L., Motulsky, A. G. (1973). Hyperlipidemia in coronary heart disease II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. The Journal of Clinical Investigation.52(7): 1544–1568.
- Gratchev, A., Sobenin, I., Orekhov, A., Kzhyshkowska, J. (2012). Monocytes as a diagnostic marker of cardiovascular diseases. Immunobiology, 217(5), 476-482.

- Griselli, M., Herbert, J., Hutchinson, W. L., Taylor, K. M., Sohail, M., Krausz, T., Pepys, M. B. (1999). C-reactive protein and complement are important mediators of tissue damage in acute myocardial infarction. Journal of Experimental Medicine. 190(12): 1733-1740.
- Grzybowski, A., and Pietrzak, K. (2013). Albert Szent-Györgyi (1893-1986): the scientist who discovered vitamin C. Clinics in Dermatology. 31(3): 327–331.
- Halliwell, B., and Gutteridge, J. M. C. (1995). The definition and measurement of antioxidants in biological systems. Free Radical Biology and Medicine. 18(1): 125–126.
- Hegele, R. A. (2009). Plasma lipoproteins: genetic influences and clinical implications. Nature reviews genetics .10(2): 109.
- Hoffer, M. J. V, Bredie, S. J. H., Boomsma, D. I., Reymer, P. W. A., Kastelein, J. J. P., de Knijff, P., Frants, R. R. (1996). The lipoprotein lipase (Asn291→ Ser) mutation is associated with elevated lipid levels in families with familial combined hyperlipidaemia. Atherosclerosis. 119(2): 159–167.
- Ikonen, E. (2008). Cellular cholesterol trafficking and compartmentalization. Nature Reviews Molecular Cell Biology. 9(2): 125.
- (IOM) Institute of Medicine. (FNB) Food and Nutrition Board. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington, DC: National Academy Press : 2000.
- Jacob, R. A., Skala, J. H., and Omaye, S. T. (1987). Biochemical indices of human vitamin C status. The American Journal of Clinical Nutrition. 46(5): 818–826.
- Jacob, R. A. (1990). Assessment of human vitamin C status. The Journal of nutrition.120(suppl_11): 1480-1485.
- Jana O., Ladislava M., Jarmila VA., Kobert V., Jiri M. Fatty acids composition of vegetebale oils and it contribution to dietry energy intake and dependence of cardiovascular mortality on dietry intake of fatty acids. Int Mol Sci.(16): 12871-12890.
- Janssen, I., Heymsfield, S. B., Wang, Z., and Ross, R. (2000). Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. Journal of Applied Physiology. 89(1): 81–88.

- Kathleen D. (2017). Hyperlipidemia: causes, diagnosis, and treatment. Medical News Today.2-7.
- Kesäniemi, Y. A., Ehnholm, C., and Miettinen, T. A. (1987). Intestinal cholesterol absorption efficiency in man is related to apoprotein E phenotype. The Journal of Clinical Investigation.80(2): 578–581.
- Knekt, P., Ritz, J., Pereira, M. A., O'Reilly, E. J., Augustsson, K., Fraser, G. E., Pietinen, P. (2004). Antioxidant vitamins and coronary heart disease risk: a pooled analysis of 9 cohorts. The American Journal of Clinical Nutrition. 80(6) : 1508-1520.
- Kosecik, M., Erel, O., Sevinc, E., and Selek, S. (2005). Increased oxidative stress in children exposed to passive smoking. International Journal of Cardiology.100(1): 61–64.
- Kou, X., Chen, Q., Li, X., Li, M., Kan, C., Chen, B., Xue, Z. (2015). Quantitative assessment of bioactive compounds and the antioxidant activity of 15 jujube cultivars. Food chemistry. 173 : 1037-1044.
- Kramer, W., Glombik, H., Petry, S., Heuer, H., Schäfer, H.-L., Wendler, W., Weyland, C. (2000). Identification of binding proteins for cholesterol absorption inhibitors as components of the intestinal cholesterol transporter. FEBS Letters. 487(2): 293–297.
- Kris-Etherton, P. M., Yu, S. (1997). Individual fatty acid effects on plasma lipids and lipoproteins: human studies. The American journal of clinical nutrition. 65(5) : 1628S-1644S.
- Kruth, H. S. (2002). Sequestration of aggregated low-density lipoproteins by macrophages. Current opinion in lipidology, 13,(5):483-488.
- Kumar, C. (1998). Collins. Robbins Pathologic Basis of Disease. Philadelphia: WB Saunders Company.
- Kumar, R., Clermont, G., Vodovotz, Y., Chow, C. C. (2004). The dynamics of acute inflammation. Journal of theoretical biology, 230(2), 145-155.
- Kurowska, E. M., Spence, J. D., Jordan, J., Wetmore, S., Freeman, D. J., Piché, L. A., & Serratore, P. (2000). HDL-cholesterol-raising effect of orange juice in subjects with hypercholesterolemia–. The American journal of clinical nutrition. 72(5): 1095-1100.

- Kurzchalia, T. V., Partan, R. G. (1999). Membrane microdomains and caveolae. Current opinion in cell biology, 11(4), 424-431.
- Lange, Y., Matthies, H. J. (1984). Transfer of cholesterol from its site of synthesis to the plasma membrane. Journal of Biological Chemistry, 259(23), 14624-14630.
- Lau, D. C., Dhillon, B., Yan, H., Szmitko, P. E., Verma, S. (2005). Adipokines: molecular links between obesity and atheroslcerosis. American Journal of Physiology-Heart and Circulatory Physiology. 288(5): H2031-H2041.
- Lecerf, J.-M., De Lorgeril, M. (2011). Dietary cholesterol: from physiology to cardiovascular risk. British Journal of Nutrition.106(1): 6–14.
- Levine, M., Conry-Cantilena, C., Wang, Y., Welch, R. W., Washko, P. W., Dhariwal, K. R., King, J. (1996). Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. Proceedings of the National Academy of Sciences. 93(8): 3704–3709.
- Levine, M., Wang, Y., Padayatty, S. J., and Morrow, J. (2001). A new recommended dietary allowance of vitamin C for healthy young women. Proceedings of the National Academy of Sciences of the United States of America. 98(17): 9842–6.
- Levy, R. C., Remer, L. A., Mattoo, S., Vermote, E. F., and Kaufman, Y. J. (2007). Second-generation operational algorithm: Retrieval of aerosol properties over land from inversion of Moderate Resolution Imaging Spectro radiometer spectral reflectance. Journal of Geophysical Research: Atmospheres. 112(13):
- Li, H., Tu, H., Wang, Y., Levine, M. (2012). Vitamin C in mouse and human red blood cells: an HPLC assay. Analytical Biochemistry. 426(2): 109–117.
- Li, Y., Schellhorn, H. E. (2007). New developments and novel therapeutic perspectives for vitamin C. The Journal of nutrition, 137(10), 2171-2184.
- Lindblad, M., Tveden-Nyborg, P., Lykkesfeldt, J. (2013). Regulation of vitamin C homeostasis during deficiency. Nutrients. 5(8): 2860-2879.
- Lintermans, L. L., Stegeman, C. A., Heeringa, P., Abdulahad, W. H. (2014). T cells in vascular inflammatory diseases. Frontiers in immunology, 5, 504.
- Loregger, A., Nelson, J. K., Zelcer, N. (2017). Assaying Low-Density-Lipoprotein (LDL) Uptake into Cells. Cholesterol Homeostasis: Methods and Protocols: 53–63.

- Loria, C. M., Whelton, P. K., Caulfiled, L. E., Szklo, M., Klag, M. J. (1998). Agreement among indicators of vitamin C status. American journal of epidemiology. 147(6): 587-596.
- Lykkesfeldt, J., Michels ,A. J., Frei B (2014). Vitamin C. Adv Nutr. 5:16-18.
- Madamanchi, N. R., Vendrov, A., Runge, M. S. (2005). Oxidative stress and vascular disease. Arteriosclerosis, thrombosis, and vascular biology. 25(1): 29-38.
- Mandl, J., Szarka, A., and Banhegyi, G. (2009). Vitamin C: update on physiology and pharmacology. British Journal of Pharmacology. 157(7): 1097–1110.
- Mantovani, A., Garlanda, C., Doni, A., Bottazzi, B. (2008). Pentraxins in innate immunity: from C-reactive protein to the long pentraxin PTX3. Journal of clinical immunology, 28(1), 1-13.
- Marnell et al., 1995).
- Mathers, C. D., Loncar, D. (2006). Projections of global mortality and burden of disease from 2002 to 2030. PLoS medicine. 3(11):442.
- Mensink, R. P., Zock, P. L., Kester, A. D., Katan, M. B. (2003). Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. The American journal of clinical nutrition. 77(5) : 1146-1155.
- Michael, T., Murayn, D. (2013). cholesterol and heart health. chapter 3 : 46.
- Moreau, R., Dabrowski, K. (1998). Body pool and synthesis of ascorbic acid in adult sea lamprey (Petromyzon marinus): an agnathan fish with gulonolactone oxidase activity. Proceedings of the National Academy of Sciences. 95(17): 10279–10282.
- Moreau, R., and Dabrowski, K. (1998). Fish acquired ascorbic acid synthesis prior to terrestrial vertebrate emergence. Free Radical Biology and Medicine. 25: 989–990.
- Mozaffarian ,D. Benjamin ,E. J., Go ,A.S., Arnett ,D.K., Blaha, M.J. (2015) Heart disease and stroke statistics--2015 update: a report from the American Heart Association. Circulation. (131):e29–322.
- (NCEP) National Cholesterol Education Program. (2001). Executive summary of the Third Report of the expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). Jama. 285(19): 2486.
- Nathan, C., Ding, A. (2010). Non resolving inflammation. Cell, 140(6), 871-882.

- Nayanatara, A.K ., Nagaraja, H.S ., Anupama B.K. (2005).The effect of repeated swimming stress on organ weights and lipid peroxidation in rats. Thai Journal of Physiological Sciences.;18(1):3-9.
- Nickolas, T. L., Radhakrishnan, J., Appel, G. B. (2003, July). Hyperlipidemia and thrombotic complications in patients with membranous nephropathy. In Seminars in nephrology WB Saunders. 23(4): 406-411..
- Nienhuis, A. W. (1981). Vitamin C and iron. 304(3):170-1.
- Noble, M., Healey, C. S., McDougal-Chukwumah, L. D., Brown, T. M. (2013). Old disease, new look? A first report of parkinsonism due to scurvy, and of refeeding-induced worsening of scurvy. Psychosomatics. 54(3): 277-283.
- oligorsky, M. S., Chen, J., Brodsky, S. (2001). Workshop: endothelial cell dysfunction leading to diabetic nephropathy: focus on nitric oxide. Hypertension. 37(2): 744-748.
- Olorunnisola, O. S., Bradley, G., Afolayan, A. J. (2012). Protective effect of T. violacea rhizome extract against hypercholesterolemia-induced oxidative stress in Wistar rats. Molecules, 17(5), 6033-6045.
- Osganian, S. K., Stampfer, M. J., Rimm, E., Spiegelman, D., Hu, F. B., Manson, J. E., Willett, W. C. (2003). Vitamin C and risk of coronary heart disease in women. Journal of the American College of Cardiology. 42(2) : 246-252.
- Ordman, A. R. (2010). Vitamin C twice a day enhances health. Health. 2(08): 819.
- Orekhov, A. N., Sobenin, I. A., Revin, V. V., Bobryshev, Y. V. (2015). Development of antiatherosclerotic drugs on the basis of natural products using cell model approach. Oxidative medicine and cellular longevity, 2015.(11):3.
- Pacier, C., Martirosyan, D. M. (2015). Vitamin C: optimal dosages, supplementation and use in disease prevention. Functional Foods in Health and Disease, 5(3), 89-107.
- Padayatty, S. J., and Levine, M. (2001). New insights into the physiology and pharmacology of vitamin C. Canadian Medical Association Journal. 164(3): 353–355
- Padayatty, S. J., and Levine, M. (2016). Vitamin C: the known and the unknown and Goldilocks. Oral Diseases. 22(6) :463–493.
- Padayatty, S. J., Katz, A., Wang, Y., Eck, P., Kwon, O., Lee, J.-H., Dutta, S. K. (2003). Vitamin C as an antioxidant: evaluation of its role in disease prevention. Journal of the American College of Nutrition. 22(1): 18–35.

- Parrow, N. L., Leshin, J. A., and Levine, M. (2013). Parenteral ascorbate as a cancer therapeutic: a reassessment based on pharmacokinetics. Antioxidants & Redox ignaling . 19(17): 2141–2156.
- Pepys, M. B., Hirschfield, G. M. (2003). C-reactive protein: a critical update. The Journal of clinical investigation. 111(12): 1805-1812.
- Pfister, R., Sharp, S. J., Luben, R., Wareham, N. J., Khaw, K. T. (2011). Plasma vitamin C predicts incident heart failure in men and women in European Prospective Investigation into Cancer and Nutrition–Norfolk prospective study. American heart journal.162(2) : 246-253.
- Phillips, C. L., Yeowell, H. N .(1997). Vitamin C, collagen biosynthesis, and aging. In Vitamin C in Health and Disease. Marcel Dekker Inc . (4) : 205-230.
- Porkka, K. V. K., Nuotio, I., Pajukanta, P., Ehnholm, C., Suurinkeroinen, L., Syvänne, M.,Ylitalo, K. (1997). Phenotype expression in familial combined hyperlipidemia. Atherosclerosis.133(2):245–253.
- Rafael, A. C., Mario R., Garcia P. (1990). Cholesterol, Triglycerides, and Associated
- Ross, R. (1999). Atherosclerosis—an inflammatory disease. New England journal of medicine. 340(2) : 115-126.
- Ross, R., Glomset, J. A. (1976). The pathogenesis of atherosclerosis (first of two parts). The New England journal of medicine. 295(7): 369.
- Roux , A.S., perraut, J. L ., Rauch, C., de Villedary, G., Kremser,A., Korth., D. T .Young .(1983). wave-particle interactions near Ω_{He^+} observed on board GEOS 1 and 2, 2.Generation of ion cyclotron waves and heating of He^+ ions, j.Geophys.Res : 87,8174.
- Ruiu, G., Pinach, S., Veglia, F., Gambino, R., Marena, S., Uberti, B., Cassader, M. (2009). Phytosterol-enriched yogurt increases LDL affinity and reduces CD36 expression in polygenic hypercholesterolemia. Lipids. 44(2): 153–160.
- Sakakura, K., Nakano, M., Otsuka, F., Ladich, E., Kolodgie, F. D., Virmani, R. (2013). Pathophysiology of atherosclerosis plaque progression. Heart, Lung and Circulation.22(6): 399-411.
- Santos-Gallego, C. G., Badimón, J. J. (2012). High-Density Lipoprotein and Cardiovascular Risk Reduction, Promises and Realities. Revista Española de Cardiología.65(04) : 305-308.

- Sereday, M.S., C. Gonzalez, D. Giorgini, L.De Lored, J. Braguinsky, C. Cobeñas, C.Libman, C.Tesone, .(2004).Prevalence of diabetes, obesity, hypertension and hyperlipidemia in the central area of Argentina. Metab. 30(4): 335-9.
- Sharrett, A. R., Ballantyne, C. M., Coady, S. A., Heiss, G., Sorlie, P. D., Catellier, D., Patsch, W. (2001). Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein (a), apolipoproteins AI and B, and HDL density subfractions: The Atherosclerosis Risk in Communities (ARIC) Study. Circulation. 104(10) : 1108-1113.
- Simmons A., Steffen K., Sanders S. (2012). Medical therapy for peripheral arterial disease. Current opinion in cardiology. 27(6): 592-597.
- Simopoulos, A. P. (2009). Evolutionary aspects of the dietary omega–6: Omega–3 fatty acid ratio: Medical implications. In A Balanced Omega-6/Omega-3 Fatty Acid Ratio, Cholesterol and Coronary Heart Disease , Karger Publishers . 21-1 : 100 .
- Singer, M. (1995). Beyond the ivory tower: Critical praxis in medical anthropology. Medical Anthropology Quarterly. 9(1) :80–106.
- Smart, E. J., Ying, Y. S., Donzell, W. C., Anderson, R. G. (1996). A role for caveolin in transport of cholesterol from endoplasmic reticulum to plasma membrane. Journal of Biological Chemistry, 271(46), 29427-29435.
- Smirnoff, N., Conklin, P. L., and Loewus, F. A. (2001). Biosynthesis of ascorbic acid in plants: a renaissance. Annual Review of Plant Biology. 52(1): 437–467.
- Smythies, J. (1996). On the function of neuromelanin. Proc. R. Soc. Lond. B, 263(1369), 487-489.
- Sniderman, A. D., Tsimikas, S., Fazio, S. (2014). The severe hypercholesterolemia phenotype: clinical diagnosis, management, and emerging therapies. Journal of the American College of Cardiology. 63(19):1935–1947.
- Soutar, A. K., Naoumova, R. P. (2007). Mechanisms of disease: Genetic causes of familial hypercholesterolemia. Nature Clinical Practice Cardiovascular Medicine. 4(4): 214–225.
- Spittle, C. R. (1972). Arteriosclerosis and Vitamin C. Lancet. (1): 798.
- Stasch, J. P., Schmidt, P., Alonso-Alija, C., Apeler, H., Dembowsky, K., Haerter, M., Schramm, M. (2002). NO-and haem-independent activation of soluble guanylyl

cyclase: molecular basis and cardiovascular implications of a new pharmacological principle. British journal of pharmacology. 136(5): 773-783.

- Sugimoto, M. A., Sousa, L. P., Pinho, V., Perretti, M., Teixeira, M. M. (2016). Resolution of inflammation: what controls its onset?. Frontiers in immunology, 7, 160.
- Takeuchi, O., Akira, S. (2010). Pattern recognition receptors and inflammation. Cell. 140(6): 805-820.
- Thompson, D., Pepys, M. B., Wood, S. P. (1999). The physiological structure of human C-reactive protein and its complex with phosphocholine. Structure.7(2) : 169-177.
- Torzewski, M., Rist, C., Mortensen R. F., Zwaka, T. P., Bienek, M., Waltenberger, J., Torzewski, J. (2000). C-reactive protein in the arterial intima: role of C-reactive protein receptor–dependent monocyte recruitment in atherogenesis. Arteriosclerosis, Thrombosis, and Vascular Biology.20(9): 2094–2099.
- Traber, M. G., Stevens, J. F. (2011). Vitamins C and E: beneficial effects from a mechanistic perspective. Free Radical Biology and Medicine. 51(5) : 1000-1013.
- Tsukaguchi, H., Tokui, T., Mackenzie, B., Berger, U. V, Chen, X.-Z., Wang, Y., Hediger, M. A. (1999). A family of mammalian Na+-dependent L-ascorbic acid transporters. Natur.399(6731): 70.
- Turley, S. D. (1999). Dietary cholesterol and the mechanisms of cholesterol absorption. European Heart Journal Supplements. 1(S): S29–S35.
- Tuzcu, E. M., Kapadia, S. R., Tutar, E., Ziada, K. M., Hobbs, R. E., McCarthy, P. M., Nissen, S. E. (2001). High prevalence of coronary atherosclerosis in asymptomatic teenagers and young adults: evidence from intravascular ultrasound. Circulation.103(22): 2705-2710.
- Urbani, L., Simoni, R. D. (1990). Cholesterol and vesicular stomatitis virus G protein take separate routes from the endoplasmic reticulum to the plasma membrane. Journal of Biological Chemistry, 265(4), 1919-1923.
- van der Wulp, M., Verkade, H., Groen, A. K. (2013). Regulation of cholesterol homeostasis. Molecular and Cellular Endocrinology .368(1–2) :1–16.
- Vanhoutte, P. M. (2009). How we learned to say NO. Arteriosclerosis, thrombosis, and vascular biology, 29(8), 1156-1160.

- Volanakis, J. E., & Kaplan, M. H. (1971). Specificity of C-reactive protein for choline phosphate residues of pneumococcal C-polysaccharide. Proceedings of the Society for Experimental Biology and Medicine. 136(2) : 612-614.
- Vollbracht, C., Schneider, B., Leendert, V., Weiss, G., Auerbach, L., Beuth, J. (2011). Intravenous vitamin C administration improves quality of life in breast cancer patients during chemo-radiotherapy and aftercare: results of a retrospective, multicentre, epidemiological cohort study in Germany. in *vivo*. 25(6): 983-990.
- Wang, Y., Russo, T. A., Kwon, O., Chanock, S., Rumsey, S. C., and Levine, M. (1997). Ascorbate recycling in human neutrophils: induction by bacteria. Proceedings of the National Academy of Sciences. 94(25): 13816–13819.
- Watson, R. R., Meester, F. De. (2017). Handbook of cholesterol Biology, function and role in health and diseases (Human Heal). The Netherlands, Wageningen Academic Publishers.(11) :20.
- Widmaier, E. P., Raff, H., Strang, K. T. (2013). Vander's human physiology: the mechanisms of body function. McGraw-Hill Science/Engineering/Math. (13).

  Widmaier, E. P., Raff, H., & Strang, K. T. (2008). Vander's human physiology: the mechanisms of body function McGraw-Hill Higher Education : 306.
- Wilson, J. X. (2005). Regulation of vitamin C transport.Annu. Rev. Nutr. (25): 105–125.
- Wouters, K., Shiri-Sverdlov, R., van Gorp, P. J., van Bilsen, M., Hofker, M. H. (2005). Understanding hyperlipidemia and atherosclerosis: lessons from genetically modified apoe and ldlr mice. Clinical Chemistry and Laboratory Medicine (CCLM), 43(5), 470-479.
- Xu, S., Liu, Z., Liu, P. (2013). HDL cholesterol in cardiovascular diseases: The good, the bad, and the ugly?. International journal of cardiology. 168(4): 3157-3159
- Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y.Masaki, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature. 332(6163): 411.
- Yanni, A. E. (2004). The laboratory rabbit: an animal model of atherosclerosis research. Laboratory animals, 38(3), 246-256.

- Young IS., Woodside JV .(2001). Antioxidants in health and disease. J Clin Pathol . (54):176-186.
- Zhou, Y., Hong, Y., Huang, H. (2016). Triptolide Attenuates Inflammatory Response in Membranous Glomerulo-Nephritis Rat via Downregulation of NF-κB Signaling Pathway. Kidney and Blood Pressure Research, 41(6), 901-910.
- Zock, P. L., de Vries, J. H., Katan, M. B. (1994). Impact of myristic acid versus palmitic acid on serum lipid and lipoprotein levels in healthy women and men. Arteriosclerosis and Thrombosis. 14: 567-575.
- Zwaka, T. P., Hombach, V., Torzewski, J. (2001). C-reactive protein–mediated low density lipoprotein uptake by macrophages: implications for atherosclerosis.Circulation. 103(9): 1194-1197.

Refrence web:

- https://bjcardio.co.uk .
- http://www.hmdb.ca/metabolites/HMDB0000067 (HMDB, 2018).
- USDA (U.S. DEPARTMENT OF AGRICULTURE) National Nutrient Database for Standard Reference, Release 28 (Adapted from Uncooked) http://www.ars.usda.gov/ba/bhnrc/ndl.
- http://pearson education.inc

Summary

Summary

Cholesterol is a component of the cell membrane and metabolites of cholesterol, such as bile acids, steroid hormones and vitamin D, serve important biologic functions in vertebrates. Cholesterol is synthesized primarily in the liver and transported to cells throughout the body by lipoproteins via the blood, However, the consumption of saturated and Trans fatty acid is the major causes of the elevation of LDL-c in plasma which is a risk factor for the Development of atherosclerosis and cardiovascular diseases (CVD).
In the present study, we evaluated the benefit of vitamin C administration on the inflammation induced by diet rich in fats during 30 days in albino rats. For this reason we have measured the hs-CRP, Total cholesterol , triglyceride, LDL-cholesterol ,HDL-Cholesterol ,glucose and enzymes such as AST and ALT.

Biochemical results showed that there is an increase in the factor of inflammation (hs-CRP) , triglyceride ,LDL-cholesterol , ALT in the group of rats fed with diet rich in fats .However the treatment with vitamin C(500mg/kg) with diet rich in fats ameliorated these changes.
The vitamin C decreased the concentration of hs-CRP and LDL-cholesterol which proves that vitamin C is an anti-inflammatory which prevented against hypercholesterolemia and cardiovascular diseases.

Key word: Diet rich in fats , Hypercholesterolemia, Atherosclerosis, cardiovascular diseases Inflammation, hs-CRP, vitamin C.

Résumé

Le cholesterol est un composant entre dans la structure de la membrane cellulaire, et les métabolites de cholesterol comme les acides biliaires, les hormones stéroïdes et la vitamine D entrent dans la fonction biologiques chez les vertèbres.

Le cholesterol est synthétisé principalement dans le foie et transporté aux cellules dans tout le corps par les lipoproteines, cependant , la consommation d'acides gras saturés et les acides gras trans est les principales des causes de l'élévation du LDL-c dans le plasma qui est un facteur de risque pour le développement de l'athérosclérose et les maladies cardiovasculaires.

Dans notre etude, nous avons évalué le bénéfice de l'administration de vitamine C sur l'inflammation induite par un régime riche en gras pendant 30jours chez les rats Albino . pour cette raison, nous avons mesuré la hs-CRP ,cholesterol total ,les triglycerides,LDL-cholesterol, HDL-cholesterol, glucose et les enzymes ALAT et ASAT.

Les résultats biochimiques ont montré qu'il ya une augmentation du facteur d'inflammation (hs-CRP, triglycerides ,LDL-cholesterol,ALAT) dans le groupe des rats nourris avec un régime riche en gras . Cependant, le traitement par la vitamine C(500mg/kg) associé ou non a un régime riche en gras a diminué les concentrations de hs-CRP,cholesteroltotal , LDL – cholesterol, ce qui prouve que la vitamin C est un anti-inflammatoire qui prévient contre l'hypercholestérolémie et les maladies cardiovasculaires.

Mots clés: Régime riche en gras , Hypercholestérolémie, Athérosclérose, cardiovasculaire Inflammation, hs-CRP,Lipide,Vitamin C.

ملخص

الكولسترول عبارة عن مركب يدخل في تركيب الغشاء البلازمي للخلايا ،و إذ نواتج الكولسترول مثل : الحمض الصفراوي ، الهرمونات الستيرويدية ،و الفيتامين د التي تتدخل في الوظائف البيولوجية عند الفقاريات .

يتخلق الكولسترول أساسا في الكبد و ينتقل في الدورة الدموية إلى الأنسجة بواسطة الليبوبروتينات ، و لهذا فإن استهلاك الأحماض الذهنية المشبعة و الأحماض الذهنية المهدرجة هي التي تسبب ارتفاع الكلسترول الضار في بلازما الدم و الذي يعتبر عامل خطير في تطور تصلب الشرايين و أمراض الأوعية القلبية .

في هذه الدراسة قمنا بتقييم أهمية إعطاء جرعات من فيتامين ج على الالتهابات التي يسببها الغذاء الغني بالدهون المعطى للجرذان لمدة 30 يوم .

ولهذا قمنا بقياس تركيز hs-CRP، الكولسترول الكلي ،الغليسيريدات الثلاثية،و الكولسترول الضار و الجيد ، الجلوكوز ،و أنزيمات ALAT-ASAT

إن النتائج المتحصل عليها بينت بأن هناك زيادة في(عامل الالتهاب hs-CRP، الغليسيريدات الثلاثية ،و الكولسترول الضار و ALAT) عند مجموعة الفئران المتغذية على غذاء غني بالدهون بينما عند معالجة الجرذان بواسطة جرعات من الفيتامين ج (500 مغ /كلغ/ يوم) مع الغذاء الذهني أو غذاء طبيعي فلقد أدى إلى خفض في تراكيز hs-CRP، الكولسترول الكلي ، الكولسترول الضار، من خلال هذه النتائج أثبتت أن فيتامين ج يعتبر مضاد للالتهاب الذي يحمي الجسم من زيادة الكولسترول البلازمي و من أمراض الأوعية القلبية .

الكلمات المفتاحية:غذاء غني بالدهون، زيادة الكولسترول البلازمي،تصلب الشرايين،أمراض الأوعية القلبية ،الالتهاب، hs-CRP، اللليبيدات ، فيتامين ج .

Annex

1.Chemicals

- Chloroform
- Formol 10%
- Distilled water
- NaCl 0, 9%
- Ethanol

2.Equipments

- Capillary
- Balance (0,001)
- Dissection kit
- Centrifuge (EZ Swing 3K)
- EDTA tubes
- Hotplate Stirrer (JLabTech)
-Tube eppenderf
-petri box
-Spss logecial

3.Preparation of solutions

1-NaCl

0.9 g⟶ 100 ml

X g⟶ 1000 ml

0.9 g ×1000 ml/100 ml=9 g

2-formol 10%

(10 ml formol(37%) + 27 ml of distilled water.)

4. Dose of vitamin C used

With regard to vitamin C, studies have shown that supplementation with .500mg vitamin C per day is associated with a lower risk of CHD **(Osganian et al.,2003)** and **(Knekt et al.,2004).**

Figure 27:Nutri Power vitamin C powder pure

5.Preparation of alcoholic Bouin

- Picric acid (1%) diluted in ethanol 95%.....……...45ml
- Formol ……..…… 26ml
- Acetic Acid…… ……. 7ml
- Distilled water ……. 22ml

6.Hematoxylin eosin coloration

Staining Procedure:

1- Dip the lame in the alcohol solution for 5 minutes.

2- Rinse with tap water.

3- Immerse sections in Hematoxylin for 4 minute.

2. Rinse with tap water.

3. Exchange tap water until the water is clear.

4. Immerse sections in EOSIN stain for 10 minutes.

5. Rinse with tap water.

6. Exchange tap water until the water is clear.

7. Dehydrate in alcohol solutions for 1 min.

8. Clear with xylene.

Table 06: The average weight and diet in group (**c**) during 30 days.

Days	Weight average (g)	Average per week (g)	Diet consumed (g)	Average per week(g)
Day : 01	144.08		***	
Day : 02	145		37.5	
Day : 03	151.24		79.6	
Day : 04	153.2		87.9	
Day : 05	158.36		85	
Day : 06	160.1		88	
Day : 07	163.7	160.03	78.4	85.35
Day : 08	165.2	±5.82	88.6	±4.26
Day : 09	168.42		90	
Day : 10	171.05		88	
Day : 11	170.86		92.5	
Day : 12	172.2		87	
Day : 13	175.42		96	
Day : 14	175.08	174.58	99	91.42
Day : 15	178.38	±3.107	90.5	±27.32
Day : 16	179.08		87	
Day : 17	181.12		90	
Day : 18	180.68		80	
Day : 19	182.44		89.1	
Day : 20	184.36		98	
Day : 21	189.04	185.23	97	93.58
Day : 22	189.06	±3.73	99	±7.05
Day : 23	189.96		102	
Day : 24	190.48		94	
Day : 25	191.04		97.9	
Day : 26	193.58		95.1	
Day : 27	192.8		110	
Day : 28	192.7	192.45	101	95.05
Day : 29	192.88	±1.13	83	±8.67
Day :30	193.68		84.4	
Day : 31	Blood samples		fasted	

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Table 07: The average weight and diet in group (**ch**) during 30 days.

Days	Weight average (g)	Average per week (g)	Diet consumed (g) +45.35g fats	Average per week(g)
Day : 01	160.74		***	
Day : 02	163		137.6	
Day : 03	166.6	169.99 ±2.194	113.7	95.27 ± 19.84
Day : 04	167.4		123.7	
Day : 05	169.88		103.7	
Day : 06	170		106.7	
Day : 07	171.14		81.7	
Day : 08	171.5		58.6	
Day : 09	173.44		78.8	
Day : 10	172.48	176.52 ±2.64	70.3	66.58 ± 16.99
Day : 11	173		107.1	
Day : 12	177.36		51.7	
Day : 13	176		49.6	
Day : 14	178		60.7	
Day : 15	179.82		70	
Day : 16	179		56.7	
Day : 17	180.34	183.70 ±2.48	62.7	66.48 ± 5.37
Day : 18	181		57.7	
Day : 19	181.9		72.7	
Day : 20	184.24		63.1	
Day : 21	185		63.6	
Day : 22	186		71.9	
Day : 23	187.44		73.7	
Day : 24	187.1	188.21 ±1.97	68.7	59.49 ± 7.25
Day : 25	186		55.7	
Day : 26	186.7		49.7	
Day : 27	187.52		72.7	
Day : 28	188.1		53.1	
Day : 29	192.1		56.3	
Day :30	190		60.25	
Day : 31	Blood samples		fasted	

Table 08: The average weight and diet in group (**ch /vc**) during 30 days.

Days	Weight average (g)	Average per week (g)	Diet consumed (g) +63.7g fats	Average per week(g)
Day : 01	141.27		***	
Day : 02	143.3		152.38	
Day : 03	146.34		112.8	
Day : 04	147.33		131.4	
Day : 05	149.57		115.3	
Day : 06	153.42		104.2	103.6
Day : 07	151.57		110.4	±
Day : 08	155.17	151.91	77.3	18.09
Day : 09	160.02	±4.41	73.8	
Day : 10	159.61		75.3	
Day : 11	160.97		72.6	
Day : 12	164.24		94.9	
Day : 13	165.31		111.3	95.28
Day : 14	166.37		101.8	±
Day : 15	167.88	164.78	106.8	13.35
Day : 16	169.11	±3.22	104.3	
Day : 17	172.07		126.5	
Day : 18	175.45		117.8	
Day : 19	174.3		105.4	
Day : 20	177.72		102.9	110.68
Day : 21	179.41		119.7	±
Day : 22	179	176.79	91.4	10.26
Day : 23	179.58	±2.69	111.1	
Day : 24	185.28		110.3	
Day : 25	184.18		107.9	
Day : 26	187.6		103.1	
Day : 27	186.17		107.6	107.5
Day : 28	186.6		107.3	±
Day : 29	188	187.28	103.1	3.14
Day :30	193.17	±2.68	113.2	
Day :31	Blood samples		fasted	

Table 09: The average weight and diet in group (**vc**) during 30 days.

Days	Weight average (g)	Average per week (g)	Diet consumed (g)	Average per week(g)
Day : 01	129.17		***	
Day : 02	129		145	
Day : 03	130.1	138.46 ±5.59	148.5	133.27 ±8.15
Day : 04	133.11		136.8	
Day : 05	135.7		140	
Day : 06	139.87		126.4	
Day : 07	139.28		125.7	
Day : 08	143.58		130	
Day : 09	147.62		125.5	
Day : 10	149.18	155.39 ±3.79	151	142.25 ±13.71
Day : 11	150.7		131.6	
Day : 12	154.74		122.4	
Day : 13	156.75		144.8	
Day : 14	157.65		167.2	
Day : 15	158.64		132.8	
Day : 16	160.11		146	
Day : 17	161	167.46 ±2.84	137.8	143.24 ±12.58
Day : 18	166.65		146.7	
Day : 19	167.35		139.3	
Day : 20	169.47		131.3	
Day : 21	168.92		172.1	
Day : 22	169.82		135	
Day : 23	169.04		140.5	
Day : 24	170	175.91 ±3.38	210.1	149.68 ±26.74
Day : 25	173.61		146.4	
Day : 26	175.3		139.1	
Day : 27	175.71		134.9	
Day : 28	176.58		120.3	
Day : 29	181.47		139.9	
Day :30	178.74		157.1	
Day :31	Blood samples		fasted	

Table 10: Composition of water used in the experiemental work (www.guedila.com)

Composition	**Mg/litre**
Calcium (ca)	78
Magnésium (Mg)	37
Soduim (Na)	29
Potasuim p	2
Sulfates	95
Chlorures	40
Nitrates	4.5
Nitrites	-0.01
Ph	7.35
R.S à 180c	564

Table 11: Quatity of 100g of butter

Composant	Quantité
Lipides (g)	83
Glucids(g)	0.8
Proteines(g)	0.3
Sodium(mg)	194
Vitamin A: Palmitate (ug)	1659
Vitamin A: Betacaroténe (ug)	500
Vitamin D (ug)	7.5
Vitamin E(mg)	3

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Title:The effect of vitamin c on inflammation induced by hypercholesterolemia in rats

Thesis submitted for the degree of Master of Cellular and molecular immunology

Summary

Cholesterol is a component of the cell membrane and metabolites of cholesterol, such as bile acids, steroid hormones and vitamin D, serve important biologic functions in vertebrates. Cholesterol is synthesized primarily in the liver and transported to cells throughout the body by lipoproteins via the blood, However, the consumption of saturated and Trans fatty acid is the major causes of the elevation of LDL-c in plasma which is a risk factor for the Development of atherosclerosis and cardiovascular diseases (CVD).

In the present study, we evaluated the benefit of vitamin C administration on the inflammation induced by diet rich in fats during 30 days in albino rats. For this reason we have measured the hs-CRP, Total cholesterol , triglyceride, LDL-cholesterol ,HDL-Cholesterol ,glucose and enzymes such as AST and ALT.

Biochemical results showed that there is an increase in the factor of inflammation (hs-CRP) , triglyceride ,LDL-cholesterol , ALT in the group of rats fed with diet rich in fats .However the treatment with vitamin C(500mg/kg) with diet rich in fats ameliorated these changes. The vitamin C decreased the concentration of hs-CRP and LDL-cholesterol which proves that vitamin C is an anti-inflammatory which prevented against hypercholesterolemia and cardiovascular diseases.

Key word: Diet rich in fats , Hypercholesterolemia, Atherosclerosis, cardiovascular diseases Inflammation, hs-CRP, vitamin C.

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