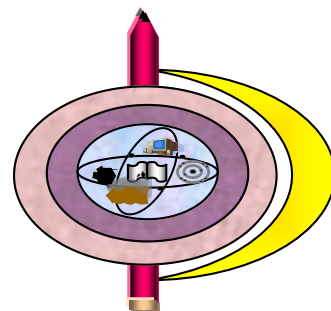


Republic of Iraq
Ministry of Higher Education and
Scientific Research
University of Thi-Qar
College of Science
Department of Biology



**Effect of Polyphenolic compounds extracted from
Rosemary leaves (*Rosmarinus officinalis*) and Grape
seeds (*Vitis vinifera*) on hypercholesterolemic female rats
(*Rattus norvegicus*)**

A Thesis

Submitted to the College of Science

University of Thi-Qar

In partial Fulfillment of the Requirements

for the Degree of master of Science in Biology /Animal Physiology

By

Yaqeen Riassan Shalaan

B.Sc. Biology (Thi-Qar University)

(2015)

Supervised by:

Prof. Dr. Khalid Al-Fartosi

2017 A. D

1439A.H

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(قُلْ لَوْ كَانَ الْبَحْرُ مَدَادًا لَّكَلِمَاتِ رَبِّي لَنَفَذَ الْبَحْرُ قَبْلَ أَنْ

تَنْفَذَ كَلِمَاتِ رَبِّي وَلَوْ جُنَا بِمِثْلِهِ مَدَدًا)

صدق الله العلي العظيم

سورة الكهف

الآية (109)

Dedication

*The light of my life ... prophet Mohammed and the
holy household (peace be upon them).*

*To dear father who removed the thorns from my
path to pave my way to science...*

*To dear my mother who makes me happy when I
see her kindest heart...*

To my brothers and sisters

To my dear husband

Yaqeen

Acknowledge

Initially I thank Almighty God for giving me the strength and the will to complete this work

My deepest affection and gratitude go to my supervisor, Prof. Dr. Khalid Al-Fartosi for his supervision, assistance and considerable scientific advice

I extend my thanks to the Department of Biology, Deanship and the Committee of Graduate Studies.

Great thank extends to my friends and classmates for their help.

Yaqeen

Tittles No.	contents	Pa ge No.
	List of Contents	I
	List of figures and Table Index	V
	List of Abbreviations	VI
	Abstract	VII
Chapter one		
1.Introduction and literature review		
1	Introduction	1
1.2	literature review	4
1.2.1	Cholesterol	4
1.2.1.1	Biochemistry of Cholesterol	4
1.2.3.	Lipid Profile	5
1.2.3.1	Total Cholesterol	5
1.2.3.2	Triglycerides	5
1.2.3.3	HDL Cholesterol	6
1.2..3.4	LDL Cholesterol	6
1.2.4	LDL Oxidation	6
1.2.5	Lipids Profile and Chronic Diseases	7
1.2.6	Hypercholesterolemia	8
1.2.6.1	Etiology of hypercholesterolemia	10
1.2.7	Polyphenols	12
1.2.7.1	physiological effects of Phenolic	12
1.2.7.2	Polyphenols as Antioxidant	13
1.2.8	Rosemary	14
1.2.8.1	History	15
1.2.8.2	Description	16
1.2.8.3	Rosemary polyphenole	16
1.2.8.4	Active Constituents	17
1.2.8.5	Hepatoprotective effects	17
1.2.9	Grape Seeds	18
1.2.9.1	History	19
1.2.9.2	Description	21
1.2.9.3	Grape Seed Polyphenol	21
1.2.9.4	Active constituents	22
1.2.9.5	Hepatoprotective effects	23

1.2.9.6	Toxicity.	23
1.2.10	Aim of the Study	25
Chapter two		
2. Materials & Methods		
2.1	Materials	26
2.1.1	Chemicals	26
2.1.2	Kit	27
2.1.3.	Equipments and Apparatus	27
2.1.4	Plants Collection	28
2.1.5	Experimental Animal	28
2.2	Methods	28
2.2.1	Extraction of Polyphenols	28
2.2.2	Qualitative Tests of Some Active Compounds in Plant Extracts	29
2.2.2.1	Detection of Phenolic Compounds in Plant Extracts	29
2.2.2.2	Detection of Flavonoid compounds in Plant Extracts	29
2.2.2.3	Detection of Tannin Compounds in Plant Extracts:	29
2.2.3	Method of Food Preparing (High Cholesterol Diet)	29
2.2.4	Laboratory Animal	30
2.2.5	Blood Sampling	31
2.2.6	Determination of hematological parameters	32
2.2.7	Determination of Biochemical Parameters	32
2.2.7.1	Determination of Serum Lipid Profile	32
2.2.7.1.1	Determination of total cholesterol (TC) level	32
2.2.7.1.2	Determination of Serum Triglyceride Concentration	34
2.2.7.1.3	Determination of Serum HDL Concentration	36
2.2.7.1.4	Calculation of LDL and VLDL	38
2.2.7.1.5	Calculation of Atherogenic Index Plasma	38
2.2.7.2	Determination of oxidative stress parameters	38

2.2.7.2.1	Determination of serum malondialdehyde Level (MDA)	38
2.2.7.2.2	Determination of Serum Ceruloplasmin Concentration	39
2.2.7.2.3	Determination of Serum Albumin Concentration	41
2.2.7.3.	Determination of liver enzymes	43
2.2.7.3.1	Determination of alanine transaminase (ALT) and aspartate transaminase (AST) levels	43
2.3	Statistical Analysis	45
Chapter Three		
3. Results		
3.1	Plant Extracts	46
3.2	Detection of Some Active Compounds in Plants Extracts	46
3.3	Effect of polyphenols extracted from ROS physiological parameters of hypercholesterolemic female rats	46
3.3.1	Effect on body Weight	46
3.3.2	Effects on hematological parameters.	48
3.3.3	Effects on lipid profile	50
3.3.4	Effect of oxidant-antioxidant markers (MDA, CP and Alb)	52
3.3.5.	Effects on liver enzymes	54
3.4	Effect of polyphenols extracted from GSE on physiological parameters of hypercholesterolemic female rats	55
3.4.1	Effects on body Weight	55
3.4.2.	Effects on hematological parameters.	56
3.4.3	Effects on the lipid profile	58
3.4.4	Effect of oxidant-antioxidant markers (MDA, CP and Alb)	60

3.4.5	Effects on Level of Liver Enzymes.	62
Chapter four		
4.Discussion		
4.1	Plant extraction	63
4.2	Effects of polyphenols extracted from (Rosemary and Grapes seeds) on body weight.	64
4.3	Effects of polyphenols extracted from (Rosemary and Grapes seeds) on the haematological parameters	64
4.4	Effects of polyphenols extracted from (Rosemary and Grapes seeds) on the lipid profile	67
4.4.1	Cholesterol	67
4.4.2	Triglycerides	68
4.4.3	High-density lipoprotein HDL	68
4.4.4	Low-density lipoprotein LDL	69
4.5	Effects of polyphenols extracted from (Rosemary and Grapes seeds) on oxidant-antioxidant markers	71
4.6	Effects of polyphenols extracted from (Rosemary and Grapes seeds) in the liver enzymes	73
Conclusions and recommendations		
	Conclusion	75
	Recommendations	76
	References	77
	Summery in arabic language	I

Tittels No.	List of figures and table index	Page No.
	Figures	
1.1	leaves and flowers of <i>Rosmarinus officinalis</i>	15
1.2	Grape seeds <i>Vitis Vinifera</i>	19
Tables		

1.1	Classification of Lipid Disorders	10
2.1	chemicals and their suppliers.	26
2.2	The kit and their company	27
2.3	The equipments and their manufactures	27
3.1	Detection of some active compounds in plant extracts	46
3.2	Effect of polyphenols extracted from ROS on body weight of hypercholesterolemic female rats.	47
3.3	Effects of polyphenols extracted from (ROS) on hematological parameters of hypercholesterolemic female rats	49
3.4	Effects of polyphenols extracted from (ROS) on lipid profile hypercholesterolemic female rats	52
3.5	Effects of polyphenols extracted from (ROS) on oxidant-antioxidant markers of hypercholesterolemic female rats	53
3.6	Effects of extract (ROS) on liver enzymes of hypercholesterolemic female rats	55
3.7	Effect of polyphenols extracted from GSE on body weight of hypercholesterolemic female rats	56
3.8	Effects of polyphenols extracted from (GSE) on hematological parameters of hypercholesterolemic female rats	58
3.9	Effects of polyphenols extracted from (GSE) on lipid profile hypercholesterolemic female rats	60
3.10	Effects of polyphenols extracted from (GSE) on oxidant-antioxidant markers of hypercholesterolemic female rats	61
3.11	Effects of extract (GSE) on liver enzymes of hypercholesterolemic female rats	62

Abbreviations	
ALT	Alanine Transaminase
APO	Apolipoprotein
AST	Aspartate Transaminase
CHD	Coronay Heart diseases
CP	Ceruloplasmin
CVD	Cardio Vascular diseases
EDTA	Ethylene Diamine tetra acetic acid
G	Gram
g/dl	Gram per deciliter
HB	Hemoglobin
HC	Hypercholesterolemia
HDL	High Density Lipoproteins
IU	International Unit
GSE	Grape Seeds Extract
LCAT	activate lecithin acyl transferase
LPO	Lipid peroxidation
LSD	Least significant difference
L	Liter
LDL	Low Density Lipoprotein
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular Volume
MDA	Malondialdehyde
Mmol	Millimol
ml	Milliliter
NADPH	Nicotinamide adinine dinucleotide phosphate

PCV	Packed Cell Volume
Pg	Picogram
ROE	Rosemary extract
ROS	Reactive Oxygen Species
S.E.	Standard Error
SOD	Superoxide dismutase
SPSS	Statistical package social science
TBA	Thiobarbituric acid
TC	Total Cholesterol
TCA	Trichloro acetic acid
TG	Triglyceride
U	Unit
VLDL	Very Low Density Lipoprotein
WBC	White Blood Cells

Summary

The present study aimed to investigate the effect of phenolic compounds extracted from (*Rosmarinus officinalis*) and (*Vitis vinifera*) on some hematological and biochemical parameters of normal and hypercholesterolemic female rats.

(60) mature female rats (*Rattus norvegicus*) were used, the average weight (180-210gm) and (10-12) mean old. The rats were put in the animal house of the Biology Department/ Science College /Thi-Qar University, under the standard conditions of temperature, light and nutrition. The study included 2 main experiments:

The first experiment included: (36) mature female rats were divided randomly into six equal groups, each group consist of (6) rats. The first group (negative control) was treated orally with (0.5ml/animal) from normal physiological saline (0.9% NaCl). The second group (positive control-Hypercholesterolemic group, the high fat diet), the third group (normal+50mg/kg (*Rosmarinus officinalis*) and fourth group(normal+100mg/kg ROE) were treated orally with (0.5ml/animal) of (50&100) mg/kg of phenolic compounds extracted from (*Rosmarinus officinalis*) respectively. The five group (HC+50 mg/kg ROE) and six group (HC+100 mg/kg ROE) (induced hypercholesterolemia for 30 days) then were treated orally with (0.5ml/animal) of (50&100) mg/kg of phenolic compounds extracted from (*Rosmarinus officinalis*) respectively. The duration of the treatment was 30 days.

The second experiment included: (24) mature female rats They were divided randomly into four equal groups, each group consist of (6) rats, the first group (negative control) was treated orally with (0.5ml/animal) from normal physiological saline (0.9% NaCl) the same group in the first experimental. The second group (positive control-HC group, the high fat diet) the same group in the first experimental. The third group (normal+50mg/kg (*Vitis vinifera*) and fourth group(normal+100mg/kg GSE) were treated orally with (0.5ml/animal)

of (50&100) mg/kg of phenolic compounds extracted from (*Vitis vinifera*) respectively. The five group (HC+50 mg/kg GSE) and six group (HC+100 mg/kg GSE) (induced hypercholesterolemia for 30 days) then were treated orally with (0.5ml/animal) of (50&100) mg/kg phenolic compounds extracted from (*Vitis vinifera*) respectively. The duration of the treatment was 30 days.

The body weight of animals in all groups was determined in the first day and the last day of experiment. All groups in each experiment were sacrificed, the blood samples were collected and the hematological and biochemical parameters which included hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells(WBC) and the level of Total cholesterol(TC), triglycerides(TG), high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL), atherogenic index, malondialdehyde(MDA), ceruloplasmin(CP), albumin(Alb) and liver enzymes (alanine transaminase (ALT) and aspartate transaminase (AST)), were measured.

The results showed a significant increase ($P \leq 0.05$) in body weight of the second group compared with group (1), while other groups don't show any significant ($p > 0.05$) deference compared with group (1). The results recorded a significant decrease ($P \leq 0.05$) in Hb, PCV and WBC in second group compared with group (1) while these parameters(MCV, MCH and MCHC) doesn't show any significant($p > 0.05$) deference in all groups treated with phenolic compounds of(rosemary and grape seeds) compared with group(1). Also there was a significant increase ($P \leq 0.05$) in cholesterol, triglyceride, LDL, vLDL, atherogenic index MDA, CP and liver enzymes (ALT and AST) in the second group compared with group (1) and these parameters showed a significant ($P \leq 0.05$) decrease in all groups treated with phenolic compounde extracted from (rosemary and grape seeds).

The same time, the results recorded a significant decrease at ($P \leq 0.05$) in HDL and Alb in the second group compared with groups (1,3,4,5 and 6), while,

these parameters increased significantly($P \leq 0.05$) when animals treated with phenolic compounds of (rosemary and grape seeds).

Chapter one

Introduction and literature Review

1.1 **Introduction**

Cholesterol is a waxy substance made by liver and also supplied in diet through animal products such as meats, poultry, fish and dairy products. It is mainly incorporated into the plasma membranes of cells and regulates membrane fluidity conferring a higher degree of rigidity. Furthermore, it is the precursor of all steroid hormones and bile acids produced (Hongbao, 2006).

Hypercholesterolemia (HC) is the most significant factor in causing coronary heart disease (CHD) and stroke which is implicated in 56% of stroke deaths. (Tirschwell *et al.*, 2004).

Environmental factors are also a has critical rols in the development of obesity and hyperlipidemia, which is more likely the result of interaction between genes and environments with varying contributions in each individual, among many environmental factors, the nutrition is a strong effective factor, consuming food with high fat content is well known to promote obesity and hyperlipidemia (Marais 2013; Doo and Kim 2015).

There includes clinical significance to cholesterol the (hypercholesterolemia and hypocholesterolemia), hypercholesterolemia, is the presence of high levels of cholesterol in the blood (Durrington, 2003).

Hypocholesterolemia is the presence of abnormally low levels of cholesterol in the blood, (Moutzouri *et al.*, 2011). Hypercholesterolemia is one of the risk factors for coronary heart diseases. Therefore, treatment of hypercholesterolemia may reduce the risk or development of cardiovascular disease (Doha *et al.*, 2010). Presently, Cardiovascular disease is a growing health problem throughout the world and represents a leading cause of death and disease in humans (Crews, 2007). Several factors, such as a high caloric diet, age, lack of exercise, smoking, alcohol consumption, and genetic predisposition have been linked with cardiovascular disease (Asaolu *et al.*, 2010). Elevated cholesterol levels predispose patients to a condition known as hypercholesterolemia (Durrington, 2003), which increases the risk of deadly

and non-deadly coronary heart disease in people over the age of 50 years (Marks *et al.*, 2003).

The existing drugs use in the management of cardiovascular disease is associated with side effects such as liver disease, diarrhea, gastric irritation and nausea (Doha *et al.*, 2010).

Several herbs have been reported to reduce high blood cholesterol with less negative side effect and they are relatively affordable (William and John, 2010).

Studies indicate that plant phenolic, flavonoids, flavonolignans and phenolic acids act as antioxidants and exhibits health-promoting properties. Furthermore, there are data suggesting the capability of phenolic to modulate and positively affect lipoprotein metabolism. Phenolic compounds have been proven to be successful in attenuating hypercholesterolemia (Bok *et al.*, 1999; Kumar *et al.*, 2005; Rehrah *et al.*, 2007).

Both human and animal *in vivo* studies have shown that the level of which LDL oxidizes, decrease with increasing phenolic concentration (Marrugat *et al.*, 2004; Weinbrenner *et al.*, 2004; Covas *et al.*, 2006).

Rosemary (*Rosmarinus officinalis* L., Lamiaceae) is considered one of the most important sources for the extraction of phenolic compounds with strong antioxidant activity. This species grows worldwide and has been cultivated since long ago, in ancient Egypt, Mesopotamia, China and India (Bradley, 2006). Also, rosemary extracts considered enriched in phenolic compounds are effective antioxidants due to their phenolic hydroxyl groups but they also possess plenty of other beneficial effects like antimicrobial, antiviral, anti-inflammatory, anticarcinogenic activities and is also known to be an effective chemopreventive agent (Aherne *et al.*, 2007). Studies indicted that taking grape seed extract substantially increased blood levels of antioxidants. Antioxidants are substances that destroy free radicals, which are harmful compounds in the body that damage DNA (genetic material) and even cause

cell death. Scientists believe free radicals contribute to aging, as well as the development of a number of health problems, including heart disease and cancer (Bagchi *et al.*, 2003).

1.2 literature review

1.2.1. Cholesterol

Cholesterol is a waxy substance in the blood plasma and in animal tissues. It is an organic compound ($C_{27}H_{46}O$) in the steroid family and it is a white, crystalline substance that is odorless and tasteless in its pure state. Cholesterol is the most abundant sterol in animals; it is synthesized by the liver and consumed through diet. Cholesterol is essential for life, serving as a building block for steroid hormones as (testosterone and estrogen), and for cell walls (Nicole, 2007).

However, cholesterol is not required in the diet because the body can synthesize all it needs for essential functions. In order for individuals to check their cholesterol, they must get a blood test done to measure their total cholesterol to determine if it is high or low. It is recommended that our total cholesterol be under 200 mg/dL and anything over this number would be considered too high level (Groff *et al.*, 2005). Cholesterol as well as other fats are not able to dissolve in blood, thus they are transported to cells by carriers called lipoproteins. There are two main types of lipoproteins are low-density lipoprotein (LDL) and high density lipoprotein (HDL).

1.2.1.1 Biochemistry of Cholesterol

Increased cholesterol levels or hypercholesterolemia can result either from overproduction or defective clearance of very low density lipoproteins (VLDL), or from increased conversion of VLDL to low density lipoproteins (LDL). Overproduction of VLDL by the liver may be caused by obesity, diabetes mellitus, alcohol excess, nephrotic syndrome or genetic disorders (Beers and Berkow, 2005).

1.2.3 Lipid Profile

A lipid profile usually consists of total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and total triglycerides (TG). A desirable lipid profile is a total cholesterol level less than 200 mg/dl, LDL cholesterol less than 130 mg/dl, HDL cholesterol greater than 40 mg/dl, and triglyceride level less than 150 mg/dl (Beers and Berkow, 2005).

1.2.3.1 Total Cholesterol

Total cholesterol is the accumulation of cholesterol found in all lipoproteins circulating in the blood stream. This includes low-density lipoprotein (LDL), highdensity lipoprotein (HDL), and very low density lipoprotein (VLDL). Increased levels of total cholesterol are directly related to coronary heart disease (CHO). Age, diets high in saturated fat and cholesterol, genetics, increased body weight, and decreased physical activity are all major factors that contribute to elevated levels of total cholesterol (Beers and Berkow, 2005).

1.2.3.2Triglycerides

Triglycerides are a highly concentrated source of energy. The majority of fat in the body is in the form of triglycerides. The lipoproteins that are rich in triglycerides include chylomicrons and VLDLs (Groff *et al.*, 2005).

1.2.3.3 HDL Cholesterol

HDL cholesterol is often known as the "good cholesterol". HDL cholesterol helps transfer the LDL cholesterol to the liver where it then is excreted out of the body through bile acids (Groff *et al.*, 2005). It is believed that HDL cholesterol plays a role in protecting the heart. Physical activity and pharmacological treatment of fibrates and nicotinic acid have been shown to increase levels of HDL concentrations (Couillard *et al.*, 2005). Fibrates are a class of carboxylic acids. Fibrates have been shown to be effective at increasing HDL cholesterol. Nicotinic acid is a form of the water soluble vitamin B³. Nicotinic acid has been shown to improve all HDL concentrations in the blood (National Heart Lung and Blood Institute, 2007).

1.2.3.4 LDL Cholesterol

LDL cholesterol is often known as the "bad cholesterol" and is the primary carrier of cholesterol in the bloodstream. Increased levels of LDL cholesterol are often linked to an increase risk for CHD (Escott and Mahan, 2004). An accumulation of circulating LDL cholesterol in the blood can lead to increased levels of oxidation. (Escott and Mahan, 2004).

1.2.4 LDL Oxidation

Under normal conditions, LDL cholesterol circulates in the blood, but is not oxidized. Many environmental factors and stress to the body can affect the oxidation of LDL in the blood. Macrophage cells take up oxidized LDL cholesterol. When there is an abundance of oxidized LDL cholesterol, macrophage cells become overloaded with lipids, they then start to form foam cells. Foam cells create fatty streaks that can accumulate and cause plaque formation in the arteries, which lead to atherosclerosis (McEnemy and Young, 2001). Supplementations of antioxidants, such as vitamin E,

have been suggested by some health care professionals in order to slow down the LDL_oxidation process and ultimately reduce the risk for developing cardiovascular disease (Groff *et al.*, 2005).

1.2.5 Lipids Profile and Chronic Diseases

The term hyperlipidemia refers to hypercholesterolemia, hypertriglyceridemia and hypolipoproteinemia, is a major risk factor for the development of cardiovascular diseases. Hyperlipidemia, mainly an increased level of total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C) along with a decrease in high-density lipoprotein cholesterol (HDL-C), is the predictor of coronary artery disease, fatty liver disease, and carcinogenesis, which is associated with the formation of reactive oxygen species (ROS) (Roberts *et al.*, 2006).

Hyperlipidemia is a disorder characterized by increased levels of blood lipids and is a well-established risk factor for CVD.(sneha and Bhatnagar, 2017).

The lipoma is a result of hypertriglyceridemia, but not hypercholesterolemia (Ford, 1996; Johnson, 2005). Lipoma is apparent when serum triglyceride concentrations exceed 2.26 mmol/l (200 mg/dl) (Bauer, 1995).

Atherosclerosis is a chronic inflammatory condition that commonly affects coronary arteries, leading to coronary heart disease (CHD). It is characterized by foam cell formation in the sub endothelial space of large and medium arteries (Ross, 1993). The coronary arteries are especially susceptible to atherogenesis atherosclerosis of the coronary arteries may lead to angina pectoris and myocardial infarction (Falk, 2005). Atherosclerosis is a multifactorial disease, exacerbated by external factors such as dietary foods high in cholesterol, smoking and stress (Cooper *et al.*, 2000).

Atherosclerosis develops when LDL become oxidized (ox-LDL) by free radicals, particularly (ROS) (Ross *et al.*, 1999). The body's immune system responds to the damage to the artery wall caused by oxidized LDL by sending specialized white blood cells (macrophages and T-lymphocytes) to absorb the ox-LDL forming specialized foam cells (Vanderwal *et al.*, 1989).

1.2.6 Hypercholesterolemia

Hypercholesterolemia is defined as elevated levels of cholesterol. Cholesterol is a lipid which, together with cholesterol esters, phospholipids, and triglycerides, is transported in the blood as part of larger molecules called lipoproteins. They can be assigned to different categories and the five major families of lipoproteins are low-density lipoproteins (LDL), highdensity lipoproteins (HDL), very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), and chylomicron (Hegele, 2009).

LDL cholesterol normally makes up 60-70% of total serum cholesterol and contains a single apolipoprotein, apo B-100 (apo B) that surrounds the fatty acids, keeping them soluble in the aqueous environment. In general, LDL transports cholesterol and triglycerides from the liver to peripheral tissues and regulates cholesterol synthesis at these sites. LDL cholesterol is well known to increase the risk of coronary heart disease (CHD) because it can be retained in arteries by arterial proteoglycans starting the formation of arterial plaques (Mensink *et al.*, 2003).

Increased levels are associated with artherosclerosis, and thereby heart attack, stroke, and peripheral vascular disease, HDL cholesterol is inversely correlated to the risk of CVD. HDL normally carries around 20-30% of total serum cholesterol. Apo A-I and apo A-II are the major apolipoproteins of HDL (Brewer and Rader, 1991).

Another lipoprotein subclass is VLDL, which is rich in triglycerides. They account for 10-15% of total serum cholesterol and are assembled in the

liver from cholesterol and apolipoprotein. In the bloodstream it is converted to LDL, therefore it is a precursor of LDL.

The intermediate lipoproteins IDL, reside between VLDL and LDL, but are included in LDL measurements in clinical practice (Brewer and Rader, 1991 ; Vance and Vans, 2008). The fifth class of blood lipoproteins is chylomicrons, which are triglyceride-rich lipoproteins formed in the intestine from dietary fat and which appear in the blood after a fat containing meal (Vance, 2008).

Table (1-1) presents different types of hypercholesterolemia classified according to Fredrickson (Fredrickson and Lees ,1965), based on the pattern of lipoproteins on electrophoresis or ultracentrifugation. This classification system has been adopted by the WHO.

Hypercholesterolemia does not lead to specific symptoms and is usually discovered during routine medical tests or examinations for atherosclerotic cardiovascular disease. Sometimes xanthomas, deposits of cholesterol, can be found in individuals with hereditary forms of the disorder or in people with high levels of cholesterol.

Deposits are especially found around the eyes or along the achilles tendon (James *et al.*, 2006).

Table (1-1): Classification of Lipid Disorders (Fredrickson and Lees ,1965)

Type	Associated clinical disorders	Labs description
Type I	decreased lipoprotein lipase (LPL) or altered ApoC2	elevated chylomicrons
Type IIa	LDL receptor deficiency	elevated LDL only
Type IIb	decreased LDL receptor and increased ApoB	elevated LDL, VLDL and triglycerides
Type III	defect ApoE synthesis	increased IDL
Type IV	increased VLDL production and decreased elimination	increased VLDL
Type V	increased VLDL production and decreased lipoprotein lipase	increased VLDL and chylomicrons

1.2.6.1 Etiology of hypercholesterolemia

There are both primary (genetic) and secondary causes of elevated cholesterol levels in the blood. Primary causes are single or multiple genetic mutations that result in an over production or defective clearance of triglycerides and LDL cholesterol, or in an underproduction or excessive clearance of HDL. Primary lipid disorders are common in patients who develop physical signs of hypercholesterolemia such as xanthomas, onset of premature atherosclerotic disease (age<60), a family history of atherosclerosis, or elevated serum cholesterol >240 mg/dL (>6.20 mmol/L), in children, primary disorders are the most common cause (Merck, 2008).

In contrast, secondary causes contribute to most cases of hypercholesterolemia in adults. The most important secondary cause in developed countries is a sedentary lifestyle with excessive dietary intake of

saturated fat, cholesterol, and trans fatty acids (Merck, 2008). Other secondary causes are diabetes mellitus and risk factors in the metabolic syndrome, nephrotic syndrome, hypothyroidism, anorexia nervosa, Zieve's syndrome, excessive alcohol intake and drugs such as thiazides, blockers, retinoids, active antiretroviral agents and glucocorticoids (Merck, 2008).

Among these factors, diabetes is of special concern, because diabetics tend to have an atherogenic combination of increased levels of triglycerides, elevated small, dense LDL fractions and low HDL. One explanation may be that this combination of lipoproteins is caused by obesity and/or poor control of diabetes, which increases circulating fatty acids, leading to an increased hepatic VLDL production. Triglyceride-rich VLDL transfers triglycerides and cholesterol to LDL and HDL cholesterol, initiating the formation of triglyceride rich, small dense LDL cholesterol and clearance of triglyceride-rich HDL cholesterol. Diabetic related hypercholesterolemia is often associated with increased caloric intake and physical inactivity, a high prevalence of hypercholesterolemia in insulin-dependent diabetes mellitus has been documented in several clinical studies (Winocour *et al.*, 1989; Thomas and Nestel, 2007).

1.2.7 Polyphenols

Polyphenols are a class of antioxidants found in a variety of plants. They are quite abundant and play an important role in the human diet (Manach *et al.*, 2004). Increased intake of polyphenols has been thought to prevent a variety of diseases that are linked to oxidative stress including CVD. Interest in polyphenols has been increasing rapidly due their strong antioxidant potential. Polyphenols help protect plants against biological and environmental stresses. Fruits such as apples, cranberries, grapes, raspberries, and strawberries are rich in polyphenolic compounds (Vattem *et al.*, 2005).

Polyphenols can be broken down into phenolic acids, flavonoids, lignans, and stilbenes (Manach *et al.*, 2004).

1.2.7.1 physiological effects of Phenolic

Plant extracts and their components have been known to exhibit biological activities, especially antimicrobial (Iskan *et al.*, 2002), antifungal (Sokovic *et al.*, 2009), antibacterial (Kanatt *et al.*, 2008) and antioxidant activities (Seun *et al.*, 2010). In general, a diet rich in fruit and vegetables has been linked to various beneficial effects on human health, such as reducing the risk of developing coronary heart disease (Dauchet *et al.*, 2006), cancer (Zhang *et al.*, 2009), hypertension (Mignone *et al.*, 2009), diabetes, and inflammatory processes (Zafra *et al.*, 2007).

The constituents responsible for these protective effects include some vitamins (*e.g.* A, C, E), minerals (*e.g.* potassium, zinc, selenium), carotenes and phenolic compounds (Muhammad *et al.*, 2006). Chlorogenic acid one of the most abundant polyphenol compounds in the human diet, is a group of phenolic secondary metabolites produced by certain plant species (Clifford, 1999; Olthof *et al.*, 2001). Accumulating evidence has demonstrated that Chlorogenic acid exhibits many biological properties, including antibacterial, antioxidant, and anticarcinogenic activities, particularly hypoglycemic and hypolipidemic effects (Kono *et al.*, 1997; Rodriguez *et al.*, 2002). It has been recently claimed to modulate glucose and lipid metabolism *in vivo* in both healthy and genetically metabolic disordered conditions (Rodriguez *et al.*, 2002; Nicasio *et al.*, 2005).

1.2.7.2 Polyphenols as Antioxidant

The bioactivity of phenolic compounds depends largely of the number of hydroxyl groups linked to the aromatic ring and their relative orientation in the molecular structure. Different assays have been used to investigate the antioxidant activity of plants extracts in several matrices such as foods, natural

products and body fluids (Guo *et al.*, 2016). The antioxidant activity of polyphenols is very important for human health and food stability. Recent research is mainly focused on the study of the antioxidants in fruits extracts, particularly their characteristics and protecting effects against free radicals. Free radicals are responsible for human ageing and food deterioration.

The only way to slow down or prevent such effects is to use the antioxidants, such as polyphenols (Prior, 2003). Basically, polyphenols are compounds used to delay free radical accumulation and strengthen oxidative stability of food and human body (Jeszka *et al.*, 2010). Phenolic compounds have been shown to possess an antioxidant activity based on their (hydroxyl group) donation to free radicals (Marinova *et al.*, 2005; Ibrahim and Jaafar, 2011).

The antioxidant properties of phenolic and flavonoid compounds are mediated by the following mechanisms:

- (1) scavenging radical species such as reactive oxygen species / reactive nitrogen species.
- (2) suppressing reactive oxygen species / reactive nitrogen species, formation by inhibiting some enzymes or chelating trace metals involved in free radical production.
- (3) up regulating or protecting antioxidant defense (Cotelle, 2001).

Antioxidant activities of fruits correlate with total contents of phenolic compounds (Wang and Lin, 2000), and the activity of fruits such as strawberry, plum, orange, and grapefruit is high (Wang *et al.*, 1996). Among the molecules exerting antioxidant activity in plums, the contribution of phenolic compounds was found much greater than that of vitamin C and carotenoids (Gil *et al.*, 2002). Polyphenols are the most abundant antioxidants in the diet; their intake is 10 times greater than that of vitamin C and 20 times that the carotenoids (Mar *et al.*, 2013).

1.2.8 Rosemary

Kingdom:	<u>Plantae</u>
Order:	Lamiales
Family:	Lamiaceae
Genus:	<i>Rosmarinus</i>
Species:	<i>Rosmarinus officinalis</i> (USDA, 2008)



Figure (1.1): leaves and flowers of *Rosmarinus officinalis*

1.2.8.1 History

Rosemary has been named the Herb of the Year in 2001 by the International Herb Association. It was introduced to Britain by the Romans and is still particularly loved today by the Italians and the British, who use it frequently in their cooking. In ancient Greece and Rome rosemary was believed to strengthen the memory, which accounts for its being known as the herb of remembrance and fidelity, Rosemary was an essential part of the apothecary's repertoire during the Renaissance, Hippocrates, Galen, and Dioscorides prescribed rosemary for liver problems,

it was introduced by the Europeans as a garden plant due to its pleasant fragrant scented leaves (Rao *et al.*, 2013).

1.2.8.2 Description

The rosemary is an aromatic plant commonly used as condiment and for medicinal purposes (Jonatas *et al.*, 2017). Leaves are leathery, opposite, strongly recurved, fringed margins and with prominent midrib, Size of the leaf is 1.0-2.5 cm long and 4 cm width. The upper surface of the leaf is green colored and the lowered surface is grey somewhat wooly due to numerous trichomes. The margins are entire and strongly revolute with obtuse apex, tapering and non petiolate base (Edqm, 2005). Typical labiates hairs contain the volatile oil, of which the BP specifies a minimum content of 1.2% calculated on the anhydrous drug. It has spiciform inflorescences of white or blue flowers, with the two stamens projecting far beyond the corolla (Youngken 1950, Boulos 1983, Bruneton 1995).

1.2.8.3 Rosemary polyphenole

Rosemary extracts, enriched in phenolic compounds are effective antioxidants due to their phenolic hydroxyl groups but they also possess plenty of other beneficial effects like antimicrobial, antiviral, anti-inflammatory, anticarcinogenic activities and is also known to be an effective chemopreventive agent (Al- Sereiti *et al.*, 1999; Aherne *et al.*, 2007).

Currently, the antioxidant properties of rosemary are well recognized. It is a free radical scavenger possibly due to metal chelator and superoxide radical inhibition (Moreno *et al.*, 2006).

The lipid labile antioxidant activity of rosemary leaf extracts is mainly due to the presence of phenolic diterpens carnosol and carnosic acid, Other phenolic diterpenes such as rosmanol, epirosmanol and methyl-epirosmanol

are present in smaller quantities and also contribute to the antioxidant activity of rosemary extracts (Ayranci, 2008).

1.2.8.4 Active Constituents

The active constituents of rosemary are caffeic acid, rosmarinic acid, carnosol, and carnosic acid, which are thought to have antioxidant activity (Al Sereiti *et al.*, 1999). The essential oil also contains alpha pinene, borneol, (-)-camphene, 1,8-cineole, camphor, verbenone, and bornyl acetate (Angioni *et al.*, 2004). Rosemary also contains phenolic diterpenes (carnosic acid, carnosol, and 12-O-methylcarnosic acid), caffeoyl derivatives (rosmarinic acid), triterpenes (lupane, oleanane, and ursane triterpenes, ursolic acid, and rofficerone), monoterpenes (e.g., myrcene and camphor), and flavones (isoscutellarein 7-O-glucoside and genkwanin) (Backleh *et al.*, 2003).

Also, suggested that rosemary's food preservation effects may be attributed to its phenolic constituents (Schwarz & Ternes, 1992a), including carnosol and carnosic acid (Smith *et al.*, 1992). This preservative effect may be due to rosemary's antioxidant capability (Bhale *et al.*, 2007) possibly through the maintenance of alpha-tocopherol or the inhibition of lipid peroxidation (LPO) (Chang & Chen, 1998).

1.2.8.5 Hepatoprotective effects.

Hyperlipidemia is known to be a cause of lipid peroxidation and atherosclerosis. The lipid-lowering and anti-oxidant activities of aqueous rosemary extract (Rosemary acid was the most important active constituent), essential oil, and crude plant powder (mixture of hydrophilic and lipophilic compounds are similar to the aqueous extract and essential oils, respectively) were studied in high-fat-diet-fed SD rats (hyperlipidemia rat model).

The effects of these three forms were different on hyperlipidemic rats. While essential oil in all doses resulted in higher cholesterol levels, the

aqueous extract and the plant powder with greater extent decreased the serum cholesterol levels. The lowering effect on TG levels was obvious with the essential oil and more significant with the powder. The aqueous extract, essential oil, and crude plant powder reduced MDA levels, increased serum HDL-C levels and SOD activity, and improved anti-oxidant capacity (Wu *et al.*, 2011).

In another attempt Al Sheyab *et al* (2012) found that administration of 100 mg/kg aqueous rosemary extract to the mice on a high fat diet reduced the plasma levels of total cholesterol, TG, and LDL-C and increased the HDL-C levels.

1.2.9 Grape Seeds:

Kingdom:	Plantae
Order:	Vitales
Family:	Vitaceae.
Genus:	Vitis
Species:	<i>Vitis vinifera</i> . (Carl, 1778)



Figure (1.2) Grape seeds *Vitis vinifera*

1.2.9.1 History

The grapevine (*Vitis vinifera*) is indigenous to southern Europe and Western Asia and is cultivated to day in all temperature regions of the world (Baydar *et al.*, 2007) which comprises about 60 inter-fertile wild *Vitis* species distributed in Asia, North America and Europe under subtropical, Mediterranean and continental. temperate climatic conditions (Nawaz *et al.*, 2006). Egyptians consumed this fruit at least 6,000 years ago, and several ancient Greek philosophers praised it, Grape Seeds are

One of the richest sources of proanthocyanidins; a class of biologically active flavonoids (Bagchi *et al.*, 2007). The study has recently shown that Grape seed extract significantly reduces plasma cholesterol in rabbits fed a high cholesterol diet, this action reduces the risk of atherosclerosis and coronary heart disease (Yamakoshi *et al.*, 1999). Many attempts to find the responsible mechanisms for anti-hyperlipidemia activities of GSE have been studied, particularly in the inhibition of lipase (Moreno *et al.*, 2003), and cellular cholesterol uptake (Leifert and Abeywardena, 2008).

Grape antioxidants help to prevent the risk of several chronic and age related diseases including dementia, cancer, heart disease and cataracts (Castillo *et al.*, 2000).

Quercetin and other polyphenols in grape have also been reported to be active against oxidation process (Osuagwu and Ernenike, 2007). Grape seed proanthocyanidins may have therapeutic potential in the prevention and treatment of vascular complications in diabetic patients (Abeer *et al.*, 2008).

The medicinal and nutritional value of grapes (*Vitis vinifera*) has been heralded for thousands of years, Among other beneficial effects of parts of a grape, grape seeds are believed to have a powerful antioxidant property due to its rich source of polyphenol compounds. The polyphenol compounds are as much as 60-70 % in grape seeds compared only 10 % in the fruit and 28 - 35

% in the peels (Garcia-Marino *et al.*, 2006; Nawaz *et al.*, 2006). Most of the polyphenol compounds found in grape seeds are flavonoids, monomeric, flavan-3ols, catechin and epicatechin.

These compounds are of interest in pharmaceutical and food factories for medical treatments and health supplements (Garcia *et al.*, 2006). It is likely that the demand for using natural antioxidants such as GSEs has greatly increased in recent years. GSE are substantially constituted with proanthocyanidins, they can react with free radicals and catalyzed metal ions necessary for the oxidation reaction then terminate chain reactions by removing radical intermediates, and inhibit other oxidation reactions by being oxidized themselves (Shahidi, Wanasundara, 1992; Sanchez *et al.*, 1999). The GSEs were used for inhibition of rancidity in cooked beef (Ahn *et al.*, 2002).

1.2.9.2 Description

All *Vitis* are deciduous and are characterized by woody, climbing vines that are generally (12- 20) meters long. Leaves grow alternatively on sides, and vary in shapes and sizes depending on the species. Some are round and unlobed while some are cordate and lobed.

Grapes are small, round, edible berries that grow in dangling clusters on the grape vine. The skin of the grape is generally thin and smooth and is often coated with a fine layer of wax. Berries have a juicy pulp with up to four seeds. The colors of the grapes vary in red, blue, purple and black. Seeds carry the embryo of genetic material from two parents. Every seedling is genetically unique. Purposeful and natural crosses lead to improvements in color and flavor, quality, and disease resistance. (Cindy and Nico, 2008)

1.2.9.3 Grape Seed Polyphenol

Polyphenols act like antioxidants that protect blood cells from oxidation with the added ability of preventing iron from producing hydroxyl radicals

from peroxide. Polyphenols are plant-based and can be Found in tea, coffee, grains, oregano, red wine, fruits, chocolate, and grapes (Whitney and Rdfes, 2011). Grape seed polyphenol (GSP) is the extracted oil from grape seed. Many studies have found that polyphenols have benefits towards improving health, reported that polyphenols may inhibit cancer cell growth, decrease vascularization, protect cells from oxidation, and improve vasodilation and insulin secretion (Del-Rio *et al.*, 2010).

Polyphenols are absorbed through the gut barrier allowing for greater antioxidant capabilities inside of the plasma. Polyphenol is converted to O-glucuronides and then transferred to the liver where metabolism continues. If polyphenols are not fully absorbed, they will travel to the colon and are metabolized by the microflora (Scalbert *et al.*, 2002). Grape seed polyphenol has resveratrol, procyanidins and proanthocyanidins, which are antioxidants that fight against free radicals and oxidation. Grape seed extracts (GSE) have been found to decrease inflammation and apoptotic cell death. To test the ability of grape seed polyphenol, (Sehirli *et al.*, 2008).

1.2.9.4 Active constituents

Chemical structures of grape phenolic compounds. (A) Anthocyanidins; (B) Catechins (structure on the left is a (+)-catechin skeleton, while the structure on the right is an (-)- epicatechin skeleton); and (C) Quercetin, gallic acid, and *trans*-resveratrol. Polyphenolics generally contain two or more hydroxyl groups attached to a conjugated ring system such as a benzene ring. Polyphenols contribute to the overall antioxidant properties of grapes and may have important health benefits, including possible preventative effects against cancer (Fan & Lou, 2004) and cardiovascular diseases (Zern *et al.*, 2003; 2005).

Anthocyanins have been shown to protect against lipid peroxidation and DNA damage in rat hepatoma cells *in vitro*, demonstrating potential

anticarcinogenic properties (Lazzé *et al.*, 2003). Flavanols, such as catechin, have demonstrated inhibitory effects on platelet reactivity *in vitro*, a property which may reduce the risk of cardiovascular disease (Pearson *et al.*, 2005). Gallic acid has been shown to exhibit selective cytotoxicity *in vivo* against a variety of human and mouse cancer cells (Inoue *et al.*, 1996). Additionally, grape seed extracts rich in polyphenolics have been found to reduce biomarkers of Alzheimer's in a rat model (Thomas *et al.*, 2009). This result was supported by a separate finding suggesting that catechins from grape seed extracts fed in a controlled diet to rats were able to cross the blood-brain barrier, as evidenced by heightened levels of catechin and epicatechin detected in the brain of rats fed the grape seed extract enriched diet (Prasain *et al.*, 2009).

1.2.9.5 Hepatoprotective effects.

It has been shown that pre-exposure of grape seed extract (3 or 7 days, 100 mg/kg, p.o.), followed by hepatotoxic doses of acetaminophen (400 and 500 mg/kg, i.p.) significantly attenuated acetaminophen-induced hepatic DNA damage, apoptotic and necrotic cell death of liver cells, and counteracted the influence of acetaminophen-induced changes in bcl- XL expression in mice (Ray *et al.*, 2001). In one study, grape seed extract (50 mg/kg a day orally for 28 days) protected the liver from oxidative damage following bile duct ligation in rats (Dulundu *et al.*, 2007).

Also, in another study, administrations of grape seed extract at a dose of 50 mg/kg/day orally for 15 days before ischemia/reperfusion

Injury and repeated before the reperfusion period, reduced hepatic ischemia/reperfusion injury in rats (Sehirli *et al.*, 2008).

1.2.9.6 Toxicity

Acute oral toxicity, dermal toxicity, dermal irritation, and eye irritation studies have been performed with GSE. The LD50 of GSE was found to be greater than 5000 mg/kg when administered once orally via gastric intubation to rats. The dose-dependent chronic effects of GSE in mice were evaluated and it was found that GSE did not cause any detrimental effects (Ray *et al.*, 2001).

Furthermore, administration of the grape seed extract activating to rats in the feed at levels of 0.5, 1.0, or 2.0% for 90 days did not induce any significant toxicological effects (Wren *et al.*, 2002). Similarly, it was reported that there was no observed adverse effect of the dietary concentration of grape seed extract or grape skin extract in rats (Bentivegna and Whitney, 2002).

1.2.10. Aim of the Study

According to the increase of hypercholesterol as a well-established risk factor for CVD and many diseases the present study aimed to assess the effect of polyphenolic compounds extracted from Rosemary and Grapes seeds in the treatment of hypercholesterolemia female rats as bellow:

- 1- Extraction of polyphenols from leaves of Rosemary and Grape seeds.
- 2- Study the effect of extracted polyphenol of (Rosemary and Grapes seeds) on body weight of normal and hypercholesterolemia female rats.
- 3- Study the effect of extracts (Rosemary and Grapes seeds) on the blood parameters of normal and hypercholesterolemia female rats including:- hemoglobin (Hb), packed cell volume(PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), Total number of white blood cells (WBC).
- 4- Study the effect of extracts (Rosemary and Grapes seeds) on biochemical parameters of normal and hypercholesterolemia female rats including: - total cholesterol (TC), triglyceride (TG), high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL), atherogenic index.
- 5- Study the effect of extracts (Rosemary and Grapes seeds) on oxidant-antioxidant markers of normal and hypercholesterolemia female rats including: - malondialdehyde (MDA), ceruloplasmin (Cp), albumin (Alb).
- 6- Study the effect of extracts (Rosemary and Grapes seeds) on liver enzymes of normal and hypercholesterolemia female rats including: - alanine transaminase (ALT) and aspartate transaminase (AST).

Chapter Two

Materials & Methods

2.1. Materials

2.1.1. Chemicals

The study used specific chemicals which were shown with their supplier's in table(2-1):

Table (2 – 1): Chemicals and their suppliers.

No	Material	Supplied company
1.	Acetic acid	BDH(England)
2.	Acetic acid anhydrate	BDH(England)
3.	Ammonia	G.C.C(U.K)
4.	Cholesterol	BDH(England)
5.	Ferric chloride	BDH(England)
6.	Lead acetate	BDH(England)
7.	Filter papers Whattman No.1	TEAR(India)
8.	n-propanol	BDH(England)
9.	Methanol	BDH(England)
10.	Para pheneline di amine(PPD)	BDH(England)
11.	Potassium hydroxide	BDH(England)
12.	Sodium acetate	BDH(England)
13.	Sodium azide	Reidledelhain(Germany)
14.	Sodium chloride	BDH(England)
15.	Thio barbituric acid (TBA)	BDH(England)
16.	Trichloro acetic acid (TCA)	BDH(England)

2.1.2. kits

Table (2 –2) explained the kits and their companies which were used in this study.

Table (2 –2): The kit and their companies

No	Kits	Company
1.	Alanine Transaminase (ALT) Kit	Biolabo(France)
2.	Albumin Kit	Biolabo (France)
3.	Aspartate Transaminase (AST) Kit	Biolabo (France)
4.	Cholesterol Kit	Biolabo (France)
5.	HDL kit	Biolabo (France)
6.	Triglyceride Kit	Biolabo (France)

2.1.3. Equipments and Apparatus

Table (2 –3) explained the equipments and apparatus which were used in this study.

Table (2 –3): The equipments and their manufactures.

No	Equipments	Supplied company
1	Animals balance__Stanton 461	(Germany)
2	Autoclave	YX-2803, (Germany)
3	Centrifuge	Universal 16 A, (Germany)
4	Incubator	Memmert , (Germany)
5	Magnetic sterrer	England
6	Oven	Gallen Kamp, (England)
7	Rotary evaporator	Buchi (switzland)
9	Sensitive balance	Denver(Germany)
10	Separator funnel	
11	Soxhlet apparatus	England
12	UV/VIS spectrophotometer	T 60,PG Instrumente Ltd, (Germany)
13	Water bath	Germany

2.1.4 Plants Collection:

The plants (Grape seeds, Rosemary leaves) were obtained from local markets in the city of Al-Nasiriyah, Thi- Qar province, Iraq, 360 Km south of Baghdad, washed with distal water and dried in air then collected in glass container at room temperature

2.1.5 Experimental Animal

Used 60 female rats (*Rattus norvegicus*) their weight (180-210gm) and aged (10-12) weeks were used in the curent study. Animals were kept in the animal house of Biology Department, Sciences College, Thi- Qar University, Iraq. They were placed in plastic cages (six rats per each cage) which have lid metal buckle provider bottle of water and a place to put food has brushed the carpeted floor cages sawdust that was replaced a week to maintain the cleanliness of rats. Animals were fed on standard rat pellet (*ad libitum*), and tap water under standard laboratory conditions of (12h light: 12h dark photoperiod cycle (LD) at (20 ±2C°) and relative humidity (45-55 %) (El-Missiry and El-Gindy, 2000).

2.2 Methods

2.2.1 Extraction of Polyphenols

Each (20g) of plant powdered material were used. then (200 ml) of %2 acetic acid were added. The mixture was placed in conceal flask (200 ml) and put in water bath for 8h, heated the mixture for 70C then left to cool. The suspension was filtered by Buchner funnel and Whitman (no.1) filter paper and used vacuum pump. Then added the same volume of n-propanol was added into filter paper, then added (NaCl)until super saturated was added. Then the mixture placed in separated funnel and shake well and left for a

period time, took the upper class that contain polyphenols, then used a rotary evaporated at temperature 50 C, the material was preserved frozen until used.

2.2.2:Qualitative Tests of Some Active Compounds in Plant Extracts:

2.2.2.1 : Detection of Phenolic Compounds in Plant Extracts:

Filter paper (Witman No.1) was wetted in (2mL) of extract and some drops of ferric chloride (1%) were added to it. After that it was exposed to ammonia vapor, appearance blue or green color indicates presence of phenolic compounds (Gayon, 1972).

2.2.2.2 : Detection of Flavonoid compounds in Plant Extracts:

Alcohol Potassium hydroxide reagent (Ethanolic KOH (5N))

(1mL) from this reagent was added to (1mL) of extracts, the yellow appearance of precipitates indicate presence of Flavonoids. (Harbone, 1984).

2.2.2.3: Detection of Tannin Compounds in Plant Extracts:

Lead acetate hydrate (1%) reagent: (1mL) of lead acetate hydrate was added to (1mL) of extracts, appearance of white or light brown precipitate indicated the presence of tannins. (Harbone, 1984).

2.2.3: Method of Food Preparing (High Cholesterol Diet)

5% of high cholesterol diet prepared from 50 g of cholesterol dissolved in 200 g of olive oil and heated in a water bath, then soluble cholesterol in the oil were added to 1 kg of feed. It was cut into small pieces fit with the size of the holes in the lid iron to boxes, to facilitate the process taken up by rats (Cook and Thomson *et al.*, 1950).

2.2.4 Laboratory Animals:

This study included two experiments, (60) female rats were used and divided into two groups; (36) female for each experiment.

-The first experiment: The effect of polyphenolic compounds extracted from rosemary (*Rosmarinus officinalis*) in some hematological and biochemical parameters of normal and hypercholesterolemic female rats were investigated. (36) female were used and divided into six groups (6 animals for each groups)

- ❖ Group (1): (negative control) treated with (0.5ml/animal) from normal physiological saline (0.9% NaCl)
- ❖ Group (2): (hypercholesterolemia group) treated with daily high cholesterol diet (Cook *et al.*, 1950).
- ❖ Group (3): normal rats were treated with 0.5 ml (50mg/kg) of (ROS polyphenolic).
- ❖ Group (4): normal rats were treated with 0.5 ml (100 mg/kg) of (ROS polyphenolic)
- ❖ Group (5): hypercholesterolemic rats were treated with 0.5 ml (50mg/kg) of (ROS polyphenolic).
- ❖ Group (6): hypercholesterolemic rats were treated with 0.5 ml (100mg/kg) of (ROS polyphenolic).

The extract was dissolved in 0.5 mL of normal physiological saline (0.9% NaCl), and the duration of each experiment was 30 days

-The second experimental: The effect of polyphenolic compounds extracted from Grape seeds (*Vitis vinifera*) in some hematological parameters and biochemical parameters of normal and hypercholesterolemic female rats was investigated.

(24) female rats were used and divided into four (6 animals for each groups)

- ❖ Group (1): (negative control) treated with (0.5ml/animal) from normal physiological saline (0.9% NaCl) the same group used in the first experiment.
- ❖ Group (2): (Hypercholesterolemia group) treated with daily high cholesterol diet (Cook et al., 1950) the same group used in the first experiment.
- ❖ Group (3): normal rats were treated with 0.5 ml (50 mg/kg). of (GSE polyphenolic).
- ❖ Group (4): normal rats were treated with 0.5 ml (100 mg/kg). of (GSE polyphenolic).
- ❖ Group (5): hypercholesterolemic rats were treated with 0.5 ml (50 mg/kg). of (GSE polyphenolic).
- ❖ Group (6): hypercholesterolemic rats were treated with 0.5 ml (100 mg/kg). of (GSE polyphenolic).

The extract was dissolved in 0.5 mL of normal physiological saline (0.9% NaCl), and the duration of each experiment was 30 days.

Measuring the weight of animals was measured in the first and last day of the experiment using animals balance_stanton461, then calculating the difference was the weight of the animals.

2.2.5 Blood Sampling

5mL of blood is drawn from each animal by heart puncture method, (2 ml) was put in clean tube with EDTA for measuring of blood picture, and the other blood (3ml) is transferred into clean tube, left at room temperature for 30 minutes for clotting, centrifuged at 3000 rpm for 10 minutes, and then the serum is separated and kept in a clean tube in the refrigerator at 2-8°C until the time of assay.

2.2.6 Determination of hematological parameters

Hematological parameters of females rats which were measured in the present study included the concentration of hemoglobin (Hb), the packed cells volume (PCV), the rate of the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), and the mean corpuscular hemoglobin concentration (MCHC) and white blood cells (WBCs) by using hematological analyzer (Nihon Kohden) in center of heart in Al-Nasiriyah city, Thi-Qar province, Iraq was used for measuring of these parameters.

2.2.7 Determination of Biochemical Parameters

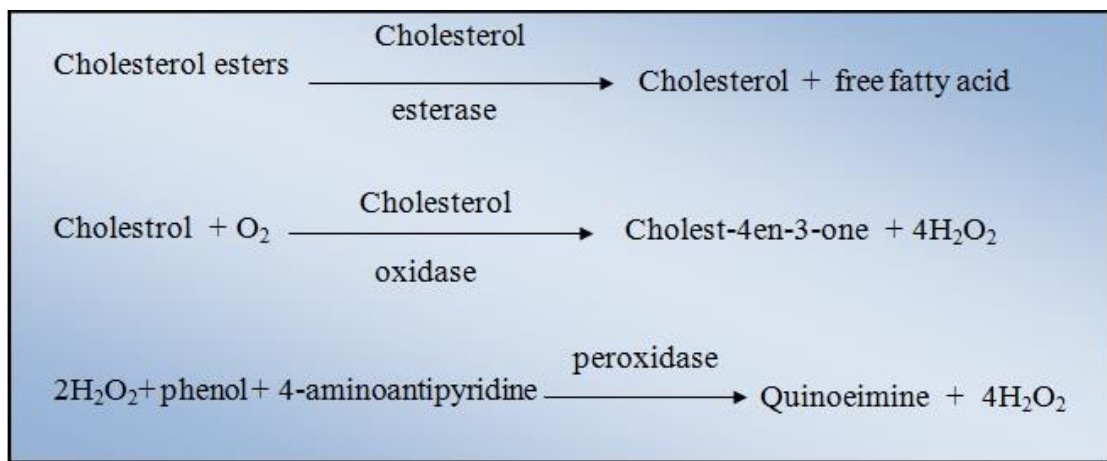
2.2.7.1. Determination of Serum Lipid Profile

2.2.7.1.1. Determination of total cholesterol (TC) level

The used reagents are supplied by Biolabo (France), and Serum total cholesterol.

Principle:

Enzymatic method described by (Allian *et al.*, 1974) which the reaction scheme is as follows: -



Reagents composition:

Reagent	Composition	
Reagent 1 Buffer	Phosphate buffer	100 mmol/L
	Chloro-4-phenol	5 mmol/L
	Sodium cholate	2.3 mmol/L
	Triton x 100	1.5 mmol/L
	Preservative	
Reagent 2 Enzymes	Cholesterol oxidase	≥ 100 IU/L
	Cholesterol estrase	≥ 170 IU/L
	Peroxydase	≥ 1200 IU/L
	4-aminoantipyridine	0.25 IU/L
	PEG 6000	167 µmol/L

Manual

Procedure:

1. Reagent ₁ and reagent ₂ were mixed to form a working solution .
2. Reagent and sample were left stand at room temperature.

	Blank	Standard	Assay
Reagent	1 mL	1 mL	1 Ml
D.W	10µL		
Standard		10µL	
Sample			10µL

Waiting for 10 minutes at room temperature and absorbance was recorded at 500 nm against reagent blank.

Calculation:

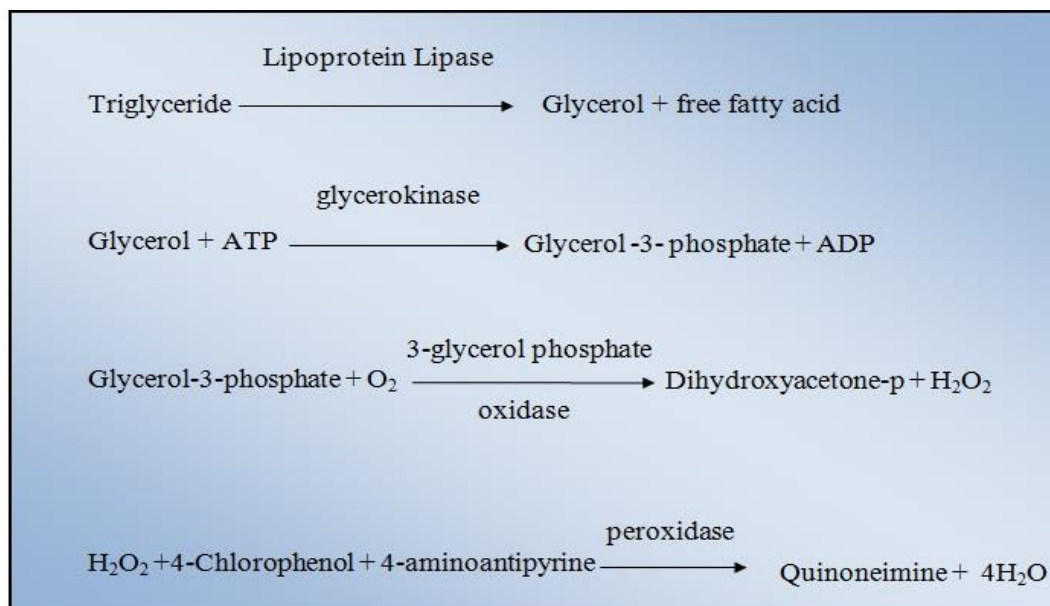
$$\text{Result(mg/dl)} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times 200 (\text{standard concentration})$$

2.2.7.1.2Determination of Serum Triglyceride Concentration

The used reagents were supplied by Biolabo (France) ,and Serum TG was measured according to (Fossati and Prencipe, 1982).

Principle:

Fossati and Prencipe method associated with Trinder reaction scheme is as follows (Fossati and Prencipe ,1982):



Reagents composition:

Reagent	Composition	
Reagent1	Pipes	100 mmol/L
Buffer	Magnesium chloride	9.8 mmol/L
	Chloro-4-phrnl	3.5 mmol/L
Reagent2	Lipase	≥ 1000 IU/L
Enzymes	Peroxidase	≥ 1700 IU/L
	Glycerol-3-phosphate oxidase	≥ 3000 IU/L
	Glycerol kinase	≥ 660 IU/L
	4-aminoantipyrine	0.5 mmol/L
	ATP	1.3 mmol/L
Reagent3	Glycerol	2.28 mmol/L
Standard	(200 mg/dl)	

Manual procedure:

1. Reagent ₁ and reagent ₂ to were mixed form working solution .
2. Reagent and sample were left stand at room temperature.

	Blank	Standard	Assay
Reagent	1 Ml	1 Ml	1 mL
D.W	10 µL		
Standard		10 µL	
Sample			10 µL

Waiting for 10 minutes at room temperature and absorbance was recorded at 500 nm against reagent blank.

Calculation : The result was calculated as follows:

$$\text{Result(mg/dl)} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times 200 (\text{standard concentration})$$

2.2.7.1.3. Determination of Serum HDL Concentration

The used reagents were supplied by Biolabo (France), and serum HDL was measured according to (Tietz, 1999).

Principle:

According to (Tietz, 1999), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons from the sample are precipitated by phosphotungstic acid and magnesium chloride . HDL obtained in supernatant after centrifugation is then measured with total cholesterol reagent.

Reagents composition:

Reagent	Composition
Reagent 1	Phosphotungstic acid 13.9 mmol/L
Precipitant	Magnesium chloride 570 mmol/L
Reagent 2	Cholesterol 2.58 mmol/L
Standard	

Manual procedure:

Sample preparation (without treating standard):

Pipetted in centrifuge	Micromethod
Sample	500 μ L
Precipitant	50 μ L

Mixed vigorously, wait for 10 minutes at room temperature, centrifuged for 15 minutes at 3500 – 4000 RPM, aspirate the supernatant. Reagent and supernatant was left stand at room temperature.

	Blank	Standard	Assay
Reagent2(Cholesterol assay)	1 mL	1 Ml	1 mL
D.W	25 μ L		
Standard		25 μ L	
Supernatant (HDL)			25 μ L

Wait for 10 minutes at room temperature and absorbance was recorded at 500 nm against reagent blank.

Calculation:

Calculate the result as follows:

$$\text{Result(mg/dl)} = \frac{\text{Abs (assay)}}{\text{Abs (standard)}} \times \text{standard concentration} \times 1.1$$

2.2.7.1.4. Calculation of LDL and VLDL

According to (Friedwald *et al.*,1972), LDL and VLDL concentrations were measured as follows:

$$\text{LDL(mg/dl)} = \text{Total cholesterol} - (\text{HDL} + \text{VLDL})$$

$$\text{VLDL(mg/dl)} = \text{serum TG} / 5$$

2.2.7.1.5. Calculation of atherogenic index plasma

Atherogenic Index was calculated by the following equation: -

$$\text{Atherogenic Index} = \text{LDL} / \text{HDL} \text{ (Wilson } et al., 1998).$$

○ 2.2.7.2 Determination of oxidative stress parameters

○ 2.2.7.2.1: Determination of serum malondialdehyde Level (MDA)

LPO is determined by using the thiobarbituric acid method. In this method, MDA formed from the breakdown of polyunsaturated fatty acids, is identified as the product of LPO that reacts with thiobarbituric acid (TBA), in coexisting with trichloro acetic acid (TCA) (Fong *et al.*, 1973). The level of serum MDA is determined spectrophotometrically with a thiobarbituric acid (TBA) solution. In brief, to 150µl serum sample, the following elements were added:

- 1- 1ml (17.5%) trichloroacetic acid (TCA) and 1ml of 0.66%TBA.
 - 2- Mixing well by vortex and incubated in boiling water for 15 minutes, and then is allowed to cool.
 - 3- One ml of 70% TCA is added and the mixture is allowed to stand at room temperature for 20 minutes ,centrifuged at 2000 rpm for 15 minutes, and the supernatant is taken out for scanning spectrophotometrically at (532nm) (Muslih *et al.*,2002).
- MDA level is calculated as follows:

$$\text{MDA}(\mu\text{mol/L}) = \frac{\text{Absorbance at } 532\text{nm}}{\text{L} \times \text{EO}} \times \text{D} \times 10^6$$

L: light path (1cm)

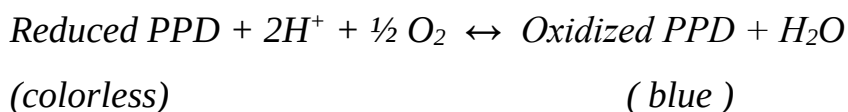
Eo: Extinction coefficient $1.56 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$

D: Dilution factor = 1 ml vol. Used in ref./0.15=6.7

2.2.7.2.2. Determination of serum ceruloplasmin concentration

Principle :-

Ceruloplasmin concentration in serum was measured by Menden *et al.*, (1977). It is based on the ceruloplasmin-catalyzed oxidation of colorless para-phenylene diamine (PPD) to blue-violet oxidized form. The reaction is followed photometrically and the blank value is determined after inhibition of the enzyme with sodium azide at (0°C)



A mixture of serum, substrate and acetate buffer at pH = 6.0 is incubated at 37°C for 15 min. The reaction is stopped by the addition of sodium azide, and the absorbance of the purple color formed (Oxidized PPD) in the

diluted test mixture is read at 525 nm against blank solution. The corrected absorbance is directly related to the concentration of Cp.

Reagents :-

● *Substrate solution :-*

50 mg of PPD was dissolved in 5 mL (4 mL of D.W and 1 mL of glacial acetic acid) . In another container 8.15gm sodium acetate trihydrate was dissolved in 30 mL of D.W then added to the first solution, mixed and completed the volume to 50 mL with D.W. This solution is stable for three hours and is kept refrigerated in a dark bottle.

● *Inactivating solution :-*

100 mg of sodium azide was dissolved in (500 mL) of D.W then was kept cold in refrigerator prior to use, this solution must be prepared every week.

Procedure :-

- 1 mL of substrate was pipetted into test tubes, incubated at 37°C for 1 min.
- 0.1 mL of plasma was added, incubated at 37°C for 15 min, then tubes were removed and placed in iced-bath for 30 min.
- 5 mL of cold inactivating solution was added, mixed and the temperature was brought to 25°C in water bath.
- Blank was prepared by combining the substrate, inactivating solution, and 0.1 mL of D.W, then the mixture was incubated as above.

The absorbance of test and blank was read in spectrophotometer at 525nm.

Calculations:-

$$\text{Serum Cp (g/L)} = \frac{A_{\text{test}} - A_{\text{blank}}}{\epsilon_{\text{Cp}}} \times 10 = \frac{\Delta A \times 10}{0.68} \epsilon_{\text{Cp}} = \text{The}$$

extinction coefficient of Cp equal to 0.68

2.2.7.2.3. Determination of serum albumin concentration

Principle: -

The concentration of albumin in serum sample was measured by the bromcresol green a colorimetric method. Albumin in the presence of bromcresol green at a slightly acid pH, produces a colour change of the indicator from yellow-green to green-blue. The absorption of the colour formed is proportional to the albumin concentration in the sample(Webster,1974).

Reagents:

Reagent	Contents	Concentrations of Solutions
R1 (Bromocresol Green)	Succinic acid Bromocresol green Sodium hydroxide Polyoxyethylene monolaurylether	83mmol/L 167µmol/L 50mmol/L 1gm/L
R2(standard)	Bovine albumin	750µmol/L

Procedure:-

The following reagents are pipetted into a cuvette:

	Blank	standard	Assay
Reagent	2.5Ml	2.5Ml	2.5Ml
D.W	5μL		
Sample			5Ml
Standard		5Ml	

It is mixed and incubated for 10 min at room temperature (15-25°C) then it reads the absorbance (A) of the samples and standard against the blank at (630) nm. The color is stable for 1 hour at room temperature.

Calculation:

Concentration of serum Alb was calculated according to equation:

$$\text{Serum Alb (g / L)} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times 50$$

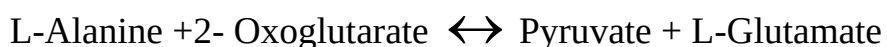
2.2.7.3. Determination of liver enzymes

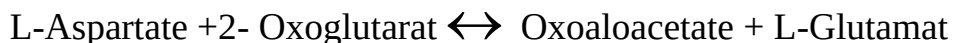
2.2.7.3.1 Determination of alanine transaminase (ALT) and aspartate transaminase (AST) levels

To determination of alanine transaminase (ALT) and aspartate transaminase (AST) levels reagents were supplied by Biolabo (France) were used.

Principle:-

Enzymatic method described by (Frankel, 1957) which the reaction scheme is as follows:-





Oxaloacetate and pyruvate are reacted with the 2,4-DNPH to produce 2,4-Dinitrophenylhydrazones which absorbance at 505 nm in alkaline medium and absorbance proportional with an activity of AST or ALT.

Reagents composition :-

No. of reagent	Contents	Concentration
R1 AST substrate	Phosphate buffer pH 7.5 L- Aspartate 2- Oxoglutarate	85 mmol/L 200 mmol/L 2 mmol/L
R2 ALT substrate	Phosphate buffer pH 7.5 L- Alanine 2- Oxoglutarate	100 mmol/L 200 mmol/L 2 mmol/L
R3 Coloration	2,4 dinitrophenylhydrazones HCl	1 mmol/L 1 mol/L
R4 Standard Solution	Phosphate buffer pH 7.5 Sodium pyruvate Sodium Mercurothiolate	100 mmol/L 2 mmol/L 0.1%

Procedure:

The number of curves recorded for each of the enzymes AST and ALT follows the steps added to the table below. Also it attends to the solution 0.4 M sodium hydroxide from dissolving 8 g of material sodium hydroxide in a small amount of distilled water and fuller-sized to pint (Size unit per milliliter), and it follows the action steps as in the table below

Tube No.	1	2	3	4	5	6
Dis. Water	0.2	0.2	0.2	0.2	0.2	0.2
R1 or R2	1	0.9	0.8	0.7	0.6	0.5
R4 standard	---	0.1	0.2	0.3	0.4	0.5
R3 colorant	1	1	1	1	1	1
NaOH 0.4 M	10	10	10	10	10	10
Mixed and left for 5min and then measured the absorbance at 505 nm						
AST Units	0	30	70	135	225	350
ALT Units	0	40	80	140	225	325

The standard curve for each enzyme uses graph paper, making the number of units of the enzyme enzymatic on the X-axis (unit \ l). The absorbance (or transmittance) is on the y-axis.

Analysis:

The samples and reagents are placed at room temperature. By using suitable test tubes and by repeating the steps followed for each sample are added in the following table:

Pipette into test tubes	AST	ALT
Reagent R1	1 Ml	
Reagent R2		1 mL
Incubated for five minutes at 37 °C		
Serum	200 µL	200 µL
Then mixed and incubated at 37 °C	Exactly 60min	Exactly 30min
Reagent R3	1 mL	1 mL
Mixed and left for 20min at room temperature, and added		
NaOH 0.4 M	10 mL	10 mL
Mixed and left for 5min and then measured the absorbance at 505 nm		

2.3 Statistical Analysis

Data were expressed as the means values $\pm$ standard deviation. Mean of minimum six measurements were compared by analysis of variance (ANOVA) using SPSS ver.19. Significant differences ($P < 0.05$) between means were determined by *Least Significant Difference* (L.S.D.) (Bryman and Cramer, 2012).

Chapter Three

Results

3.The results

3.1. Plant Extracts

result of the present study showed content plants extract of grape seeds & Rosemary on (1,2 g) Respectively from phenolic compound in each (20 g) of dry powder plants

3.2. Detection of some active compounds in plants extracts:

Results obtained in table (3-1) showed that chemical detection indicated the presence of phenolic compounds, flavonoids and tannins in all extracts plants which contained.

Table (3-1): Detection of some active compounds in plants extracts.

Chemical compounds	Reagent used	Indication	Rosemary	Grape seeds
Phenolic compounds	1%follin reagent	Dark color	+Ve	+Ve
Tannins	(1%)Lead acetate hydrate	Dark color	+Ve	+Ve
Flavonoid	KOH 5N	Dark color	+Ve	+Ve

Note: +Ve indicate the presence of active compounds.

3.3. Effect of polyphenols extracted from ROS on physiological parameters of hypercholesterolemic female rats

3.3.1 Effect on body weight

The results listed in table (3-2) indicated a significant increase in body weight of the second group (hypercholesterol group) at ($p \leq 0.05$) compared with the first group (negative group) and with all other groups, while the third group

(normal+50mg/kg, ROS) and the fourth group (normal+100mg/kg, ROS) showed a significant decrease compared with groups (2 and 5) but doesn't show any significant difference at ($p>0.05$) compared with groups (1 and 6), also the fifth group (Chole+50mg/kg, ROS) didn't show any significant difference at ($p>0.05$) compared with group (2 and 6) but showed significant increase at ($p>0.05$) compared with group (1,3 and 4), also the sixth group (Chole+100mg/kg, ROS) showed a significant increase compared with groups (1) but didn't show any significant difference at ($p>0.05$) compared with groups (3,4 and 5)

Table (3-2): Effect of polyphenols extracted from ROS on body weight of hypercholesterolemic female rats (mean $\pm$ SD)

Parameters Groups	Difference in body weight(g)
Group (1) Normal control	+15.35 $\pm$ 0.21 ^{eb}
Group (2) HC-group	+32.00 $\pm$ 0.28 ^a
Group (3) Normal+50 mg/kg of ROS	+20.00 $\pm$ 0.70 ^{cd}
Group (4) Normal+100 mg/kg of ROS	+19.34 $\pm$ 0.35 ^{cd}
Group (5) HC+50 mg/kg of ROS	+26.66 $\pm$ 0.14 ^{ab}
Group (6) HC+100 mg/kg of ROS	+23.17 $\pm$ 0.07 ^{bc}
L.S.D	5.85

*The different letters refer to a significant difference at ($p\leq 0.05$).

*The same letters refer to non significant difference at ($p\leq 0.05$).

3.3.2. Effects on hematological parameters

The result in table (3-3) showed a significant decrease at ($p \leq 0.05$) in Hb level of groups (2,3,4,5 and 6) compared with control group, and this groups dose not showed any significant difference at ($p > 0.05$) between them. The PCV showed in the group (2,3,4,5and 6) a significant decrease at ($p \leq 0.05$) compared with group (1), but this groups does not show any significant difference at ($p > 0.05$) between them, table (3-3).

The results in the table (3-3) showed that the groups (2,5 and 6) recorded a significant increase at ($p \leq 0.05$) in the MCV compared with group (1), while group (3) show significant decrease at ($p \leq 0.05$) compared with group (1) and all other groups, while group (4) didn't showed significant difference at ($p \leq 0.05$) compared with group (1). There was a significant increase at ($p \leq 0.05$) in MCH in group (2 and 5) compared with the group (1) and other groups, while groups (3 and 4) showed significant decrease at ($p \leq 0.05$) compared with group (1 and 2) but no significant difference at ($p > 0.05$) between them, group (5) showed a significant increase at ($p \leq 0.05$) compared with groups (1,3,4 and 6), while group (6) did not recorded any significant difference at ($p > 0.05$) compared with group(1) but increased significantly at ($p \leq 0.05$) compared with groups (3 and 4) and significant decrease compared with groups (2 and 5), MCHC showed a significant decrease at ($p \leq 0.05$) in group (2) compared with(1 and 4) but didn't showed any significant difference compared with groups (3,5 and 6) (Table 3-3).

Table (3-3) showed that the group (2) recorded a significant decrease at ($p \leq 0.05$) in total WBC compared with group (1) and all other group, while group (3,4 and 5) didn't showed significant difference at ($p > 0.05$) compared with group (1), group (6) recorded a significant decrease at ($p \leq 0.05$) compared with group (1,3,4 and 5).

Table (3-3): Effects of polyphenols extracted from (ROS) on hematological parameters of hypercholesterolemic female rats (mean± SD).

Parameters Groups	Hb (g/dl)	PCV (%)	MCV (pg.)	MCH (g/dl)	MCHC (g/dl)	WBC (10³/mm³)
Group (1) Normal control	16.11± 0.57 ^a	46.50± 5.71 ^a	58.86± 1.02 ^b	19.63± 0.44 ^b	33.08± 0.58 ^a	13.10± 2.77 ^a
Group (2) HC-group	12.95± 1.30 ^b	40.83± 4.66 ^b	66.43± 1.02 ^a	21.26± 0.43 ^a	31.90± 0.46 ^{bc}	5.10± 1.59 ^c
Group (3) Normal+50 mg/kg of ROS	11.83± 0.75 ^c	42.83± 3.31 ^{ab}	56.28± 2.68 ^c	19.03± 0.08 ^c	32.15± 1.17 ^{ab}	12.28± 2.53 ^a
Group (4) Normal+10 0 mg/kg of ROS	11.16± 1.47 ^c	39.16± 6.49 ^b	60.15± 0.73 ^b	19.05± 0.12 ^c	33.02± 0.44 ^a	13.03± 0.70 ^a
Group (5) HC+50 mg/kg of ROS	12.50± 1.04 ^{bc}	38.16± 5.26 ^b	64.96± 1.66 ^a	20.96± 0.68 ^a	32.21± 0.41 ^b	12.16± 3.31 ^a
Group (6) HC+100 mg/kg of ROS	12.66± 1.21 ^{bc}	38.16± 5.49 ^b	64.46± 5.36 ^a	20.05± 0.76 ^b	32.03± 1.47 ^{ab}	8.46± 1.39 ^b
L.S.D	1.08	5.14	2.57	0.48	0.84	2.19

*The different letters refer to a significant difference at (p≤0.05).

*The same letters refer to non-significant difference at (p≤0.05).

3.3.3 Effects on lipid profile

The results indicated a significant increase in TC in group (2) at (p≤0.05) compared with group (1) and all other groups, while groups (1,3 and 4) showed a significant decreased at (p≤0.05) compared with (2 and 5) and

showed a significant increase compared with (6), group(5) didn't showed a significant difference at ($p>0.05$) with groups(1,3 and 4) but showed a significant increase compared with (6), while group (6) didn't showed a significant difference at ($p>0.05$) with groups(1,3 and 4) but showed a significant increase compared with (2 and 5). Table (3-4)

A significant increase of TG in the results listed in table (3-4) indicated in group (2) at ($p\leq 0.05$) compared with group (1) and all other groups, while there was non-significant difference between the groups groups (3, 4 and 6) compared with the group (1) and between them, while group (5) increased significantly compared with groups (1,3,4 and 6).

while the HDL in table (3-4) in group (2) significant decreased at ($p\leq 0.05$) compared with group (1) and with all other groups, while group (3) showed a significant increase at ($p\leq 0.05$) compared with group (1), while group (4) showed increased significantly at ($p\leq 0.05$) compared with all groups, while group (5) showed decrease significant compared with all groups, whereas group (6) didn't recorded a significant difference compared with group (1).

The results showed the LDL in the group (2) increase significantly at ($p\leq 0.05$) compared with group (1) and with the other groups, while group (3 and 5) showed non significant difference between them and with group (1), while group (4 and 6) show significant decrease the LDL level compared with group (1), there non a significant difference between groups (4 and 6), as showed in the table (3-4)

Table (3-4) indicated that VLDL level in-group (2) increased significantly at ($p\leq 0.05$) when compared with group (1) and other groups, while groups (3,4 and 6) do not recorded a significant difference compared with group (1) and between them, while group (5) showed significant increase in compared with group (1).

While group (2) recorded a significant increase at($p\leq 0.05$) in Atherogenic Index in table (3-4) compared with the group (1) and with other groups, while

group (3 and 6) showed a significant decrease at ($p \leq 0.05$) compared with groups (1 and 2) and dose not significant differences between them, while group (4) showed a significant decrease at ($p \leq 0.05$) Compared with all groups, whereas group (5) showed a significant increase at ($p \leq 0.05$) Compared with all groups, except group (2).

Table (3-4):-Effects of polyphenols extracted from (ROS) on lipid profile of hypercholesterolemic female rats (mean $\pm$ SD).

Parameters Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Atherog enicInde x
Group (1) Normal control	86.16 $\pm$ 6.94 ^{bc}	81.33 $\pm$ 5.27 ^c	47.83 $\pm$ 6. 01 ^c	30.13 $\pm$ 2. 99 ^b	16.26 $\pm$ 1. 05 ^c	0.60 $\pm$ 0.0 6 ^{bc}
Group (2) HC-group	138.66 $\pm$ 10.38 ^a	108.16 $\pm$ 5.41 ^a	25.66 $\pm$ 5. 16 ^e	80.33 $\pm$ 7. 56 ^a	21.63 $\pm$ 1. 08 ^a	2.25 $\pm$ 0.4 5 ^a
Group (3) Normal+50 mg/kg of ROS	89.66 $\pm$ 11.96 ^{bc}	81.0 $\pm$ 8. 83 ^c	53.16 $\pm$ 6. 36 ^b	30.50 $\pm$ 4. 59 ^b	16.20 $\pm$ 1. 76 ^c	0.51 $\pm$ 0.0 4 ^{cd}
Group (4) Normal+100 mg/kg of ROS	83.33 $\pm$ 11.30 ^{bc}	75.33 $\pm$ 5.27 ^c	59.66 $\pm$ 3. 55 ^a	19.66 $\pm$ 5. 85 ^c	15.06 $\pm$ 1. 05 ^c	0.25 $\pm$ 0.1 0 ^e
Group (5) HC+50 mg/kg of ROS	90.66 $\pm$ 6.25 ^b	94.0 $\pm$ 5. 58 ^b	40.66 $\pm$ 2. 87 ^d	29.83 $\pm$ 3. 60 ^b	18.80 $\pm$ 1. 11 ^b	0.70 $\pm$ 0.0 ^b
Group (6) HC+100 mg/kg of ROS	81.50 $\pm$ 4.32 ^c	78.50 $\pm$ 12.81 ^c	47.66 $\pm$ 4. 03 ^c	21.0 $\pm$ 4.2 8 ^c	15.70 $\pm$ 2. 56 ^c	0.40 $\pm$ 0.12 ^d
L.S.D	8.80	7.57	4.74	4.94	1.51	0.19

*The different letters refer to a significant difference at ($p \leq 0.05$).

*The same letters refer to non significant difference at ($p \leq 0.05$).

3.3.4. Effect of oxidant-antioxidant markers (MDA, CP and Alb)

Table (3-5) indicated that MDA level in-group (2) significant increase at ($p \leq 0.05$) compared with group (1) and other groups. Whereas (3 and 5) doesn't record a significant difference in comparison with group (1), while groups (4 and 6) showed a significant decrease at ($p \leq 0.05$) in compared with other groups, but does not record a significant difference between them.

The results of ceruloplasmin showed a significant increase at ($p \leq 0.05$) in group (2) compared with group (1) and with all groups, while groups (3 and 6) did not recorded a significant difference compared with group (1) and between them, while groups (4 and 5) showed a significant increase at ($p \leq 0.05$) in compared with all groups, (table 3-5).

The results recorded a significant decrease at ($p \leq 0.05$) of Alb in group (2) compared with group (1) and all groups, whereas group (3) showed a significant increase at ($p \leq 0.05$) in compared with group (1) and all other groups, while groups (4,5) recorded a significant decrease compared with group (3) increase with groups(1,2 and 6), while group (6) didn't showed a significant difference at ($p > 0.05$) in compared with group (1) but decreased significantly compared with group (3,4 and 5). (table 3-5)

Table (3-5) Effects of polyphenols extracted from (ROS) on oxidant-antioxidant markers of hypercholesterolemic female rats (mean± SD).

Parameters Groups	MDA(mg/dl)	CP (mg/dl)	Albumin (mg/dl)
Group (1) Normal control	3.56±0.49 ^b	0.51±0.04 ^{cd}	4.05±0.28 ^c
Group (2) HC-group	6.01±2.31 ^a	1.63±0.53 ^a	2.65±0.33 ^d
Group (3) Normal+50 mg/kg of ROS	3.90±2.13 ^b	0.30±0.14 ^d	5.06±0.24 ^a
Group (4) Normal+100 mg/kg of ROS	2.04±0.36 ^{cd}	0.81±0.44 ^{bc}	4.55±0.18 ^b
Group (5) HC+50 mg/kg of ROS	3.02±0.05 ^{bc}	0.92±0.55 ^b	4.60±0.35 ^b
Group (6) HC+100 mg/kg of ROS	1.68±0.53 ^d	0.65±0.40 ^{bcd}	3.83±0.35 ^c
L.S.D	1.29	0.39	0.29

*The different letters refer to a significant difference at ($p \leq 0.05$).

*The same letters refer to non significant difference at ($p \leq 0.05$).

3.3.5. Effects on liver enzymes

The results listed in the table (3-6) showed the ALT level in groups (2,3,4 and 6) at ($p \leq 0.05$) was non-significant difference in compared with group (1) and between them, while group (5) showed significant decrease at ($p \leq 0.05$) in compared with group (1) and other groups.

AST level in group (2) showed a significant increase at ($p \leq 0.05$) in comparison with all groups, while groups (3 and 6) showed a significant decrease in compared with groups (1 and 2) but did not recorded a significant difference between them, while group (4) showed a significant decrease at ($p \leq 0.05$) in compared with groups (1,2,3 and 6), while group (5) showed a significant decrease at ($p \leq 0.05$) in compared with all groups.(table 3-6)

Table (3-6):- Effects of extract (ROS) on liver enzymes of hypercholesterolemic female rats (means± SD).

Parameters Groups	ALT (U/L)	AST (U/L)
Group (1) Normal control	45.0 ± 3.57 ^a	164.50 ± 17.39 ^b
Group (2) HC-group	41.50 ± 1.76 ^{ab}	186.83 ± 4.75 ^a
Group (3) Normal+50 mg/kg of ROS	42.50 ± 3.61 ^{ab}	134.16 ± 11.08 ^c
Group (4) Normal+100 mg/kg of ROS	43.0± 11.94 ^{ab}	105.33 ± 7.20 ^d
Group (5) HC+50 mg/kg of ROS	38.83±4.16 ^b	86.83± 2.31 ^e
Group (6) HC+100 mg/kg of ROS	44.0±4.38 ^{ab}	128.0 ± 24.60 ^c
L.S.D	5.77	13.32

*The different letters refer to a significant difference at (p≤0.05).

*The same letters refer to non significant difference at (p≤0.05).

3.4. effect of polyphenols extracted from GSE on physiological parameters of hypercholesterolemic female rats

3.4.1Effects on body weight

The results listed in the table (3-7) showed a significant increase in body weight in group (2) at (p≤0.05) in comparison with group (1) and all other groups, while there was non-significant difference in groups (3,4,5 and 6) compared with group(1)and between them .

Table (3-7): Effect of polyphenols extracted from GSE on body weight of hypercholesterolemic female rats (mean $\pm$ SD)

Groups \ Parameters	Difference in body weight
Group (1) Normal control	+15.35 $\pm$ 0.21 ^b
Group (2) HC-group	+32.00 $\pm$ 0.28 ^a
Group (3) Normal+50 mg/kg of GSE	+16.33 $\pm$ 13.37 ^b
Group (4) Normal+100 mg/kg of GSE	+15.00 $\pm$ 10.95 ^b
Group (5) HC+50 mg/kg of GSE	+17.84 $\pm$ 3.50 ^b
Group (6) HC+100 mg/kg of GSE	+21.67 $\pm$ 1.69 ^b
L.S.D	7.05

*The different letters refer to a significant difference at ($p \leq 0.05$).

*The same letters refer to non significant difference at ($p \leq 0.05$).

3.4.2. Effects on hematological parameters

The result in table (3-8) showed that the groups (2,3,4 and 6) records a significant decrease at($p \leq 0.05$) in Hb compared with group (1), whereas groups (5) showed a significant decrease compared with all groups.

The PCV in the groups (2,3,5 and 6) records a significant decrease at($p \leq 0.05$) in compared with group (1) but doesn't record a significant difference between them, whereas groups (4) showed a significant decrease compared with all groups.

Table (3-8) indicate that MCV in all group record a decrease significantly at ($p \leq 0.05$) compared with group (2) but doesn't record a significant difference between them.

Also, the result of this study showed there were a significant increase in MCH at ($p \leq 0.05$) between the group (2) and (1,3,4 and 6) But didn't record a significant difference when compared with group (6).

The results in the table (3-8) record a significant decrease at ($p \leq 0.05$) in MCHC in group (2 and 5) compared with all groups, while groups (3,4 and 6) did not record a significant difference when compared with group (1) and between them.

Table (3-8) showed that the group (2) records a significant decrease at ($p \leq 0.05$) in total WBC compared with group (1) and all other group, whereas groups (3,4 and 6) doesn't record a significant difference compared with group (1) and between them, group (5) show decrease significant compared with all groups.

Table (3-8): Effects of polyphenols extracted from (GSE) on hematological parameters of hypercholesterolemic female rats (mean $\pm$ SD).

Parameters Groups	Hb(g/dl)	PCV(%)	MCV (pg)	MCH (g/dl)	MCHC (g/dl)	WBC (10³/mm³)
Group (1) Normal control	16.11 $\pm$ 0.57 ^a	46.50 $\pm$ 5.71 ^a	58.86 $\pm$ 1.02 ^b	19.63 $\pm$ 0.44 ^b	33.08 $\pm$ 0.58 ^a	13.10 $\pm$ 2.77 ^a
Group (2) HC-group	12.95 $\pm$ 1.30 ^b	40.83 $\pm$ 4.66 ^b	66.43 $\pm$ 1.02 ^a	21.26 $\pm$ 0.43 ^a	31.90 $\pm$ 0.46 ^{bc}	5.10 $\pm$ 1.59 ^c
Group (3) Normal+50 mg/kg of GSE	11.83 $\pm$ 0.75 ^c	42.83 $\pm$ 3.31 ^{ab}	56.28 $\pm$ 2.68 ^c	19.03 $\pm$ 0.08 ^c	32.15 $\pm$ 1.17 ^{ab}	12.28 $\pm$ 2.53 ^a
Group (4) Normal+100 mg/kg of GSE	11.16 $\pm$ 1.47 ^c	39.16 $\pm$ 6.49 ^b	60.15 $\pm$ 0.73 ^b	19.05 $\pm$ 0.12 ^c	33.02 $\pm$ 0.44 ^a	13.03 $\pm$ 0.70 ^a
Group (5) HC+50 mg/kg of GSE	12.50 $\pm$ 1.04 ^{bc}	38.16 $\pm$ 5.26 ^b	64.96 $\pm$ 1.66 ^a	20.96 $\pm$ 0.68 ^a	32.21 $\pm$ 0.41 ^b	12.16 $\pm$ 3.31 ^a
Group (6) HC+100 mg/kg of GSE	12.66 $\pm$ 1.21 ^{bc}	38.16 $\pm$ 5.49 ^b	64.46 $\pm$ 5.36 ^a	20.05 $\pm$ 0.76 ^b	32.03 $\pm$ 1.47 ^{ab}	8.46 $\pm$ 1.39 ^b
L.S.D	1.08	5.14	2.57	0.48	0.84	2.19

*The different letters refer to a significant difference at ($p \leq 0.05$).

*The same letters refer to non significant difference at ($p \leq 0.05$).

3.4.3. Effects on the lipid profile

The results listed in table (3-9) indicated a significant increase in TC in group (2) at ($p \leq 0.05$) compared with group (1) and all other groups, while groups (3 and 4) didn't showed a significant difference compared with group (1), whereas groups (5 and 6) showed a significant increase compared with group (1).

In TG a significant increase in group (2) at ($p \leq 0.05$) when compared with group (1) and all other group, while groups (3 and 4) didn't recorded any significant difference in compared with group (1), while groups (5 and 6) showed increase significant in compared with groups (1,3 and 4) and showed decrease significant in comparison with group (2), table (3-9).

The HDL in the group (2) decreased significantly at ($p \leq 0.05$) compared with group (1) and with all other groups, whereas groups (3,4,5 and 6) didn't showed significant deference compared with group (1), (table 3-9)

Table (3-9) indicated that LDL level in-group (2) increased significantly at($p \leq 0.05$)compared with group (1) and other groups, group (3) showed a significant decrease at ($p \leq 0.05$) in compared with group (1) and other groups, whereas (4) didn't recorded a significant difference in compared with group (1), while groups (5 and 6) showed a significant increase in compared with groups (1,3 and 4) but showed a significant decrease in compared with other groups.

While VLDL level in-group (2) increased significantly at($p \leq 0.05$) when compared with group (1) and other groups. Whereas (3 and 4) didn't recorded a significant difference in comparison with group (1) and between them.

groups (5 and 6) showed a significant increase in compared with all groups except group (2), (table 3-9).

The results in the table (3-9) showed a significant increase in Atherogenic Index in group (2) at ($p \leq 0.05$) in comparison with all groups, while groups (3 and 4) does not showed any significant difference compared with group (1), while groups (5 and 6) showed a significant increase compared with groups (1,3 and 4).

Table (3-9): Effects of polyphenols extracted from (GSE) on lipid profile hypercholesterolemic female rats (mean $\pm$ SD).

Parameters Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Athero genic index
Group (1) Normal control	86.16 $\pm$ 6.94 ^c	81.33 $\pm$ 5.27 ^c	47.83 $\pm$ 6.01 ^b	30.13 $\pm$ 2.99 ^c	16.26 $\pm$ 1.05 ^d	0.60 $\pm$ 0.63 ^c
Group (2) HC-group	138.66 $\pm$ 10.38 ^a	108.16 $\pm$ 5.41 ^a	25.66 $\pm$ 5.16 ^c	80.33 $\pm$ 7.56 ^a	21.63 $\pm$ 1.08 ^a	2.25 $\pm$ 0.45 ^a
Group (3) Normal+50 mg/kg of GSE	89.83 $\pm$ 8.54 ^c	80.50 $\pm$ 9.95 ^c	62.50 $\pm$ 3.67 ^a	22.0 $\pm$ 4.47 ^d	17.30 $\pm$ 1.99 ^d	0.30 $\pm$ 0.08 ^d
Group (4) Normal+100 mg/kg of GSE	84.50 $\pm$ 6.77 ^c	73.16 $\pm$ 16.61 ^c	49.50 $\pm$ 3.88 ^b	30.66 $\pm$ 3.98 ^c	13.83 $\pm$ 3.50 ^e	0.60 $\pm$ 0.04 ^c
Group (5) HC+50 mg/kg of GSE	120.83 $\pm$ 6.30 ^b	98.0 $\pm$ 13.98 ^b	46.83 $\pm$ 4.70 ^b	55.83 $\pm$ 10.75 ^b	20.0 $\pm$ 2.79 ^b	1.28 $\pm$ 0.47 ^b
Group (6) HC+100 mg/kg of GSE	115.16 $\pm$ 4.87 ^b	93.33 $\pm$ 12.73 ^b	47.66 $\pm$ 4.80 ^b	52.0 $\pm$ 4.64 ^b	18.66 $\pm$ 2.54 ^{bc}	1.15 $\pm$ 0.21 ^b
L.S.D	7.35	11.24	4.67	6.18	2.29	0.28

*The different letters refer to a significant difference at ($p \leq 0.05$).

*The same letters refer to non significant difference at ($p \leq 0.05$).

3.4.4.Effect of oxidant-antioxidant markers

The result in this study indicated that MDA in-group (2) showed a significant increase at ($p \leq 0.05$) compared with group (1) and other groups, groups (3,4 and 6) doesn't showed a significant different at($p > 0.05$) compared with group (1) and between them, while group (5) showed a significant decrease at($p \leq 0.05$) when compared with all groups, table (3-10).

The Cp in-group (2) showed a significant increase at ($p \leq 0.05$) in compared with group (1 and 6) and other groups, whereas groups (3) doesn't show any significant difference at ($p > 0.05$) when compared with group (1,2,4 and 5) and between them, group (6) showed significant decrease at ($p \leq 0.05$) in comparison with all groups, (table 3-10).

Table (3-10) showed a significant decrease at ($p \leq 0.05$) in albumin level in group (2) compared with group (1) and all other groups, groups (3and 6) showed a significant increase at ($p \leq 0.05$) in comparison with groups (1,2 and 5) and showed decreased significantly compared with group (4), group (4)recorded a significant increase compared with group (1) and all other groups, group(5) don't showed any significant difference at ($p > 0.05$) when compared with group (1) but decreased significantly with groups (3,4 and 6).

Table (3-10):-Effects of polyphenols extracted from (GSE) on oxidant-antioxidant markers of hypercholesterolemic female rats (mean± SD).

Parameters Groups	MDA(mg/dl)	CP(mg/dl)	Albumin(mg/dl)
Group (1) Normal control	3.56±0.49 ^b	0.51±0.04 ^{bc}	4.05 ± 0.28 ^c
Group (2) HC-group	6.98±2.86 ^a	1.28±0.59 ^a	2.65 ± 0.33 ^d
Group (3) Normal+50 mg/kg of GSE	2.33±0.54 ^{bc}	0.87±0.50 ^{ab}	5.48 ±0.43 ^b
Group (4) Normal+100 mg/kg of GSE	3.02±0.05 ^b	1.08±0.49 ^a	5.95 ± 0.61 ^a
Group (5) HC+50 mg/kg of GSE	1.65±0.72 ^c	1.14±0.66 ^a	4.38 ± 0.07 ^c
Group (6) HC+100 mg/kg of GSE	2.43±0.77 ^{bc}	0.30±0.14 ^c	5.31 ± 0.24 ^b
L.S.D	1.25	0.46	0.36

*The different letters refer to a significant difference at ($p \leq 0.05$).

*The same letters refer to non significant difference at ($p \leq 0.05$).

3.4.5 Effects on level of liver enzymes.

The ALT in-group (2 and 5) does not showed any significant difference compared with group (1), whereas groups (3 and 4) showed significant decreases compared with all groups, but doesn't show any

Significant difference between them, group (6) showed significant increases compared with all groups, in table (3-11)

Table (3-11) indicated that AST in-group (2) increased significantly at($p \leq 0.05$) when compared with group (1) and all other groups, whereas groups (3,4and 6) showed significant decreases compared with groups (1,2) but didn't showed any significant difference between them, group (5) showed significant decrease compared with group (1,2) showed a significant increase compared with other groups.

Table (3-11):-Effects of extract (GSE) on liver enzymes of hypercholesterolemic female rats (mean± SD).

Parameters Groups	ALT(U/L)	AST(U/L)
Group (1) Normal control	45.0 ± 3.57 ^b	164.50± 17.39 ^b
Group (2) HC-group	41.5 ± 1.76 ^{bc}	186.83 ± 4.75 ^a
Group (3) Normal+50 mg/kg of GSE	36.66 ± 5.68 ^d	84.0 ± 10.37 ^e
Group (4) Normal+100 mg/kg of GSE	38.83 ± 5.41 ^{cd}	107.16 ± 3.31 ^d
Group (5) HC+50 mg/kg of GSE	41.33±4.22 ^{bc}	151.5 ± 7.86 ^c
Group (6) HC+100 mg/kg of GSE	50.50 ±3.08 ^a	111.0 ± 2.09 ^d
L.S.D	4.09	9.03

*The different letters refer to a significant difference at ($p \leq 0.05$).

*The same letters refer to non significant difference at ($p \leq 0.05$).

Chapter four

Discussion

4.1 Plant extraction

The results of this study showed that rosemary and grape seeds contains on (2, 1 g) respectively, from phenolic compound in each (20 g) of dry powder plant. Then qualitative tests were used to detection their compound. The result agrees with (Lo *et al.*, 2002). It was founded that rosemary contains a variety of polyphenolic compounds including carnosol, carnosic acid, methyl carnosol, rosmarinic acid, ursolic acid, and many others. Approx- imately 5% of the dry weight of rosemary leaves is comprised of the phenolic diterpenes carnosol and carnosic acid (Zhu *et al.*, 1998).

On the other hand, regarding the result of qualitative tests on grape seeds agree with (Fan and Lou, 2004), it was found that grape seeds are rich in phenolic compounds such as anthocyanin, the glucosides of anthocyanidins, catechin, and gallic acid. Polyphenolics gene- rally contain two or more hydroxyl groups attached to a conjugated ring system such as a benzene ring. Polyphenols contribute to the overall antioxidant properties of grape and may have important health benefits, including possible preventative effects against cancer (Fan and Lou, 2004) and cardiovascular diseases (Zern *et al.*, 2003; 2005). Anthocyanins have been shown to protect against lipid per- oxidation and DNA damage in rat.

Hepatoma cells in vitro, demonstrating potential anticarcinogenic properties (Lazzé *et al.*, 2003). Flavanols, such as catechin, have demonstrated inhibitory effects on platelet reactivity in vitro, a property which may reduce the risk of cardiovascular disease (Pearson *et al.*, 2005).

4.2 Effects of polyphenols extracted from (Rosemary and Grapes seeds) on body weight.

The result showed a significant increase in the body weight in the second group (HC) when compared with the first group (control group) These results were consistent with the results of (Onyeike *et al.*, 2012). who reported that

there was a significant increase in the weight of the hypercholesterolemia rats after two weeks of feeding.

The increase body weight in the second group, which results from intake of high fat diet and other metabolic disorders, is rising at an alarming rate and is becoming a major public health concern with incalculable social cost. Indeed, obesity facilitates the development of metabolic disorders such as diabetes, hypertension, and cardiovascular diseases such as stroke, osteoarthritis, apnea, some cancers and inflammation-bases pathology (Gonzalez *et al.*, 2001).

The different in body weight in groups that treated with polyphenols extract is due to the role of phenolic compounds, which have the ability to reduce the synthesis of fatty acids and increase oxidation, which increases the mechanism of reducing fat in the body of animals, and also by increasing secretions of the bile because it activates the enzymes of cytochrome and thus reduce the level of cholesterol in body of animal (Carlo *et al.* , 1992).

4.3 Effects of polyphenols extracted from (Rosemary and Grapes seeds) on the hematological parameters

The results indicated a significant decrease of Hb level in all group compared with group (1) and does not show any significant difference between this groups in the first experiment.

This decrease due to the effect of phenolic extracts on the amount of iron, which is the basic material for building red blood cells (Hurrell *et al.*, 1999). Phenolic compounds released from food and vegetables during digestion are associated with iron in a complex form and thereby prevent iron from absorption by the body. This result agrees with (Abdulkarimi *et al.*, 2011) who was found that the level of red blood cells and hemoglobin in chicken blood treated with plant extract (*Mentha piperita*) was low.

These plants possess phenolic compounds that are composed of an aromatic ring and one or more groups of hydroxyl, which are associated with

iron, a complex component that prevents the absorption of iron by the small intestine and also effects on its permeability. This result also was agreed with (Akdogan *et al.*, 2004), but in the second experience there was a slight increase in Hb in groups that treated with phenolic compound. The reason may be due attributed mainly to its antioxidant ability or its immune system stimulatory effects (Scaglione *et al.*, 2001).

The result showed the effects of extract (rosemary and grapes seeds) on PCV percentage in both experiments PCV percentage in the group (2) and other groups decreased as compared with group (1)

The low volume of hemoglobin in most groups leads to a decrease in red blood cells and therefore the reduced volume of blood cells compressed (PCV) if there is a direct relationship between them (Ganong, 1990).

This decrease due to the effect of some compound from extract on the secretion of the hormone erythropoietin from the kidney, which is the main catalyst for the production of red blood cells. (Akdogan, 2000) reported that the treatment of laboratory rats with plant extract cause toxic effects of renal tissue Nephrotoxicity, that the production of red cells occurs under the influence of stimulating the factor of erythropoietin, that is excreted from the kidney (renal erythropoietin factor) clonidine is associated with the secretion of the liver to be (erythropoietin stimulating factor) which carries blood to the bone marrow and stimulates stem cells to produce blood cells and the inability of renal units to produce this hormone leads to renal anemia (Akdogan , 2000).

The result showed the effects of extract (rosemary and grapes seeds) on MCV, MCH and MCHC did not differ significantly among the groups in both experiments when compared with controls group this result agrees with (Antai *et al.*, 2009) who found that polyphenol has little or non-effected on (MCV, MCH, MCHC).

The result from this study in the both experiments show that the effects of extract (rosemary and grapes seeds) on total WBC where showed that the group (2) records a significant decrease at ($p \leq 0.05$) in total WBC compared with group (1) and all other groups in. This significant decrease in total WBC may return to stress which cause by the animal's dosage where this result also was agreed with (Bouzidi *et al.*, 2011).

The increase in other groups in total WBC is compared with group (1). The result agrees with (Essien *et al.*, 2012). It was found that the treatment of laboratory rats with water extract of some plants containing phenols such as Basil (*Occimum basilicum*) leading to an increase in the total number of white blood cells. Phenol was also found to be effective in stimulating the immune response and helps to produce white blood cells supportive of the body's natural mechanical defense (Wright, 2002).

4.4 Effects of polyphenols extracted from (Rosemary and Grapes seeds) on the lipid profile

4.4.1 Cholesterol

The results in this study indicated a significant increase in TC in group (2) when compared with group (1) and all other group, this increase was due to rats were treated with daily high cholesterol diet for 30 days. These results were similar to the result of (Nishi *et al.*, 2013) reported that more pronounced effect was found with low-dose chlorogenic acid than with high dose in elevated plasma cholesterol, while groups that treated with plant extracts showed a significant reduction in TC level; the reduced effect of plant extracts may be attributed to the possibility of containing compounds that may inhibit the hydroxymethyl glutaryl coA reductase enzyme responsible for the synthesis of cholesterol. Some phenolic compounds such as flavonoids inhibit the synthesis of hepatic cholesterol by inhibiting HMG-CoA reductase, the

main enzyme that regulates the liver's cholesterol (Bok *et al.*, 2000; Lee *et al.*, 2003).

The high content of saturated fatty acids in high cholesterol diet caused lipid metabolism disorder. Compared with control group (Chang *et al.*, 2016). After treatment with polyphenolic extracted, the formation of fatty acids was inhibited, indicating that polyphenolic extracted inhibited the synthesis of fatty acids in rats, these results agree with those of (Su *et al.* (2017). This contrast may be related to different phenolic compounds and model systems.

4.4.2 Triglycerides

The results indicated a significant increase in TG in group (2) when compared with group (1) and all other groups. While groups that treated with extract plant show a decrease in level of TG compared with group (2).

The reduction may be due to the role of the extracts in the activation of Lipoprotein lipase, which works to divide triglycerides into lipids that are absorbed by fat cells. The result was agreed with (Barbalho *et al.*, 2011), where extracts of some plants containing phenolic compounds were found to reduce cholesterol and triglycerides in the serum of male laboratory rats.

4.4.3 High-density lipoprotein (HDL)

The results showed a significant decrease in the level of high-density lipoproteins in group (2) at ($p \leq 0.05$) compared with group (1), this due to most important of which increase the effectiveness of cholesterol ester transfer protein which transfer cholesterol ester from HDL to VLDL leaving the HDL rich in glycerides and less affinity to (Apolipoprotein A). The major component protein of HDL and remain free which facilitates filtered by the kidney and resultant changes in the liver function, which leads to the inhibition

of the production of (Apo A) (Giubergl, 1996). The results showed a significant increase in the level of high-density lipoprotein (HDL) in the groups treated with rosemary extract compared with groups (1 and 2), and in second experiment most of the results were stable compared to control group (1).

This may be due to the fact that phenolic compounds, especially flavonoids, activate lecithin acyl transferase (LCAT), which combines free cholesterol with high-density proteins and thus raises HDL in blood (Ghule, 2006). That phenols have increased the effectiveness and activation of the enzyme lipoprotein lipase and this enzyme participates in the metabolism of high-density lipoproteins (Hemilä, 1992)

4.4.4 Low-density lipoprotein (LDL)

The results in this study indicated a significant increase in TG in group (2) when compared with group (1) and all other group in both experiments, this increase was due to rats were treated with daily high cholesterol diet for 30 days, this agree with (Endo *et al* ., 1999)

The results in both experiments showed a significant decrease level of LDL in groups that treated with (rosemary and grape seed) compared with group (2). The decrease of LDL levels that reported in this study was compatible with finding of (Nishi *et al.*, 2013). On the other hand, several studies have confirmed the predominant phenolic compounds inhibited LDL oxidation the strong antioxidant activity of chlorogenic acid toward LDL (Nardini *et al.*, 1995; Rice-Evans *et al.*, 1996). Other phenolic components such as caffeic acid have also been reported to be active inhibitors of human LDL oxidation (Nardini *et al.*, 1995; Rice-Evans *et al.*, 1996; Teissedre *et al.*, 1996).

Polyphenols were shown to exhibit strong antioxidant properties that could protect LDL from oxidation (Vinson *et al.*, 2001). In a rat model, caffeic acid spared vitamin E and enhanced the resistance of LDL towards oxidative stress (Nardini *et al.*, 1997). Also, polyphenols can activate paraoxonase enzyme that runs inhibition of oxidized LDL uptake by the cells.

The best reduction of LDL level in group that treated with extracted rosemary this due to extract of rosemary causes a decrease in the level of cholesterol in the serum and consequently LDL does not transfer cholesterol from liver to tissue through blood, and this leads to the decrease of LDL level in blood. This effect might be related to the antioxidative properties of rosemary this agree with (Block and Mead, 2003).

Also, extract of rosemary in the first experiments was the best effect in group (3) and of course in group (4) leads to the decreasing in the level of VLDL.

The decrease may be due to antioxidant effect of caffeic acid and its derivatives such as rosmarinic acid, which are among phytochemical constituents of rosemary leaves (Al-Sereiti *et al.*, 1999) or because dried rosemary leaf powder might change the rate of fatty acids oxidation in the liver and reduced the rate of triglycerides biosynthesis in rats. Also rosmarinic acid or carnosic acid (derived from rosemary) inhibited LDL oxidation in a dose dependent manner as reported by (Fuhrman *et al.*, 2002).

This reduce in general groups is because extract of rosemary and Grapes seeds reduces the triglycerides level in the blood, therefore VLDL level is reduced because VLDL contains a large proportion of triglycerides, the result agrees with (Barbalho *et al.*, 2011).

Atherogenic index level has given the best effect of extract of rosemary and Grapes seeds in both experiments after treatment was in group (4 and 6) in case of the first experiments, while in the second experiments the

best effect were in group (3 and 4), this may be due to the effect of rosemary on HDL and LDL where the rosemary and grapes seeds decrease LDL and increase HDL and this leads to a decrease in the level of atherogenic index, the result agrees with (Howell *et al.*, 1998; Abdulkarim *et al.*, 2012). In addition, polyphenolic extracts of red wine have antiatherogenic properties (Vinson *et al.*, 2001).

4.5 Effects of polyphenols extracted from (Rosemary and Grapes seeds) on oxidant-antioxidant markers

Monounsaturated aldehyde (MDA) is known as the final product of the lipid oxidation process by converting lipid peroxid to unsaturated fatty acids to aldehyde monomers, which reflects cases of increased free radical generation and oxidative stress, it has the ability to interact with proteins and phosphorus fats, causing a change in its properties and function (Najjar, 2010).

The results of the present study showed increase the level of MDA and CP in group (2) and a slight decrease in the level of MDA and CP in most of groups that treated with plant extracted in experience, MDA is a general biomarker of biological oxidative stress (Kadiiska *et al.*, 2005).

The increase of MDA levels that has been reported in the present study in group (2) agrees with the results of (Hung *et al.*, 2006), the high level of MDA referring to the increase in the generation of free radicals which leads to increased (LPO) that back to analysis phospholipids by phospholipase which is produced arachidonic acid that forms reactive oxygen species, detrimental aldehyde, free radical and MDA (Adibhatla *et al.*, 2003), and the increase in the concentration of serum ceruloplasmin in group (2) was probably due to catalysis of the liver cells synthesis of Cp against iron overload status (Tobe *et al.*, 2002) and elevation in serum copper level (Butris and Ashood, 1996) as a defiance function.

Moreover, Phenol compounds act to reduce fat oxidation because they are effective antioxidants by inhibiting the formation of effective oxygen varieties and preventing the formation of free radicals. In addition, phenolic compounds, especially Flavonoids, reduce the level of the antioxidant enzymes such as Lipogenase, Myeloperoxidase, Xanthine- oxidase, NAD (P) H oxidase, Cytochrome enzymes P-450) (Evans *et al.*, 1996; Amorati *et al.*, 2006; Cetkovic, 2008). This leads to a reduction in the level of MDA and CP and the result was agreed with (Pakdeechote *et al.*, 2011), It was pointed out that the extract of mint plant *Mentha cordifolia* showed a significant decrease in the level of (MDA) in the serum of laboratory rats.

The cause of the decline is due to the presence of phenolic compounds in this extract, extract (rosemary and grape seeds)leaves high quantities of phenols flavonoids and flavons, which give the plant an important protection against the risk of radiation because these compounds are considered natural antiperspirants and works to inhibit free radicals this agree with (Samarth & Samarth, 2009). Flavonoids are natural antioxidants which give the body protection from the excessive oxidation of fat and fat proteins, production of atherosclerosis (Fuhrman *et al.*, 2002).

This result agreed with (Al-Ghazi *et al.*, 2010) in MDA and CP levels in diabetic rabbits after treatment with phenolic extracts of green tea, grape seeds and pomegranate seeds.

The albumin is a type of blood protein that is made in the liver. It makes up about 60% of the total blood proteins. It keeps fluid from leakage from blood vessels to tissue. It also nourishes tissues. It transfers hormones, vitamins, and ions as calcium ions throughout the body, and the concentration of albumin in the blood indicates the state of liver function and the nutritional status. The results in most of group (3,4,5 and 6) in both experiments were close at ($p \leq 0.05$) in comparison with group(1). This result was agreed with (Al-Ghazi *et al.*, 2010; Najjar, 2010).

4.6 Effects of polyphenols extracted from (Rosemary and Grapes seeds) in the liver enzymes

The results in both experiments showed a significant increase in the ALT and AST activity in group (2) in comparison with group (1) and all other groups, it is known that the rises of AST and ALT activities in serum are attributable to the damaged structural integrity of the liver, because these enzymes have intracellular localization and are released from liver into circulation after cell damage (Song *et al.*, 2004).

The results in both experiments showed that the groups treated with extract rosemary and grapes seeds showed a significant stability in the level of AST and ALT enzymes compared with control group.

Foods rich in antioxidants, have also been proposed as a tool to prevent liver damage (Morisco *et al.*, 2008). It is generally assumed that these antioxidant compounds may scavenge free radicals and regulate the activity and/or expression of certain enzymatic systems implicated in relevant physiological processes like the metabolism of xenobiotics in the liver (Singletary and Rokusek, 1997; Subbaramaiah *et al.*, 2002). Synergistic interactions amongst the antioxidants present in the grapes seeds and rosemary might reduce serum ALT and AST levels (Sotero-Féliz *et al.*, 2002).

The hepatoprotective action of extract grapes seeds and rosemary reported in the present study is similar to that obtained by (Abdel-Wahhab *et al.*, 2010; Cayir *et al.*, 2011) the mechanism of hepato protection of extract grapes seeds and rosemary is assumed to be through the activity of cytochrome P450 enzymes in the rat liver microsomes (Kim *et al.*, 1997).

The mechanisms which provide grapes seeds and rosemary hepatoprotective effects are closely attributed to antioxidation properties

grapes seeds and rosemary which enhances the antioxidant defense mechanism (Kim *et al.*, 2011) and also increased self-antioxidant enzyme activities of superoxide dismutase, glutathione reductase, glutathione peroxidase, catalase, glutathione-S-transferase, and heme oxygenase-1 in the aged-rat liver (Ramesh *et al.*, 2012).

Hepatoprotective plants contain a variety of chemical components such as phenols, flavonoids, xanthenes, glycosides saponins, and terpenes (Bhawna, 2009). Grapes seeds and rosemary have antioxidant activity as they contain phenolic acids and flavonoids, these properties of grapes seeds and rosemary are thought to provide many beneficial preventative effects against organ damage (He *et al.*, 2012; Ramesh *et al.*, 2012).

Conclusions and recommendations

Conclusions

The present study included the following conclusions:

1. Both Rosemary and Grapes seeds have important role in reducing hypercholesterolemia.
2. Both Rosemary and Grapes seeds have an effect on increasing body weight.
3. Both Rosemary and Grapes seeds have a positive effect on hematological parameters.
4. Both Rosemary and Grapes seeds have a positive effect on biochemical parameters of male rats, which include:
 - a- Lipid profile
 - b- Oxidant and antioxidant.
 - c- Liver enzymes.

Recommendations

The following can be recommended for the next studies:

1. Recommend patients with high hypercholesterol to eat (Rosemary and Grapes)
2. Study of other parts of this plant such as steam and root.
3. Study of more biological activities for these extracts such as anti-inflammatory, antifungal and antiviral.
4. Further works are needed to study the effect of these extracts as a hypoglycemic and hypotension.
5. Study the effect of rosemary and grape seed on histological change such as liver.

References

References

Abdel-Wahhab, M.A.; Hassan, N.S.; El-Kady, A.A.; Khadrawy, Y.A.; El-Nekeety, A.A.; Mohamed, S.R.; Sharaf, H.A. and Manna, F.A. (2010). Red ginseng extract protects against aflatoxin B1 and fumonisins-induced hepatic pre-cancerous lesions in rats. *Food Chem. Toxicol.*, 48:733-742.

Abdulkarim, R.; Aghazadeh, A. and Daneshyar, M. (2012). Effect of *Mentha* extract (*Mentha piperita*) supplementation in drinking water on performance, plasma lipoproteins, carcass characteristics and liver color index of broiler chickens. *Indian Journal of Animal Science*, 25: 411- 516.

Abdulkarimi, R.; Abdullahi, A. and Amini, M. (2011). *Mentha* extract consumption (*Mentha piperita* L) reduced blood iron concentration and increased TIBC levels in broiler chickens. *Journal of American Science*, 7(9):494-500. (ISSN: 1545-1003).

Abeer, E.; El-Wahab, A.; El-Adawi, H. and Kassem, H. (2008). Towards Understanding the Hepato protective effect of Grape Seeds Extract on Cholesterol-Fed Rats. *Australian Journal of Basic and Applied Sciences*, 2(3): 412-417.

Adibhatla, R. M.; Hatcher, J. F. and Dempsey, R. J. (2003). Phospholipase A2, hydroxyl radicals, and lipid peroxidation in transient cerebral ischemia. *Pubmed*, 5(5):647-654.

Aherne, S.A., Kerry, J.P. & O'Brien, N.M. (2007). Effects of plant extracts on antioxidant status and oxidant-induced stress in Caco-2 cells. *British J Nutr* 97: 321-328. URL:

Ahn, J.; Grun, I.U. and Fernando, L.N. (2002). Antioxidant properties of natural plant extracts containing polyphenolic compounds in cooked ground beef. *J Food Sci* 67: 1364-1369.

Akdogan, M. (2000). Investigation of biochemical and histopathological effects of *Mentha piperita* L. and *Mentha spicata* L. on kidney tissue in rats. *SAGE publication*. ISSN: 0960-3271.

Akdogan, M.; Gultekin, F. & Youtem, M. (2004). Effect of *Mentha piperita* (Labiatae) and *Mentha spicata* (Labiatae) on iron absorption in rats. *Toxicological and Industrial Health*, 20: 119-122.

AL-Gazi, S.; Al-Salih, R. & Al-Fartosi, K. (2010). Study of some plant polyphenolic extracts as hypoglycemic and antioxidant agents. Master, thesis. college of science, Thi-Qar university.

Allan, C. and Dawson, J.G. (1979). Enzymatic assay of total cholesterol involving chemical or enzymatic hydrolysis-a comparison of methods. *Clin.Chem.* 25 (6): 976-984.

Allan, C.C.; Poon, L.S.; Chan, C.S.G.; Richmond, W. and Fu, P.C. (1974). Enzymatic determination of total serum cholesterol. *Clin. Chem.* 20(4): 470-475.

Al-Sereiti, M.R., Abu-Amer K.M. & Sen P. (1999). Pharmacology of Rosemary (*Rosmarinus officinalis* Linn.) and its therapeutic potentials. *Indian J Exp Biol*, 37(2): 124-30. URL: www.pubget.com

Al-Sheyab, F.M.; Abuharfeil, N.; Salloum, L.; Hani, R.B. and Awad, D.S. (2012). The effect of rosemary (*Rosmarinus officinalis* L.) plant extracts on the Immune response and lipid profile in mice. *J Biol Life Sci* 3:31–58. doi:10.5296/jbls.v3i1.906.

Amorati, R.; Pedulli, G.; Cabrini, L.; Zambonin, L. ; Landi, L. and Agric, J. (2006). *Food Chem.* 54: 2932-2937.

Angioni A, Barra A, Cereti E, Barile D, Coisson JD, Arlorio M, Dessi S, Coroneo V, Cabras P. Chemical, (2004) composition, plant genetic differences, antimicrobial and antifungal activity investigation of the essential oil of *Rosmarinus officinalis* L. *J Agric Food Chem.* ,52:3530–3535.

Antai, A.B.; Ofem, O.E.; Ikpi, D.E.; Ukafia, S. and Agiang, E.A. (2009). phytochemistry and some haematological changes following oral administration of ethanolic root extract of *Gonglonema latifolium* in rats. *Nigerian Journal of Physiological Sciences*, 24 (1): 79 –83.

Asaolu, M.F., Asaolu, S.S., Oyeyemi, A.O. and Aluko, B.T. (2010). Hypolipemic effects of methanolic extract of *Persea americana* seeds in hypercholesterolemic rats. *J Med Sci*, 1(4):126–128.

Ayranci, E.; Erkan, N. and Ayranci, G. (2008). Antioxidant activities of rosemary (*Rosmarinus Officinalis* L.) extract, blackseed (*Nigella sativa* L.) essential oil, carnosic acid, rosmarinic acid and sesamol. *Food Chemistry*. 110, 1: 76-82.

Bagchi, D.; Sen, C.K.; Ray, S.D, et al.(2003). "Molecular mechanisms of cardioprotection by a novel grape seed proanthocyanidin extract. *Mutat" Res.* , 523-524:87-97.

Bagchi, D.; Stohs, D.K.; Das, S.D.; Ray, C.A.; Kuszynski, S. and Pruess, H. (2007). Free radicals and grape seeds proanthocyanidin extract: importance in human health and disease prevention. *Toxicology*, 148(2-3): 187-197.

Backle M, Leupold G, Parlar H.(2003). Rapid quantitative enrichment of carnolic acid from rosemary (*Rosmarinus officinalis* L.) by isoelectric focused adsorptive bubble chromatography. *J Agric Food Chem*, 51:1297–1301.

Barbalho, S.; Damasceno, D.; Spada, A.; Silva, V.; Martuchi, K.; Oshiiwa, M.; Machado, F. and Mendes, C. (2011). Metabolic Profile of Offspring from Diabetic Wistar Rats Treated with *Mentha piperita* (Peppermint). *Evid Based Complement Alternat Med*: 430237.

Bauer, J.E.(1995). Evaluation and dietary considerations in idiopathic hyperlipidemia in dogs. *Journal of the American Veterinary Medical Association.* , 206: 1684–1688.

Baydar, N.G.; Özkan, G. and Yasar, S .(2007) . Evaluation of the antiradical and antioxidant potential of grape extracts. *Food Control* ., 18: 1131-1136.

Beers, M.H. and Berkow, R. (2005). *The Merck Manual of Diagnosis and Therapy*, Seventeenth Edition, Merck Research Laboratories, New Jersey, : 200-211.

Bentivegna, S.S. and Whitney, K.M. (2002). Subchronic 3-month oral toxicity study of grape seed and grape skin extracts. *Food Chem Toxicol.*, 40: 1731–1743.

Bhale SD, Xu Z, Prinyawiwatkul W, King JM, Godber JS .(2007). Oregano and rosemary extracts inhibit oxidation of long-chain N-3 fatty acids in menhaden oil. *J Food Sci.*72:504–508.

Block, K.I. and Mead, M.N. (2003). Immune system effects of Echinacea, ginseng, and astragalus. A review. *Interg, Cancer Ther.*, 2:247-67.

Bok, S.H; Lee, S.H; Park, Y.B; Bae, K.H; Son, K.H; Jeong, T,S. and Choi, M.S.(1999). Plasma and hepatic cholesterol and hepatic activities of 3-Hydroxy MethylGlutaryl-CoA reductase and acyl CoA: cholesterol transferase are lower in rats fed citrus peel extract or a mixture of citrus bioflavonoids.*J Nutr.* 129:1182–1185.

Bok, S.H.; Shin, Y.W.; Bae, K.H.; Jeong, T.S.; Kwon, Y.K. and Park, Y.B. (2000). Effects of Naringenin and Lovastatin on plasma and hepatic lipids in high fat and high-cholesterol fed rat. *Nutr. Res.* 20 : 1007-1015 .

Boulos, L. (1983). Medicinal plants of North Africa. St. Clair River Drive Algonac, Michigan.

Bouzidi, A.; Mahdeb, N. and Kara, N.(2011). Toxicity studies of alkaloids of seeds of *Datura stramonium* and synthesis alkaloids in male rats. *Journal of Medicinal Plants Research*; 5(15), pp. 3421-3431.

Bradley, P. (2006). "A handbook of scientific information on widely used plant drugs", British herbal Medicine Association, Bournemouth.

Brewer , H.B. Jr and Rader, D.J.(1991). HDL: structure, function and metabolism. *Prog Lipid Res* ., 30 (2-3): 139-144.

Bruneton, J. (1995). Pharmacognosy, phytochemistry, medicinal plants. Lavoisier Pubs, Paris.

Bryman, A. and Cramer, D. (2012). Quantitative Data Analysis with Ibm Spss 17, 18 & 19: A Guide for Social Scientists. Routledge.

Butris, G. A. and E. R. Ashood. (1996). Tetiz fundamental of clinical chemistry. 4th ed., Sunders W.S and Philadephia,: 361.

Carl, von. and Linné .(1778). botanist, professor (Alexander Roslin) - Nationalmuseum - 15723.tif.

Carlo, G.; Eisenberg, N. and Knight, G. P. (1992). An objective measure of adolescents' prosocial moral reasoning. *J. Res., Adolesc.* 2: 331– 349.

Carr, A.; McCall, M . and Frei, B. (2000). "Oxidation of LOL by myeloperoxidase and reactive nitrogen species". *Arteriosclerosis, Thrombosis, And Vascular Biology*, 20(7),1716-1723.

Castillo, J.; Benavente-Garcia, J.; Lorente, M.; Alcaraz, A.; Redondo, A. and Del- Rio, J. (2000). Antioxidant activity and radioprotective effects against chromosomal damage induced *in vivo* by X-rays of flavan-3-ols (Procyanidins) from grape seeds (*Vitis vinifera*): comparative study versus other phenolic and organic compounds. *J Agric Food Chem.*, 48(5): 1738-1745.

Cayir, K.; Karadeniz, A.; Simsek, N.; Yildirim, S.; Karakus, E.; Kara, A.; Akkoyun, H.T. and Sengul, E. (2011). Pomegranate Seed Extract Attenuates Chemotherapy-Induced Acute Nephrotoxicity and Hepatotoxicity in Rats. *J. Med. Food.*, 14:1254-1262.

Ćetković, G. (2008). The chemistry of natural products, Faculty of Technology, University of Novi Sad, in Serbian.

Chang, M.H and Chen, T.C. (1998).“Hotness” stability of chicken hot-wing products as affected by preparation methods and storage. *Poult Sci*,77:627–631.

Chang, Y. C., Yang, M. Y., Chen, S. C., & Wang, C. J. (2016). Mulberry leaf polyphenol extract improves obesity by inducing adipocyte apoptosis and inhibiting preadipocyte differentiation and hepatic lipogenesis. *Journal of Functional Foods*, 21, 249–262.

Cholesterol Management Health Center.” 26 August 2005. 25 October 2007. <http://www.webmd.com/cholesterol-management/>.

Cindy Liang “08 and Nico Drohojowski_(2008). in College Seminar 235 Food for Thought: The Science, Culture, & Politics of Food Spring, The Wine Pages,"Grapes - Botanical Description - Botany.". 17 Feb. <http://www.thewinepages.com/grapes-botany.html>.

Clifford MN (1999). Chlorogenic acids and other cinnamates—nature, occurrence and dietary burden. *Journal of the Science of Food and Agriculture*. 79(3):362–372.

Cook, R. P. and Thomson, R. O. (1950). *Quart. J. exp. Physiol.* 36:61.

Cooper, R.; Cutler, J.; Desvigne-Nickens, P.; Fortmann, S.P.; Friedman, L. and Hailik, R. (2000). Trends and disparities in coronary heart diseases, stroke, and other cardiocardiовascular diseases in the United States: findings of the national conference on cardiovascular disease prevention. *Circulation* ., 102(3):137–47.

Cotelle, N. (2001). Role of flavonoids in oxidative stress. *Curr. Topics Med. Chem.* 1: 569-590.

Couillard, C.; Couture, P.; Lamarche, B.; Pomerleau, S. and Guillaume, R. (2005). "Changes in plasma antioxidant capacity and oxidized low-density lipoprotein levels in men after short-term cranberry juice consumption". *Metabolism -Clinical and Experimental.*, 54(7),856-861.

Covas, M.I., Nyyssonen, K., Poulsen, H.E., Kaikkonen, J., Zunft, H.J., Kiesewetter, H., Gaddi, A., de la Torre, R., Mursu, J., Baumler, H., Nascetti, S., Salonen, J.T., Fito, M., Virtanen, J., Marrugat, J. and Group, E.S. (2006). The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial. *Ann. Int. Med.* 145: 333–341.

Crews, D.E. (2007). Senescence, aging, and disease. *J Physiol Anthropol* . 26(3):365–372.

Dauchet, L.; Amouyel, P.; Hercberg, S. and Dallongeville, J. (2006). Fruit and vegetable consumption and risk of coronary heart disease: A meta-analysis of cohort studies. *Journal of Nutrition* ., 136(10): 2588-2593.

Del -Rio, D.; Costa, L.G.; Lean, M.E. and Crozier, A. (2010). Polyphenols and Health: What Compounds Are Involved? *Nutr., Metab. & Cardiovas.*, 20: 1-6.

Doha, A.; Mohamed; Thanaa, E.; Hamed; Sahar, Y. and Al-Okbi (2010). "Reduction in hypercholesterolemia and risk of cardiovascular diseases by mixtures of plant food extracts: a study on plasma lipid profile, oxidative stress and testosterone in rats". *Grasas Y aceites.*, 61(4): 378-389.

Doo, M. and Kim, Y. (2015). Obesity: interactions of genome and nutrients intake. *Prev Nutr Food Sci*, 20(1):1–7

Dulundu E, Ozel Y, Topaloglu U, Toklu H, Ercan F, Gedik N, Sener G. (2007). Grape seed extract reduces oxidative stress and fibrosis in experimental biliary obstruction. *J Gastroenterol Hepatol. Jun*; 22(6):885-92. PMID:17565645.

Durrington, P. (2003). Dyslipidaemia. *Lancet*. 362(9385):717–731.

El- Missiry, M. and El-Gindy, A. (2000). Amelioration of alloxan induced diabetes mellitus and oxidative stress in rats by oil of *Eruca sativa* seeds. *Annals of Nutrition and Metabolism Journal*, 44: 97-100.

Escott-Stump, S. and Mahan, L. K. (2004). Krause's Food, Nutrition, & Diet Therapy (11th edition). Philadelphia: Saunders.

Essien, E.; Onyeike, E.; Ugbeyide, D. and Eneke, I. (2012). Effects of Aqueous Extract Of *Occimum basilicum* Leaves on some Hematological and Biochemical Parameters of Wistar Albino Rats. *Canadian Journal on Scientific and Industrial Research.*, 3.(5).

Endo, T. ; Nakano, M. ; Shimizu, S. ; Fukushima, M. and Miyoshi, S. (1999). Effects of a probiotic on the lipid metabolism of cocks fed

on a cholesterol – enriched diet. *Biosci. Biotechnol. Biochem*, 63 (9)
: 1569 – 1575.

EDQM (2005) European Pharmacopoeia .(2005). Direct. Qual. Med. Counc.
Eur. (EDQM), Strasbourg.

El- Missiry, M. and El-Gindy, A. (2000). Amelioration of alloxan induced
diabetes mellitus and oxidative stress in rats by oil of *Eruca sativa* seeds.
Annals of Nutrition and Metabolism Journal, 44: 97-100.

Evans, C.; Miller, N. and Paganga, G. (1996) . Free Radical Bio. Med. 20:
933-956.

Falk ,E. (2005) : Pathogenesis of atherosclerosis . *Journal of the American
College of Cardiology* , 47 (8) : C7 – C12.

Fan, P. and Lou ,H. (2004) . Effects of polyphenols from grape seeds on
oxidative damage to cellular DNA. *Molecular and Cellular Biochemistry* .,
267 (1-2), 67-74.

Ford, R.B. (1996). Clinical management of lipemic patients. Compendium on
Continuing Education for the Practicing Veterinarian., 18: 1053–1060.

Fossati, P. and Prencipe, L. (1982). Serum triglycerides determined
colorimetrically with an enzyme that produces hydrogen peroxide. *Clin.
Chem.* , 28: 2077-2080.

Fredrickson ,D.S. and Lees, R.S.(1965). "A system for phenotyping
hyperlipidemia. *Circulation*., 31: 321-327.

Friedwald ,W.T. ; Levy, R.I. and Fredrickson ,D.S. (1972) .Estimation of concentration of LDL-C in plasma without the use of preparative ultracentrifuge .*Clinical Chemistry*, 18 : 499-502.

Fuhrman, B.;Oiknine, J. and Aviram, M.(2002). Iron induces lipid peroxidation in cultured macrophages, increases their ability to oxidatively modify LDL, and affects their secretory properties. *Atherosclerosis* ., 111:65–78.

Ganong , W . (1990). Review of medical physiology . Prentice-Hall International Inc. 13 (1) : 63 – 69 .

Garcia-Marino, M.; Rivas-Gonzalo, J.C.; Ibanez, E . and Garcia-Moreno, C. (2006) . Recovery of catechins and proanthocyanidins from winery by-products using subcritical water extraction. *Anal Chim Acta* ., 563: 44-50.

Gayon, T.A. (1972). Plant phenolic. Oliver and Boyed. Edinboursa . :254.

Ghule, B.; Ghante, M.; Saoji, A. and Yeole, P. (2006). Hypolipidemic and antihyperlipidemic effect of Lagenaria siceraria fruit extract . *Indian journal of experimental biology*; 44 : 905-909.

Gil, M. I.; Toma`s-Barberan, F. A.;Hess-Pierce, B. and Kader, A. A. (2002). Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of Nectarine, Peach, and Plum cultivars from California. *J. Agric. Food Chem.* 50: 4976-82.

Gonzalez-Castejon, M. and Rodriguez-Casado, A. (2001). Dietary phytochemicals their potential effects on obesity: a review. *Pharmacol Res.* Nov ;64(5):438-55. PMID 21798349.

Groff, J.;Groupper, S. S. and Smith, J. L. (2005). "Advanced nutrition and human metabolism". Belmont, CA: Thomson Wadsworth.

Guo, Q.; Gao, S.; Sun, Y.; Gao, Y.; Wang, X.& Zhang, Z. (2016). Antioxidant efficacy of Rosemary ethanol extract in palm oil during frying and accelerated storage. *Industrial Crops and Products*, 94(1), 82-88<http://dx.doi.org/10.1016/j.indcrop.2016.08.032>.

Harbone, J.B. (1984). Phytochemical methods agude to moden techniques of plant analysis.2nd (edn) champman and hall, London, NewYork.

He, H.; Xu, J.; Xu, Y.; Zhang, C.; Wang, H.; He, Y.; Wang, T. and Yuan, D. (2012). Cardioprotective effects of saponins from *Panax japonicas* on acute myocardial ischemia against oxidative stress-triggered damage and cardiac cell death in rats. *J. Ethnopharmacol.*, 140:73-82.

Hegele, R.A. (2009). Plasma lipoproteins: genetic influences and clinical implications. *Nat Rev Genet* ., 10 (2): 109-121.

Heml , H.(1992). Vitamin C and Plasma Cholesterol. *Critical Reviews in Food Science and Nutrition.*, 32(l):33-57.

Hongbao, M. (2006)." Cholesterol and Human Health", Department of Medicine, Michigan State University, East Lansing, Michigan, *The Journal of American Science*, 2(1);_(517) 432-0623.

Howell,T. ; MacDougall, D. and Jones,P. (1998) Phytosterols partially explain differences in cholesterol metabolism caused by corn or olive oil feeding. *J Lipid Res*; 39: 892-900.

Hung, M.Y.; Fu, T.Y.; Shih, P.H.; Lee, C.P. and Yen, G.C. (2006). Du-Zhong (*Eucommia ulmoides* Oliv.) leaves inhibit CCl₄ induced hepatic damage in rats. *Food Chem Toxicol.*, 44:1424–31.

Hurrell, R.; Reddy, M. and Cook, J. (1999). Inhibition of non-heme iron absorption in man by polyphenolic-containing beverages. *British Journal of Nutrition.*, 81:289-295.

Ibrahim, M.H. and Jaafar, H.Z.E. (2011). Enhancement of leaf gas exchange and primary metabolites, up-regulate the production of secondary metabolites of *Labisia Pumila* Blume seedlings under carbon dioxide enrichment. 16: 3761–3777.

Inoue, M.; Suzuki, R.; Sakaguchi, N.; Li, Z.; Takeda, T.; Ogihara, Y.; Jiang, B.Y. and Chen, Y. (1996) . Selective induction of cell death in cancer cells by gallic acid. *Biological and Pharmaceutical Bulletin.*, 20 (5), 1526-1530.

Iskan, G.; Demirci, F.; Kirimer, N.; Kürkcüoğlu, M . and Baser, K. H. C. (2002). Antimicrobial screening: *Mentha piperita* essential oil. *Journal of Agricultural and Food Chemistry*. 50: 3943-3946 .

James, W.; Berger, T. and Elston, D. (2006). "Andrew's diseases of the skin": Clinical dermatology. 10th.ed, Saunders Elsevier.

Jeszka, M.; Flaczyk, E.; Kobus-Cisowska, J. and Dziedzic, K. (2010). Phenolics – characteristic and significance in food technology. *Nauka Przyr. Technol.*, 4: 2- 19 .

Johnson, M.C. (2005). Hyperlipidemia disorders in dogs. *Compendium on Continuing Education for the Practicing Veterinarian*. 27: 361–364.

Jonatas Rafael de Oliveira, Daiane de Jesus, Leandro Wagner Figueira, Felipe Eduardo de Oliveira, Cristina Pacheco Soares, Samira Estves Afonso Camargo, Antonio Olavo Cardoso Jorge and Luciane Dias de Oliveira(2017). Biological activities of *Rosmarinus officinalis* L. (rosemary) extract as analyzed in microorganisms and cells. *Experimental Biology and Medicine*, 10: 1–10.

Kadiiska, M. B.; Gladen, B. C.; Baird, D. D.; Germolec, D.; Graham, L. B.; Parker, C. E.; Nyska, A.; Wachsman, J. T.; Ames, B. N.; Basu, S.; Brot, N.; Fitzgerald, G. A.; Floyd, R. A.; George, M.; Heinecke, J. W.; Hatch, G. E.; Hensley, K.; Lawson, J. A.; Marnett, L. J.; Morrow, J. D.; Murray, D. M.; Plastaras, J.; Roberts, L. J.; 2nd, hiel, J. C.; Wellner, D.; Walter, P. B.; Tomer, K. B.; Mason, R. P. and Barrett, J. C. (2005). Biomarkers of oxidative stress study II: Are oxidation products of lipids, proteins, and DNA markers of CCl₄ poisoning?. *Free Radical Biology and Medicine*., 38: 698–710.

Kanatt, S.; Chander, R. and Sharma, A. (2008). Chilean and mint mixture: A new preservative for meat and meat products. *Food Chemistry*. 107: 845–852.

Kim, H.G.; Yoo, S. R. and Park, H. J. (2011). Antioxidant effects of *Panax ginseng* C.A. Meyer in healthy subjects: a randomized, placebo-controlled clinical trial,” *Food and Chemical Toxicology*, 49. 9: 2229–2235.

Kim, J.H.; Chun, Y.J.; Park, J.D.; Kim, S.I. and Roh, J.K.(1997). Protection of rat liver microsomes against carbon tetrachloride-induced lipid peroxidation by red ginseng saponins through cytochrome P450 inhibition. *Planta Medica*., 63(5): 415- 418.

Kono, Y.; Kobayashi, K. and Tagawa, S. ; (1997). Antioxidant activity of polyphenolics in diets. Rate constants of reactions of chlorogenic acid and caffeic acid with reactive species of oxygen and nitrogen. *Biochimica et Biophysica Acta—General Subjects*. 1335(3):335–342. [[PubMed](#)]

Kumar, S.A. ; Sudhahar, V. and Varalakshmi, P. (2005). Attenuation of serum lipid abnormalities and cardiac oxidative stress by eicosapentaenoate-lipoate (EPA-LA) derivative in experimental hypercholesterolemia. *Clin Chim Acta*. 335:197–204.

Lazzé, M.C.; Pizzala, R.;Savio, M.;Stivala, L.A.; Prosperi, E. and Bianchi, L. (2003). Anthocyanins protect against DNA damage induced by *tert*-butyl-hydroperoxide in rat smooth muscle and hepatoma cells. *Mutation Research* .,535 (1), 103-115.

Lee, M.K.; Moon, S.S.; Lee, S.E. (2003) Naringenin 7-O-cetyl ether as inhibitor of HMG-CoA reductase and modulator of plasma and hepatic lipids in high cholesterol-fed rats. *Bioorgam Med Chem.*,11: 393-398 .

Leifert, WR. ; Abeywardena, MY. ; (2008). Grape seed and red wine polyphenol extracts inhibit cellular cholesterol uptake, cell proliferation, and 5-lipoxygenase activity. *Nutr. Res.*, 28: 842-850.

Lo, A.H. ; Liang, Y.C. ; Lin-Shiau, S.Y. ; Ho, C.T. and Lin, J.K. (2002). Carnosol, an antioxidant in rosemary, suppresses inducible nitric oxide synthase through down-regulating nuclear factor-B in mouse macrophages. *Carcinogenesis* 23, 983-991.

Lopes-Virella , M.F. (1977) : Cholesterol determination in high-density lipoproteins separated by three different methods. *Clin. Chem.* , 23(25) : 882-884.

Manach, C.; Schaller, A.; Morand, C.; Remesy, C. and Jimenez, L. (2004). Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition.*, 79(5), 727-747.

Mar, Q.; Marta M. and Amaya, A. (2013). Beneficial effects of polyphenols on cardiovascular disease. *Pharmacological Research.*, 68 : 125– 131.

Marais AD (2013) Dietary lipid modification for mild and severe dyslipidaemias. *Proc Nutr Soc* 72(3):337–341

Marinova, D.; Ribarova, F. and Atanassova, M. (2005). Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *J. Univ. Chem. Technol. Metall.*, 40: 255–260.

Marks, D.; Thorogood, M.; Neil, H.A. and Humphries, S.E. (2003). A review on the diagnosis, natural history, and treatment of familial hypercholesterolaemia. *Atherosclerosis.*, 168(1): 1–14.

Marrugat, J. ; Covas, M.I. ; Fito, M. ; Schroder, H. ; Miro-Casas, E. ; Gimeno, E. ; Lopez-Sabater, M.C. ; de la Torre, R. and Farre, M. (2004). Effects of differing phenolic content in dietary olive oils on lipids and LDL oxidation--a randomized controlled trial. *Eur. J. Nutr.* 43: 140–147.

Maton, D.; Hopkins, J.; McLaughlin, Ch. W.; Johnson, S.; Warner, M. Q.; LaHart, D. ; Wright, J. D. and Deep V. Kulkarni .(1997). Human Biology and

Health. Englewood Cliffs, New Jersey, US: Prentice Hall. ISBN 0-13-981176-1.

McEnemy, J. and Young, I. (2001). "Lipoprotein oxidation and atherosclerosis". *Biochemical Society*, 34(6), 358-362.

Menden, C.; Boian, J.; Murthy, L. ; Petering, H. and Anal, L. (1977). Plasma Antioxidant. *Anal Lett*, 10: 197.

Mensink ,R.P.; Aro ,A.; Den- Hond ,E.; German, J.B.; Griffin, B.A.; Ter-Meer, H.U.; Mutanen ,M.; Pannemans, D. and Stahl, W. (2003).PASSCLAIM – Diet-related cardiovascular disease. *Eur J Nutr.*, 1: I6-I27.

Merck. (2008). Merck Manuals Online Medical Library. <http://www.merck.com/mmpe/print/sec12/ch159/ch159b.html>.

Mignone, L.I.; Giovannucci, E.; Newcomb, P.A.;Titus-Ernstoff, L.; Trentham-Dietz, A.; Hampton, J.M.; Willett, W.C. and Egan, K.M. (2009). Dietary carotenoids and the risk of invasive breast cancer. *International Journal of Cancer*. 124(12): 2929-2937.

Mohammed ,A-D.; SalehAlqasoumi, Majid ;Ahmad, Mohammed ,A.Y.;Nazam ;Ansari, M. and Syed ,R .(2011). Effect of beta vulgaris oncholesterol rich diet-induced hypercholesterolemia in rats. *FARMACIA.*, 59(5): 669-678.

Moreno, DA. ; Ilic, N. ; Poulev, A. ; Brasaemle, DL. ; Fried, SK. and Raskin, I. (2003). Inhibitory effects of grape seed extract on lipases. *Nutrition*, 19: 876- 879.

Moreno, S.; Scheyer, T.; Romano, C. and Vojnov, A.(2006). Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. *Free Radical Research.*, 40, 2, : 223-231, 2006.

Morisco, F. P.; Vitaglione, D.; Amoruso, B.; Russo, V. ; Fogliano and N. Caporaso. (2008). Foods and Liver health. *Mol. Aspects Med.* 29:114-150.

Moutzouri,E.;Elisaf, M.and Liberopoulos,E.N. (2011). "Hypocholesterolemia". *Current Vascular Pharmacology*.9(2): 200–12.

Muhammad, A.S.; Theeshan, B. and Okezie, I.A. (2006). Chemopreventive actions of polyphenolic compounds in cancer. *BioFactors.*, 27(1-4): 19-35.

Najjar , S .H . (2010). Extraction and purification of the phospholipase enzyme molecule from the venomous snake with diffuse scales and studying some of its physiological and biochemical effects in male laboratory rats. M.Sc.Thesis. College of Science. Dhi Qar University.

Nardini, M.; D'Aquino, M.;Tomassi, G.; Gentili, V.; Di- Felice, M. and Scaccini, C. (1995). Inhibition of human low-density lipoprotein oxidation by caffeic acid and other hydroxycinnamic acid derivatives. *Free Radical Biol. Med.* 19: 541-552.

Nardini, M.;Natella, F.; Gentili, V.; Di- Felice, M. and Scaccini, C. (1997). Effect of caffeic acid dietary supplementation on the antioxidant defense system in rat: an in vivo study. *Arch Biochem Biophys.* 342(1): 157-60.

National Heart Lung and Blood Institute (NHBI) (2007). "Cholesterol lowering medicines". Retrieved November 15,2007, from National Heart Lung and Blood Institute: <http://www.nhlbi.nih.gov/chdlmeds.htm#statins>.

Nawaz, H.; Shi, J.; Mittal, G.S. and Kakuda, Y . (2006). Extraction of polyphenols from grape seeds and concentration by ultrafiltration. *Separation and Purification Technology* ., 48: 176-181.

Nicasio, P. ; Aguilar-Santamaría, L. ; Aranda, E. ; Ortiz, S. and González, M. (2005). Hypoglycemic effect and chlorogenic acid content in two Cecropia species. *Phytotherapy Research*. 19(8):661–664. [[PubMed](#)]

Nicole, St. and Jean .(2007). “Functional Food Fact Sheet.” April 2003. 2 October 2007.

Nishi; Amjid ,A. and Pawan, K. (2013). Hypolipidemic effect of Chlorogenic acid in Hypercholesterolemic rat model. *Int J Pharm Bio Sci*. 4(1): 582 – 586.

Olthof, MR. ; Hollman, PCH. and Katan, MB.(2001). Chlorogenic acid and caffeic acid are absorbed in humans. *Journal of Nutrition*. 131(1):66–71. [[PubMed](#)]

Onyeike, E.N.; Monanu, M. O. and Okoye, C. N. (2012). Changes in the blood lipid profile of wistar albino rats fed rich cholesterol diet .Department of Biochemistry, Faculty of Science, University of Port Harcourt, *Nigeria*., 3 (11):5186-5191.

Osuagwu, G. and Ernenike, J. (2007). Photochemical and Mineral content of four Nigerian pterocarpus. *International Journal of molecular Medicine and Advance sciences*.,3 (1):6-11.

Pakdeechote, P.; Kukongviriyapan, U.; Berkban, W.; Prachaney,P.; Kukongviriyapan,V. and Nakmareong, S.(2011). *Mentha cordifolia* extract

inhibits the development of hypertension in L-NAME-induced hypertensive rats. *Journal of Medicinal Plants Research*, 5(7), : 1175-1183.

Pearson, D.A.; Holt, R.R.; Rein, D.; Paglieroni, T.; Schmitz, H. and Keen, C. (2005). Flavanols and platelet reactivity. *Clinical and Developmental Immunology* 12 (1), 1-9.

Porth, C.T. (2009). Pathophysiology: "Concepts of altered health states". (7th ed.). Philadelphia: Lippincott Williams & Wilkins.

Prasain ,J.K.; Peng, N.; Dai, Y.; Moore ,R.; Arabshahi, A.; Wilson, L.; Barnes ,S.; Wyss, J.M.; Kim, H. and Watts, R.L. (2009) . Liquid chromatography tandem mass spectrometry identification of proanthocyanidins in rat plasma after oral administration of grape seed extract. *Phytomedicine* 16 (2-3), 233-243.

Prior ,R.L. (2003). Fruits and vegetables in the prevention of cellular oxidative damage. *Am. J. Clin. Nutr.* 78: 570S-578S.

Ramachandran, H.D.; Narasimhamurthy ,K. and Raina ,P.L. (2003). Modulation of cholesterol induced hypercholesterolemia through dietary factors in Indian desert gerbils (*Meriones hurricinae*). *Nutr. Res.*, 23:245-256.

Ramesh, T.; Kim S. W. and Sung, J. H. *et al.* (2012). "Effect of fermented *Panax ginseng* extract (GINST) on oxidative stress and antioxidant activities in major organs of aged rats," *Experimental Gerontology*., 47, 1,: 77–84.

Ray, S.; Bagchi ,D.; Lim ,P.M.; Bagchi, M.; Gross ,S.M.; Kothari, S.C.; Preuss, H.G and Stohs, S.J. (2001). Acute and long-term safety evaluation of a novel IH636 grape seed proanthocyanidin extract. *Res Commun Mol Pathol Pharmacol* .,109: 165– 197.

Rehrah, D. ; Ahmedna, M. ;Yu. J. ; Goktepe, I. ; Hurley, S. ; Anner, T. and RaoPatel, A. (2007). Enhanced cholesterol- and triglyceride lowering effect of West African green tea. *J Sci Food Agric.* 87:1323–1329.

Rice-Evans, C. A.; Miller, N. J. and Paganga, G. (1996). Structure-Antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* 20 (7): 933-956.

Roberts, C.K.; Barnard, R.J.; Sindhu, R.K.; Jurczak, M.; Ehdaie, A. and Vaziri, N.D. (2006). Oxidative stress and dysregulation of NAD(P)H oxidase and antioxidant enzymes in dietinduced metabolic syndrome. *Metabolism.* 55: 928–934.

Rodriguez, DE. ; Sotillo, DV. and Hadley M.(2002). Chlorogenic acid modifies plasma and liver concentrations of: cholesterol, triacylglycerol, and minerals in (fa/fa) Zucker rats. *Journal of Nutritional Biochemistry.* 13(12):717–726.[[PubMed](#)]

Ross, R. (1993). The pathogenesis of atherosclerosis: A perspective for 1990s. *Nature.* 362: 801–809.

Samarth, R. and Samarth, M.(2009). Protection against radiation-induced testicular damage in Swiss Albino Mice by *Mentha piperita* (Linn.) *Basic and Clinical Pharmacology and Toxicology.*, 104(4):329–334.

Sanchez-Moreno, C.; Jose, A. ; Larrauri and Saura-Calixto, F. (1999). Free radical scavenging capacity and inhibition of lipid oxidation of lipid oxidation of wine, grape juices and related polyphenolic constituents. *Food Res Int.* 32: 407-412.

Scaglione, F.; Weiser, K. and Allessandria, M. (2001). Effects of the standardized ginseng extracts G115 in patients with chronic bronchitis. *Clin. Drug Invest.*, 21:41- 5.

Scalbert, A.; Morand, C.; Manach, C. and Remesy, C. (2002). Absorption and Metabolism of Polyphenols in the Gut and Impact on Health. *Biomed. Pharmac.*, 56: 276-282.

Sehirli, O.; Ozel, Y.; Dulundu, E.; Topaloglu, U.; Ercan, F. and Sener, G. (2008). Grape Seed Extract Treatment Reduced Hepatic Ischemia-reperfusion Injury in Rats. *Phytother. Res.*, 22: 43-48.

Seun-Ah, Y.; Sang-Kyung, J.; Eun-Jung, L.; Chang-Hyun, L. S. and In-Seon, L. (2010). Comparative study of the chemical composition and antioxidant activity of six essential oils and their components. *Journal of Natural Product Research*. 24(2): 140–151.

Shahidi, F. and Wanasundara, P.K. (1992). Phenolic antioxidants. *Crit Rev. Food Sci. Nutr.*, 32: 67-103.

Schwarz, K. and Ternes, W. (1992a). Antioxidative constituents of *Rosmarinus officinalis* and *Salvia officinalis*. I. Determination of phenolic diterpenes with antioxidative activity amongst tocochromanols using HPLC. *Z Lebensm Unters Forsch.* 195:95–98.

Singletary, K. W. and Rokusek, J.T. (1997). Tissue specific enhancement of xenobiotic detoxification enzymes in mice by dietary rosemary extract. *Plant Foods Hum. Nutr.*, 50(1):47-53.

Smith, C. ; Halliwell, B. and Aruoma, OI.(1992). Protection by albumin against the pro-oxidant actions of phenolic dietary components. *Food Chem Toxicol.* 30:483–489.

Sneha Rai and Sonika Bhatnagar (2017). Novel Lipidomic Biomarkers in Hyperlipidemia and Cardiovascular Diseases: An Integrative Biology Analysis. *OMICS A Journal of Integrative Biology.*, 21. 3

Sokovic´, M. D.; Vukojevic ´, J.; Marin, P. D.; Brkic ´, D. D.; Vajs, V. and Van;Griensvon, L. J. L. D. (2009). Chemical composition of essential oils of Thymus and Mentha species and their antifungal activities. *Molecules.*, 14(1): 238–249.

Song, J.; Akhalaia, M.; Platonov, A.; Kim, H.; Jung, I.; Han, Y. and Yun, Y. (2004). Effects of Polysaccharide Ginsan from Panax ginseng on Liver Function. *Arch Pharm Res.*, 27(5):531-538.

Sotelo-Félix, J. I.; Martinez-Fong ,D.and Muriel,P.(2002). Protective effect of carnosol on CCl4-induced acute liver damage in rats. *Eur. J.Gastroenterol. Hepat.* 14:1001-1006.

Su, D. ; Zhang, R. ; Hou, F. ; Chi, J. ; Huang, F. ; Yan, S. and Zhang, M. (2017). Lychee pulp phenolics ameliorate hepatic lipid accumulation by reducing miR-33 and miR-122 expression in mice fed a high-fat diet. *Food & Function.*, 8: 808–815.

Subbaramaiah, K.; Cole,P.A. and Dannenberg,A.J.(2002). Retinoids and carnosol suppress cyclooxygenase-2 transcription by CBP/p300-dependent and independent mechanisms. *Cancer Res.*, 62 (9):2522–2530.

Teissedre, P. L.; Frankel, E. N.; Waterhouse, A. L.; Peleg, H. and German, J. B. (1996). Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wine. *J. Sci. Food Agric.* 90: 55-61.

Thomas, M.C. and Nestel, P.J. (2007). Management of dyslipidaemia in patients with type 2 diabetes in Australian primary care. *MJA.*, 186 (3): 128-130.

Thomas, P.; Wang, Y.; Zhong, J.; Kosaraju, S.; O'Callaghan, N.J.; Zhou, X. and Fenech, M. (2009). Grape seed polyphenols and curcumin reduce genomic instability events in a transgenic mouse model for Alzheimer's disease. *Mutation Research* 661 (1-2), 25-34.

Tietz, N.W. ; Burtis, C.A. ; Ashwood, E.R. and Saunderson, W.B. (1999) . Text book of clinical chemistry, 3rd Ed. : 809-857.

Tirschwell, D.L.; Smith, N.L.; Heckbert, S.R.; Lemaitre, R.N.; Longstreth, W.T. and Psaty, B.M.(2004). Neurology. Nov 23; 63(10):1868-75.[PubMed] [Ref list).

Tobe, S. W.; P. A. McFarlane and D. M. Naimark. (2002). Microalbuminuria in diabetes mellitus. *Canadian Medical Association.*, 167:499-503.

Trinder, P. (1969) . Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor. *Ann. Clin. Biochem.*, 6: 24-27.

United States Department of Agriculture (USDA). (2008). "*Rosmarinus officinalis*". Germplasm Resources Information Network (GRIN). Agricultural Research Service (ARS), -03-03.

U.S. Department of Health and Human Services (2001), Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), NIH Publishers, :.2-4

Vance, D.E. and Vance, J.E. (2008). Biochemistry of lipids, lipoproteins and membranes (New Comprehensive Biochemistry). Elsevier Science, 5th edition.

Vanderwal, A.C.; Das, P.K.; Bentz; VandeBerg, D.; Vander;Loos, C. M. and Becker, A.E. (1989). Atherosclerotic lesions in Humans: in situ immunophenotypic analysis suggesting an immune mediated response. *Lab. Invest.* 61: 166–70.

Vattem, D.; Ghaedian, R. and Shetty, K. (2005). Enhancing health benefits of berries through phenolic antioxidant enrichment: focus on cranberry. *Asia Pacific Journal of Clinical Nutrition*, 14(2), 120-130.

Vinson, J. A.; Teufel, K. and Wu, N. (2001). Red wine, dealcoholised red wine, and especially grape juice, inhibit atherosclerosis in hamster model. *Atherosclerosis*. 156: 67–72.

Wang, H.; Cao, G. and Prior, R. L. (1996). Total antioxidant capacity of fruits. *J. Agric. Food Chem.* 44: 701-705.

Wang, S. Y. and Lin, H.S. (2000). Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *J. Agric. Food Chem.* 48: 140-146.

Webster, D. (1974). Interaction of bromocresol green with isolated serum Globulin fractions. *International journal of clinical chemistry*. 53: 109-115.

Weinbrenner, T., Fito, M., de la Torre, R., Saez, G.T., Rijken, P., Tormos, C., Coolen, S., Albaladejo, M.F., Abanades, S., Schroder, H., Marrugat, J. and Covas, M.I. (2004). Olive oils high in phenolic compounds modulate oxidative/antioxidative status in men. *J. Nutr.* 134: 2314–2321

Whitney, E. and Rolfes, S.R.(2011). *Understanding Nutrition*. 12th ed. Wadsworth Cengage Learning: Belmont, CA, USA, 134, 214-217, 368-369, 427.

Wilson, P.; Ordovas, J. and Namara, J. (1998). "Clin.Chem." Blackwell–Scientific publication, London, 44: 1224-1232.

William, R.L. and John, M.S. (2010). "Overview of the LDL receptor: relevance to cholesterol metabolism and future approaches for the treatment of coronary heart disease". *J. Receptor Ligand Channel Res.*, 3: 1-14.

Winocour, P.H.; Durrington, P.N.; Ishola, M.; Hillier, V.F .and Anderson, D.C.(1989). The prevalence of hyperlipidaemia and related clinical features in insulin-dependent diabetes mellitus. *QJM* ., 70 (3): 265-276.

Wren ,A.F.; Cleary, M.;Frantz ,C.; Melton, S. and Norris ,L. (2002). 90-day oral toxicity study of a grape seed extract (IH636) in rats. *J Agric Food Chem.*, 50: 2180–2192.

Wright ,T. (2002). Simple Essential Oil Remedies to The Most Common Ailments.C.F. Internet at www.findarticles.com/cf-o/mOHL/4-168/743334691/print.html.

Wu ,Y.N.; Huang, J.; Zuo, A.L. and Yao, L .(2011). Research on the effects of rosemary (*Rosmarinus officinalis* L.) on the blood lipids and antilipid peroxidation in rats. *J Essential Oil Res.*, 23:26–34.

Yamakoshi, J.; Kataoka, S.; Koga, T. and Ariga T. (1999). Proanthocyanidin rich extract from grape seeds attenuates the development of aortic atherosclerosis in cholesterol-fed rabbits. *J Atherosclerosis*. 142:139–149.

Youngken, H.W.(1950). Textbook of pharmacognosy. Blakiston, Philadelphia, PA.

Zafra-Stone, S.; Yasmin, T.; Bagchi, M.;Chatterjee, A.; Vinson, J.A. and Bagchi, D. (2007). Berry anthocyanins as novel antioxidants in human health and disease prevention. *Molecular Nutrition & Food Research*. 51(6): 675-683.

Zern, T.L.; West, K.L . and Fernandez ,M.L .(2003). Grape polyphenols decrease plasma triglycerides and cholesterol accumulation in the aorta of ovariectomized guineapigs. *The Journal of Nutrition.*, 133 (7), 2268-2272.

Zern, T.L.; Wood, R.J.; Greene, C.; West, K.L.; Liu, Y.; Aggarwal ,D.; Shachter, N.S. and Fernandez, M.L . (2005). Grape polyphenols exert a cardioprotective effect in pre- and postmenopausal women by lowering plasma lipids and reducing oxidative stress. *The Journal of Nutrition.*, 135 (8), 1911-1917.

Zhang, C.X.; Ho, S.C.;Chen, Y.M.; Fu, J.H.; Cheng, S.Z. and Lin, F.Y. (2009). Greater vegetable and fruit intake is associated with a lower risk of breast cancer among Chinese women. *International Journal of Cancer.*, 125(1): 181-188.

Zhu, B.T. ; Loder, D.P. ; Cai, M.X. ; Ho, Ch.T. ; Huang, M.T. and Conney, A.H. (1998). Dietary administration of an extract from rosemary leaves enhances the liver microsomal metabolism of endogenous estrogens and decreases their uterotrophic action in CD-1 mice. *Carcinogen.*, 19: 1821-1827.

الخلاصة

هدفت الدراسة الحالية إلى التعرف على تأثير المركبات الفينولية المستخلصة من *Vitis vinifera* و *Rosmarinus officinalis* L في بعض العايرير الدموية والمعايرير البيوكيميائية لأناث الجرذان الطبيعية والمصابة بارتفاع الكولسترول.

استخدمت 60 من اناث الجرذان الناضجة تراوحت اوزانها ما بين (180-210) غم، اما اعمارها فكانت بين (10-12) اسبوعا، فترة التجربة كانت 30 يوم، في البيت الحيواني قسم علوم الحياة / كلية العلوم / جامعة ذي قار في ظل الظروف القياسية لدرجة الحرارة، الضوء، التغذية وتتضمن الدراسة تجربتين رئيسيتين:

التجربة الأولى:- (36) من الجرذان الناضجة تم تقسيمهن عشوائيا إلى ست مجموعات متساوية، كل مجموعة تتكون من (6) جرذان، تم معاملة المجموعة الأولى (السيطرة السالبة) فمويا مع (0.5 مل / حيوان) من محلول ملحي فسيولوجي طبيعي (0.9% كلوريد الصوديوم)، المجموعة الثانية (مجموعة السيطرة الإيجابية- Hypercholesterolemic، نظام غذائي عالي الدهون)، تم معاملة المجموعتين الثالثة والرابعة (المجموعات الطبيعية) فمويا مع (0.5 مل / حيوان) من (50 و 100) ملغم / كغم من (*Rosmarinus officinalis*) المستخلص الفينولي على التوالي. تم معاملة المجموعتين الخامسة والسادسة (المجموعات المصابة بارتفاع الكولسترول لمدة 30 يوم) فمويا مع (0.5 مل / حيوان) من (50 و 100) ملغم / كغم من (*Rosmarinus officinalis*) المستخلص الفينولي على التوالي. وكانت مدة المعاملة 30 يوما.

التجربة الثانية:- (24) من الجرذان الناضجة تم تقسيمها عشوائيا إلى ست مجموعات متساوية، كل مجموعة تتكون من (6) جرذان، تم معاملة المجموعة الأولى (السيطرة السالبة) فمويا مع (0.5 مل / حيوان) من محلول ملحي فسيولوجي طبيعي (0.9% كلوريد الصوديوم) استخدمت هذه المجموعة في كلا التجربتين، المجموعة الثانية (مجموعة السيطرة الإيجابية- HC، النظام الغذائي عالي الدهون) استخدمت هذه المجموعة في كلا التجربتين، تم معاملة المجموعات الثالثة والرابعة فمويا مع (0.5 مل / حيوان) من (50 و 100) ملغم / كغم (*Vitis vinifera*) الفينول من المستخلص على التوالي. وتم معاملة المجموعات الخامسة والسادسة (المجموعات المصابة بارتفاع الكولسترول لمدة 30 يوم) فمويا مع (0.5 مل / حيوان) من (50 و 100) ملغم / كغم (*Vitis vinifera*) الفينول من المستخلص على التوالي. وكانت مدة المعاملة 30 يوما.

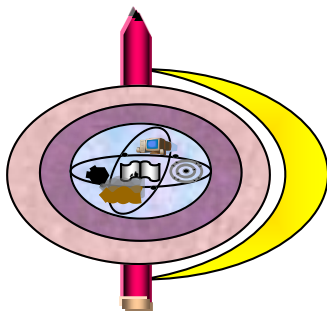
تم تحديد وزن الجسم للحيوانات في جميع المجموعات في اليوم الأول واليوم الأخير من التجربة في نهاية الفترة التجريبية، وتم تشريح ستة مجاميع من كل تجربة وتم جمع عينات الدم وقياس معاملات الدم (Hb, PCV, MCV, MCH, MCHC and WBC)، ومستوى الكولسترول (TC)، الدهون الثلاثية (TG)، البروتينات الدهنية عالية الكثافة (HDL)، البروتينات الدهنية منخفضة الكثافة (LDL)، البروتينات الدهنية منخفضة الكثافة جدا (vLDL)، وعامل التصلب، ومستوى مالونديالدهيد (MDA) سيرولوبلازمين (CP)، الألبومين (alb)، وانزيمات الكبد (ALT و AST).

أظهرت النتائج في الدراسة الحالية زيادة معنوية عند ($P \leq 0.05$) في وزن الجسم في المجموعة الثانية مقارنة مع مجموعة (1)، في حين لم تظهر المجاميع الأخرى فروقا معنوية ($p > 0.05$) مقارنة مع مجموعة السيطرة.

سجلت النتائج انخفاضا معنويا ($P \leq 0.05$) في كل من HB، PCV و WBC في المجموعة الثانية مقارنة مع مجموعة السيطرة بينما لم تظهر هذه المعاملات (MCV, MCH, MCHC) بشكل واضح فروقات معنوية ($p > 0.05$) في جميع المجموعات التي تم معاملتها باستخدام المستخلصات النباتية (بذور العنب والروزماري).

كما لوحظ وجود زيادة معنوية ($P \leq 0.05$) في الكوليسترول والدهون الثلاثية و LDL و VLDL وعامل التصلب، MDA، CP و انزيمات الكبد (AST و ALT) في المجموعة الثانية مقارنة مع المجموعة السيطرة وانخفضت معنويا ($P \leq 0.05$) هذه المعاملات بشكل ملحوظ في جميع المجموعات المعالجة بالمستخلصات النباتية (بذور العنب و روزماري)

في نفس الوقت سجلت النتائج انخفاضا معنويا في HDL و Alb عند مستوى معنوية ($P \leq 0.05$) في المجموعة الثانية مقارنة مع جميع المجموعات، في حين زادت هذه المعاملات معنويا ($P \leq 0.05$) عند معاملة الحيوانات مع مستخلص نبات (بذور العنب وروزماري).



جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة ذي قار / كلية العلوم
قسم علوم الحياة

تأثير المركبات الفينولية المستخلصة من نباتي اوراق
Rosmarinus officinalis اكليل الجبل
Vitis vinifera وبذور العنب
في اناث الجرذان المختبرية عالية الكولسترول

رسالة مقدمة إلى

مجلس كلية العلوم / جامعة ذي قار

كجزء من متطلبات نيل درجة الماجستير في علوم الحياة / فسلجة حيوان

من قبل

يقين ريسان شعلان

بكالوريوس علوم الحياة (جامعة ذي قار)

2015

باشراف

الاستاذ الدكتور

خالد الفرطوسي

2017 م

1439 هـ