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Biochemical studies on the effect of methanolic extract of *Arbutus pavarii* "*leaves*" as an antioxidant in treatment of hypercholesterolemia in albino rats

M. Sc. – Dissertation By

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LIST OF ABBREVIATIONS

ABBREVIATION	The word
Abs	Absorbance.
ADP	Adenosine Diphosphate.
ALP	Alkaline phosphatase.
ALT	Alanine aminotransferase.
AMP	Adenosine monophosphate.
AST	Aspartate aminotransferase.
Ator.	Atorvastatin.
ATP	Adenosine Tri phosphate.
BCG	Bromcresol green.
b.w.	Body weight.
°C	Degree Celsius.
CAT	Catalase.
CHD	Coronary heart disease.
CO_3^{-2}	Carbonate.
Cu	Copper
Cu^{+1}	Copperous
Cu^{+2}	Copperic
Cu-Zn SOD	Copper-zinc SOD.
d.f.	Degree of freedom.
dl	Deciliter.
DNA	Deoxyribonucleic acid.
$DPPH^{\cdot}$	1,1-diphenyl-2-picrylhydrazyl.
E-Coli	Escherichia coli
E.C.SOD	Extra cellular super oxide dismutase.
e.g.	Exampli gratia.
ET	Electron transfer.
FA	Ferulic acid.
Fe (II)	Ferrous.
Fe (III)	Ferric.
$Fecl_3$	Ferric chloride.
Fig.	Figure.

Figs.	Figures.
g	gram.
G-GT	Gama glutamyl transferase.
GPx	Glutathione peroxidase.
GR	Glutathione reductase.
GSH	Reducing glutathione.
GS-SG	Oxidizing glutathione.
h	Hour.
H	Hydrogen.
HAT	Hydrogen atom transfer.
HCL	Hydrochloric acid.
HCD	High cholesterol diet.
HClO	Hypo chlorous acid.
HDL-C	High density lipoprotein- Cholesterol.
HFD	High fat diet.
HMG-CoA	Hydroxy methyl glutaryl- CoA
HQ	Hydroquinone.
HNO_2	Nitrous acid.
H_2O_2	hydrogen peroxide.
$HOO^{\bullet}$	Hydroperoxyl radical.
i.e.	It mean.
iu	International unit.
kg	Kilogram.
$L^{\bullet}$	Lipid centered radical
l	Liter.
LCAT	Lecithin cholesterol acyl transferase.
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein.
$LOO^{\bullet}$	Lipid peroxyl
LPO	Lipid peroxidation.
M	Molar.
MDA	Malondialdehyde.
MDH	Malate dehydrogenase.

Meth. Ext.	Methanolic Extract.
MEAPL	Methanolic extract of Arbutus Pavarii leaves.
mg	Milligram.
min.	Minute.
ml	Milliliter
mM	Milimolar.
Mn	Manganese
Mn-SOD	Manganese-SOD.
Mo	Molybdenum.
Mol	Mole.
MPO	Myeloperoxidase.
mu	Milliunit
µg	Micro gram
µl	Micro liter
n	Nano.
n	Number of measurements.
NACLAR	National Advisory Committee For Laboratory Animal
Na_2CO_3	Sodium carbonate.
NAD^+	Nicotine amide adenine dinucleotide, oxidized.
NADH	Nicotine amide adenine dinucleotide, reduced.
NADPH	Nicotine amide adenine dinucleotide phosphate, reduced.
N.B.	Nota bene.
NH_4^+	Ammonium.
nm	Nanometer.
No.	Number.
NO	Nitric oxide.
$NO^{\cdot}$	Nitric oxide radical.
$NO_2^{\cdot}$	Nitrogen dioxide radical.
4-NPP	4-Nitrophenyl phosphate.
ns	Nano second.
O_2	Oxygen.

$O_2^{\cdot}$	Superoxide anion.
$^{\cdot}OH$	Hydroxyl radical.
$ONOO^-$	Peroxynitrite anion.
ONOOH	Peroxynitrous acid.
P	Probability.
PUFA	Polyunsaturated fatty acid.
Redox	Reduction oxidation reaction.
RNS	Reactive nitrogen species.
PON	Paraoxonase.
$ROO^{\cdot}$	Peroxyl radical.
ROS	Reactive oxygen species.
RPA	Reducing power assay.
rpm	Round per minute.
RSA	Radical scavenging activity.
S	Serum.
S.D.	Standard deviation.
Sec.	Second.
S.E.	Standard error.
SGOT	Serum glutamate oxaloacetate transferase.
SOD	Superoxide dismutase.
TBA	Thiobarbituric acid.
T. C	Total cholesterol.
TFC	Total flavonoid content.
T. G	Triglyceride.
T. lipid	Total lipid.
TPC	Total phenolic content.
U/L	Unite per liter.
UV-visible	Ultra violet-visible.
VLDL-C	Very low density lipoprotein-Cholesterol.
X	Sum of the individual values.
Zn	Zinc.
$(\bar{X})$	Arithmetic mean.
+Ve	Positive.

-Ve	Negative.
$\sum d^2$	Sum of squared deviations of the values of the variant from the arithmetic mean of series.
α	Alpha.
β	Beta.
δ	Sigma.

ABSTRACT

The study was designed to investigate the antioxidant activity of methanolic extract of *Arbutus pavarii* leaves (MEAPL) and its role in treatment the hypercholesterolemia. The phytochemical screening of (MEAPL) indicates the presence of Phenolic and flavonoid compounds. Total Phenolic content and flavonoids were found to be 63 µg/ml of pyrogallol and 66 µg/ml of quercetin which equivalent per 100 µg/ml of (MEAPL) respectively. The antioxidant activity of (MEAPL) ranged from 39% at 100 µg/ml to 85% at 500 µg/ml. The reducing capacity of (MEAPL) found to be 94.5 µg/ml of ascorbic acid equivalent to 100 µg/ml of (MEAPL).

The hypercholesterolemia induced by cholesterol and cholic acid at 3:1 ratio in male albino rats every 24 h for 60 days. The effect of (MEAPL) against hypercholesterolemia in male rats carried out by various types of lipoproteins exist, but the two most abundant are Low-density Lipoprotein (LDL) and High-density Lipoprotein (HDL). Total cholesterol (TC) and triglycerides (TG) in addition to liver marker enzymes were assayed such as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Gama glutamyl transferase (G-GT), Lactate dehydrogenase (LDH) and Alkaline phosphatase (ALP) in addition to proteins and Malondialdehyde (MDA). Antioxidant enzymes activity Superoxide dismutase (SOD), Glutathione reductase (GR), Glutathione peroxidase (GPx) and Catalase (CAT), were measured in control and high fat diet (HFD) rats.

We found that the level of TC, LDL, TG and liver marker enzymes were increased but the level of HDL decreased in HFD rats compared with control rats. Lipid peroxidation determined according to the level of MDA, where the MDA was increased in HFD rats compared with control rats while antioxidant enzymes SOD, GR, GPx and CAT activity were decreased. The study cared out in two groups prophylactic and curative. The results also compared with two doses of (MEAPL) and vitamin C were used as standard antioxidant. The histopathological studies also supported the prophylactic and curative effects of (MEAPL).

The results obtained revealed that these herbs has a prophylactic and curative effect against hypercholesterolemia compared with the reference standard hypercholesterolemia agent ″Atorvastatin″ and standard antioxidant vitamin C.

INTRODUCTION

Many of the chronic diseases that affect human have an uneven geographic distribution. Although the general perception that several diseases, specially the various types of cancer, kidney and liver diseases as well as coronary heart disease (CHD) often result from an exposure to pollutants and toxic environmental such as chemicals, pesticides, fungicides and food additives. The high incidence of CHD is often correlated with high fat, high cholesterol and low fiber diets and also the consumption of fried foods **[1]**.

Cholesterol presents a great health hazard when its consumption is unduly increased. Hypercholesterolemia arteriopathies whether coronary or cerebral take the biggest tool of middle aged and elderly deaths. Intake of fatty diets, lack of exercise, smoking habits and mental stress participate in hypercholesterolemia and the resultant arteriopathies. Atherosclerosis, a chronic inflammatory disease which Is characterized by the accumulation of plasma lipoproteins that carry cholesterol and triglycerides in the arteries, Atherosclerosis results in coronary heart disease (CHD), one of the major cause of morbidity and mortality worldwide **[2]**.

Although the focus of research so far has been mainly on the vascular effects of hyperlipidemia, i.e. arteriosclerosis, it is now quite evident that hyperlipidemia exerts direct effects on the myocardium in addition to the development of atherosclerosis.

Hyperlipidemia is often linked to oxidative/nitrosative stress in the vasculature and in the myocardium. We have previously shown an increased formation of peroxynitrite, a toxic reaction product of superoxide and nitric oxide, in the rat myocardium in cholesterol-enriched diet -induced hyperlipidemia **[3]**.

Strong evidences have been put forward by various investigators for the involvement of free radicals production and lipid peroxidation in the onset of atherosclerosis **[4]**.

One of the initial events in the development of atherosclerosis is the accumulation of cells contained excess lipids within the arterial wall.

Hypercholesterolemia, especially elevated level of serum cholesterol and low-density lipoprotein (LDL), has been implicated in the initiation of atherosclerosis. Furthermore, oxidative stress is also suggested as a mechanism underlying hypercholesterolemia, which is an important factor in atherosclerosis. According to the oxidative modification hypothesis, oxidation of LDL is crucial to the cellular uptake of LDL in the first stages of atherosclerotic plaque development **[5]**.

Large acute doses of chronic exposure to toxic agents can overpower to the antioxidant defense system which cause hepatic cell damage. Natural or synthetic compounds having antioxidant properties can scavenge the free radicals which damage lipid, protein, DNA molecules and cell membrane. Their removals prevent the development of certain diseases **[6]**.

Antioxidants had important role in decreasing serum lipids and retarding atherosclerosis. The observational epidemiological studies have suggested that individuals with high dietary antioxidant intake have lower risks of CHD which remains the leading cause of death in most countries **[7]**.

Diet rich in fruits and vegetables are associated with decreased risk of CHD. Biochemical functions of naturally occurring antioxidants in biological systems such as flavonoids, polyphenols, vitamin C and E have been reported to protect the body system against reactive oxygen species. Antioxidant compounds, various efforts are now concentrated on many herbal plant extracts because of their potential to induce antioxidant effects **[8]**.

Plant products are widely used in testing because of their low toxicity and great medicinal value. Much research has concentrated on different plant extracts' abilities to induce antioxidant effects **[9]**.

This thesis aimed to investigate the potency of methanolic extract of *Arbutus Pavarii* leaves. To achieve this purpose three main experiments were carried out i.e.

(1) The antioxidant activity of methanolic extract of *Arbutus pavarii* leaves.

(2) The prophylactic effect of these herbs against hypercholesterolemia.

(3) The curative effect against hypercholesterolemia.

Experimental induction of hypercholesterolemia was induced in order to study the effect of this plant against hypercholesterolemia.

Review of literature

It is widely accepted that free radicals and related reactive oxygen and nitrogen species, ROS/RNS, play an important role in the pathogenesis of various disorders and diseases, although, like oxygen molecule, they are double edged word and under certain circumstances they exert important physiological functions to protect the host from foreign compounds and to maintain homeostasis. Many lines of evidence suggest involvement of free radicals in the pathogenesis of various diseases and in fact numerous observations have been reported which suggest the correlation between the increase in free radical-mediated oxidation products and the progression of diseases. One of such examples is the oxidation of low density lipoprotein (LDL) and atherosclerosis. Since the first proposal of the LDL oxidation hypothesis for atherosclerosis by Steinberg and his colleagues in 1989, ample evidence has been presented supporting the hypothesis that oxidative modification of LDL is the key initial event for the progression of atherosclerosis **[10]**.

Antioxidants are believed to play a very important role in the body defense system against ROS. Antioxidant is a chemical that delays the start or slows the rate of lipid oxidation reaction. It inhibits the formation of free radical and hence contributes to the stabilization of the lipid specially unsaturated fatty acids. Natural antioxidants are constituents of many fruits and vegetables and they have attracted a great deal of public and scientific attention **[11,12]**.

The most important and well characterized natural antioxidants in the animal body are vitamins (E) and (C). In fact in the body, all antioxidants are working in concert as a team, the (antioxidant system), responsible for prevention of the damaging effects of free radicals and toxic products of their metabolism. However, the antioxidant (team) acts to control levels of free radical formation as a coordinated system where deficiencies in one component impact the efficiency of others **[13]**.

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. Many of these indigenous medicinal plants are used as spices and food plants **[14]**.

Plants are potent biochemists and have been components of phytomedicine since times immemorial. Many plant species have been utilized as traditional medicines but it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs **[15]**.

Herbs and spices have been extensively used as food additives for natural antioxidants. Spices and aromatic herbs are considered to be essential in diets or medical therapies for delaying aging and biological tissue deterioration **[16]**.

The usage of herbal drugs for the treatment of diseases has increased all over the world. According to therapeutic success of modern medicine has increased the usage of alternative medicine including herbal preparations **[17]**.

1. *ARBUTUS PAVARII* Pamp. (*Ericaceae*)

1.1. General

Arbutus pavarii Pamp. (Ericaceae) is one of the endemic species in El-Jabel El-Akhdar. As it described in the Libyan flora **[18]**.

It is an evergreen shrub or small tree, 1.5 to 3 m tall. The bark is reddish brown fissured and peeling in small flakes. The self-fertile flowers are drooping bells approximately 5 cm, generally white. They appear from late October to February. The fruit takes around 8 months to ripen, which is why they are still on the tree when it flowers. The fruits are spherical and warty, and turn from yellow to orange to scarlet as the autumn progresses. These "strawberries" are edible directly as fruits or can be made into jam but the taste is somewhat insipid. The fruits are irregularly with 15-20 mm in diameter and they have many seeds.

The flowers have an attractive scent like honey (a good source of nectar and pollen for bees) and the honey, which is locally called "ASHMARI", is very expansive and widely used for medicinal purposes. It is used for relief and protects against a number of diseases in folk medicine, in addition to the plant leaves. Some compounds used in the medicinal industry were isolated from the leaves and stems of *Arbutus pavarii* **[19]**.

1.2. Active constituents of *Arbutus pavarii:*

The methanolic extract of the leaves of *Arbutus pavarii* were subjected to flash chromatography to yield α-amyrin, lupeol, and oleanic acid. In addition to catechin, quercitin-3-o-rhamnoside, isoquercitrin, myricetin, ferulic acid and arbutin **[20]**.

1.3. Phytochemistry of *Arbutus pavarii*:

The medicinal herbs belonging to the subfamily *Ericaceae* are rich sources of antioxidants. The crude extracts can be fractionated in order to obtain highly efficient concentrated antioxidant mixtures that can be used for further purification or provide antioxidant protection from oxidative stress and prevent deterioration of food ingredients. Were the *Arbutus pavarii Pamp* consider as a part of this subfamily **[21]**.

The polar fractions of the extract were further separated using sephadex column to yield catechin, quercitin-3-o-rhamnoside, isoquercitrin, myricetin, ferulic acid and arbutin **[20]**.

1.3.1. Ferulic acid (FA)

Ferulic acid is a natural antioxidant found especially in fruits and vegetables such as tomatoes ,sweet corn, rice bran, etc. FA reduces the risk of chronic diseases including cardiovascular diseases **[22]**.

Ferulic acid (FA), a naturally found phenolic acid, is a potent antioxidant able to neutralize free radicals, such as reactive oxygen species (ROS). Recent studies suggest that FA has antitumor activity against breast cancer **[23]**.

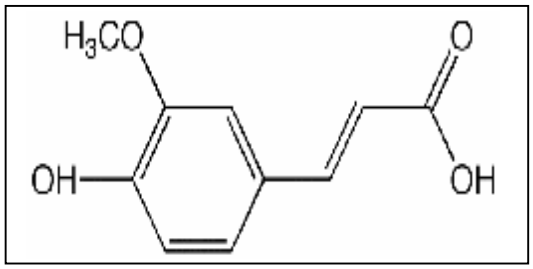


Fig. (1): Chemical structure of Ferulic acid

1.3.2. Arbutin

Arbutin is found in the dried leaves of a number of different plant species. The Arbutin is used in non-prescription medicinal products mainly to treat urinary tract infection, cystitis, kidney stones, and as a diuretic. The active component, arbutin, is converted to hydroquinone (HQ) which has antimicrobial, astringent, and disinfectant properties **[24]**.

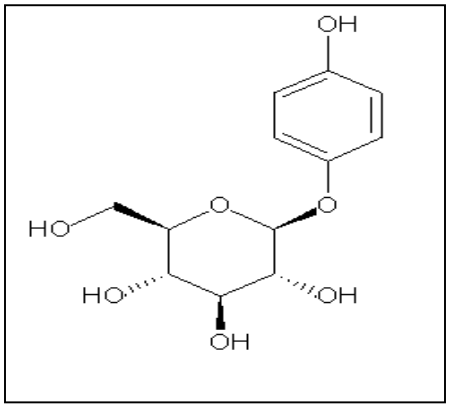


Fig. (2): Chemical structure of Arbutin

1.3.3. Quercetin

Polyphenols such as quercetin, is linked to lower rates of stomach, pancreatic, lung and possibly breast cancer **[25]**.

Some studies indicate that a diet high in flavonoids ,particularly quercetin ,may help prevent blood clots and blocked arteries, significantly reducing the chance of death

from stroke or heart. It should be noted that while most flavonoids and phenolic compounds possess antioxidant properties and are considered safe **[26]**.

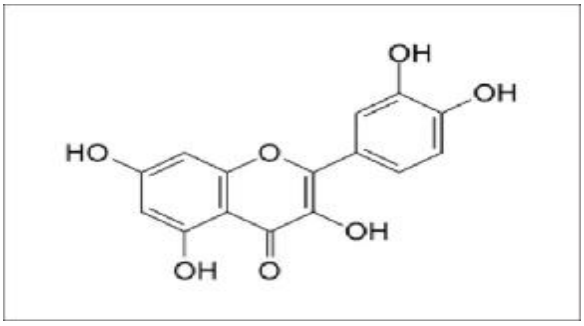


Fig. (3): Chemical structure of Quercetin

1.3.4. Catechin

Catechin in particular, phenolic compounds have been reported to be associated with antioxidative action in biological systems, acting as scavengers of free radicals and several polyphenolic compounds including flavonoids such as catechins **[27]**.

Several observations have shown that catechins have hypocholesterolemic activity in experimental animals. Some studies have found that catechins significantly increases fecal excretion of cholesterol in rats. These observations suggest that catechins reduce plasma cholesterol concentrations by inhibiting cholesterol absorption **[28]**.

In particular, it has been found that polyphenols exert protective effects against the development of cardiovascular diseases **[29]**.

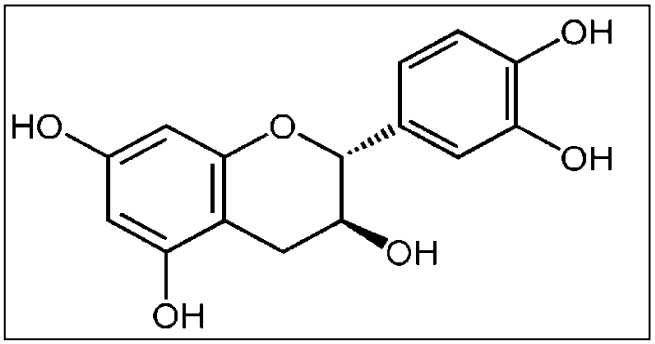


Fig.(4): Chemical structure of Catechin

1.3.5. α-amyrin

Chemical investigations have revealed the presence of α, β-amyrin, a pentacyclic triterpene, and pharmacological studies have revealed anti-inflammatory, antioxidant, gastro protective and hepatoprotective effects.

The cholesterol fractions VLDL and LDL were significantly increased by a high-fat diet. α, β-amyrin (30 and 100 mg/kg) significantly reduced the LDL level and VLDL levels but significantly increased the HDL levels **[30]**.

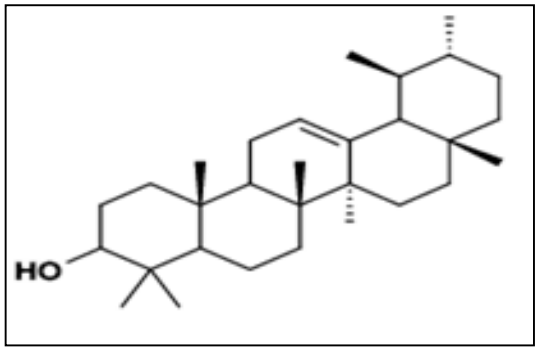


Fig. (5): Chemical structure of α-amyrin

2. Atorvastatin (ATOR)

2.1. Chemistry

[R-(βR,δR)]-2-(4-Fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-1H-pyrrol-1-heptanic acid $C_{33}H_{35}FN_2O_5$ = 558.7 **[31]**.

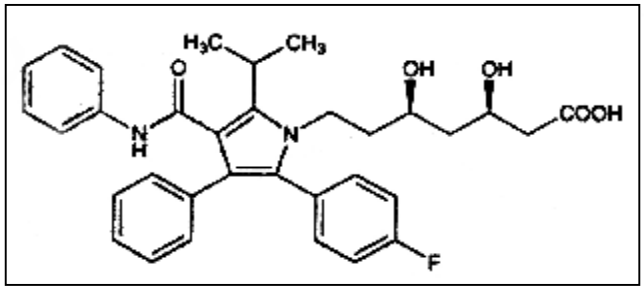


Fig. (6): Chemical structure of Atorvastatin.

2.2. Pharmacokinetics

Atorvastatin is administrated as a calcium salt. It is rapidly absorbed from the gastrointestinal tract after oral administration. It is strongly bound to plasma proteins, and is extensively transformed in the liver to active metabolites. Atorvastatin and its active metabolites are metabolized principally in the liver, where they are excreted primarily in the bile. Atorvastatin has prolonged half-life of about 20 hours, which is thought to play a role in the greater efficacy of Atorvastatin compared with the other statins **[32, 33]**.

2.3. Therapeutic effect

Atorvastatin is a lipid-regulating drug. It is used to reduce LDL-cholesterol, apolipoprotein B, and triglycerides, and to increase HDL-cholesterol in the treatment of hypercholesterolemia **[34]**.

Atorvastatin is an HMG-CoA reductase inhibitor (statin) that lowers plasma cholesterol levels. Statins are first line for the treatment of hypercholesterolemia **[35]**.

2.4. Dose

Normal dose of 10 mg (one tablet) once daily independent of mealtimes is sufficient to control primary hypercholesterolemia and combined hyperlipidemia to give an evident response within 2 weeks with a maximum response within 4 weeks **[36]**.

3. Hypercholesterolemia

Abnormal lipid metabolism is a main cause of dyslipidemia, which is a major risk factor for cardiovascular disease, obesity, cholesthiasis and overall mortality **[37]**.

The concentration of plasma cholesterol can be regulated by cholesterol biosynthesis, removal of cholesterol from the circulation, absorption of dietary cholesterol and excretion of cholesterol via bile and feces. In liver, such lipid accumulation initially results in fatty liver that develops fatty infiltration and in chronic stages results in damage of hepatocytes. It is well known that diet plays an important role in the control of cholesterol homeostasis **[38]**.

Although humans synthesize cholesterol to maintain minimum levels for biological functioning, diet also is known to play a role in serum cholesterol levels. The extent of influence varies significantly from person to person. HDL cholesterol, known as good cholesterol, is an important scavenger of surplus cholesterol by transporting it from cell membrane to the liver, where it is degraded or converted into bile acids. The increase in HDL cholesterol level is known to have protective effect on the risk of coronary heart disease.

A number of mechanisms that regulate the synthesis of cholesterol, uptake and metabolism of cholesterol. HMG-CoA Reductase is a key reaction in the biosynthesis of cholesterol **[39]**.

Hypercholesterolemia occurs when there is an elevated level of total cholesterol in the bloodstream. It is the result of high levels of low-density lipoprotein (LDL) as compared to high-density lipoprotein (HDL) cholesterol. LDL, the 'bad' cholesterol, leaves behind fatty deposits or plaques in the blood vessels. Accumulation of these plaques congests blood vessels and blocks blood supply to the organs. HDL, the 'good' cholesterol, cleans up excess cholesterol from the body, thus minimizing the amount of congestion and blockage. Hypercholesterolemia hardens and narrows blood vessels in various parts of the body, leading to fatal diseases such as chest pains, heart attack and stroke. Blocked blood vessels in the limbs can cause pain, ulcers, infections and gangrene **[39]**.

Hypercholesterolemia is a major risk for coronary artery diseases. In the development of atherosclerosis, ROS are produced by endothelial cells, and macrophages oxidize LDL in the subendothelial space **[40]**.

It was reported that hypercholesterolemia may be responsible for oxidative modification of LDL with excess production of free radicals and lipid peroxidation products **[41]**.

The current hypothesis suggests oxidative stress as an underlying mechanism by which dyslipidemia, particularly hypercholesterolemia, induces tissue damage or provokes several human diseases **[42]**.

A lot of studies have reported that increased aldehydes such as malondialdehyde (MDA) and conjugated dienes are involved in hyperlipidemia-provoked free radical

attacks on membrane lipoproteins and polyunsaturated fatty acids. One of the initial events in the development of atherosclerosis is the accumulation of cells contained excess lipids within the arterial wall **[41,43]**.

4. FREE RADICALS

Free radicals are defined as molecules or molecular fragments that contain one or more unpaired electrons in atomic or molecular orbitals. The occurrence of unpaired electron results in high reactivity and affinity to donate/obtain another electron to attain stability **[44]**.

Accordingly, oxidants are being classified based on their chemical nature (radical or non radical), production site and reactivity towards biological targets. These free radicals being very reactive can potentially damage the biological architecture either alone or as a source for the generation of more reactive/damaging species **[45]**.

The radicals are likely to take part in chemical reactions, taking electrons from vital components and leaving them damaged. Radicals also play an important role in biochemistry, and many other chemical processes, including human physiology **[46]**.

Oxygen free-radicals as the name implies, contain oxygen, and are highly reactive chemical species with the potential to react with almost every type of molecule in living cells. The highly reactive hydroxyl radical is especially toxic because it can react with proteins, polysaccharides, nucleic acids, and polyunsaturated fatty acids and cause alteration of their structure and functions **[47]**.

Reactive oxygen species can be classified into oxygen-centered radicals and oxygen-centered non radicals. Oxygen-centered radicals are superoxide anion, hydroxyl radical, alkoxyl radical, and peroxyl radical. Oxygen centered non radicals are hydrogen peroxide and singlet oxygen **[48]**.

4.1. Reactive Oxygen Species (ROS)

The cellular toxicity of oxygen has been attributed to its affinity in accepting electrons from other molecules. Reactive oxygen species category includes both oxygen radicals/oxygen derived non-radicals that possess high energy oxidizing potential e.g. superoxide anion, hydrogen peroxide, peroxyl and the very reactive hydroxyl radicals.

Most of these free radicals including NO and $ONOO^-$ anion have been considered to play a significant role in the pathogenesis of variety of diseases **[45]**.

4.1.1. Superoxide anion ($O_2^{\cdot -}$)

Superoxide anion is a negatively charged free radical produced by a one electron reduction of molecular oxygen either by autooxidation or by the action of various oxidases. Superoxide exists as $O_2^{\cdot -}$ at physiological pH and as hydroperoxyl ion HO_2^- at lower pH **[49]**. Being very reactive, the hydroperoxyl radical, easily penetrates the biological membrane than the charged form $O_2^{\cdot -}$ and reduces ferric iron.

The most important reaction of superoxide radical is dismutation, where two superoxide molecules undergoes redox reaction, where one gets oxidized to oxygen and the other being reduced to hydrogen peroxide **[50]**.

$$O_2 + 1e^- \xrightarrow{\text{Oxidase}} O_2^{\cdot -}$$

$$O_2^{\cdot -} + 2H^+ \xrightarrow{\text{SOD}} H_2O_2$$

The source for the generation of $^{\cdot}OH$ by reacting with superoxide anion (Haber-Weiss reaction) or with free iron (Fenton reaction) **[51]**.

$$O_2^{\cdot -} + H_2O_2 \longrightarrow O_2 + {}^{\cdot}OH + {}^{-}OH \quad \text{" Haber-Weiss reaction "}$$

$$Fe\ (II) + H_2O_2 \longrightarrow Fe\ (III) + {}^{\cdot}OH + {}^{-}OH \quad \text{" Fenton reaction "}$$

4.1.2. Hydroxyl radicals ($HO^{\cdot}$)

Hydroxyl radicals are extremely energetic, short lived (10^{-9} sec). Being a powerful oxidizing agent, it can react at a faster rate with most organic/inorganic molecules in the cell including DNA, protein, lipids, amino acids and metals. Production of $^{\cdot}OH$ close to DNA could lead to this radical reacting with DNA bases or the deoxyribosyl backbone of DNA to produce damaged bases or strand breaks. Hydroxyl radicals are formed *In vivo* when metal ions such as iron and copper reacts with hydrogen peroxide **[52]**.

4.1.3. peroxyl radicals ($ROO^{\bullet}$)

Peroxyl radicals are high-energy species. The simplest peroxyl radical is the dioxyl (hydroperoxyl) radical $HOO^{\bullet}$, which is the conjugate acid of superoxide, $O_2^{\bullet -}$. The chemistry of this type of molecule varies according to the nature of the R group, the local environment, and the concentration of oxygen and of other reactants **[53]**.

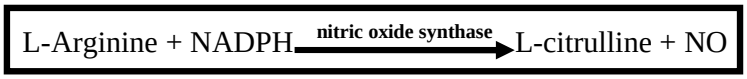
Perhaps the most interesting feature of peroxyl radicals is the diversity of those biological reactions in which they participate **[54]**.

4.2. Reactive Nitrogen Species (RNS)

Overproduction of reactive nitrogen species is called nitrosative stress **[55]**. This may occur when the generation of reactive nitrogen species in a system exceeds the system's ability to neutralise and eliminate them. Nitrosative stress may lead to nitrosylation reactions that can alter the structure of proteins and so inhibit their normal function **[54]**.

4.2.1. Nitric oxide (NO)

Nitric oxide is an important small molecule that is involved in various biochemical pathways such as neurotransmission and the immune response. Under normal conditions, NO is produced by nitric oxide synthase at low concentrations. However, the production of excess amounts of NO can have detrimental effects via the formation of reactive nitrogen species (RNS) such as dinitrogen trioxide (N_2O_3) and peroxynitrite ($ONOO^-$). The formation of these RNS has been considered to be the basis for NO related cytotoxicity in biology. The formation of $ONOO^-$ is particularly important because it can cause both oxidative and nitrosative stress under physiological conditions.



4.2.2. Peroxynitrite anion ($ONOO^-$)

Peroxynitrite anion can be produced biologically at levels high enough to play a significant role in $NO^{\cdot}$-dependent pathology. Because peroxynitrite is a transient species with a biological half-life even shorter than that of $NO^{\cdot}$ **[56]**.

The formation of $ONOO^-$ is particularly important because it can cause both oxidative and nitrosative stress under physiological conditions. The protonation of peroxynitrite generates peroxynitrous acid (ONOOH) **[57]**.

$$NO^{\cdot} + O_2^{\cdot -} \longrightarrow ONOO^-$$

$$ONOO^- \longrightarrow ONOOH$$

The damage of the tissues that occurs through a number of biochemical mechanisms, all of which have in common the formation of highly reactive compounds "free radical" that can oxidize protein, lipid, and nucleic acids. According to the imbalance between these reactive molecule "Reactive species" and defense system of the body i.e. "Antioxidant" the oxidative stress occurs **[58]**.

4.3. OXIDATIVE STRESS:

Oxidative stress results from an imbalance between excessive formation of reactive oxygen species (ROS) and/or reactive nitrogen species and limited antioxidant defenses.

Oxidative stress has been considered a mechanism involved in the pathogenesis of ischemic heart disease and atherogenesis, in cancer and other chronic diseases, and it also plays a major role in the aging process. Oxidative stress describes various deleterious processes resulting from an imbalance between the excessive formation of reactive oxygen and/or nitrogen species and limited antioxidant defenses **[59]**.

Oxidative stress can result in injury to all the important cellular components like proteins, DNA and cell membrane lipids, which can cause cell death. Oxidative stress has been demonstrated to be involved in various physiological and pathological processes, including DNA damage, proliferation, and survival. Moreover, there are several experiments and clinical data providing compelling evidence for the involvement of oxidative stress in large number of pathological states including carcinogenesis **[60]**.

There is substantial evidence that oxidative stress is a causative or at least ancillary factor in the pathogenesis of major neurodegenerative diseases, including Parkingson and Alzheimer disease **[61]**.

Postprandial oxidative stress has been described in postprandial hyperglycemia and/or hyperlipidemia and is associated with a higher risk for atherosclerosis, diabetes, and obesity. Additionally, endothelial function is impaired in the postprandial state of hyperlipidemic and hyperglycemic subjects **[62]**.

Oxidant/antioxidant balance in neutrophils of hyperlipidemic human subjects converts in favor of oxidants; therefore, neutrophils may contribute to the atherosclerotic process due to increased O_2 production **[63]**.

4.4. Free radicals and hypercholesterolemia

Oxidation of LDL may be induced by radicals and enzymes of various cells. The abstraction of hydrogen from fatty acids such as polyunsaturated fatty acids (PUFAs) in LDL particles results in the formation of lipid-centered radicals ($L^{\cdot}$). Not only fatty acids but also apolipoprotein B-100 is modified during LDL oxidation. ($L^{\cdot}$) lead to the formation of lipid peroxyl ($LOO^{\cdot}$) and alkoxyl radicals which in turn can initiate a radical chain reaction in neighboring fatty acids **[64]**.

The oxidation of HDL is inherently capable of exerting anti-atherogenic effect by metabolizing and transporting lipid oxidation products as well as cholesterol from the cells to the liver by virtue of paraoxonase- 1 (PON-1), lecithin-cholesterol acyltransferase (LCAT), phospholipase **[65,66]**.

The oxidation of LDL particle contains several hundred molecules each of phospholipids, cholesteryl esters, and triglycerides together with free cholesterol. LDL oxidation proceeds by multiple mechanisms induced by different oxidants. LDL may be oxidized within artery wall and also in peripheral sites of inflammation, polyunsaturated fatty acids (PUFAs) are quite vulnerable to free radical attack and readily oxidized to give versatile products including aldehydes, which react with lysine and tyrosine in apo-lipoprotein B-100 resulting in their modification and functional loss **[67,68]**.

The oxidation of LDL lipids has been studied extensively and the products of phospholipids and cholesteryl esters have been studied in detail for their levels and roles in atherosclerosis **[69]**.

Polycyclic ring and side chain of cholesterol are also an important substrate and oxidized to give various products named oxysterols **[70]**.

The oxidants responsible for LDL oxidation in vivo have been the subject of extensive studies and arguments. Various compounds have been shown to induce LDL oxidative modification to such a form observed in atherosclerotic lesions. Metals such as iron and copper may be a potent candidate, although they are sequestered by proteins in vivo.

The redox active transition metal ions such as iron and copper contribute to the formation of free radicals and in the initiation of LDL oxidation. It has been observed that LDL is oxidized when incubated with several types of cells such as endothelial cells, neutrophils, and macrophages. Various enzymes may contribute in the oxidation such as myeloperoxidase, NADPH oxidases and nitric oxide synthases. Myeloperoxidase (MPO) together with hydrogen peroxide and chloride ion produces hypochlorite, which is a strong oxidant and oxidizes proteins and lipids by radical and non-radical mechanisms. Although hypochlorite reacts with protein components more rapidly than with lipids **[71]**.

There is now ample evidence showing that MPO modifies both LDL and HDL into pro-atherosclerotic forms. As described above, LDL may be oxidized in vivo by different oxidants by either free radical or non-radical mechanisms. Specific products of lipid oxidation for different oxidants. An increase in lipid oxidation products in atherosclerosis patients and experimental animals has been observed in many studies **[72]**. It is known that the oxidative modification of LDL increases electronegativity of LDL particles. specific products of free radical oxidation, increased with an increase in LDL electronegativity of human LDL, both in absolute concentration and in the ratio relative to respective parent substrate **[10]**.

5. Induction of hypercholesterolemia

hypercholesterolemia is considered to be one of the most widespread disease all over the world. This disease is occurring due to some factors e.g. genetic, fatty diets, smoking habits, alcohols and diabetes. If this disease is left uncontrolled, it leads to a variety of complications which can comprise some of the most important functioning system of the human body.

hypercholesterolemia was attained in rats using cholesterol/cholic acid mixture (3:1) calculated in the basis that each rat was received 0.5g of this mixture/kg b.w daily for 8 weeks in order to accelerate and potentiate the incidence of hypercholesterolemia in adult male albino rats **[73,74]**.

induced hypercholesterolemia in adult albino rats using diet containing 1% cholesterol, 0.25% bile salts and 20% animal fats for 2 months **[75]**.

Reported that supplementation of adult male albino rats with 25% saturated fat, 1% cholesterol and 0.1% cholic acid for 26 days was efficient in producing hypercholesterolemia and hypertriacylglycerolemia in the experimental animals, provoking a significant increase in both serum total cholesterol and triglycerides **[76]**.

6. ANTIOXIDANTS

Antioxidant is a chemical that delays the start or slows the rate of lipid oxidation reaction. It inhibits the formation of free radical and hence contributes to the stabilization of the lipid sample **[77]**.

Naturally, there is a dynamic balance between the amount of free-radicals generated in the body and antioxidants to quench and/or scavenge them and protect the body against their deleterious effects. However, the amounts of these protective antioxidant principles present under the normal physiological conditions are sufficient only to cope with the physiological rate of free radical generation **[78]**.

The ROS affect many cellular functions by damaging nucleic acids, oxidizing proteins, and causing lipid peroxidation and it is important to note that whether ROS will act as damaging, protective or signaling factors depends on the delicate equilibrium between ROS production and scavenging at the proper site and time **[79]**.

Damage to cells caused by free radicals is believed to play a central role in the aging process and in disease progression. Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and well being. The need for antioxidants becomes even more critical with increased exposure to free radicals. Pollution, cigarette smoke, drugs, illness, stress, and even exercise can increase free radical exposure. Because so many factors can contribute to oxidative stress, individual assessment of susceptibility becomes important. Free radical scavenger may prevent the oxidative stress by peroxidation, inhibiting free radicals and also by other mechanism can prevent disease **[80]**.

Due to continuous generation of partially reduced forms of oxygen by constitutive metabolic pathways, a number of protective antioxidant enzyme, such as SOD, CAT, GPx and non- enzymatic antioxidants have involved to deal with toxic species.

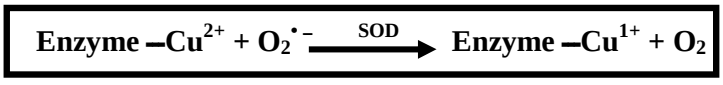
Antioxidant molecules can be divided into different categories based on their functions (enzymatic or nonenzymatic), their physical properties (water-soluble or lipid-soluble), their sources (endogenous or exogenous).

6.1. Enzymatic antioxidants

6.1.1. Superoxide dismutase (SOD)

One of the most effective intracellular enzymatic antioxidants is superoxide dismutase (SOD). Superoxide dismutase is the antioxidant enzyme that catalyzes the dismutation of $O_2^{\bullet -}$ to O_2 and to the less-reactive species H_2O_2 **[81]**.

Superoxide dismutase exists in several isoforms, differing in the nature of the active metal centre and amino acid constituency, cofactors and other features. In humans there are three forms of SOD cytosolic (Cu, Zn-SOD), mitochondrial (Mn-SOD), and extra cellular SOD (EC-SOD) **[82]**.

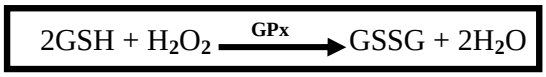


6.1.2. Catalase (CAT)

Catalase is an enzyme presents in the cells of plants, animals and aerobic (oxygen requiring) bacteria. Catalase is located in a cell organelle called the peroxisome. The enzyme very efficiently promotes the conversion of hydrogen peroxide to water and molecular oxygen. One molecule of catalase can convert 6 million molecules of hydrogen peroxide to water and oxygen each minute **[81]**.

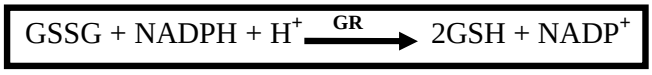
6.1.3. Glutathione peroxidases (GPx)

Localized to the cytoplasm is known to be another H_2O_2 scavenger, are a family of selenocysteine-containing enzymes that can convert H_2O_2 to water **[83]**. via the oxidation of reduced glutathione. Glutathione peroxidase competes with catalase for H_2O_2 and is the major source of protection against low levels of oxidative stress **[84]**.



6.1.4. Glutathione reductase (GR)

An enzyme belonging to the flavoprotein disulfide oxidoreductase family. It reduces GSSG to GSH at the expense of NADPH, which is provided via the glucose monophosphate shunt. The reaction of converting GSSG to GSH is fast and irreversible, thus the enzyme can provide cells with a high intracellular GSH/GSSG ratio **[85]**.



6.2. Non-enzymatic antioxidants

They include lipophilic and hydrophilic antioxidants as illustrated below.

6.2.1. Lipid-soluble antioxidants (Lipophilic)

6.2.1.1. Vitamin E

Vitamin E is a lipophilic antioxidant that scavenge $LOO^{\cdot}$ and inhibits the lipid peroxidation process in biological membranes. Following interaction, tocopherol is converted to tocopherol quinine/ quinone. As with other scavengers, the tocopheryl radical is recycled to its active form by ascorbate and/or GSH **[86]**.

Recent evidence suggests that α-tocopherol and ascorbic acid function together in a cyclic type of process. During the antioxidant reaction, α -tocopherol is converted to an α - tocopherol radical by the donation of a labile hydrogen to a lipid or lipid peroxyl radical. The α-tocopherol radical can thus be reduced to the original α - tocopherol form by ascorbic acid **[86]**.

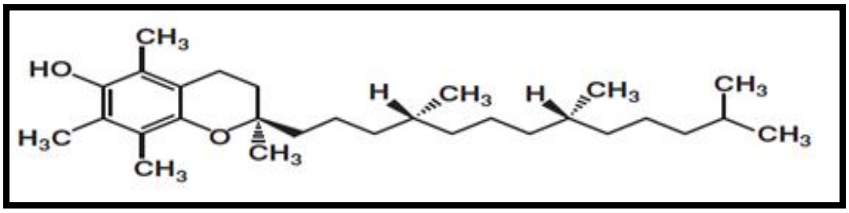


Fig. (7): Chemical structure of Vitamin E

6.2.2.Water-soluble antioxidants

6.2.2.1. Vitamin C

Vitamin C is a water soluble antioxidant synthesized from glucose by plants and few animals. The antioxidant property of ascorbate is attributable to its inherent capability to donate electrons ($2e^-$) and form ascorbyl radical, which is stable and a powerful antioxidant. The radical may lose its electron and gets transformed to dehydroascorbic acid or regenerated to the reduced form either by GSH or NADH **[87]**.

Majority of in vivo studies showed a reduction in markers of oxidative DNA, lipid and protein damage after supplementation with vitamin C. vitamin predominantly reduces in vivo oxidative damage **[88]**.

Recent studies indicate the ability of ascorbic acid to regulate factors that may influence gene expression, apoptosis and other cellular functions **[89]**.

In many studies vitamin C protects against cell death triggered by various stimuli and a major proportion of this protection has been linked with its antioxidant ability **[81]**.

MATERIALS AND METHODS

1. Materials

The following materials were used in the present study

1.1. *Arbutus pavarii* Pamp. (Ericaceae)

The leaves of *Arbutus pavarii*, were collected from Al-Jabal Al Akhdar (wadi el kof) area in Libya during 2012.

The leaves of *Arbutus pavarii* Pamp were dried in laboratory and powder in a mixture grinder. The powder of leaves was extracted by hot continuous extraction, Soxhletion process was used for the extraction of the leaves with methanol. The methanolic extract was evaporated in rotary evaporator.

1.2.Chemicals

1,1-Diphenylpicrylhydrazyl radical (DPPH˙), Atorvastatin were obtained from Sigma Aldrich Company, ascorbic acid, Folin-Ciocalteu reagent, ferric chloride, potassium ferricyanide, monobasic dihydrogen phosphate, dibasic monohydrogen phosphate, trichloro acetic acid ,sodium carbonate, quercetin and pyrogallol obtained from biochemistry laboratory of chemistry department.

1.3. Experimental animals

A total of 66 adult male albino rats weighting 80-100 g were used in this study. Rats were provided from the animal house in faculty of medicine and acclimatized for 2 weeks in the animal house under normal conditions. Animals allowed free access of water and fed on a standard synthetic diet according to **N.A.C.L.A.R**.,2004 **[90]**.

2. Methods

2.1. Preparation of *Arbutus pavarii* Pamp leaves extract

The leaves of *Arbutus pavarii* Pamp were dried in laboratory and powder in a mixture grinder. The powder of leaves was extracted by hot continuous extraction, Soxhletion process was used for the extraction of the leaves with methanol. The methanolic extract was evaporated under vacuum by rotary evaporator.

2.2.Antioxidant activities assays and quantitative analysis.

2.2.1. Total phenolic content (TPC)

Total phenolic was estimated using the colorimetric method based on Folin-Ciocalteu reagent **[91]**. "100,200,300,400,500 µg/ml" of (MEAPL) where 2 ml of (MEAPL) was diluted by was diluted by 2 ml of distilled water and mixed with 600 µl of Folin-Ciocalteu reagent the mixture was allowed to stand for 5 min. and then 2 ml of 20% Na_2CO_3 was added and kept at boiling water bath for 1 minute after cooling the blue colour formed measured at 765 nm by UV-visible spectrophotometer. Quantification was done with respect to stander calibration curve of Pyrogallol the results were expressed as pyrogallol "µg/ml"

2.2.2. Total flavonoids content (TFC)

Aluminum chloride colorimetric method was used for flavonoids determination **[92]**. "100,200,300,400,500 µg/ml" of (MEAPL) where 2 ml of (MEAPL) mixed with 0.1 ml of 10 % aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a UV-visible spectrophotometer. The calibration curve was prepared by preparing quercetin solution in methanol at concentrations "100 to 500 µg/ml".

2.2.3. Reducing power assay (RPA)

This assay was carried out as described previously **[93]**. "100,200,300,400,500 µg/ml" of (MEAPL) where 2 ml of (MEAPL) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide then mixture was incubated in water bath at 50 C^0 for 20 minutes and 2.5 ml of trichloroacetic acid was added to the mixture which was then centrifuged at 3000 rpm for 10 minutes. Finally 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 1 ml $Fecl_3$ and absorbance of Prussian blue colour measured at 700 nm by UV-Visible spectrophotometer. Quantification was done with respect to stander calibration curve of ascorbic acid the results were expressed as ascorbic acid "µg/ml".

2.2.4. DPPH$^{\cdot}$ Radical Scavenging activity (RSA)

Solution of DPPH$^{\cdot}$ (0.2 mM) in methanol was prepared by dissolving 0.008 mg of DPPH$^{\cdot}$ in methanol and volume was made up to 100 ml with methanol. The DPPH$^{\cdot}$ scavenging activity of (MEAPL) was estimated according to **[94]**. After mixing "100, 200,300, 400, 500 µg/ml" of (MEAPL) where 2 ml of (MEAPL) with 2 ml of 0.2 mM DPPH$^{\cdot}$ in methanol was kept in darkness at room temperature for 30 minutes the absorbance of the sample was measured at 517 nm by UV-Visible spectrophotometer. Radical scavenging activity was expressed as percent of inhibition and was calculated using the following formula:-

%DPPH "RSA" = [Abs. of Control - Abs. of Sample / Abs. of Control] x 100

2.3. Induction of hypercholesterolemia

hypercholesterolemia was attained to rats using cholesterol/cholic acid mixture (3:1) and mixed with the synthetic diet in a dose calculated in the basis that each rat was received 0.5g of this mixture/kg b.w. daily for 8 weeks. 10% Saturated fat was used in the diet instead of the corn oil. Also, 50% of sucrose (as source of carbohydrate) was used in the composition of the diet in order to accelerate the incidence of hypercholesterolemia (Table1).

Table (1): Composition of the standard synthetic normal and hypercholesterolemia diet calculated as g/100g diet

Ingredient	Standard diet	hypercholesterolemia diet
Casein	10	10
Corn oil	4	--
Salt mixture[(a)]	4	4
Vitamins mixture[(b)]	1	1
Sucrose	40.4	50
Fiber	40.4	20.8
Choline	0.2	0.2
Cholesterol	--	3
Cholic acid	--	1
Saturated Fat	--	10

(a) Composition of 1kg of salt mixture (American Institute of Nutrition, 1980)

139.3g Sodium Chloride, 0.79g Potassium Iodide, 389g Potassium Dihydrogen Phosphate, 57g Magnesium Sulphate, 381.852g Calcium Carbonate, 27g Ferrous Sulphate, 4.01g Manganese Sulphate, 0.023g Cobalt Chloride, 0.548g Zinc Sulphate, 0.477g Copper Sulphate

(b) Composition of 1kg of vitamin mixture (American Institute of Nutrition, 1980)

2000 I.U. Vitamin A, 200 I.U. Vitamin D, 10 I.U. Vitamin E, 4.0g Nicotinamide, 0.8g Riboflavin, 0.5g Thiamine, 0.5g Pyridoxine HCl, and dextrose to make one Kg, 0.5g Vitamin K, 10.0g p-Aminobenzoic acid, 10.0g Inositol, 4.0g Calcium Pentothionate, 0.2g Folic acid, 0.04g Biotin, 0.003g Vitamin $B_{12\ and}$ dextrose to make one kg.

2.4. Experimental design

2.4.1. The prophylactic effect of different treatments against hypercholesterolemia

To study the protective effect of the methanolic extract of *Arbutus pavarii* leaves against hypercholesterolemia, a total of 30 rats were used and the experiment lasted for 8 weeks. Animals were divided into five groups (6 rats each) as follows:

Group 1: Rats were fed on the standard synthetic diet and served as negative control (- ve) for 8 week.

Group 2: Rats were daily attained to the hypercholesterolemia diet (H.C.D) and served as positive control group (+ ve).

Group 3: Rats were daily administered vitamin C at a dose of 300 mg/Kg b.w. (oral) and hypercholesterolemia diet.

Group 4: Rats were daily administered methanolic extract of leaves of *Arbutus pavarii* at a dose of 300 mg/kg b.w. (oral) and hypercholesterolemia diet.

Group 5: Rats were daily administered methanolic extract of leaves of *Arbutus pavarii* at a dose of 500 mg/kg b.w. (oral) and hypercholesterolemia diet.

2.4.2.The curative effect of different treatments on hypercholesterolemic rats

In this experiment, a total of 36 rats were used. six rats were fed on the standard synthetic diet and served as negative control (- ve) "**group1**". The other rats were subjected to the induction of experimental hypercholesterolemia for 8 weeks as described before and treated for 8 weeks.

The hypercholesterolemia rats (30 rats) were divided randomly into equal 5 sub-groups (6 rats each) as follows:

Group 2: Rats were served as hypercholesterolemic animals (+ ve).

Group 3: Rats were daily received hypercholesterolemia diet and vitamin C at a dose of 300 mg/kg b.w.(oral).

Group 4: Rats were daily hypercholesterolemia diet and received methanolic extract of leaves of *Arbutus pavarii* at a dose of 300 mg/kg b.w. (oral).

Group 5: Rats were daily received hypercholesterolemia diet and methanolic extract of leaves of *Arbutus pavarii* at a dose of 500 mg/kg b.w. (oral).

Group 6: Rats were daily received hypercholesterolemia diet and 1mg/kg b.w. of Atorvastatin as a standard hypolipaemic agent (oral).

2.5. Blood sampling

Blood samples were collected before treatment and then after 4 and 8 weeks. Blood samples were obtained from the animal (in clean and dry centrifuge tubes) the tubes were centrifuged and clear serum and plasma were carefully withdrawn and kept in eppendrof tube for the determination the biochemical parameters.

2.6. Biochemical analysis

2.6.1. Determination of serum total lipids

Serum total lipids were reacted with vanillin in the presence of sulphuric and phosphoric acids. The produced colored complex was measured at 525 nm **[95]**.

2.6.2. Determination of serum total cholesterol

Serum total cholesterol was determined using the enzymatic method Quinone imine was colourimetrically measured at 500 nm. **[96]**.

$$\text{Cholesterol ester} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesteol} + \text{Fatty Acids}$$

$$\text{Cholesterol} \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest} - 4 - \text{en} - 3 - \text{one} + H_2O_2$$

$$2H_2O_2 + 4 - \text{Aminoantipyrine} \xrightarrow{\text{Peroxidease}} \text{Quinoneimine} + 4H_2O$$

2.6.3. Determination of serum HDL-Cholesterol

In the presence of magnesium sulphate and dextran sulphate, water-soluble complexes with LDL, VLDL, and chylomicrons are formed which are resistant to PEG-modified enzymes. The cholesterol concentration of HDL-cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approximately 40%). Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ4-cholestenone and hydrogen peroxide. The color intensity of the blue quinone imine dye formed is directly proportional to the HDL-C. concentration. It is determined by measuring the increase in absorbance at 583nm. **[97]**.

$$\text{HDL-cholesterol esters} + H_2O \xrightarrow{\textit{PEG-cholesterol esterase}} \text{HDL-cholesterol} + \text{RCOOH}$$

$$\text{HDL-cholesterol} + O_2 \xrightarrow{\textit{PEG-cholesterol oxidase}} \Delta 4\text{-cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{HSDA} + H^+ \xrightarrow{\textit{peroxidase}} \text{purple blue pigment} + 4H_2O$$

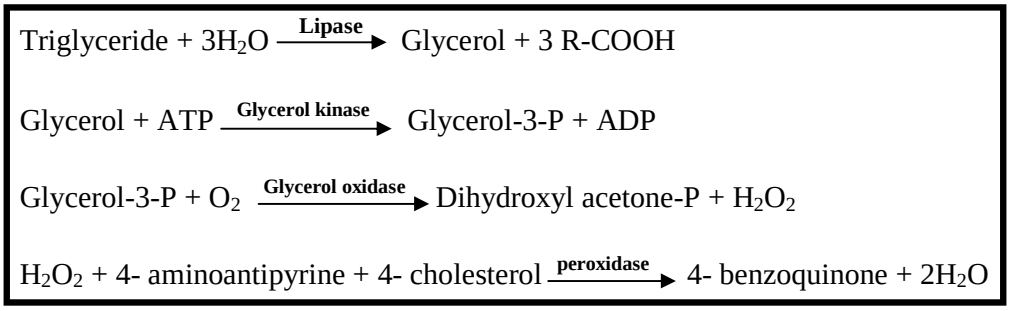
2.6.4. Determination of serum LDL-Cholesterol and VLDL-Cholesterol

LDL and VLDL and chylomicron fractions are precipitated quantitatively by the addition of phosphotungestic acid in presence or magnesium ions after centrifugation the cholesterol concentration in the high density lipoprotein (HDL) fraction **[98]**. which remains in the supernatant, is determined .

- Very low density lipoprotein (VLDL cholesterol) = ————
- LDL cholesterol = Total cholesterol – HDL cholesterol– VLDL cholesterol

2.6.5. Determination of serum triglycerides

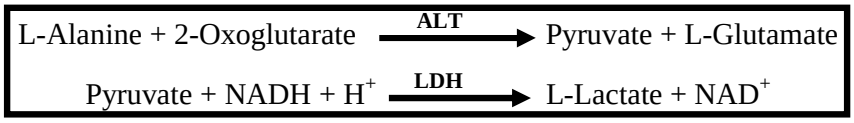
Triglycerides are hydrolyzed by lipoprotein lipase to glycerol and fatty acids. Glycerol is then phosphorylated to glycerol-3-phosphate by ATP in a reaction catalyzed by glycerol kinase. The oxidation of glycerol-3-phosphate is catalyzed by glycerol phosphate oxidases to form dihydroxyacetone phosphate and hydrogen peroxide.



In the presence of peroxidase, hydrogen peroxide effects the oxidation coupling of 4-chlorophenol and 4-aminophenazone to form a red-colored quinone imine dye, which is measured at 512 nm. The increase in absorbance is directly proportional to the concentration of triglycerides in the sample **[99]**.

2.6.6. Determination of serum ALT

ALT catalyzes the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD^+. Pyridoxal phosphate serves as a coenzyme in the amino transfer reaction. It ensures full enzyme activation.



The rate of NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance at 340 nm **[100]**.

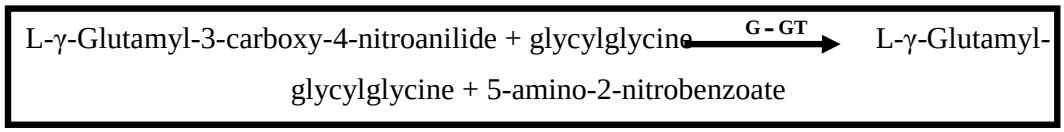
2.6.7. Determination of serum AST

AST in the sample catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH), to form NAD^+. Pyridoxal phosphate serves as a coenzyme in the amino transfer reaction.

The rate of NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance at 340 nm **[101]**.

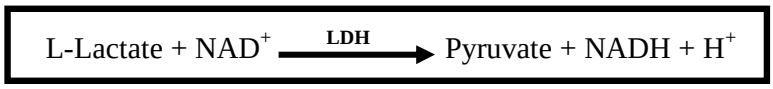
2.6.8. Determination of serum G –GT

The amount of 5-amino-2-nitrobenzoate liberated is proportional to the GGT activity in the sample. It is determined by measuring the increase in absorbance at 409 nm **[102]**.



2.6.9. Determination of serum lactate dehydrogenase

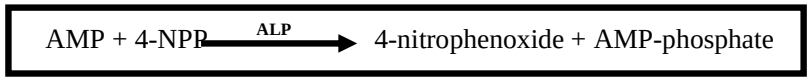
Lactate dehydrogenase catalyzes the conversion of L-lactate to pyruvate; NAD^+ is reduced to NADH in the process.



The initial rate of the NADH formation is directly proportional to the catalytic LDH activity. It is determined by measuring the increase in absorbance at 340 nm**[103]**.

2.6.10. Determination of serum alkaline phosphatase

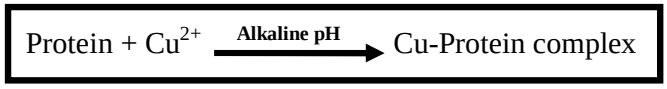
In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleaved by phosphatases into phosphate and p-nitrophenol.



The p-ntirophenol released is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance at 409 nm **[104, 105]**.

2.6.11. Determination of serum total protein

Divalent copper reacts with the peptide bonds of proteins under alkaline conditions to form the characteristic pink to purple biuret complex. Sodium potassium tartrate prevents copper hydroxide precipitation and potassium iodide prevents the autoreduction of copper.



The color intensity is directly proportional to the protein concentration. It is determined by measuring the increase in absorbance at 552 nm **[106]**.

2.6.12. Determination of serum albumin

At pH 4.3, albumin is sufficiently cationic to bind the anionic dye bromcresol green (BCG) to form a blue-green colored complex.

$$\text{Albumin + BCG} \xrightarrow{\text{pH 4.3}} \text{albumin-BCG complex}$$

The intensity of the blue-green color is directly proportional to the concentration of albumin in the sample. It is determined by monitoring the increase in absorbance at 629 nm **[107]**.

2.6.13. Determination of serum blood urea

Urea is hydrolyzed by urease to form ammonium and carbonate. In the second reaction 2-oxoglutarate reacts with ammonium in the presence of glutamate dehydrogenase and the coenzyme NADH to produce L-glutamate. In this reaction two moles of NADH are oxidized to NAD.

$$\text{Urea + 2 } H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_3^{2-}$$

$$NH_4^+ + \text{2-oxoglutarate + NADH} \xrightarrow{\text{GLDH}} \text{L-glutamate + } NAD^+ + H_2O$$

The rate of decrease in the NADH concentration is directly proportional to the urea concentration in the specimen. It is determined by measuring the absorbance at 340 nm **[108]**.

2.6.14. Determination of serum creatinine

In alkaline solution creatinine reacts with picrate to form a yellow-red adduct. The rate of the dye formation is directly proportional to the creatinine concentration in the specimen. It is determined by measuring the increase in absorbance at 512 nm **[109, 110]**.

$$\text{Creatinine + picric acid} \xrightarrow{\text{Alkaline pH}} \text{yellow-red complex}$$

2.6.15. Determination of superoxide dismutase (SOD)

Superoxide dismutase assay (SOD) activity was assayed according the assay relies on the ability of enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye **[111]**.

2.6.16. Determination of malondialdehyde (MDA)

According to Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in acidic medium at temperature of $95C^0$ for 30 min to form thiobarbituric acid reactive product the absorbance of resultant pink product can be measured at 534nm **[112,113]**.

2.6.17. Determination of glutathione reductase (GR)

In method of enzymatic analysis Glutathione reductase catalyses the reduction of glutathione (GSSG) in the presence of NADPH, which is oxidized to $NADP^+$. The decrease in absorbance at 340 nm is measured **[114].**

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

2.6.18. Determination of glutathione peroxidase (GPx)

Glutathione peroxidation (GPx) activity was determined by The assay is an indirect measure of the activity of GPx. oxidized glutathione (GSSG). Produced upon reduction of organic peroxide by GPx, is recycled to its reduced state by the enzyme glutathione reductase (GR**) [115]**.

$$R\text{-}O\text{-}O\text{-}H + 2\ GSH \xrightarrow{c\text{-}GPx} R\text{-}O\text{-}H + GSSG + H_2O$$

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

2.6.19. Determination of Catalase (CAT)

Catalase (CAT) activity was assayed colorimetrically. Catalase reacts with known quantity of H_2O_2. The reaction is stopped after exactly one minute with catalase inhibitor **[116, 117]**.

$$H_2O_2 \xrightarrow{Catalase} 2H_2O + O_2$$

2.7. Histopathological studies.

At the end of experiments, animals in all groups were scarified dislocation. For histopathological studies, liver was removed and fixed in 10% neutral formalin. The slides were coded and were examined by a histopathologist in Al. Noun center. after which photographs were taken.

2.8. Statistical analysis of the data:

The results obtained were statistically analyzed. The probability "P" was deduced from table of "t" test according to the degree of freedom **[118]**.

2.8.1. Arithmetic mean

$(\bar{X}) = \sum \frac{(X)}{n}$ where:

$(\bar{X})$ = Arithmetic mean

(X) = Sum of the individual values of the variant

n = Number of measurements

2.8.2. Standard Deviation (S.D.)

S.D. $= \sqrt{\frac{\sum d^2}{n-1}}$ where

S.D. = standard deviation.

$\sum d^2$ = Sum of squared deviations of the individual values of the variant from the arithmetic mean $(\bar{X})$ of the series.

n = Number of observations

2.8.3. Standard Error (S.E)

S.E = $\sqrt{\frac{\sum d^2}{n(n-1)}}$ where:

S.E. = standard error

$\sum d^2$ = Sum of squared deviations of the values of the variant from the arithmetic mean of series.

n = Number of observation.

"t" calculated = $\frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{\sum d_1^2 + \sum d_2^2}{n_1 + n_2 - 2}\left[\frac{1}{n_1} - \frac{1}{n_2}\right]}}$ where

$\bar{X}_1 - \bar{X}_2$ = difference between the sample means.

$\sum d_1^2 + \sum d_2^2$ = stand for the sum of the squared deviations of the individual values of sample (1) and (2) respectively from their corresponding mean values.

n_1 and n_2 = number of observations in each of the two samples.

The statistical significance can then be assessed by comparing the magnitude of "t" with reference to the theoretical values in students "t" table located at the points of intersection of horizontal row for the degree of freedom (d.f.) of the two samples combined with successive vertical columns representing the gradations in probability levels.

† Insignificant at P> 0.1**;**

* Significant at P<0.05**;**

** Highly significant at P<0.01**;**

*** Very highly significant at P<0.001.

RESULTS

1. Phytochemical screening of methanolic extract of *Arbutus Pavarii* leaves

Based on the absorbance values of the methanolic extract of *Arbutus Pavarii* leaves and compared with the standard solutions of pyrogallol, quercetin and vitamin C as described below.

Results obtained in table (2) and figs. (8, 9) referred to total phenolic content of methanolic extract of *Arbutus Pavarii* leaves where compared with pyrogallol as a standard phenolic compound were each 100 µg/ml of methanolic extract of *Arbutus Pavarii* leaves equivalent to 63 µg/ml. Total flavonoids content also determined in methanolic extract of *Arbutus Pavarii* leaves were compared with Quercetin and as illustrated in table (3) and figs. (10, 11) the flavonoid content in methanolic extract of *Arbutus Pavarii* leaves was 66 µg/ml of Quercetin in each 100 µg/ml.

The reducing capacity of methanolic extract of *Arbutus Pavarii* leaves has been compared with the ascorbic acid according to the results that mentioned in table (4) and figs. (12, 13) were notice each 100 µg/ml of methanolic extract of *Arbutus Pavarii* leaves equivalent to 94.5 µg/ml. The results of the DPPH$^{\bullet}$ radical scavenging activity of methanolic extract of *Arbutus Pavarii* leaves are shown in table (5) and fig. (14, 15).

These results are compared with the well known antioxidant ascorbic acid the percent of inhibition started from 39% at 100 µg/ml to 85% at 500 µg/ml and recorded from 45% at 100 µg/ml to 84% at 500 µg/ml in ascorbic acid.

Table(2): Total phenolic content of methanolic extract of *Arbutus Pavarii* leaves according to vitamin C

Concentration of Pyrogallol "µg/ml"	Mean ± Standard Deviation	Concentration of *Arbutus Pavarii* leaves "µg/ml"	Mean ± Standard Deviation
100	0.481 ± 0.0036	100	0.335 ± 0.0213
200	0.718 ± 0.0085	200	0.525 ± 0.0046
300	0.977 ± 0.011	300	0.707 ± 0.0035
400	1.283 ± 0.0194	400	0.922 ± 0.0068
500	1.462 ± 0.0693	500	1.261 ± 0.0072

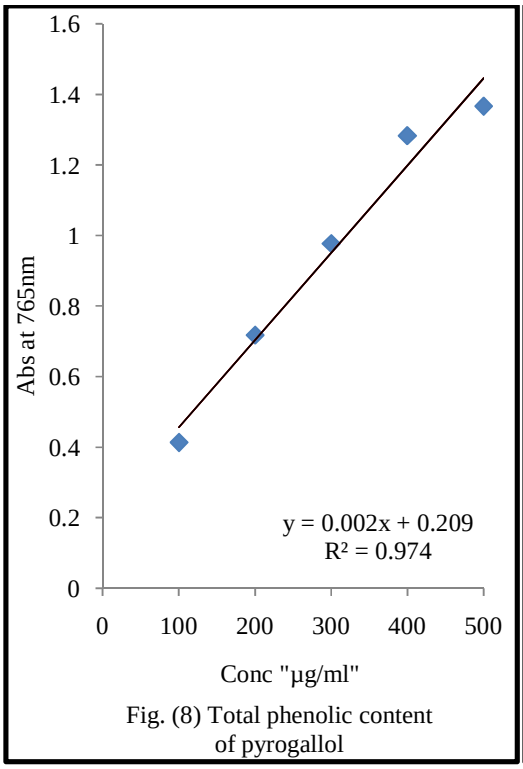


Fig. (8) Total phenolic content of pyrogallol

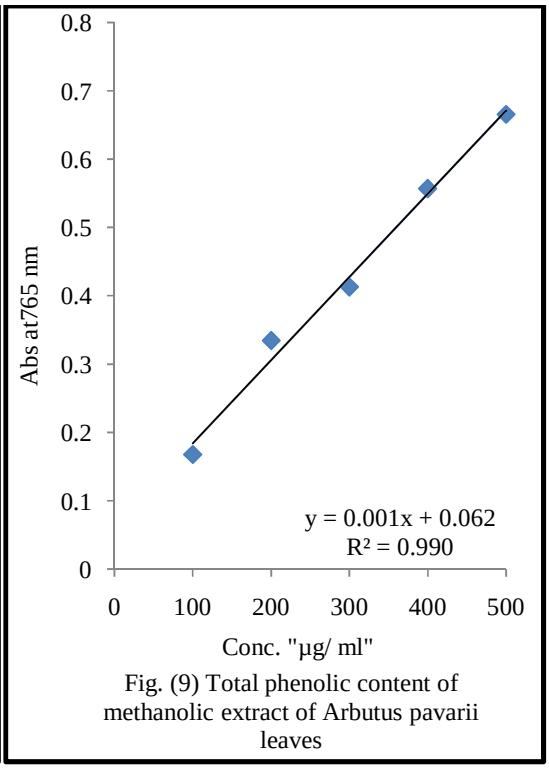


Fig. (9) Total phenolic content of methanolic extract of Arbutus pavarii leaves

Table (3): Total flavonoid content of methanolic extract of *Arbutus Pavarii* leaves according to quercetin.

Concentration of *Arbutus Pavarii* leaves "µg/ml"	Mean ± Standard Deviation	Concentration of quercetin"µg/ml"	Mean ± Standard Deviation
100	0.205 ± 0.055	100	0.307±0.025
200	0.487 ±0.045	200	0.587 ±0.075
300	0.715±0.075	300	0.974 ±0.074
400	0.903 ±0.071	400	1.203 ±0.056
500	1.145 ±0.085	500	1.511 ±0.026

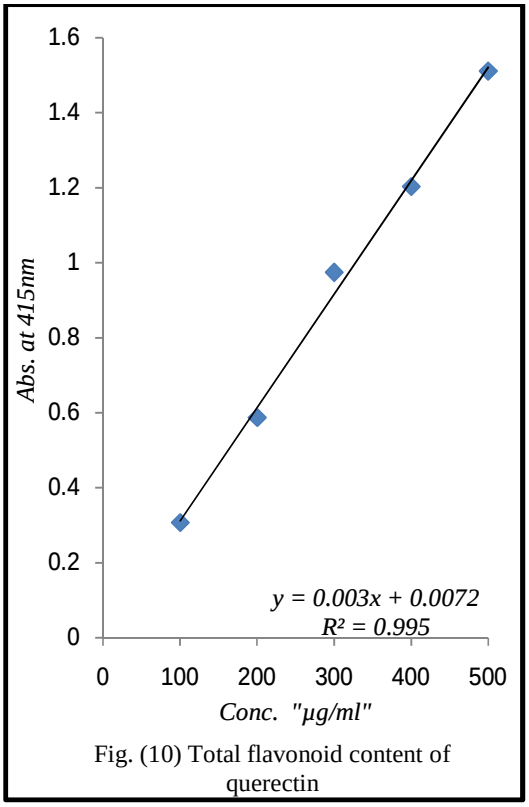


Fig. (10) Total flavonoid content of querectin

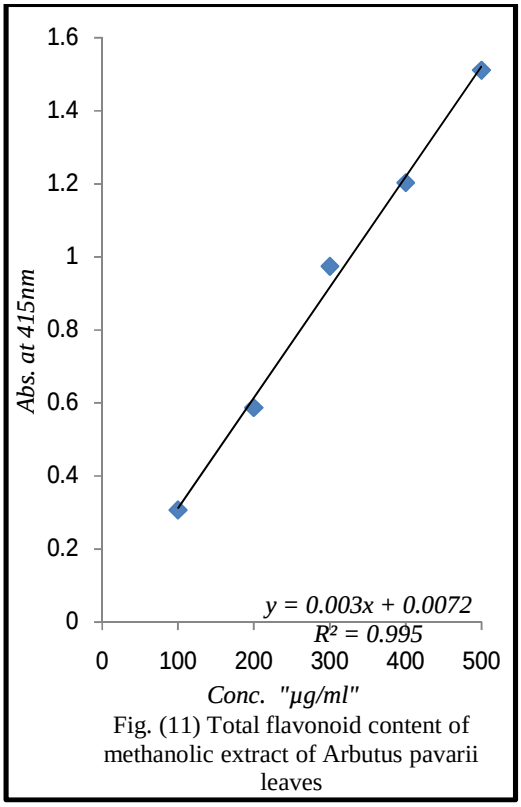


Fig. (11) Total flavonoid content of methanolic extract of Arbutus pavarii leaves

Table(4): Reducing power assay of methanolic extract of *Arbutus Pavarii* leaves according to vitamin C

Concentration of Vitamin C "µg/ml"	Mean ± Standard Deviation	Concentration of *Arbutus Pavarii* leaves "µg/ml"	Mean ± Standard Deviation
100	0.488 ± 0.0125	100	0.466 ± 0.0217
200	0.943 ± 0.0038	200	0.884 ± 0.0173
300	1.247 ± 0.0354	300	1.315 ± 0.0045
400	1.612 ± 0.0166	400	1.738 ± 0.0162
500	2.163 ± 0.0546	500	2.194 ± 0.0198

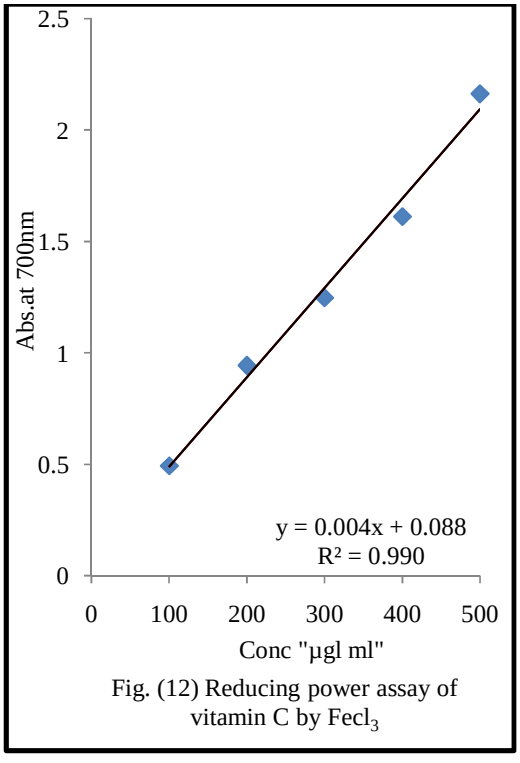


Fig. (12) Reducing power assay of vitamin C by $Fecl_3$

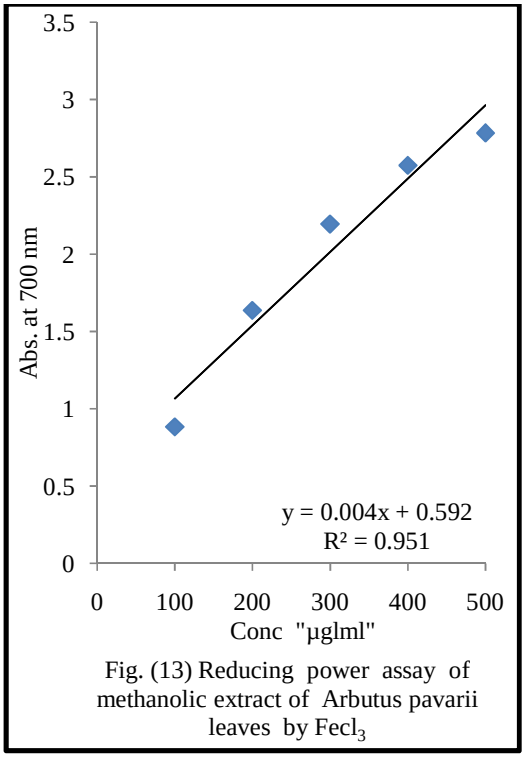


Fig. (13) Reducing power assay of methanolic extract of Arbutus pavarii leaves by $Fecl_3$

Table(5): DPPH decolouration test of methanolic extract of *Arbutus Pavarii* leaves according to vitamin C

Concentration of Vitamin C "µg/ml"	% of inhibition of vitamin C	Concentration of *Arbutus Pavarii* leaves "µg/ml"	% of inhibition of *Arbutus Pavarii* leaves
100	45 %	100	39 %
200	56 %	200	50 %
300	66 %	300	68 %
400	79 %	400	72 %
500	84 %	500	85 %

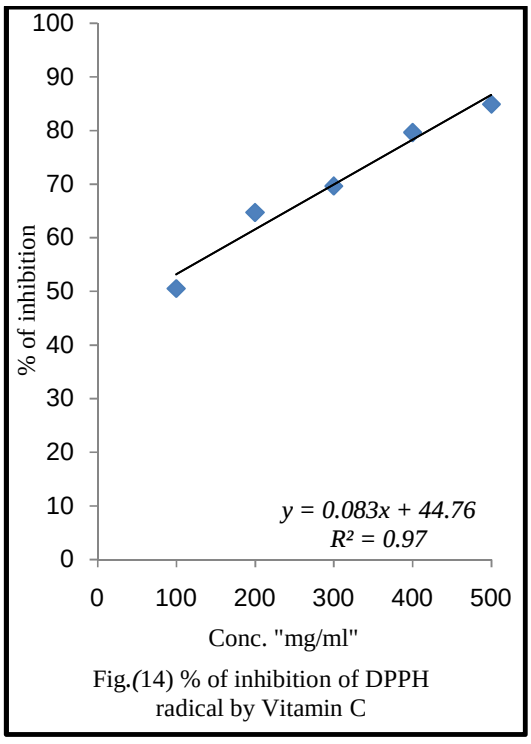


Fig.(14) % of inhibition of DPPH radical by Vitamin C

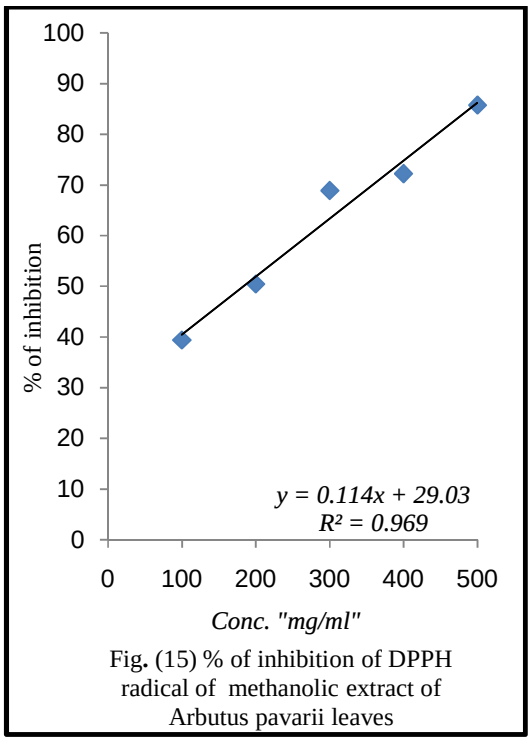


Fig. (15) % of inhibition of DPPH radical of methanolic extract of Arbutus pavarii leaves

2. Effect of different treatments in prophylactic groups:

2.1. Effect of different treatments on serum of T. lipid ,T. Chol. ,HDL-C. , T. Chol./HDL-C. , LDL.C.,VLDL-C .and T.G.

Oral administration of hypercholesterolemic diet significantly increased the activities of the serum of T. lipid, T. Chol., T. Chol./HDL-C., LDL.C., VLDL-C. and T. G. by (117%, 147%, 366.7%, 554%, 122.4 and 122%), respectively. As illustrated in tables (6, 7, 9, 10, 11, and 12), and figs. (16, 17, 19, 20, 21 and 22), but the level of HDL-C. decreased by 46.9% as illustrated in table (8) and fig. (18)

2.2.Effect of different treatments on serum ALT, AST, G-GT, LDH and ALP

The effects of hypercholesterolemic diet on the serum enzymes ALT, AST, G-GT, LDH and ALP, illustrated in tables (13, 14, 15, 16, and 17), and figs. (23, 24, 25, 26, and 27) lead to significantly increased the activities of these enzymes by (148.4%, 128%, 345.2%, 39.3% and 30.5%), respectively.

2.3. Effect of different treatments on serum T. proteins and albumin

The level of total protein and albumin significantly decreased by (35.2%, 45.5%), respectively as illustrated in tables (18, 19) and figs. (28, 29).

2.4. Effect of different treatments on serum blood urea. and creatinine

Serum blood urea and creatinine significantly increased by (33.4%, and 50%), respectively, where noted in tables (20, 21) and figs. (30, 31).

2.5. Antioxidant enzymes in prophylactic group:

After the induction of rats to hypercholesterolemic diet only a significant decrease in the activities of the antioxidant enzymes SOD, GR, GPx and CAT, and, in comparison to the control group by (48.3%, 47.9%, 33%, and 23.4%), respectively in tables (22, 24, 25, and 26), and figs. (32, 34, 35, and 36), but the MDA level shows significant increase by 56% table (23) and fig. (33).

Table (6) : Prophylactic effect of different treatments on S. T. Lipids (mg/dl ± S. D) and % variation from the corresponding control during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Animal groups	Time intervals (Weeks)				
	0	4	% var.	8	% var.
Control	273 ± 15	299 ± 15 †	9.5 ↑	301 ± 16 †	10.2 ↑
Positive control	268 ± 13	423 ± 19 [a], ***	57.8 ↑	581 ± 23 [a], ***	117 ↑
vitamin C (300 mg/Kg.b.w)	266 ± 20	322 ± 17 [b], ***	21.0 ↑	340 ± 21 [b], ***	28 ↑
Meth. Ext. of Arbutus pavarii leaves (300 mg/Kg.b.w)	272 ± 17	355 ± 19 [b], ***	30.5 ↑	315 ± 20 [b], ***	16 ↑
Meth. Ext. of Arbutus pavarii leaves (500 mg/Kg.b.w)	269 ± 18	354 ± 23 [b], ***	31.5 ↑	306 ± 17 [b], ***	14 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with positive group.

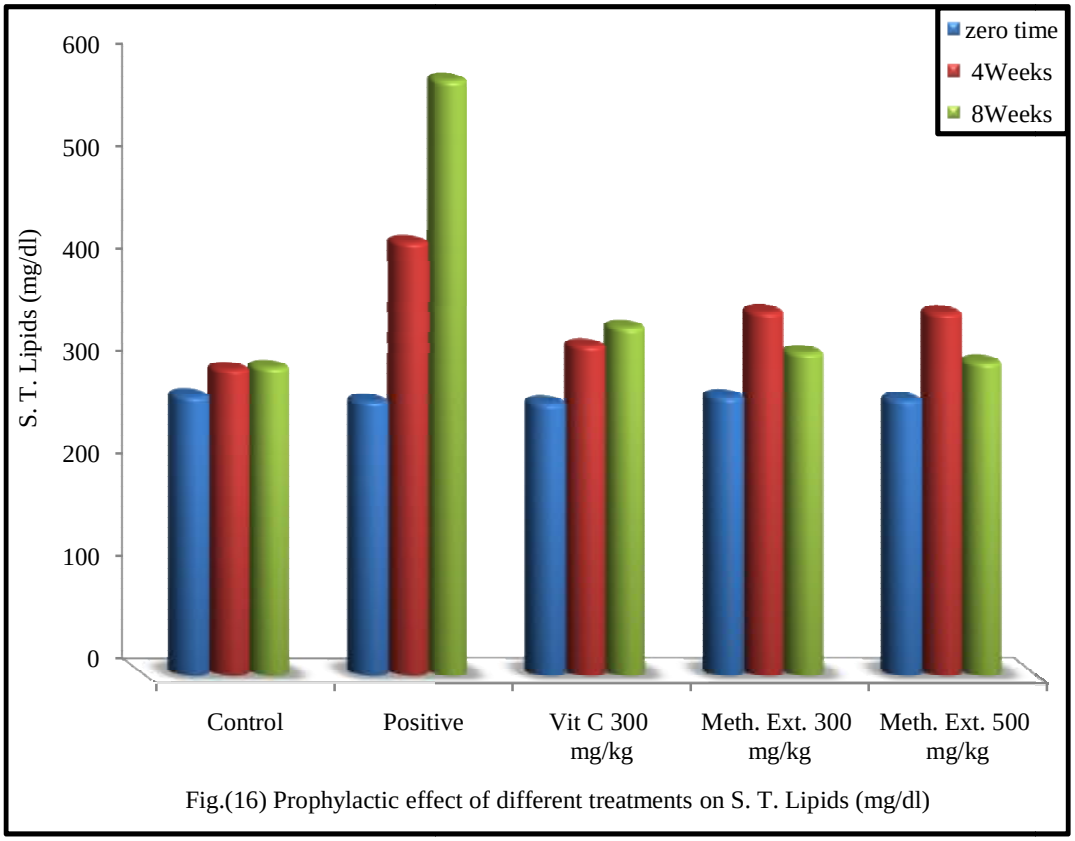


Fig.(16) Prophylactic effect of different treatments on S. T. Lipids (mg/dl)

Table (7): prophylactic effect of different treatments on S. T. Cholesterol (mg/dl ± S.D) and % variation from the corresponding control during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Animal groups	Time intervals (Weeks)				
	0	4	% var.	8	% var.
Control	62.5 ± 10	66.7 ± 12 †	6.7 ↑	54.9 ± 13 †	12 ↓
Positive control	60.8 ± 13	125 ± 15 [a], ***	106 ↑	150.5 ± 9 [a], ***	147 ↑
vitamin C (300 mg/Kg.b.w)	63.8 ± 17	111.6 ± 13 [b], *	75 ↑	82.9 ±13 [b], ***	30 ↑
Meth. Ext. of Arbutus pavarii leaves (300 mg/Kg.b.w)	66.8 ± 11	106.5 ± 12 [b], *	59.4 ↑	79.3 ± 15 [b], ***	19 ↑
Meth. Ext. of Arbutus pavarii leaves (500 mg/Kg.b.w)	65.0 ± 12	100.4 ± 13 [b], **	54.5 ↑	63.5 ±13 [b], ***	2.3 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with positive group.

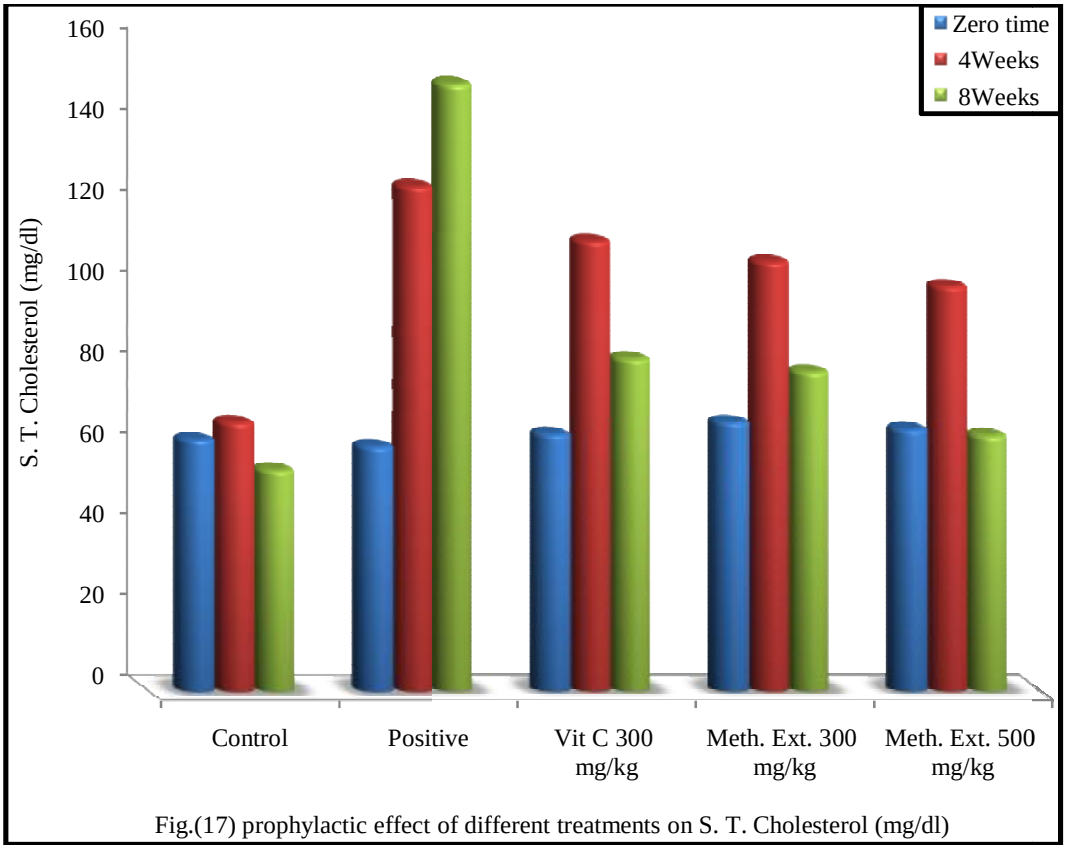


Fig.(17) prophylactic effect of different treatments on S. T. Cholesterol (mg/dl)

Table (8): prophylactic effect of different treatments on S. HDL-Cholesterol (mg/dl ± S.D) and % variation from the corresponding control during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Animal groups	Time intervals (Weeks)				
	0	4	% var.	8	% var.
Control	34.6 ± 8	35 ± 9 †	1.2 ↑	35.3 ± 5 †	2.0 ↑
Positive control	33.7 ± 6	23 ± 7 [a], **	31.8 ↓	17.9 ± 8 [a], **	46.9 ↓
vitamin C (300 mg/Kg .b.w)	34.1 ± 9	26 ± 9 [b], †	24 ↓	30.0 ± 9 [b], *	12.0 ↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/Kg.b.w)	34.8 ± 7	28 ± 5 [b], †	19.5 ↓	32 ± 8 [b], *	8.0 ↓
Meth. Ext. of Arbutus pavarii leaves (500 mg/ Kg.b.w)	36.9 ± 9	30 ± 7 [b], †	18.6 ↓	34.2 ± 9 [b], *	7.3 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with positive group.

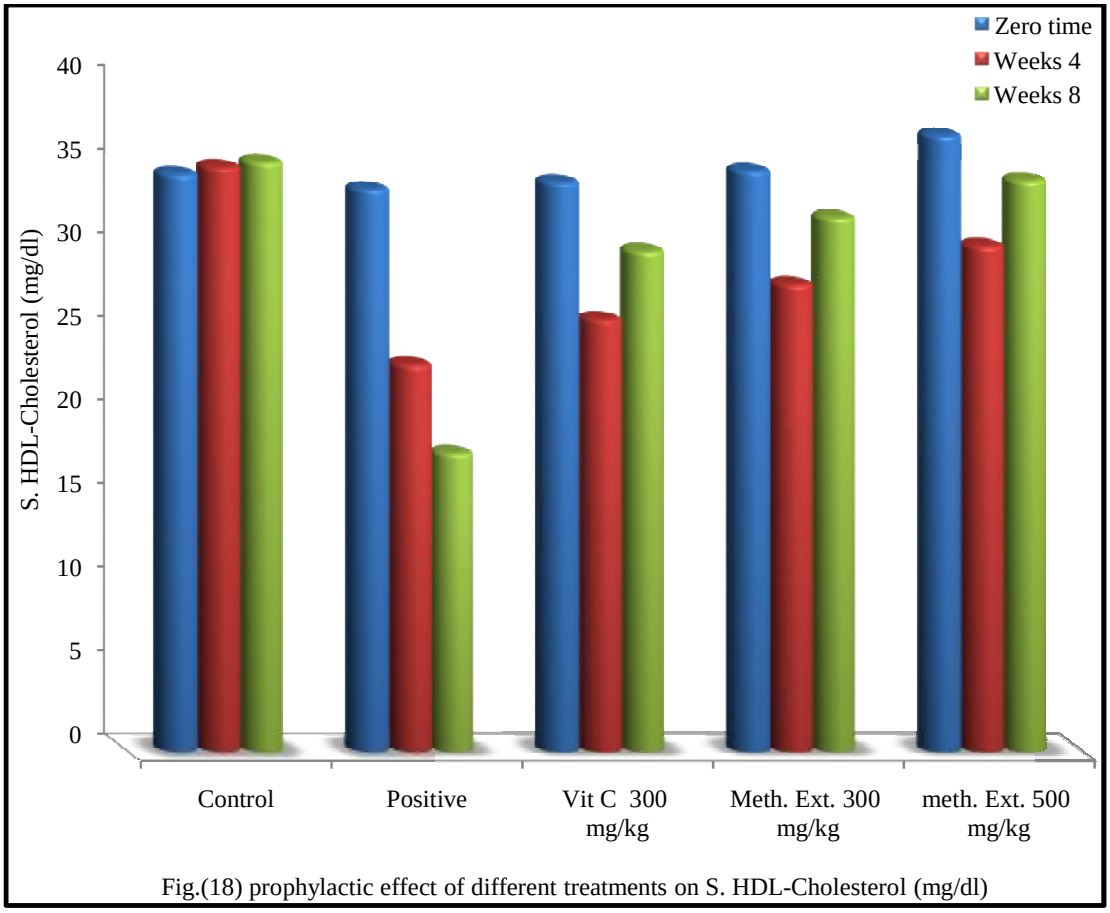


Fig.(18) prophylactic effect of different treatments on S. HDL-Cholesterol (mg/dl)

Table (9): prophylactic effect of different treatments on (T. Chol./HDL. Chol.) (mean value ± S.D) and % variation from the corresponding control during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Animal groups	**Time intervals (Weeks)**				
	0	**4**	**% var.**	**8**	**% var.**
Control	1.81 ± 0.1	1.91 ± 1.2 †	5.5 ↑	1.6 ± 3 †	11.6 ↓
Positive control	1.8 ± 0.4	5.4 ± 2.1 [a], ***	200 ↑	8.4 ± 2.5 [a], ***	366.7 ↑
vitamin C (300 mg/Kg.b.w)	1.87 ± 0.2	4.5 ± 2.2 [b], *	141 ↑	2.8 ± 1.3 [b], ***	49.7 ↑
Meth. Ext. of Arbutus pavarii leaves (300 mg/Kg .b.w)	1.9 ± 0.3	3.8 ± 1.9 [b], *	100 ↑	2.5 ± 1.2 [b], ***	31.6 ↑
Meth. Ext. of Arbutus pavarii leaves (500 mg/Kg.b.w)	1.77 ± 0.2	3.3 ± 1.3 [b], *†	86.4 ↑	1.9 ± 1 [b], ***	7.3 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with positive group.

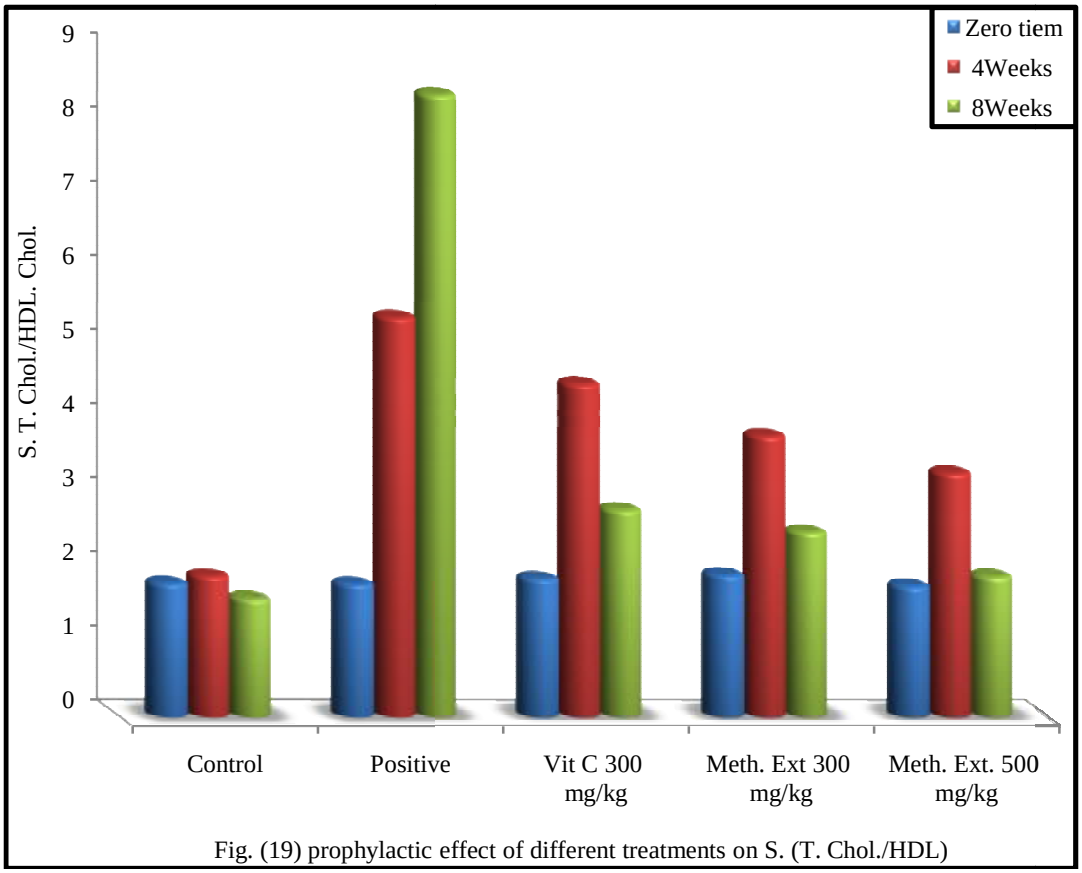


Fig. (19) prophylactic effect of different treatments on S. (T. Chol./HDL)

Table (10): prophylactic effect of different treatments on S. LDL-Cholesterol (mg/dl ± S.D) and % variation from the corresponding control during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Animal groups	Time intervals (Weeks)				
	0	4	% var.	8	% var.
Control	18 ± 8	21.3 ± 10 †	18 ↑	19.14 ± 8 †	6.3↑
Positive control	16.6 ± 5	83.5 ± 15 [a], ***	403 ↑	108.6 ± 10 [a], ***	554 ↑
vitamin C (300 mg/kg .b.w)	19.7 ± 7	74.27 ± 13 [b], *	277 ↑	42.42 ± 13 [b], ***	115.5 ↑
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.g.b.w)	20.8 ± 7	66 ± 12 [b], **	217↑	35.8 ± 8 [b], ***	72.1 ↑
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg.g.b.w)	17.6 ± 9	58.3 ± 13 [b], **	231↑	19.61 ±7 [b], ***	11.4 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with positive group.

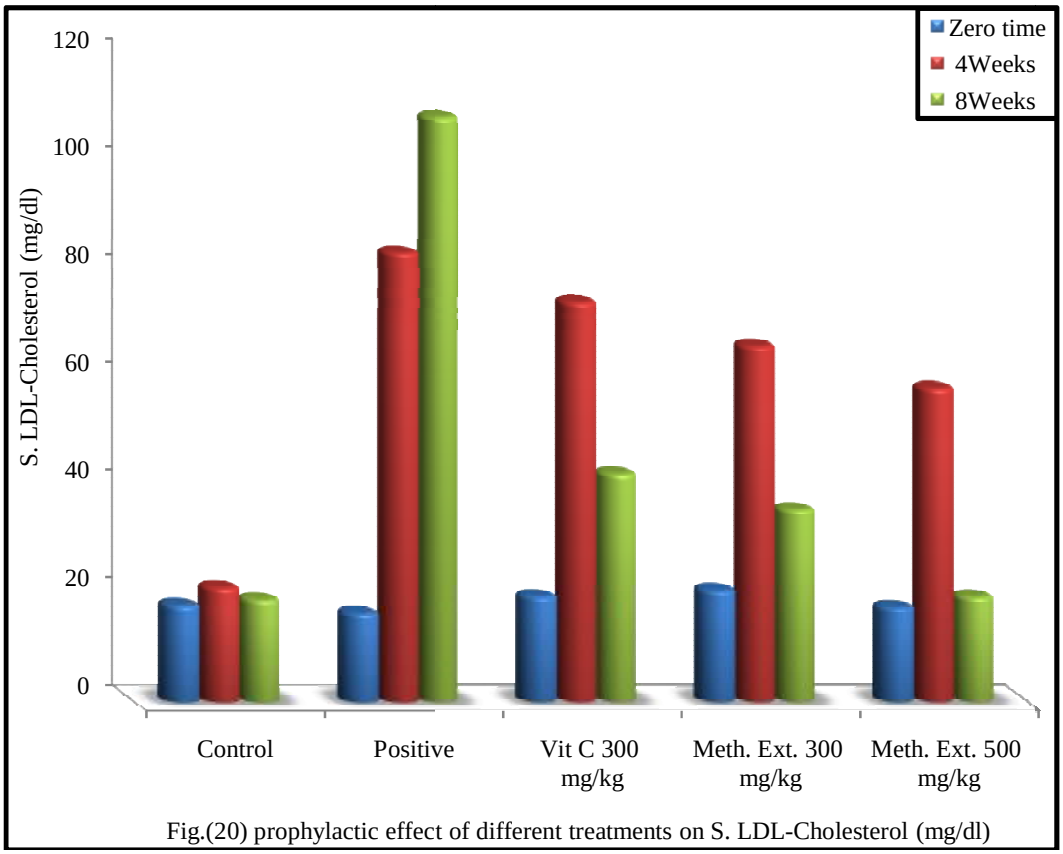


Fig.(20) prophylactic effect of different treatments on S. LDL-Cholesterol (mg/dl)

Table (11): prophylactic effect of different treatments on S. VLDL-Cholesterol (mg/dl ± S.D) and % variation from the corresponding control during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Animal groups	**Time intervals (Weeks)**				
	0	**4**	**% var.**	**8**	**% var.**
Control	10.4 ± 3	10.38 ± 4 †	0.19 ↓	10.46 ± 2 †	0.58 ↑
Positive control	10.8 ± 2	18.4 ± 5 [a], [*]	70 ↑	24.02 ± 6 [a], [**]	122.4 ↑
vitamin C (300 mg/kg.b.w)	10 ± 3	11.4 ± 3 [b], [*]	14 ↑	10.48 ± 3 [b], [*]	4.8↑
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.b.w)	11.16 ± 4	12.4 ± 2 [b], [*]	11.1 ↑	11.52 ± 4 [b], [*]	3.2 ↑
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg.b.w)	10.47 ± 3	11.8 ± 4 [b], [*]	12.7 ↑	9.68 ±1 [b], [*]	7.5 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; [*] Significant difference at P < 0.05; [**] highly sig. difference at P < 0.01; [***] Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with positive group.

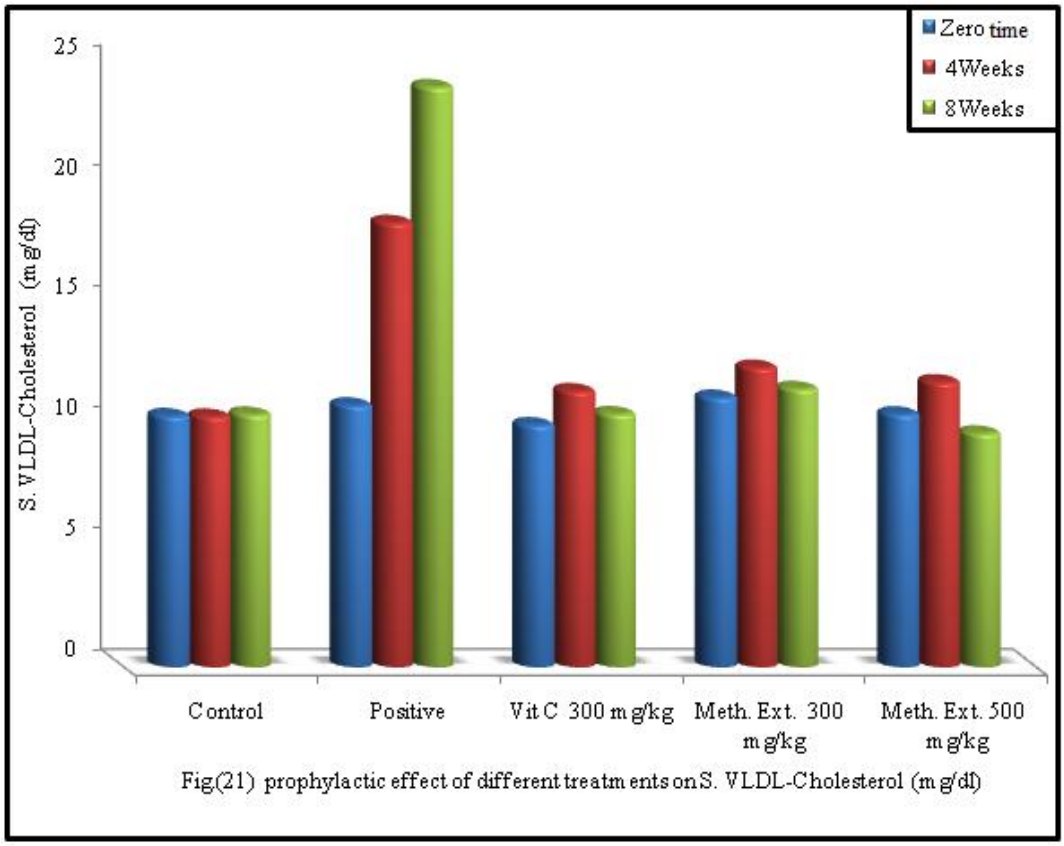


Fig.(21) prophylactic effect of different treatments on S. VLDL-Cholesterol (mg/dl)

Table (12): prophylactic effect of different treatments on S. Triglycerides (mg/dl ± S.D) and % variation from the corresponding control during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Animal groups	**Time intervals (Weeks)**				
	0	**4**	**% var.**	**8**	**% var.**
Control	52.0 ±12	51.9 ± 17 †	0.19 ↓	52.3 ± 8 †	0.58 ↑
Positive control	54.2 ± 10	92 ± 14 [a], ***	69.7 ↑	120 ± 13 [a], ***	122 ↑
vitamin C (300 mg/kg.b.w.)	50.1± 15	56.7 ± 19 [b], ***	13.2 ↑	52.4 ± 15 [b], ***	4.6 ↑
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.b.w.)	55.8 ± 11	62 ± 16 [b], ***	11.1 ↑	57.6 ± 10 [b], ***	3.2 ↑
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg.b.w)	53.7 ± 16	59.2 ± 12 [b], ***	10.2 ↑	48.4 ± 11 [b], ***	10 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with positive group.

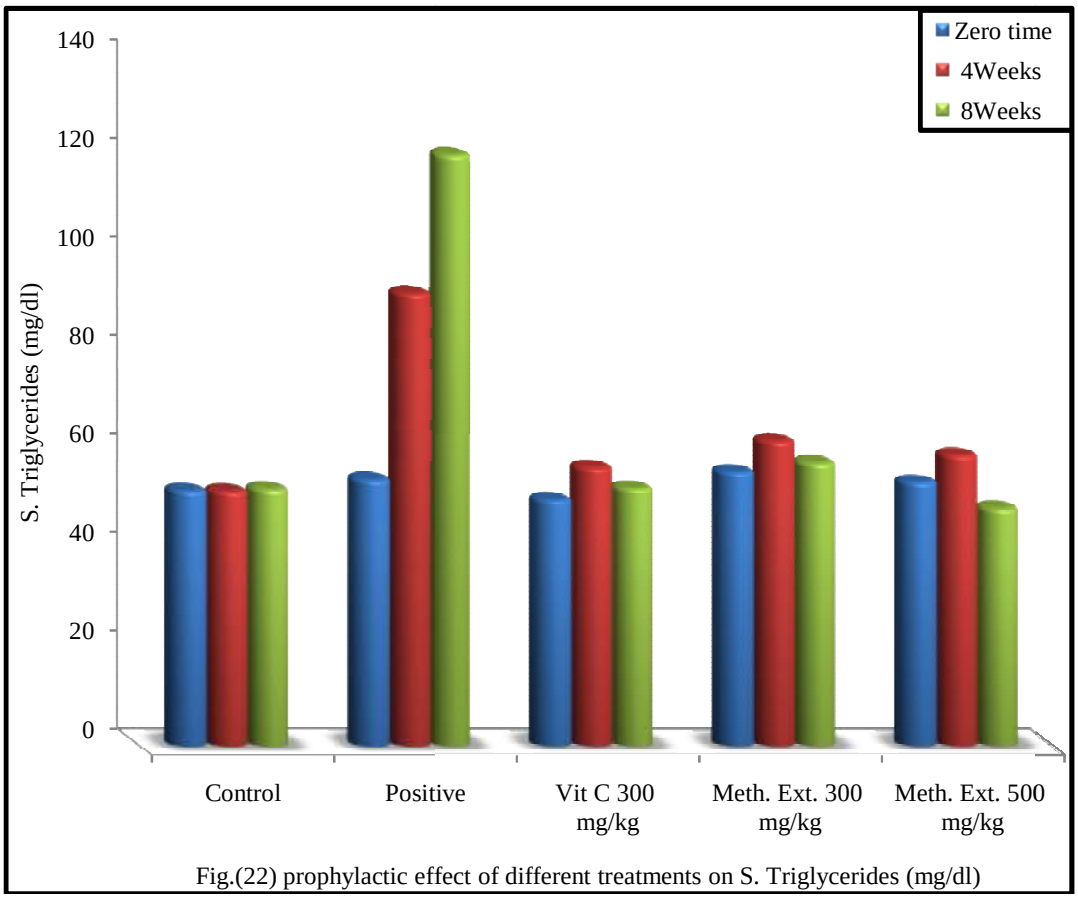
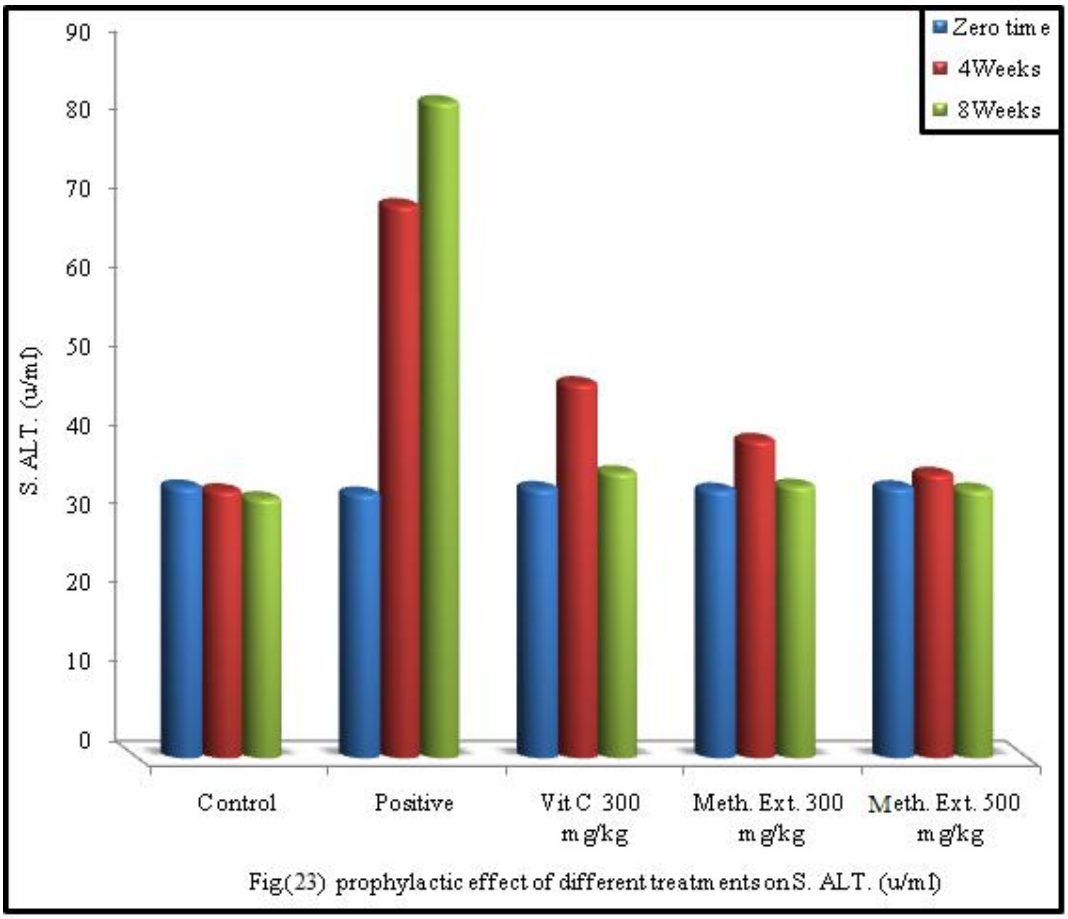


Fig.(22) prophylactic effect of different treatments on S. Triglycerides (mg/dl)

Table (13): prophylactic effect of different treatments on S. ALT. (u/ml ± S.D) and % variation from the corresponding control during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Animal groups	Time intervals (Weeks)				
	0	4	% var.	8	% var.
Control	34.4 ± 10	34 ± 12 †	1.2 ↓	33 ± 11 †	4.4 ↓
Positive control	33.5 ± 12	70 ± 15 [a], ***	109.3 ↑	83.2 ± 13 [a], ***	148.4↑
vitamin C (300 mg/kg.b.w.)	34 ± 11	47.5 ± 13 [b], ***	39.7 ↑	36.2 ± 13 [b], ***	6.5↑
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.b.w)	34.2 ± 12	40.3 ± 10 [b], ***	17.8 ↑	34.5 ± 10 [b], ***	0.88 ↑
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg.b.w)	34.3 ± 13	36 ± 12 [b], ***	4.95 ↑	34 ± 13 [b], ***	0.88 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with paracetamol positive group.



Fig(23) prophylactic effect of different treatments on S. ALT. (u/ml)

Table (14): prophylactic effect of different treatments on S. AST. (u/ml ± S.D) and % variation from the corresponding control during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Animal groups	Time intervals (Weeks)				
	0	4	% var.	8	% var
Control	67.2 ± 8	63.2 ± 11 †	5.9 ↓	65.4 ± 12 †	2.7 ↓
Positive control	65 ± 6	130.4 ± 9 [a], ***	101 ↑	148 ± 9 [a], ***	128 ↑
vitamin C (300 mg/kg)	64.7 ± 9	112.2 ± 13 [b], *	73.4 ↑	94.3 ± 11[b], ***	45.7 ↑
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.b.w.)	63.4 ± 8	117 ± 14 [b], *	84.5 ↑	90.6 ± 14 [b], ***	42.9 ↑
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg.b.w.)	65 ± 12	109 ± 11 [b], **	67.6 ↑	86.7 ± 13 [b], ***	33.2 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with positive group.

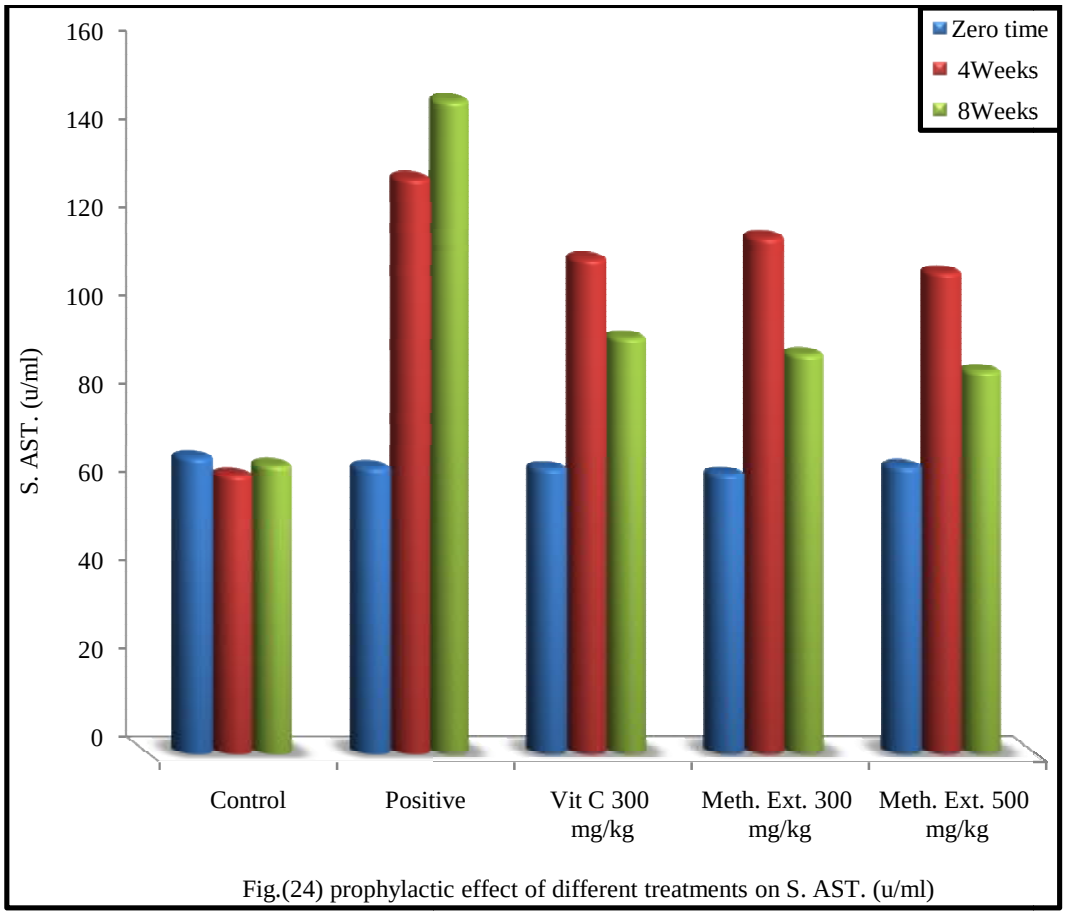


Fig.(24) prophylactic effect of different treatments on S. AST. (u/ml)

Table (15): prophylactic effect of different treatments on Gama-Glutamyl transferase S. GGT (U/L ± S.D) and % variation from the corresponding control during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Animal groups	Time intervals (Weeks)				
	0	4	% var.	8	% var.
Control	3 ± 1.2	3.01 ± 0.10 †	0.33 ↑	2.94 ± 0.8 †	2 ↓
Positive control	3.01 ± 1	11.6 ± 2 [a], ***	285.4 ↑	13.4 ± 3 [a], ***	345.2 ↑
vitamin C (300 mg/kg)	2.87 ± 0.9	8.03 ± 1.8 [b], *	179.8 ↑	6.23 ± 2 [b], **	117.1 ↑
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg)	3 ± 1.3	7.73 ± 2 [b], *	158.5 ↑	6.83 ± 1 [b], **	128.4 ↑
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg)	2.96 ± 0.8	7.23 ± 2 [b], *	144.3 ↑	6.59 ± 2 [b], **	122.6 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with positive group.

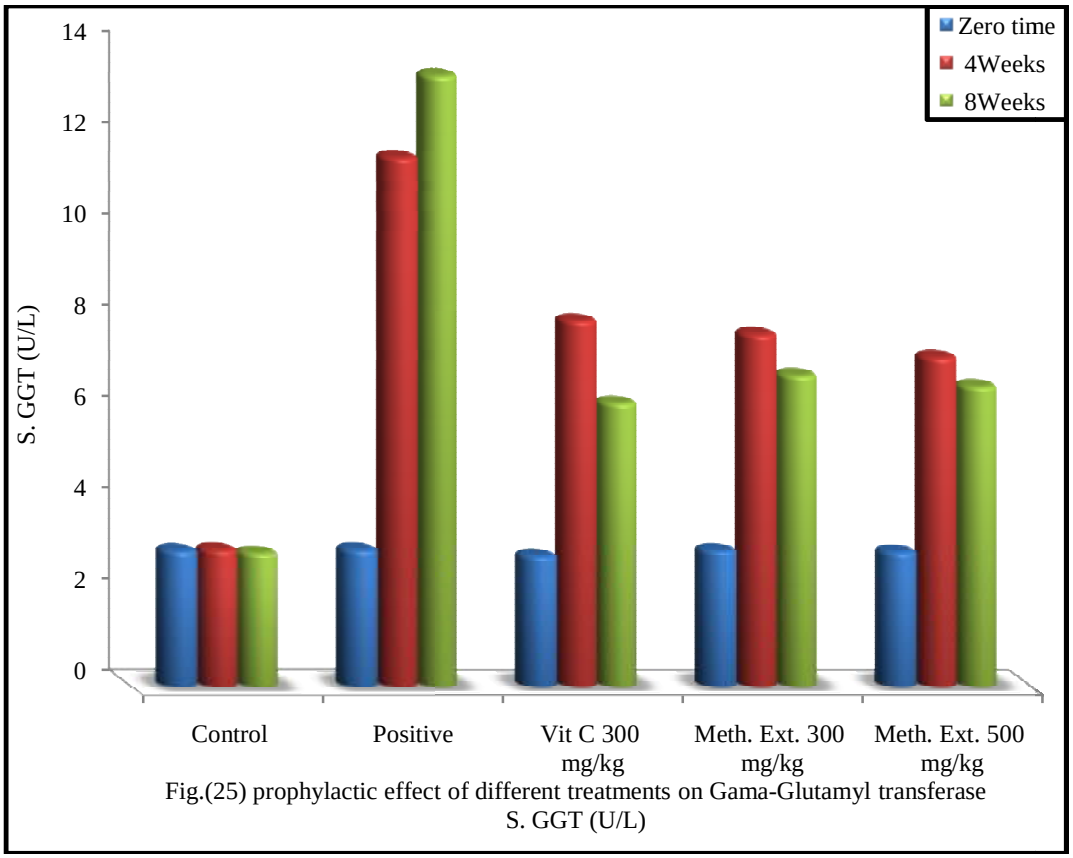


Fig.(25) prophylactic effect of different treatments on Gama-Glutamyl transferase S. GGT (U/L)

Table (16): prophylactic effect of different treatments on S. LDH. (u/l ± S.D) and % variation from the corresponding control during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Animal groups	Time intervals (Weeks)				
	0	4	% var.	8	% var.
Control	620 ± 40	622 ± 66 †	0.3 ↑	627 ± 86 †	1.1 ↑
Positive control	628 ± 50	794 ± 64 [a], ***	26.4 ↑	875 ± 47 [a], ***	39.3 ↑
vitamin C (300 mg/kg)	618 ± 66	700 ± 54 [b], ***	13.2 ↑	670 ± 31 [b], ***	8.4 ↑
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg)	626 ± 61	688 ± 43 [b], ***	9.9 ↑	663 ± 41 [b], **	5.9 ↑
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg)	628 ± 58	670 ± 65 [b], ***	6.7 ↑	645 ± 37 [b], ***	2.7 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with positive group.

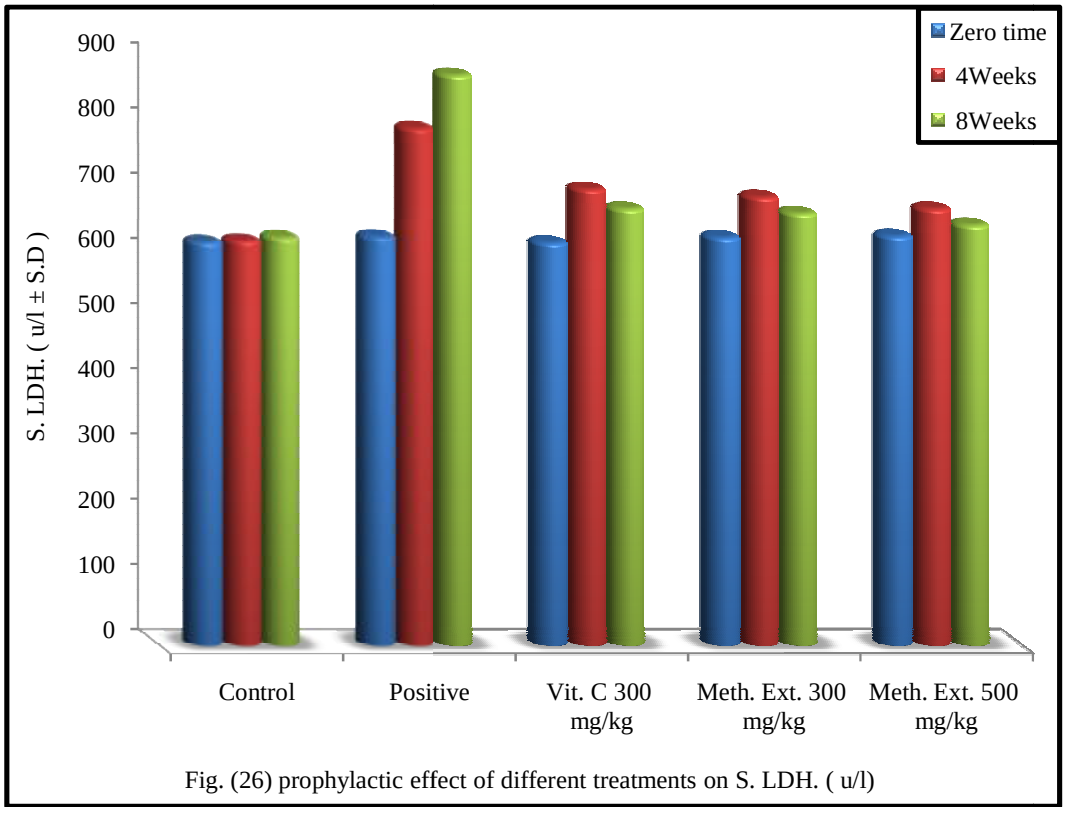


Fig. (26) prophylactic effect of different treatments on S. LDH. (u/l)

Table (17): prophylactic effect of different treatments on S. ALP. (iu/l ± S.D) and % variation from the corresponding control during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Animal groups	**Time intervals (Weeks)**				
	0	**4**	**% var.**	**8**	**% var**
Control	132 ± 20	130 ± 19 †	1.5 ↓	131.4 ± 25 †	0.45 ↓
Positive control	130 ± 16	150 ± 23 [a], **	15.4 ↑	170 ± 22 [a], ***	30.5 ↑
vitamin C (300 mg/kg)	130 ± 17	146 ± 15 [b], †	12.5 ↑	154 ± 28 [b], *	18.6 ↑
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.)	131 ± 22	138 ± 21 [b], *	5.3 ↑	145 ± 18 [b], **	10.5 ↑
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg.)	130 ± 21	135 ± 22 [b], *	3.7 ↑	141 ± 20 [b], **	8.5 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with positive group.

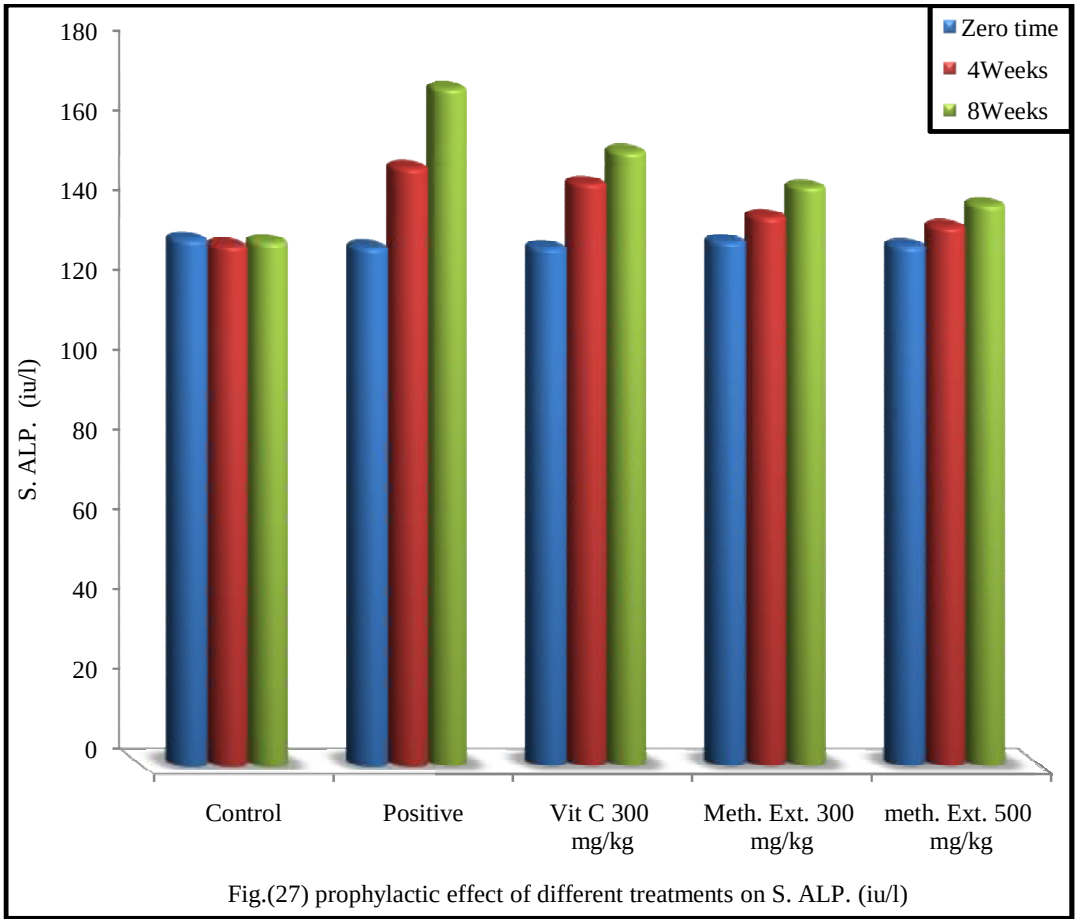


Fig.(27) prophylactic effect of different treatments on S. ALP. (iu/l)

Table (18): prophylactic effect of different treatments on S. T. Proteins (g/dl ±S.D) and % variation from the corresponding control during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Animal groups	**Time intervals (Weeks)**				
	0	**4**	**% var.**	**8**	**% var**
Control	6.1 ± 2	6 ± 1.3 †	1.6 ↓	6.1 ± 2 †	zero ↓
Positive control	6 ± 1.2	4.1 ± 1 a, *	31.8 ↓	3.9 ± 1 a, **	35.2 ↓
vitamin C (300 mg/kg.b.w.)	6 ± 2.4	4.3 ± 1 b, †	28.3 ↓	4.8 ± 1.8 b, †	20 ↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.b.w.)	5.9 ± 1.6	4.7 ± 2 b, †	20.6 ↓	5.15 ± 1 b, *	13 ↓
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg.b.w.)	6 ± 2	4.9 ± 2 b, †	18.5 ↓	5.5 ± 1.3 b, *	8.5 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.

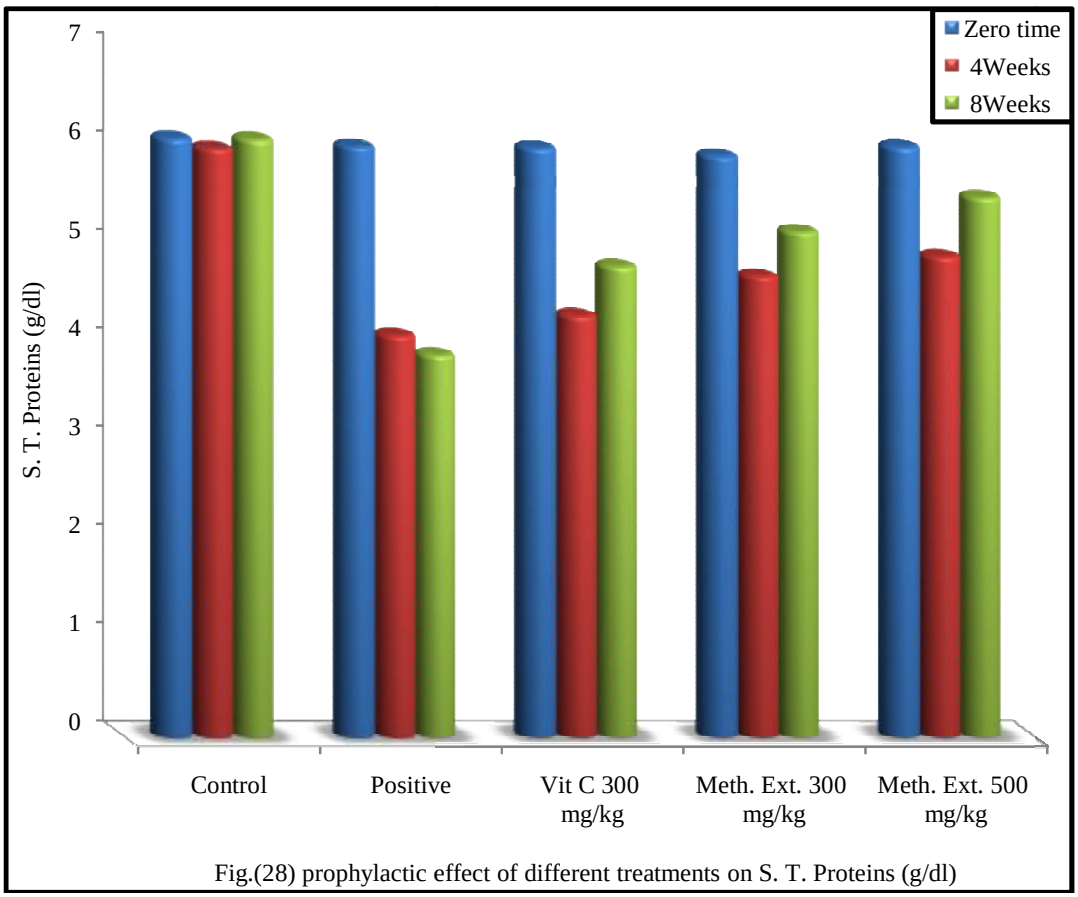


Fig.(28) prophylactic effect of different treatments on S. T. Proteins (g/dl)

Table (19): prophylactic effect of different treatments on S. Albumin (g/dl±S.D) and % variation from the corresponding control during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Animal groups	Time intervals (Weeks)				
	0	4	% var.	8	% var.
Control	5.3 ± 0.43	5.4 ± 0.24 †	1.9 ↑	5.36 ± 0.13 †	1.1↑
Positive control	5.5 ± 0.14	3.5 ± 0.4 [a], **	36.2 ↓	3 ± 0.16 [a], **	45.5 ↓
vitamin C (300 mg/kg.b.w.)	5.4 ± 0.12	3.7 ± 0.33 [b], †	31.6 ↓	4.1 ± 0.1 [b], *	24.6 ↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.b.w.)	5.6 ± 0.8	3.9 ± 0.20 [b], †	30.6 ↓	4.4 ± 0.23 [b], *	21.7 ↓
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg.b.w.)	5.5 ± 0.32	3.9 ± 0.4 [b], †	29.2 ↓	4.5 ± 0.52 [b], *	18.9 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with paracetamol positive group.

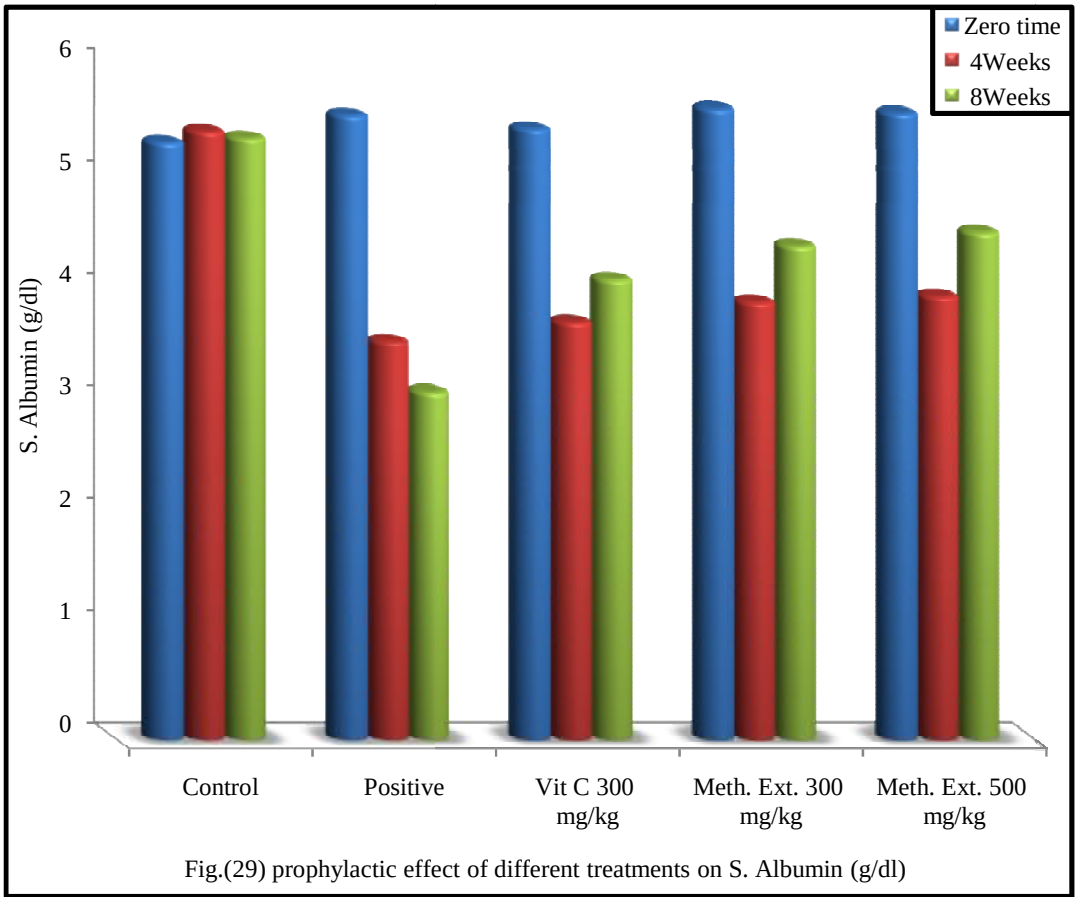


Fig.(29) prophylactic effect of different treatments on S. Albumin (g/dl)

Table (20): Prophylactic effect of different treatments on S. Blood urea (mg/dl ± S. D) and % variation from the corresponding control during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Animal groups	**Time intervals (Wk's)**				
	0	**4**	**% var.**	**8**	**% var**
Control	24.6 ± 2.03	24.0 ± 3 †	2.4 ↓	25 ± 3 †	1.6 ↑
Positive control	25.9 ± 1.59	30.0 ± 3 a, †	17.1 ↑	34.6 ± 5 a, *	33.4 ↑
vitamin C (300 mg/kg.b.w)	25 ± 3.01	27.1 ± 2 b, †	8.4 ↑	24.0 ± 3 b, *	4 ↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.b.w)	27.4 ± 1.82	29.6 ± 4 b, †	8.0 ↑	25.7 ± 2 b, *	7.3 ↓
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg.b.w)	25.6 ± 2.01	29.4 ± 3 b, †	14.8 ↑	24.7 ± 4 b, *	3.5 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.

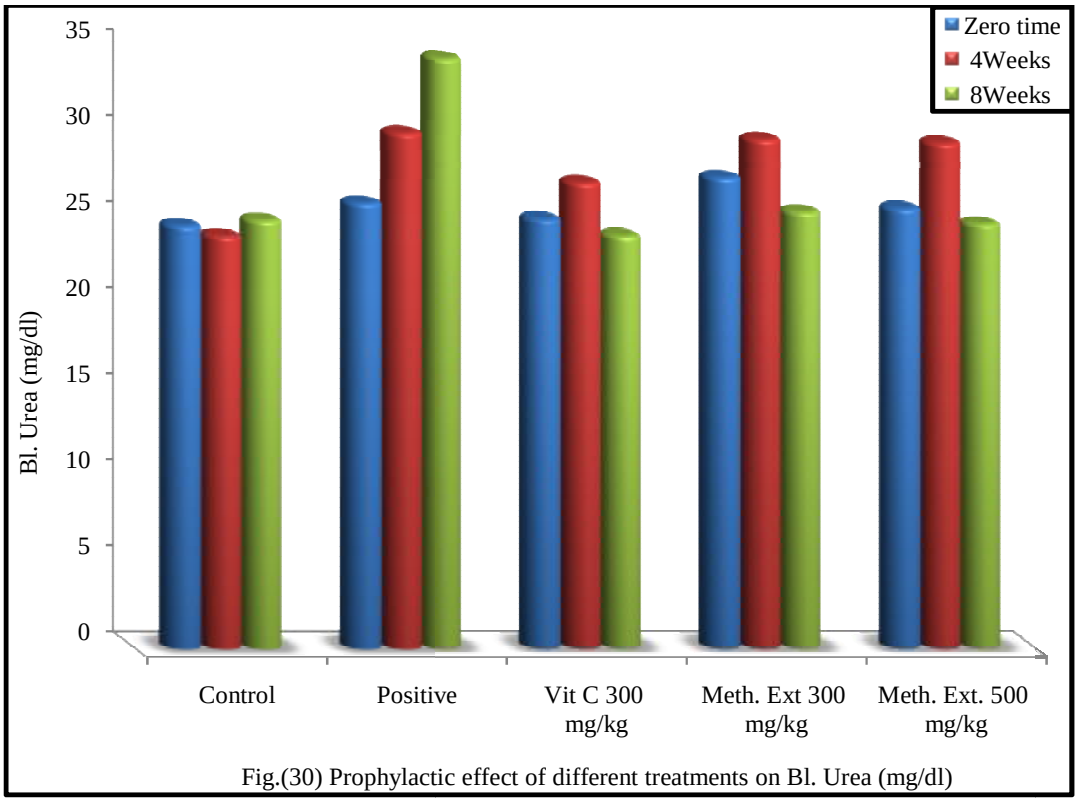


Fig.(30) Prophylactic effect of different treatments on Bl. Urea (mg/dl)

Table (21): Prophylactic effect of different treatments on S. Creatinine (mg/dl ± S. D) and % variation from the corresponding control during the induction of hypercholesterolemia for 8 weeks in male albino rats

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Animal groups	Time intervals (Weeks)				
	0	4	% var.	8	% var.
Control	0.28 ± 0.02	0.29 ± 0.05 †	3.6↑	0.29± 0.08 †	3.5↑
Positive control	0.30 ± 0.09	0.42 ± 0.02 [a], *	40↑	0.45 ± 0.02 [a], *	50↑
vitamin C (300 mg/kg.b.w)	0.31 ± 0.03	0.38 ± 0.08 [b],†	22.6↑	0.37 ± 0.01 [b],†	19.4↑
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg .b.w.)	0.30 ± 0.10	0.35 ± 0.01 [b],†	16.7↑	0.33 ± 0.03 [b], *	10↑
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg .b.w.)	0.31 ± 0.06	0.37 ± 0.04 [b],†	19.4↑	0.34 ± 0.02 [b], *	9.8↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with positive group.

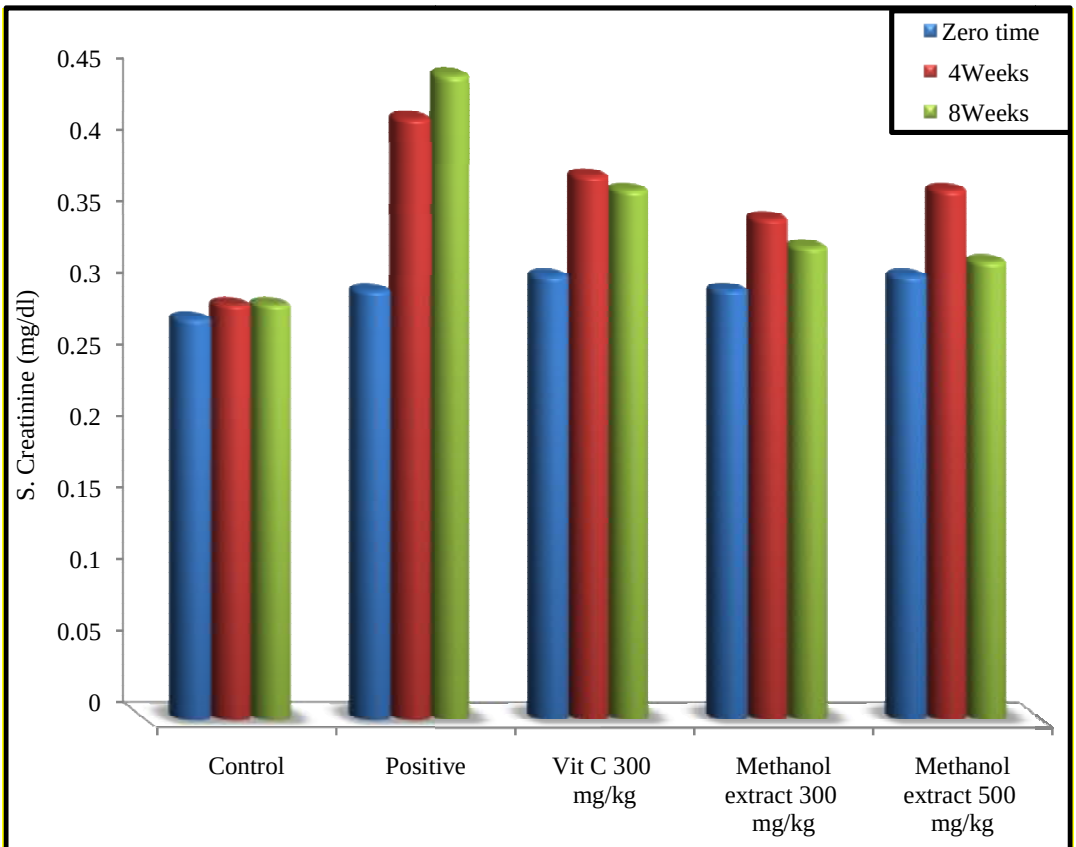


Fig.(31) Prophylactic effect of different treatments on S. Creatinine (mg/dl)

Table (22): prophylactic effect of different treatments on plasma SOD (u/mol ± S.D) and % variation from the corresponding control during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Animal groups	Time intervals (Weeks)				
	0	4	% var.	8	% var.
Control	8.23 ±2	8.28± 2 †	0.6 ↑	8.16±1.5 †	0.9↓
Positive control	8.42±1.7	5.12± 1 a, *	39.2 ↓	4.35±0.7 a, **	48.3↓
vitamin C (300 mg/kg .b.w.)	8.36±1	6.82± 1 b, *	18.4 ↓	7.18±1.9 b, **	14.1↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg .b.w.)	8.29±2	6. 81 ± 1 b, *	17.9 ↓	7.07±2 b, **	14.7↓
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg .b.w.)	8.32± 2	6. 78± 2 b, *	18.5 ↓	7.21±1.6 b, **	13.3↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.

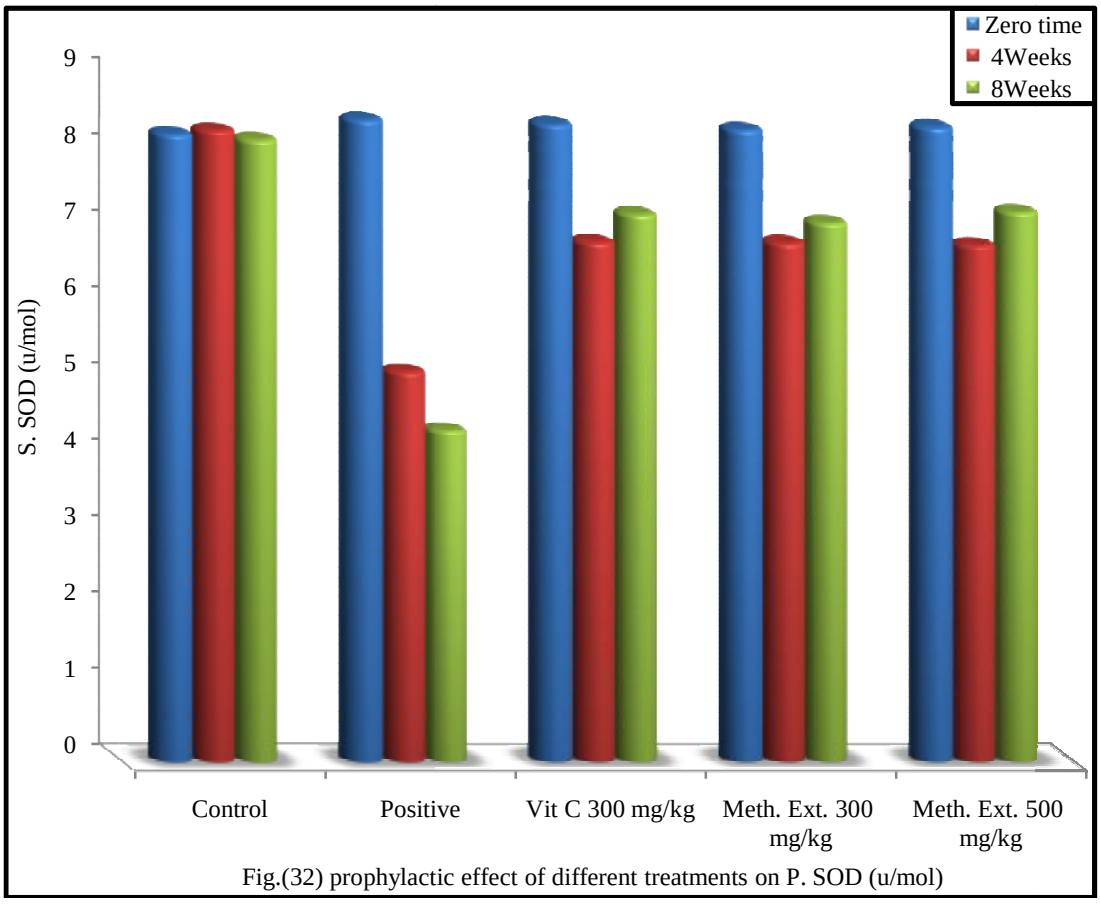


Fig.(32) prophylactic effect of different treatments on P. SOD (u/mol)

Table (23): prophylactic effect of different treatments on plasma MDA (n mol/ml ± S.D) and % variation from the corresponding control during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Animal groups	Time intervals (Weeks)				
	0	4	% var.	8	% var.
Control	15 ± 4	16 ±4.29 †	0.6 ↑	16 ± 0.38†	6.7↑
Positive control	16 ± 3	20 ± 4 a, *	25 ↑	25 ± 6.8 a, *	56↑
vitamin C (300 mg/kg.b.w.)	15 ± 2	18 ± 2.9 b,†	20 ↑	17 ± 5 b,†	13.3↑
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.b.w.)	14 ± 3	18 ± 3.6 b,†	28.5 ↑	18 ± 3 b,†	28.6↑
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg.b.w.)	15 ± 2	19 ± 4 b,†	26 ↑	17 ± 4 b,†	13.3↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.

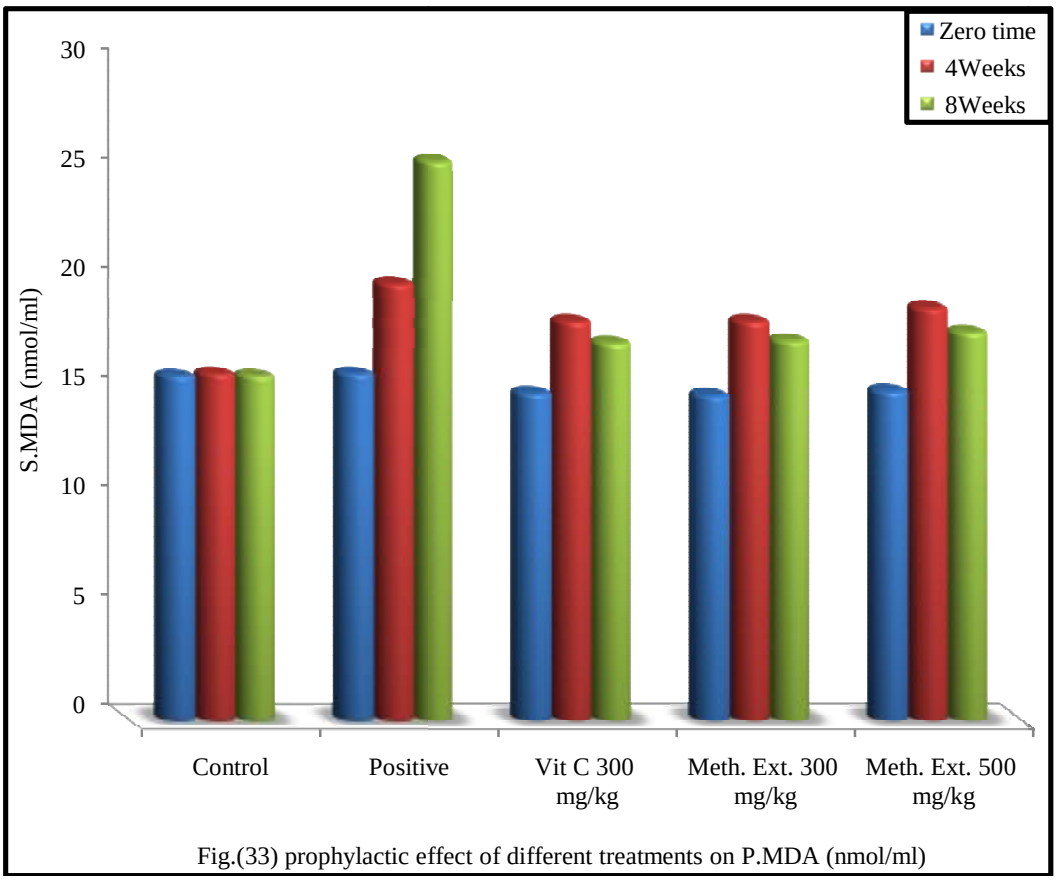


Fig.(33) prophylactic effect of different treatments on P.MDA (nmol/ml)

Table (24): prophylactic effect of different treatments on plasma GR (u/l ± S.D) and % variation from the corresponding control during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Animal groups		Time intervals (Weeks)			
	0	4	% var.	8	% var.
Control	25.3 ± 4	25.3 ± 3 †	Zero	25.8 ± 4 †	2↑
Positive control	25.3 ± 2	16.2 ± 2 a, †	36 ↓	13.2 ± 3 a, †	47.9 ↓
vitamin C (300 mg/kg.b.w.)	26.3 ± 5	19.2 ± 2 b, †	27.1 ↓	21.3 ± 2 b, †	19.1 ↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.b.w.)	24.8 ± 3	18.9 ± 4 b, †	23.7 ↓	22.2 ± 3 b, †	10.3 ↓
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg.b.w.)	25.3 ± 4	19.2 ± 3 b, †	24.0 ↓	21.3 ± 2 b, †	15.92 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.

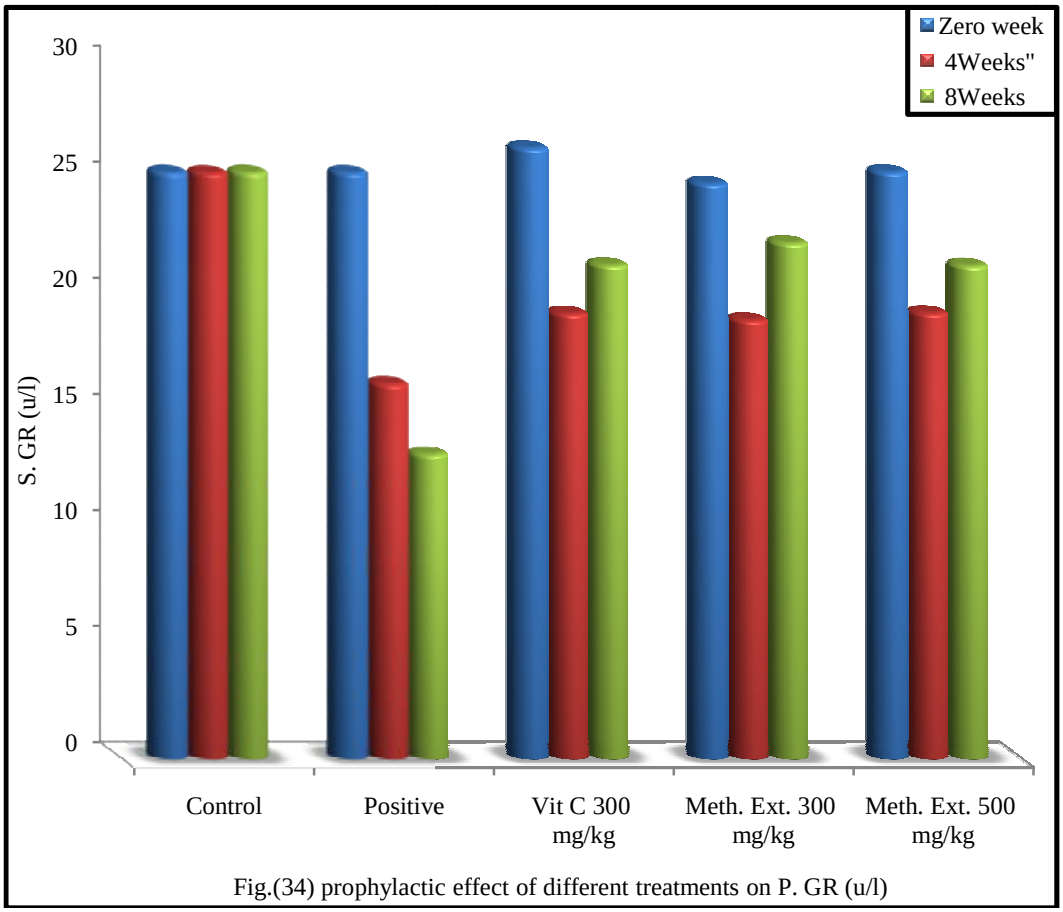
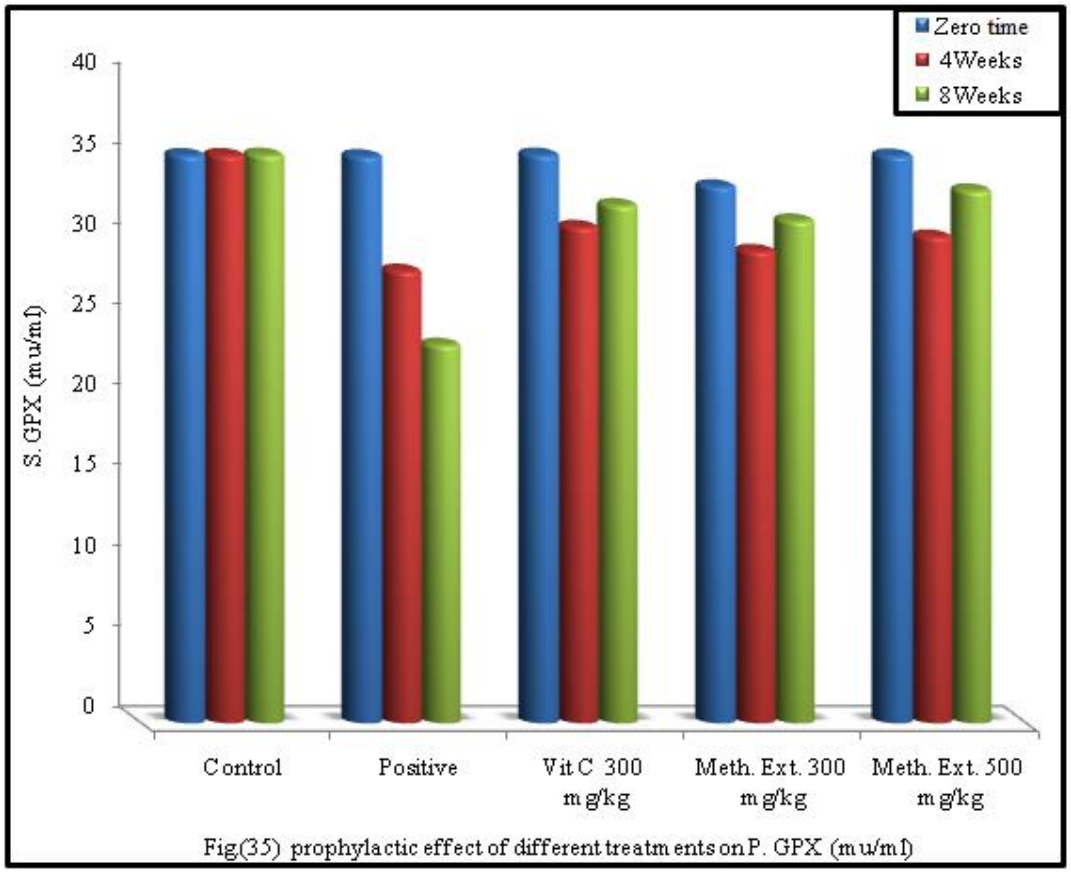


Fig.(34) prophylactic effect of different treatments on P. GR (u/l)

Table (25): prophylactic effect of different treatments on plasma GP_X (mu/ml ± S.D) and % variation from the corresponding control during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Animal groups	Time intervals (Weeks)				
	0	4	% var.	8	% var.
Control	35.5 ± 5	35.5 ± 3 †	zero	35.3 ± 4 †	0.56 ↑
Positive control	35.2 ± 4	28.1 ± 4 [a, †]	20.2 ↓	23.5 ± 2 [a, †]	33 ↓
vitamin C (300 mg/kg.b.w.)	34.3 ± 2	30.8 ± 5 [b, †]	10.2 ↓	32.2 ± 2 [b, †]	6.2 ↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.b.w.)	33.3 ± 3	29.3 ± 4 [b, †]	12.1 ↓	31.2 ±5 [b, †]	6.4 ↓
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg.b.w.)	35.2 ± 4	30.2 ± 3 [b, †]	14.3 ↓	33.1 ± 4 [b, †]	6 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; [*] Significant difference at P < 0.05; [**] highly sig. difference at P < 0.01; [***] Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with positive group.



Fig(35) prophylactic effect of different treatments on P. GPX (mu/ml)

Table (26): prophylactic effect of different treatments on plasma CAT (u/l) ± S.D) and % variation from the corresponding control during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Animal groups	Time intervals (Weeks)				
	0	4	% var.	8	% var.
Control	50.6 ± 9	50.5 ± 6 †	0.2 ↓	50.6 ± 5†	Zero
Positive control	50.3 ± 6	42.9 ± 7 a, †	14.7↓	38.5 ± 6 a, *	23.4↓
vitamin C (300 mg/kg.b.w.)	49.8 ± 8	45.5 ± 8 b, †	8.7 ↓	41.5 ± 8 b, †	16.6↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.b.w.)	48.9 ± 7	45 ± 8 b, †	8.0 ↓	42.2 ± 8 b, †	13.7↓
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg.b.w.)	50.4 ± 9	45.2 ± 5 b, †	10.2 ↓	43 ± 7 b, †	14.7↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.

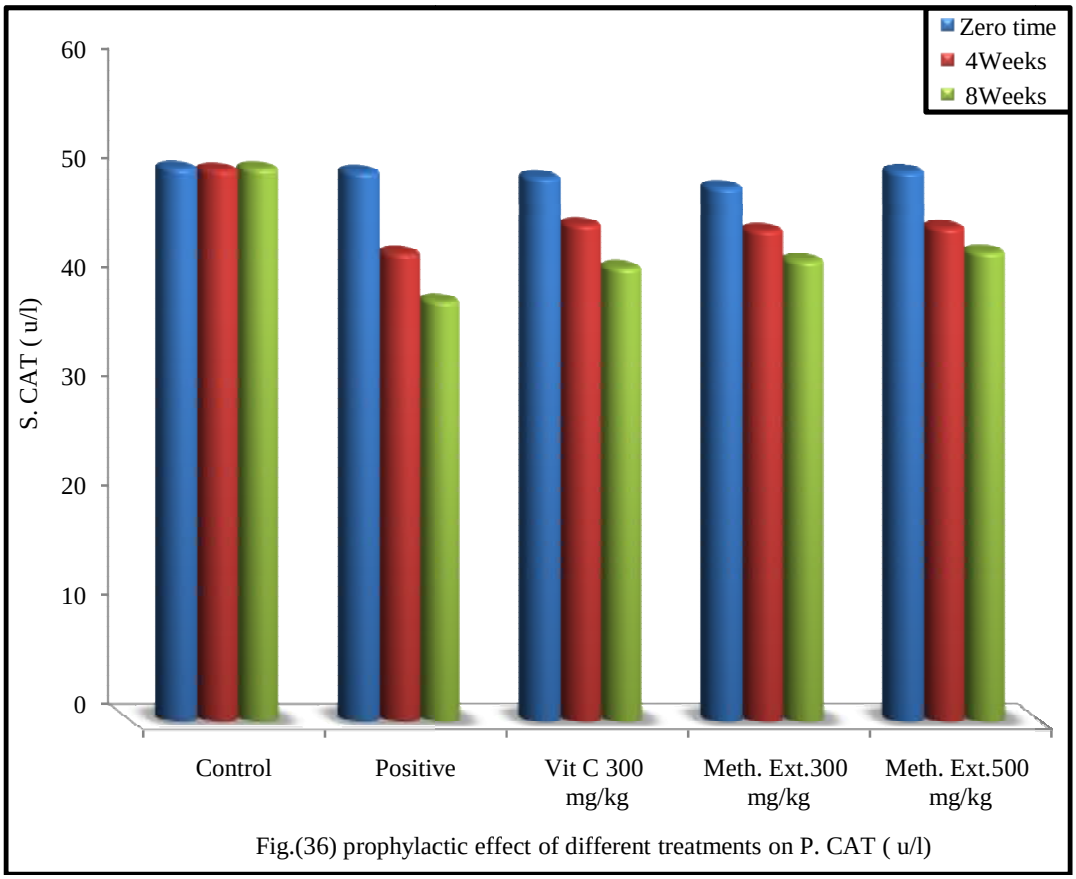


Fig.(36) prophylactic effect of different treatments on P. CAT (u/l)

Histopathological studies

Liver tissue

In control group of animals**,** the parenchyma of liver in all animals showed normal pattern regarding to size, shape, arrangement and staining characters. Also, the portal tract and control area were normal (Fig.37).

In all examined animals of the (+ve) control group, histopathological changes of liver tissue revealed diffuse vacuolar degenerative changes of hepatocytes vary from mild to marked in severity. Ballooning of hepatocytes together with focal lymphocytic cells aggregates in portal areas were seen in Fig.(38).

The hepatocytes distortion was ameliorated where near normal appearance of hepatocytes in methanolic extract of *Arbutus pavarii* leaves at (300 and 500) µg/ml in pretreated livers, also at the 300 mg/kg of vitamin C as illustrated in (figs. 39, 40, 41). The most significant ameliorating effect was recorded for 500 µg/ml of methanolic extract of *Arbutus pavarii* leaves pretreated liver (fig. 41).

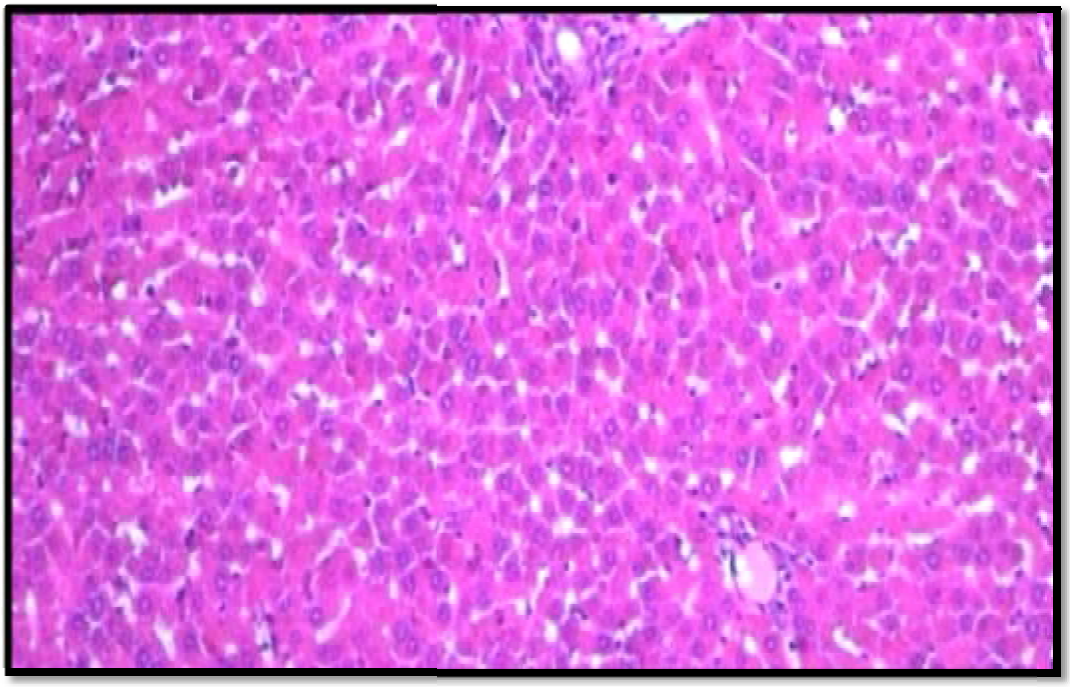


Fig. (37): control group of animals**,** the parenchyma of liver in all animals showed normal pattern regarding to size, shape.

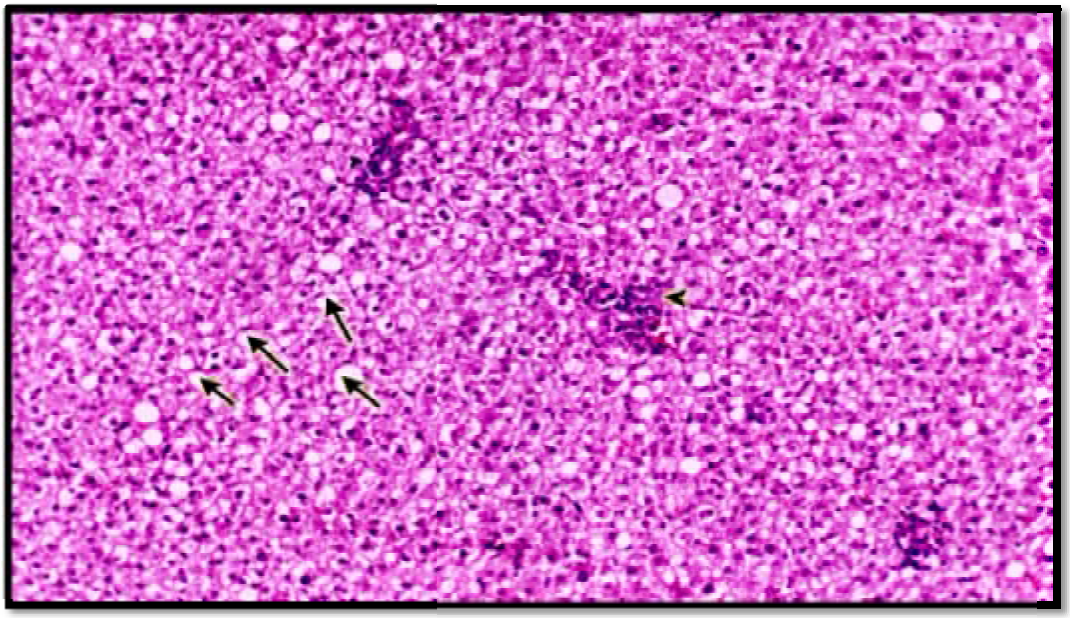


Fig. (38): The liver of positive control rat, showing marked vacuolated hepatocytes (arrow) and aggregates of mononuclear inflammatory cells (arrow head) in portal areas.

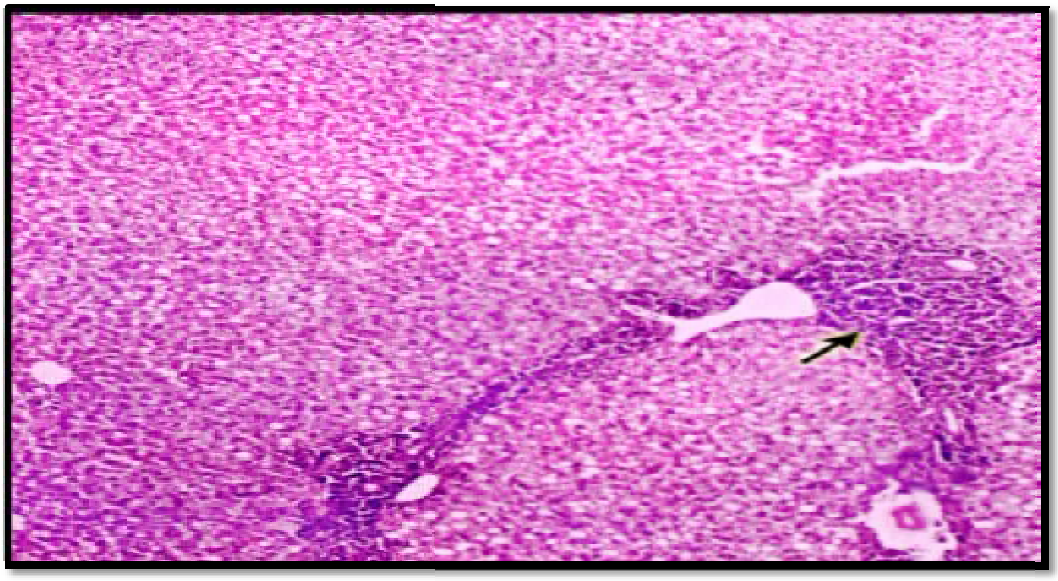


Fig. (39): The liver section of methanolic extract *Arbutus pavarii* leaves (300mg/kg. b.w.), with mild increase in lymphocytic aggregates (arrow) in portal areas.

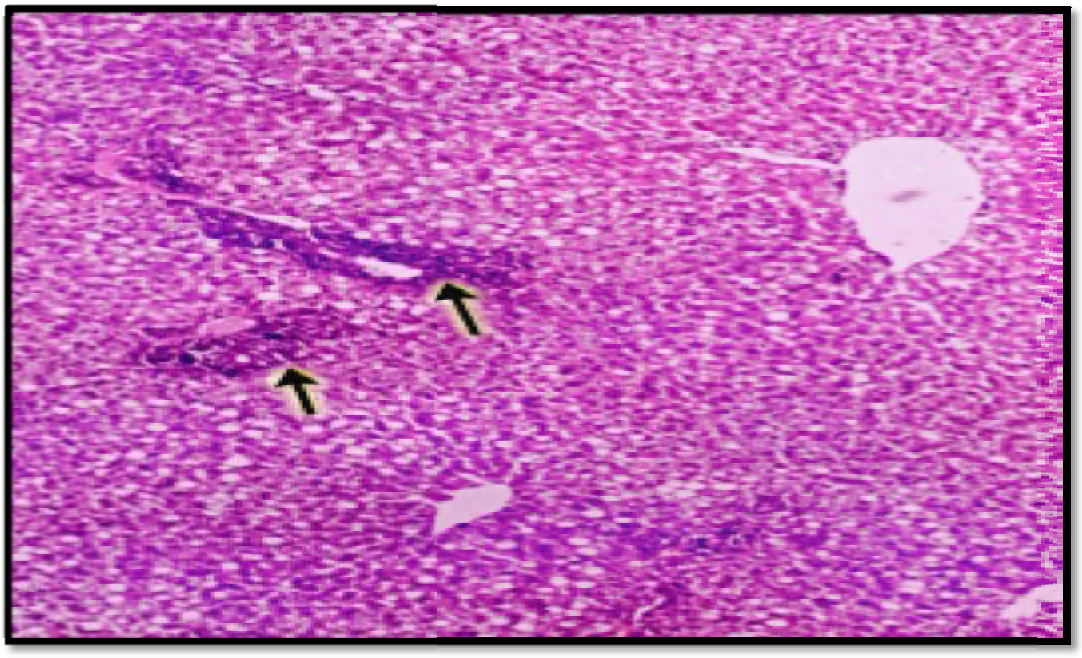


Fig. (40): The liver section of methanolic extract Arbutus pavarii□s leaves treated (500mg/kg. b.w.) with mild increase in lymphocytic aggregate (arrow) in portal areas.

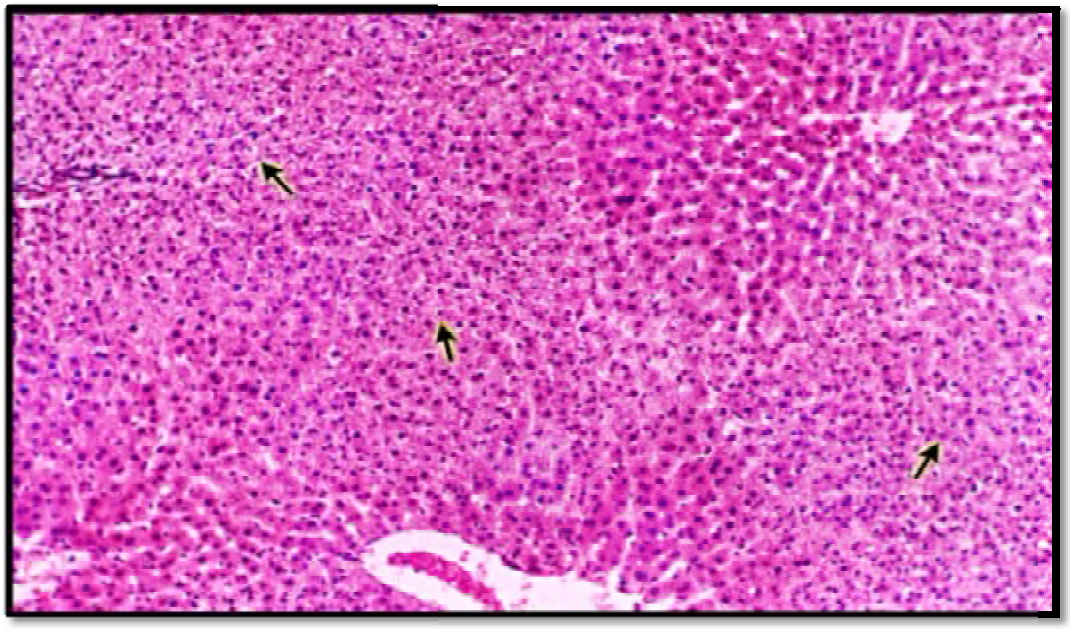


Fig. (41): The liver section of vitamin C treated (300mg/kg. b.w.), with mild increase in lymphocytic aggregates (arrow) in portal areas Stain.

Induction of hypercholesterolemia by cholesterol and cholic acid.

Table (27): Arithmetic mean values ± S.D and % changes from the corresponding control of different biochemical parameters before and after induction of hypercholesterolemia in male albino rats.

Parameter	Before induction of hypercholesterolemia	After induction of hypercholesterolemia	% change
S.T. lipid. (mg /dl)	301± 37	618 ± 43 [a], [***]	105.3 ↑
S.T. Chol. (mg /dl)	67 ± 12	171.4± 29 [a], [***]	155.8 ↑
S.HDL-Chol. (mg /dl)	23 ± 3	18.46 ± 5 [a], [†]	42.3 ↓
(T. Chol./HDL-Chol.)	2 ± 0.3	9.28 ±2 [a], [***]	364 ↑
S.LDL-Chol. (mg /dl)	22.5± 2.9	128 ±17 [a], [***]	468.9 ↑
S.VLDL-Chol. (mg/dl)	10.62 ± 2	24.92 ±6 [a], [*]	134.7 ↑
S. Triglyceride (mg /dl)	53.1 ± 10	124.64 ± 22 [a], [***]	134.7 ↑
S.ALT (u/ml)	34 ±7	85 ± 11 [a], [***]	150 ↑
S.AST (u/ml)	70 ± 13	142 ± 33 [a], [***]	102.7 ↑
G-GT (u/l)	3.25 ± 0.7	12.22 ± 3 [a], [***]	276 ↑
S. LDH (u/l)	640 ± 33	978 ± 45 [a], [***]	52.8 ↑
S. ALP (u/l)	131 ± 18	163.6 ± 22 [a], [***]	24.9 ↑
S. Total Protein (g/dl)	5.8 ± 2	3.89 ± 1 [a], [*]	32.9 ↓
S. Albumin(g/dl)	5.7 ± 1.6	3.24 ± 0.8 [a], [*]	43.2 ↓
S. Blood urea (mg/dl)	20.5 ± 4	32.8 ± 5 [a], [*]	60 ↑
S. Creatinine (mg/dl)	0.30 ± 0.02	0.41 ± 0.03 [a], [†]	36.7 ↑
P. SOD (u/mol)	7.36 ± 3	4.34 ± 0.9 [a], [*]	41.0 ↓
S.MDA (nmol/ml)	15.8 ± 4	24.7 ± 5 [a], [*]	56.3 ↑
P. GR (u/l)	25.29 ± 6	13.2 ± 3 [a], [*]	47.8 ↓
P. GPx (mu/ml)	34 ± 8	24.85 ± 4.6 [a], [*]	26.9 ↓
P. CAT (u/l)	53 ± 12	36.3 ± 7 [a], [**]	31.5 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; [*] Significant difference at P < 0.05; [**] highly sig. difference at P < 0.01; [***] Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b]

3. Effect of different treatments in curative groups:

3.1. Effect of different treatments on serum of T. lipid ,T. Chol. ,HDL-C. , T. Chol./HDL-C. , LDL.C.,VLDL-C .and T.G.

Oral administration of hypercholesterolemic diet significantly increased the activities of the serum of **T. lipid ,T. Chol. ,T. Chol./HDL-C. , LDL.C.,VLDL-C. and T.G.** by (105.3%, 155.8%, 364%, 468.9%, 134.7 and 134.7%), respectively. But the level of HDL-C decreased by 42.3% As illustrated in tables (27),

3.2. Effect of different treatments on serum ALT, AST, G-GT, LDH and ALP

The effects of hypercholesterolemic diet on the serum enzymes ALT, AST, G-GT LDH and ALP, illustrated in table (27), lead to significantly increased the activities of these enzymes by (150%, 102.7%, 276%, 52.8% and 24.9%), respectively.

3.3.Effect of different treatments on serum T. proteins and albumin

The level of total protein and albumin significantly decreased by (32.9%, 43.2%), respectively in hypercholesterolemic diet as illustrated in tables (27).

3.4. Effect of different treatments on serum blood urea. and creatinine

After induction serum blood urea and creatinine significantly increased by 60 %, and 36.7% respectively, as illustrated in tables (27).

3.5.Antioxidant enzymes in Curative group:

After the induction of rats to hypercholesterolemic diet only a significant decrease in the activities of the antioxidant enzymes SOD, GR, GPx and CAT, and, in comparison to the control group by (41%, 47.8%, 26.9%, and 31.5%), respectively in table (27), and figs. (58, 60, 61, and 62), but the MDA level shows significant increase by 56.3% table (45) and fig. (59).

Table (28): The curative effect of different treatments on S.T. Lipids (mg/dl ± S.D) and % variation from the corresponding control after induction of hypercholesterolemia as well as during 8 weeks of treatment in male albino rats.

Animal groups	Before treatment	After treatment	% var.
Control	301 ± 15	295 ± 25 †	2 ↓
positive control	620 ± 23	618 ± 33 a, ***	0.32 ↓
vitamin C (300 mg/kg. Kg.b.w)	612 ± 24	430 ± 20 b, ***	29.7↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg. . Kg.b.w)	617 ± 30	414 ±27 b, ***	32.9 ↓
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg. . Kg.b.w)	623 ± 27	408 ±26 b, ***	34.5 ↓
Atorvastatin (1 mg/kg b .w)	618 ± 33	395 ±23 b, ***	36.1 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.

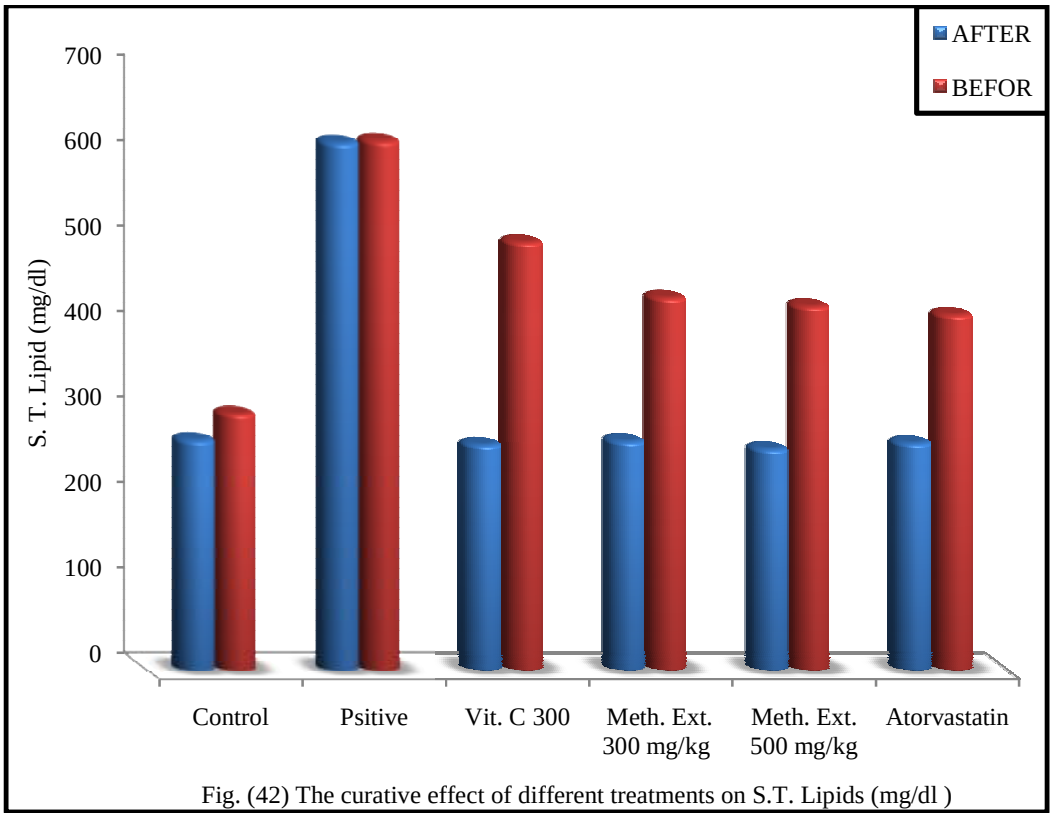


Fig. (42) The curative effect of different treatments on S.T. Lipids (mg/dl)

Table (29): The curative effect of different treatments on S. T. Cholesterol (mg/dl ± S.D) and % variation from the corresponding control after induction of hypercholesterolemia as well as during 8 weeks of treatment in male albino rats.

Animal groups	Before treatment	After Treatment	% var.
Control	66.2± 3.09	63.4±4.45†	4.23 ↓
positive control	161 ± 6.23	160 ± 23 [a, ***]	0.6 ↓
vitamin C (300 mg/kg.)	168 ±8.91	135 ± 26 [b, ***]	19.6 ↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.)	169±6.89	127 ± 29 [b, †]	24.9 ↓
Meth. Ext of Arbutus pavarii leaves (500 mg/kg.)	162±14.01	120 ± 18 [b, *]	26 ↓
Atorvastatin (1mg/kg b.w)	167±9.03	103 ± 27 [b, **]	38.3 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with positive group.

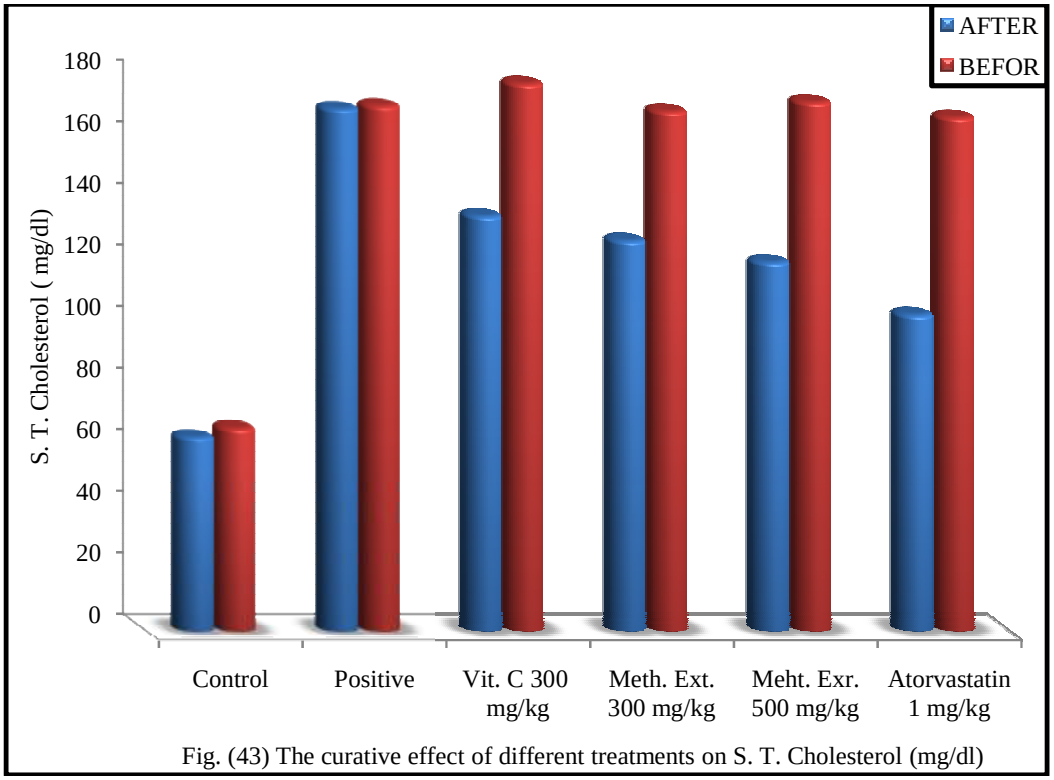


Fig. (43) The curative effect of different treatments on S. T. Cholesterol (mg/dl)

Table (30): The curative effect of different treatments on S. HDL-cholesterol (mg/dl± S.D) and % variation from the corresponding control after induction of hypercholesterolemia as well as during 8 weeks of treatment in male albino rats.

Animal groups	Before treatment	After treatment	% var.
Control	33 ± 3	35.8 ± 4 †	8.48 ↑
positive control	18.3 ± 2	19 ± 3 [a, *]	3.2 ↑
vitamin C (300 mg/kg.)	18 ± 2	24.8 ± 2 [b, †]	35.5 ↑
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.)	19 ± 3	24.3 ± 3 [b, †]	28.6 ↑
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg.)	18 ± 4	26 ± 4 [b, †]	49.4 ↑
Atorvastatin (1 mg/kg b.w)	18 ± 2	28 ± 2 [b, †]	58.2 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with positive group.

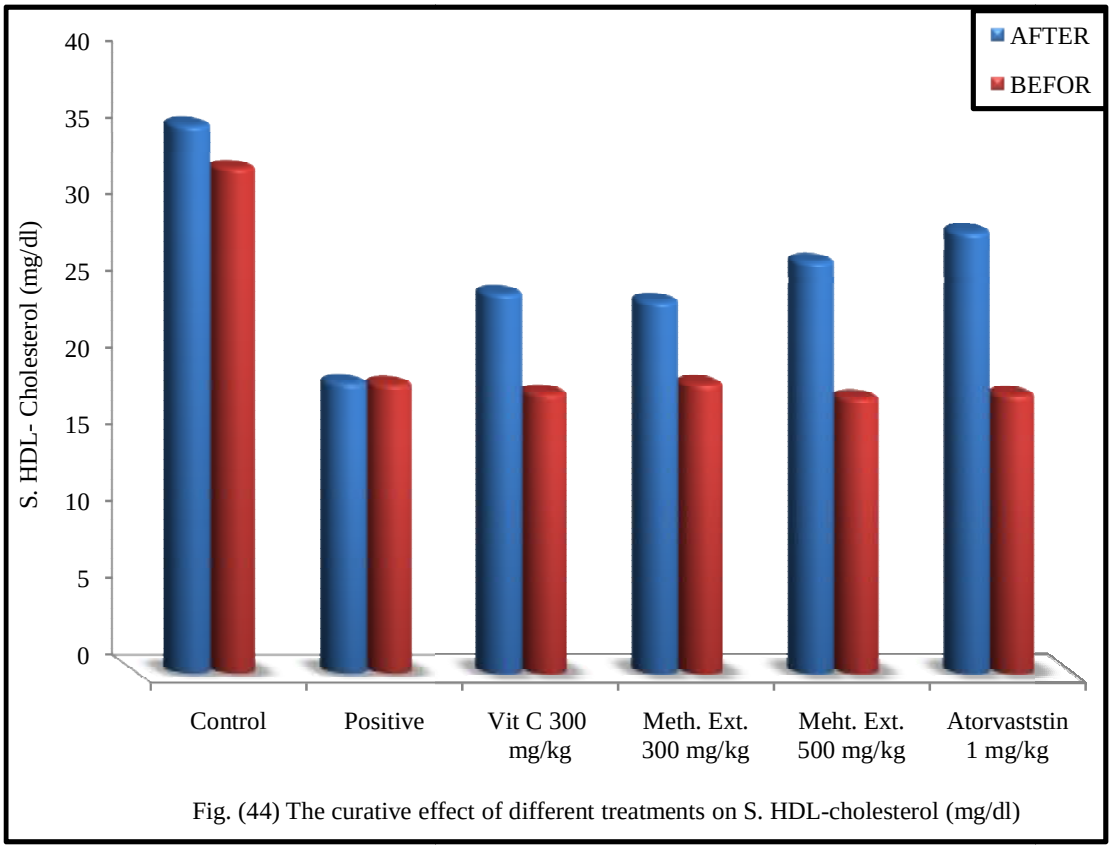


Fig. (44) The curative effect of different treatments on S. HDL-cholesterol (mg/dl)

Table (31): The curative effect of different treatments on S. R.R "T. Chol./HDL Chol." (mean value ± S.D) and % variation from the corresponding control after induction of hypercholesterolemia as well as during 8 weeks of treatment in male albino rats.

Animal groups	Before Treatment	After treatment	% var.
Control	2.0	1.8 †	10 ↓
positive control	9.0	8.9 a, ***	1.1 ↓
vitamin C (300 mg/kg. b. w.)	9.7	5.4 a, ***	44.3 ↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg. b. w.)	8.9	5.2 b, †	41.6 ↓
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg. b. w.)	9.6	4.5 b, *	43.1 ↓
Atorvastatin (1 mg/kg b.w)	9.2	3.6 b,*	60.9 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.

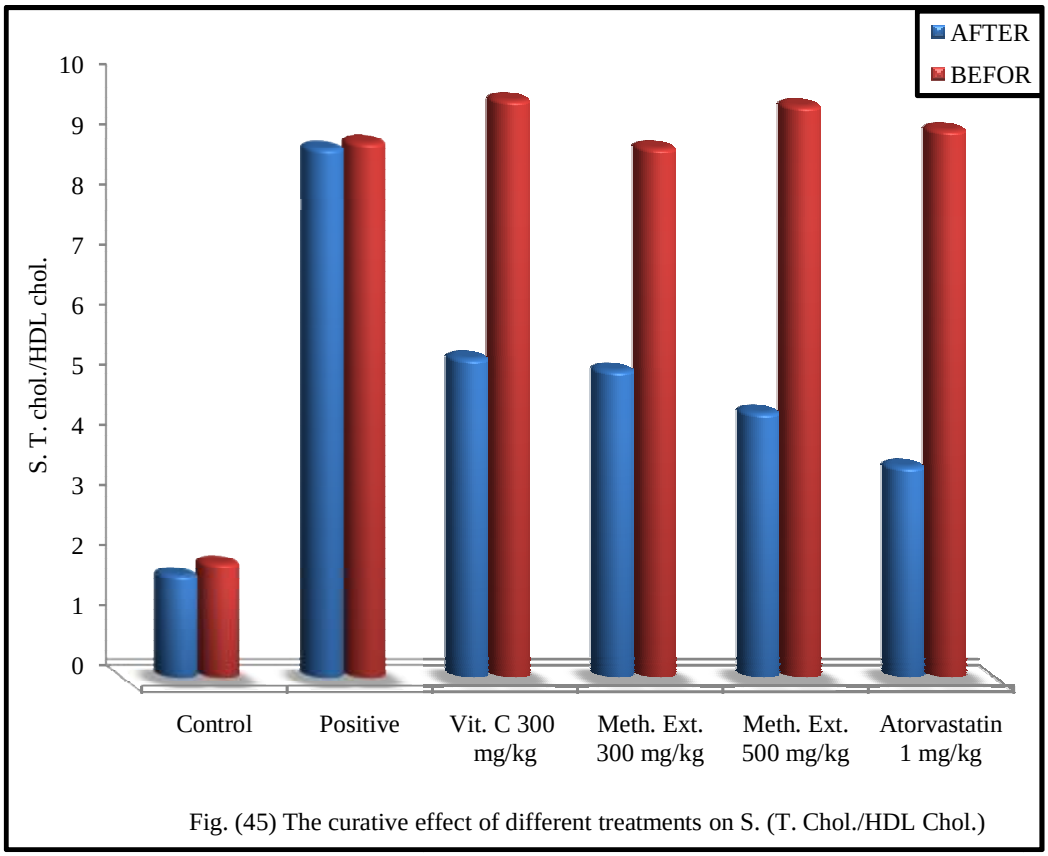


Fig. (45) The curative effect of different treatments on S. (T. Chol./HDL Chol.)

Table (32): The curative effect of different treatments on S. LDL-cholesterol (mg/dl± S.D) and % variation from the corresponding control after induction of hypercholesterolemia as well as during 8 weeks of treatment in male albino rats.

Animal groups	Before treatment	After treatment	% var.
Control	22 ± 2	17 ± 3 †	22.7 ↓
positive control	126.7 ± 12	125 ± 11 a, ***	1.3 ↓
vitamin C (300 mg/kg. b. w)	135.2 ± 9	92.3 ± 8 b, ***	31.7 ↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg. b.w)	125.3 ± 10	84.1 ± 9 b, †	32.9 ↓
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg. b. w)	128.8 ± 11	78.5 ± 8 b, *	39 ↓
Atorvastatin (1 mg/kg. b.w)	124.1 ± 16	58 ± 7 b, ***	53.2 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.

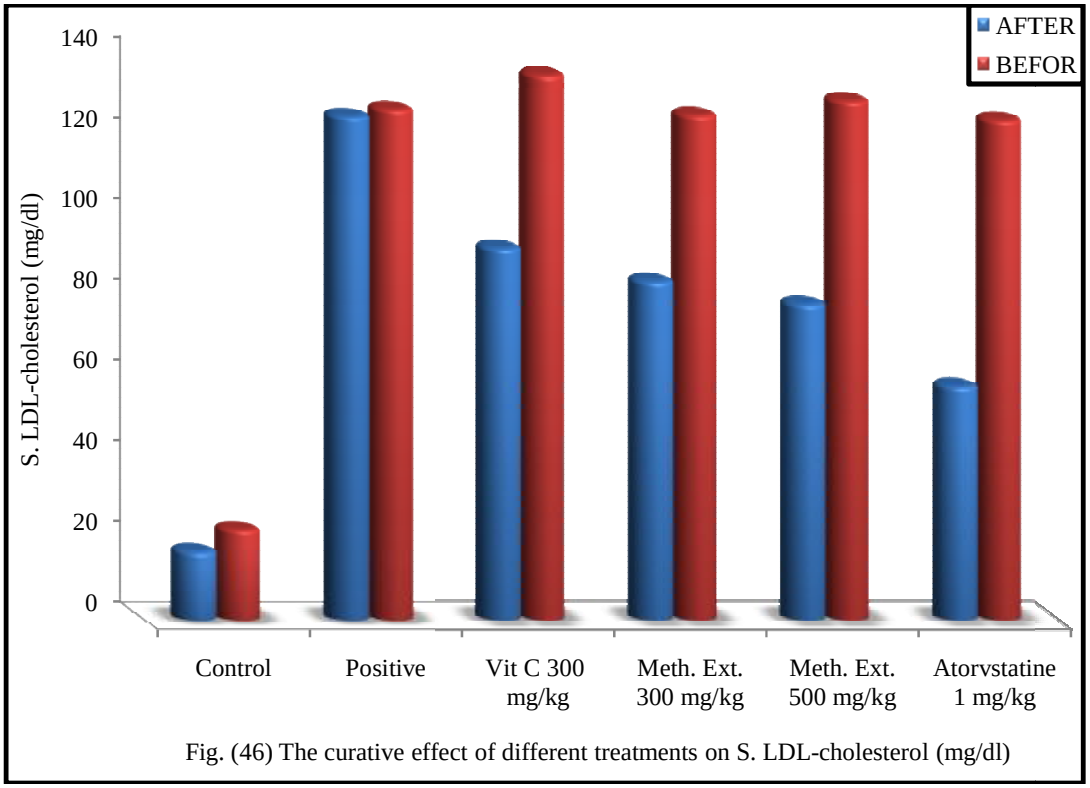


Fig. (46) The curative effect of different treatments on S. LDL-cholesterol (mg/dl)

Table (33): The curative effect of different treatments on S. VLDL-cholesterol (mg/dl ± S.D) and % variation from the corresponding control after induction of hypercholesterolemia as well as during 8 weeks of treatment in male albino rats.

Animal groups	Before treatment	After treatment	% var.
Control	10.62 ± 3	10.5 ± 2 [†]	1.13 ↓
positive control	25.4 ± 5	26.02 ± 3 [a, *]	2.4 ↑
vitamin C (300 mg/kg.)	24.5 ± 4	17.9 ± 4 [b, †]	26.9 ↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg. b. w)	24.8 ± 2	18.6 ± 2 [b, †]	25 ↓
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg. b. w)	25.2 ± 4	15.5 ± 3 [b, *]	38.5 ↓
Atorvastatin (1 mg/kg b.w)	24.7 ± 4	16 ± 5 [b, *]	35.2 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; [*] Significant difference at P < 0.05; [**] highly sig. difference at P < 0.01; [***] Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with positive group.

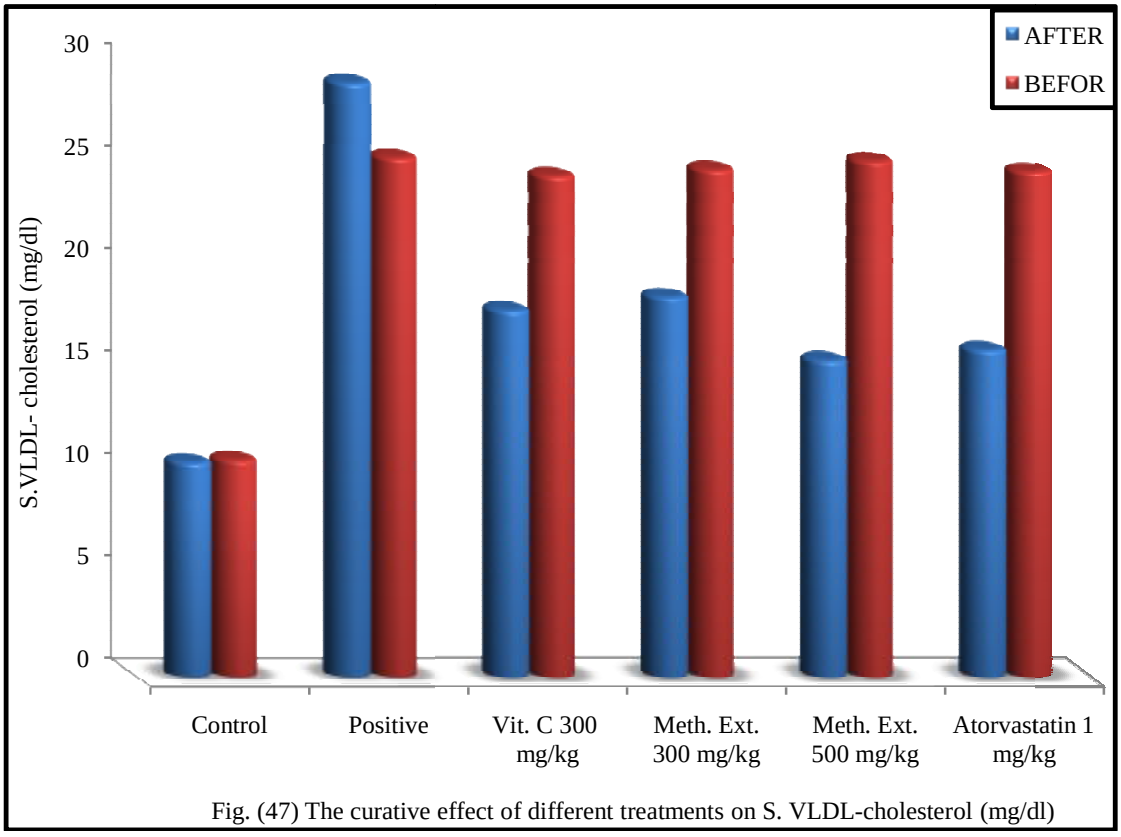


Fig. (47) The curative effect of different treatments on S. VLDL-cholesterol (mg/dl)

Table (34): The curative effect of different treatments on S. Triglyceride (mg/dl ± S.D) and % variation from the corresponding control after induction of hypercholesterolemia as well as during 8 weeks of treatment in male albino rats.

Animal groups	Before treatment	After treatment	% var.
Control	53 ± 7	52.5 ± 8 †	0.94 ↓
positive control	127 ± 9	130 ± 16 a, ***	2.4 ↑
vitamin C (300 mg/kg.)	122.5 ± 11	89.7± 11 b, ***	26.8 ↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.)	124 ± 13	92.9± 13 b, ***	25.1 ↓
Meth. Ext. of Arbutus pavarii P leaves (500 mg/kg. b. w)	126 ± 17	77.5± 17 b, ***	38.5 ↓
Atorvastatin (1 mg/kg b.w)	123.5 ± 15	79.9± 9 b, ***	35.3 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.

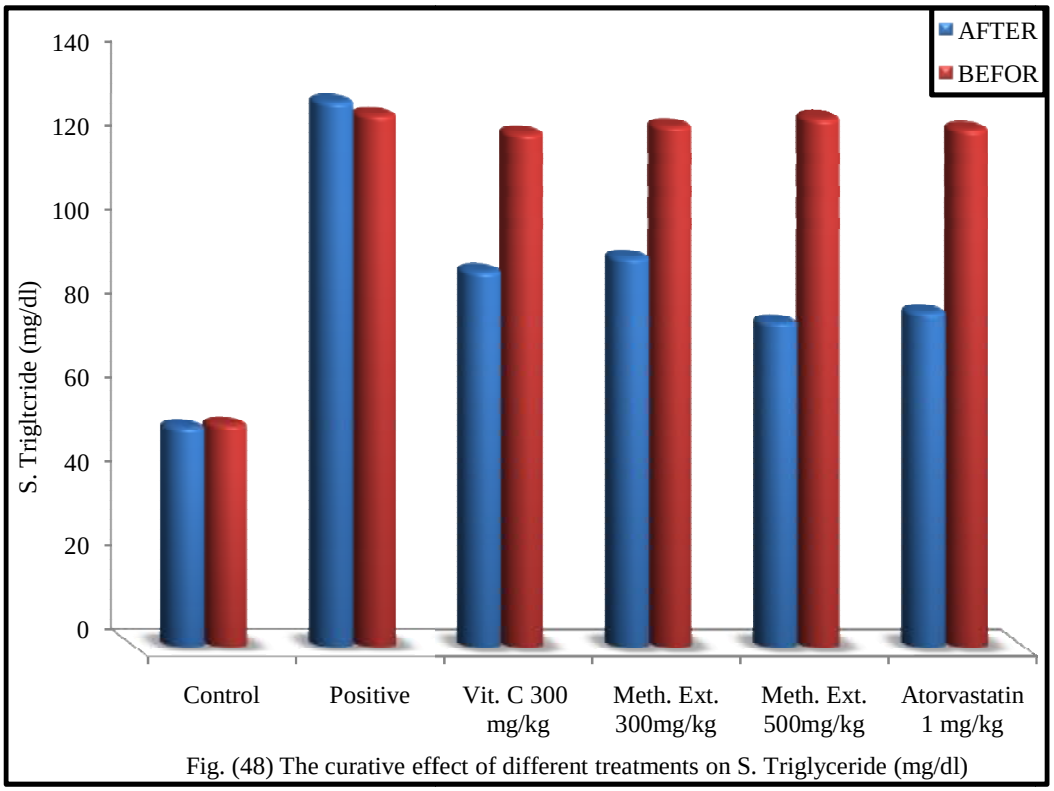


Fig. (48) The curative effect of different treatments on S. Triglyceride (mg/dl)

Table (35): The curative effect of different treatments on S. ALT (u/ml ± S.D) and % variation from the corresponding control after induction of hypercholesterolemia as well as during 8 weeks of treatment in male albino rats.

Animal groups	Before Treatment	After treatment	% var.
Control	34 ± 7	34.2 ± 7 †	0.6 ↑
positive control	90.8 ± 9	69.2 ± 9 a, ***	23.8 ↓
vitamin C (300 mg/ml.)	86 ± 6	49.5 ± 8 b, **	42.6 ↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg. b. w)	83.8 ± 8	47.3 ± 9 b, **	43.6 ↓
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg. b. w)	81.5 ± 10	44 ± 10 b, **	46 ↓
Atorvastatin (1 mg/kg b.w)	83 ± 8	50.6 ± 7 b, *	39 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.

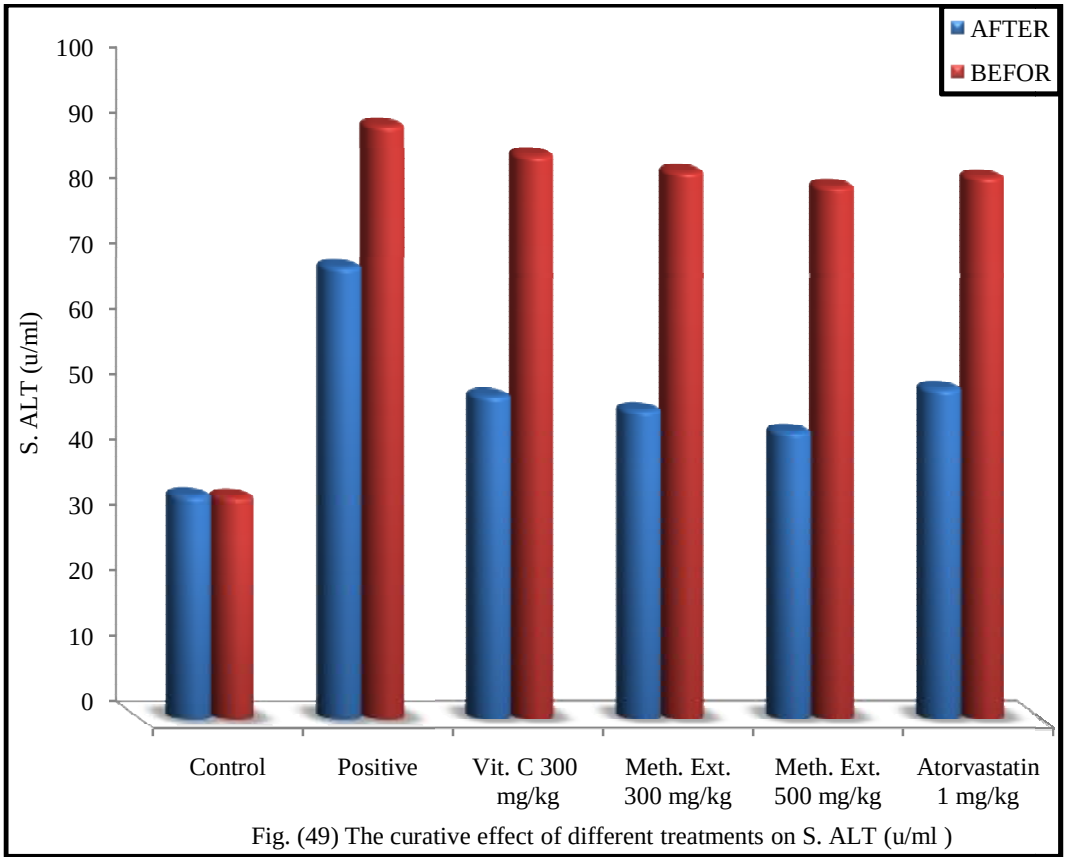


Fig. (49) The curative effect of different treatments on S. ALT (u/ml)

Table (36): The curative effect of different treatments on S. AST. (u/ml ± S.D) and % variation from the Corresponding control after induction of hypercholesterolemia as well as during 8 weeks of treatment in male albino rats.

Animal groups	Before treatment	After treatment	% var.
Control	66.9 ± 8	65.8± 8 †	1.6 ↓
positive control	145.4 ± 19	142.4± 18 a, ***	2.1 ↓
vitamin C (300 mg/kg.)	138 ± 17	115.1± 16 b, **	16.6 ↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.)	142 ± 18	112 ± 14 b, ***	21.3 ↓
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg.)	140.6 ± 17	110 ± 13 b, ***	21.8 ↓
Atorvastatin(1 mg/kg b.w)	142.9 ± 18	95.7 ±11 b, ***	33 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group

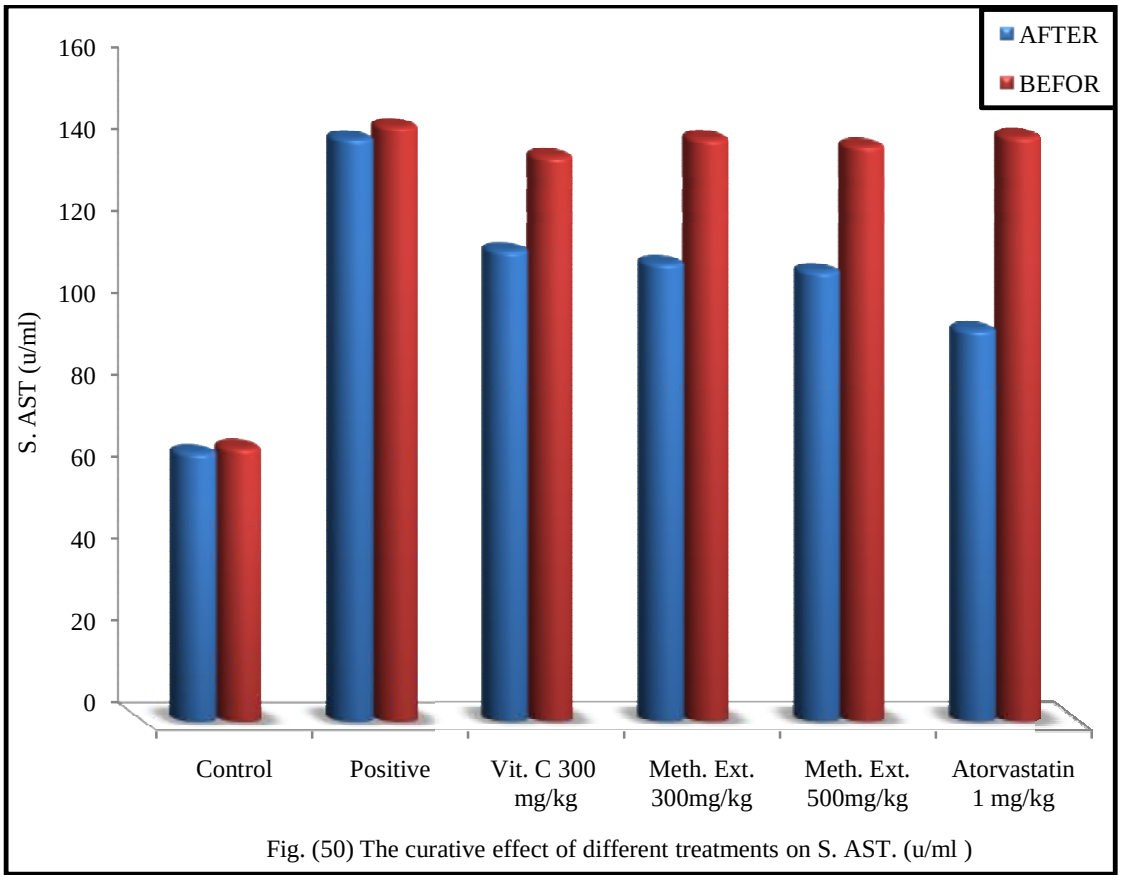


Fig. (50) The curative effect of different treatments on S. AST. (u/ml)

Table (37): The curative effect of different treatments on S. G-GT (U/L± S.D) and % variation from the corresponding control after induction of hypercholesterolemia as well as during 8 weeks of treatment in male albino rats.

Animal groups	Before treatment	After treatment	% var.
Control	3.21 ± 1	3.1 ± 0.88 †	3.4 ↓
positive control	12.6 ± 4	11 ± 2 [a, ***]	12.7 ↓
vitamin C (300 mg/kg.)	12.3 ± 3	7.44 ± 2 [b, †]	39.5 ↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.)	11.9 ± 2	8.99 ± 3 [b, †]	24.5 ↓
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg.)	12.0± 3	6.58 ± 2 [b, †]	45.2 ↓
Atorvastatin (1 mg/kg. b.w)	12.3± 4	8.56 ± 3 [b, †]	30.4 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; [a] compared with control group; [b] compared with positive group.

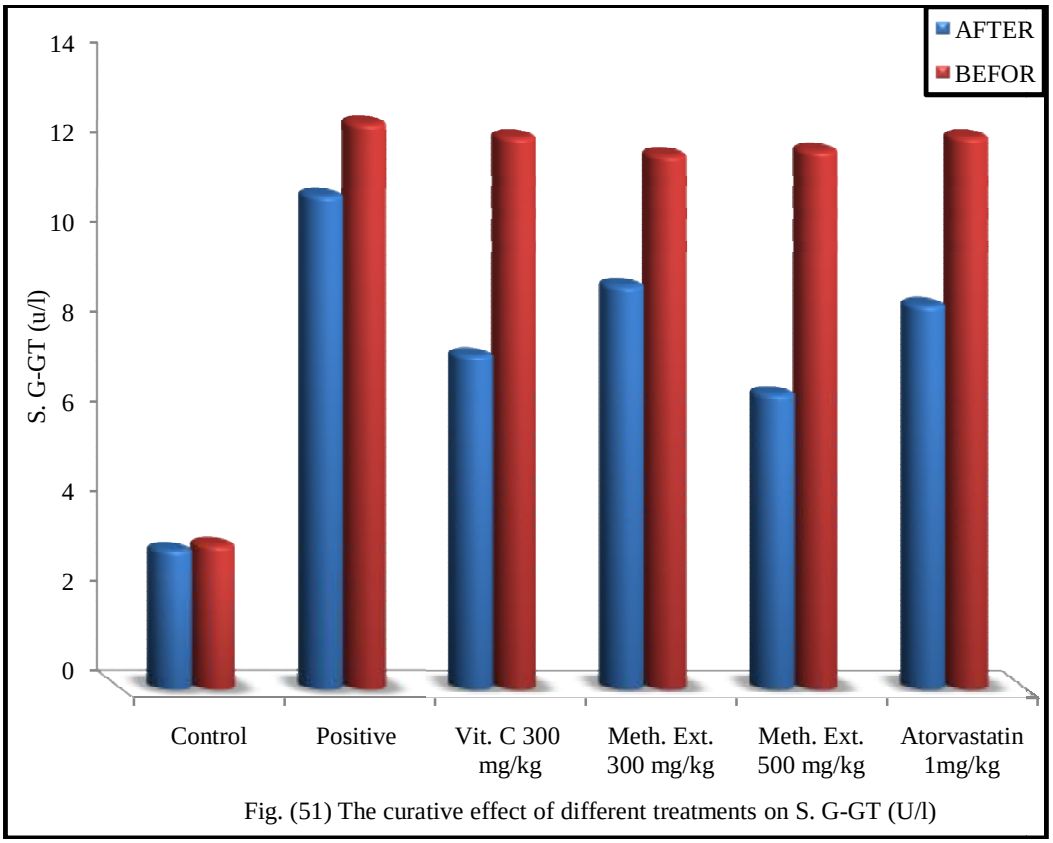


Fig. (51) The curative effect of different treatments on S. G-GT (U/l)

Table (38): The curative effect of different treatments on S.LDH. (u/l ± S.D) and % variation from the corresponding control after induction of hypercholesterolemia as well as during 8 weeks of treatment in male albino rats.

Animal groups	Before Treatment	After treatment	% var.
Control	639 ± 51	631 ±48 †	1.3 ↓
positive control	881 ± 62	871.4 ± 72 a, ***	0.97 ↓
vitamin C (300 mg/kg.)	876.8 ± 74	748 ± 63 b, ***	23.4 ↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.)	881.6 ± 77	752 ± 67 b, ***	23.39 ↓
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg.)	878 ± 73	721 ± 63 b, ***	26.3 ↓
Atorvastatin (1 mg/kg b.w)	872.4 ± 71	744 ± 64 b, ***	23.5 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.

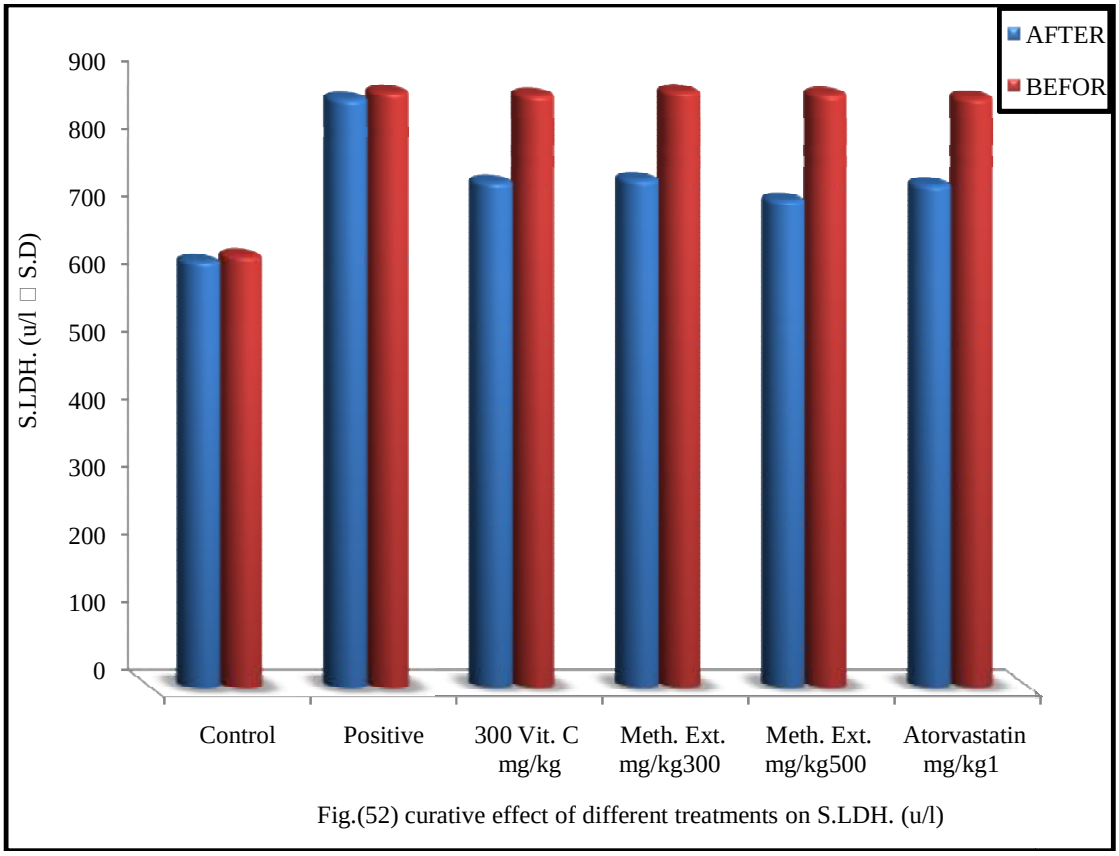


Fig.(52) curative effect of different treatments on S.LDH. (u/l)

Table (39): The curative effect of different treatments on S. ALP. (iu/l ± S. D) and % variation from the corresponding control after induction of hypercholesterolemia as well as during 8 weeks of treatment in male albino rats.

Animal groups	Before treatment	After treatment	% var.
Control	131.1 ± 18	133.1 ± 17 †	1.5 ↑
positive control	164.9 ± 27	162.9 ± 27 a, **	1.2 ↓
vitamin C (300 mg/kg.)	162.7 ± 24	142.1 ± 23 b, **	12.7 ↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.)	164 ± 20	148.9 ± 25 b, *	9.2 ↓
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg.)	163.5 ± 21	144.9 ± 24 b, *	11.4 ↓
Atorvastatin(1 mg/kg b .w)	162.9 ± 21	149.2 ± 21 b, *	8.4 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.

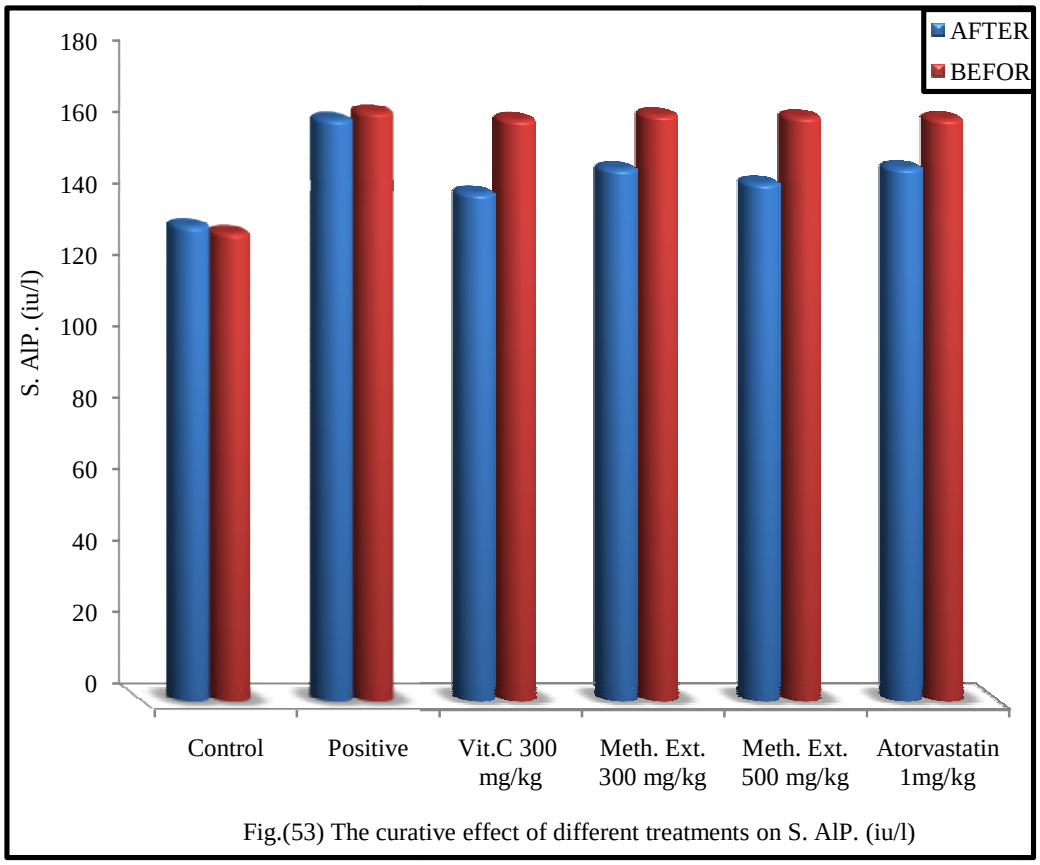


Fig.(53) The curative effect of different treatments on S. AlP. (iu/l)

Table (40): The curative effect of different treatments on S.T. Proteins (g/dl c ± S.D) and % variation from the corresponding control after induction of hypercholesterolemia as well as during 8 weeks of treatment in male albino rats.

Animal groups	Before treatment	After treatment	% var.
Control	5.80 ± 1	5.83 ± 2 †	0.5 ↑
positive control	3.80 ± 0.8	3.92 ± 1.1† a. †	3.2 ↑
vitamin C (300 µg/ml.)	3.85 ± 1	4.12 ± 1 b, †	7.0 ↑
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.)	4.00 ± 1	4.18 ± 1.3 b, †	4.5 ↑
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg)	3.93 ± 0.75	4.30 ± 1 b, †	9.4 ↑
Atorvastatin (1 mg/kg b .w)	3.88 ± 1	4.35 ± 1.5 b, †	12.1 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.

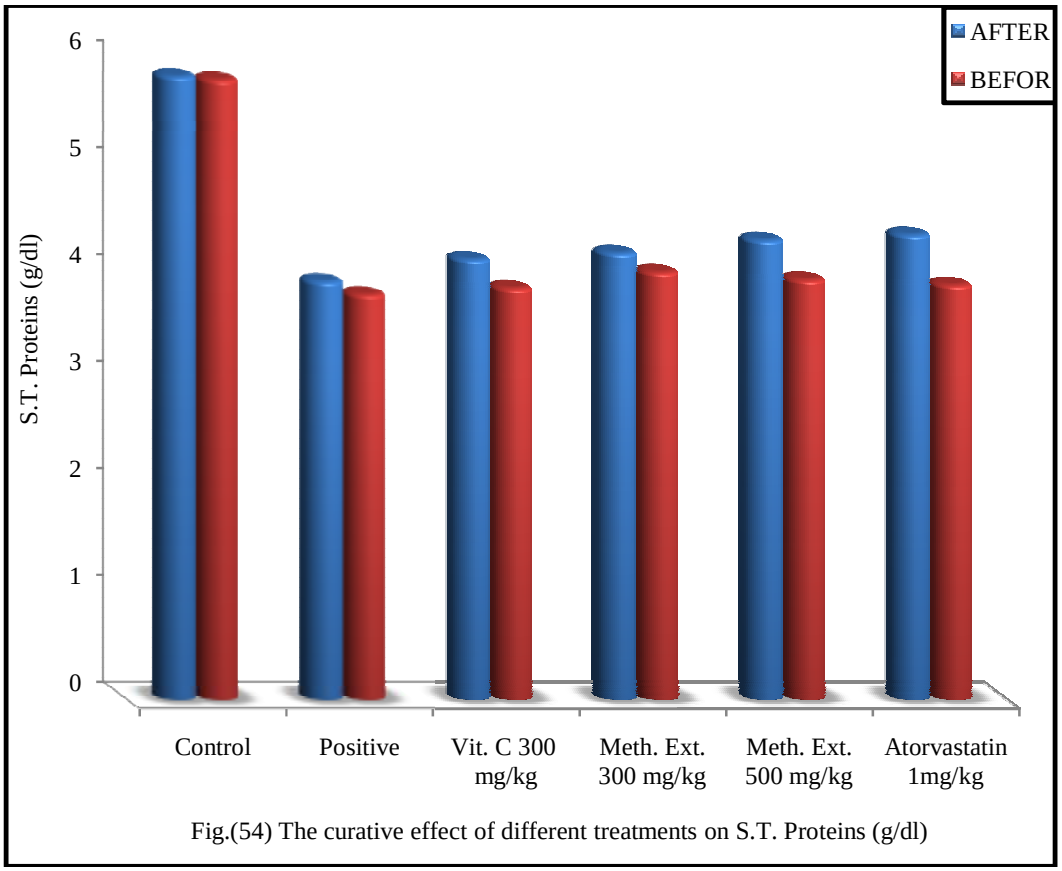


Fig.(54) The curative effect of different treatments on S.T. Proteins (g/dl)

Table (41): The curative effect of different treatments on S. Albumin (g/dl ± S.D) and % variation from the corresponding control after induction of hypercholesterolemia as well as during 8 weeks of treatment in male albino rats.

Animal groups	Before treatment	After treatment	% var.
Control	5.69 ± 2	5.69 ± 2 †	Zero
positive control	3.20 ± 1	3.22 ± 1.2 a, †	0.63 ↑
vitamin C (500 mg/kg)	3.18 ± 1.2	3.52 ± 1 b, †	10.7 ↑
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg.)	3.29 ± 1.3	3.63 ± 1.5 b, †	10.3 ↑
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg)	3.40 ± 1	3.69 ± 1.1 b, †	8.5 ↑
Atorvastatin (1 mg/kg b .w)	3.12 ± 1.5	3.75 ± 1.3 b, †	20.2 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.

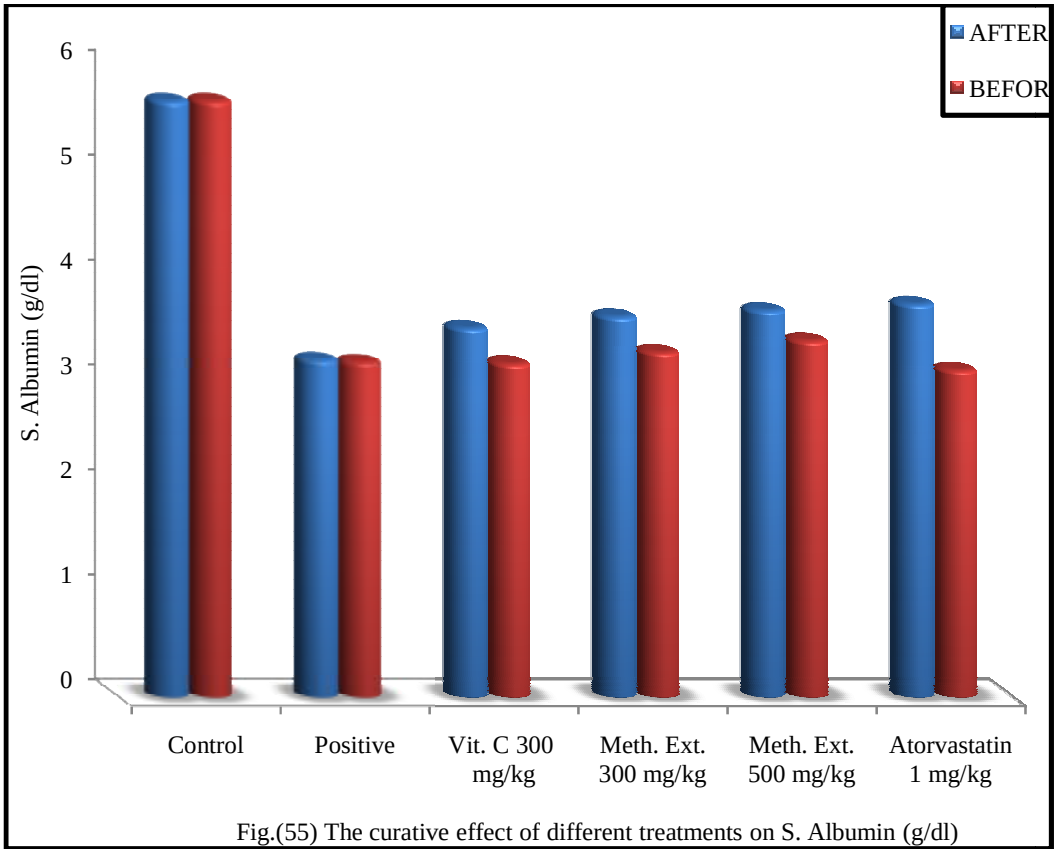


Fig.(55) The curative effect of different treatments on S. Albumin (g/dl)

Table (42): The curative effect of different treatments on B1.Urea (mg/dl± S.D) and % variation from the corresponding control after induction of hypercholesterolemia as well as during 8 weeks of treatment in male albino rats.

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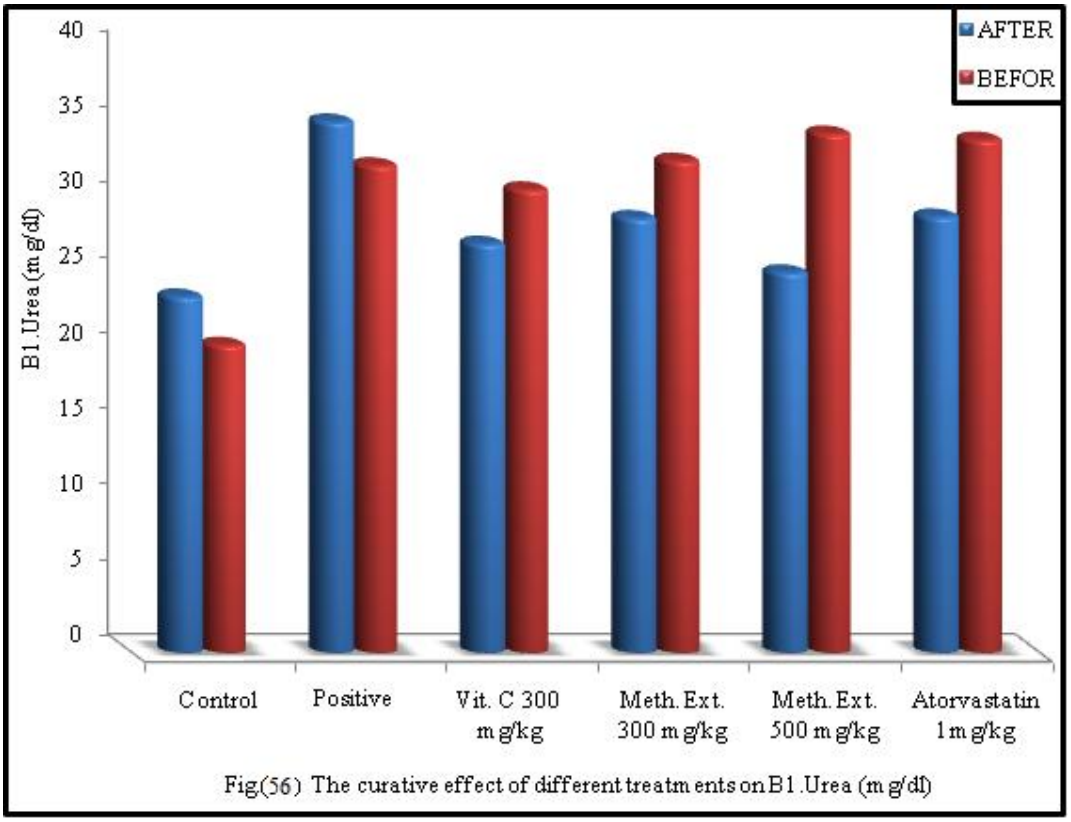
Animal groups	Before treatment	After treatment	% var.
Control	20.4 ± 2	23.6 ± 2 †	15.7 ↑
positive control	32.3 ± 3	35.2 ± 3 a, *	51 ↑
vitamin C (300 mg/kg)	30.7 ± 2	27.1 ± 5 b, †	11.7 ↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg)	32.6 ± 2	28.8 ± 3 b,*	11.7 ↓
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg)	34.4 ± 4	25.2 ± 2 b,*	26.7 ↓
Atorvastatin (1 mg/kg b .w)	34 ± 2	28.9 ± 4 b,*	15 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.



Fig(56) The curative effect of different treatments on B1.Urea (mg/dl)

Table (43): The curative effect of different treatments S. Creatinine (mg/dl ± S.D) and % variation from the corresponding control after induction of hypercholesterolemia as well as during 8 weeks of treatment in male albino rats.

Animal groups	Before treatment	After treatment	% var.
Control	0.30 ± 0.02	0.29 ± 0.05 †	3.33 ↓
positive control	0.43 ± 0.01	0.46 ± 0.03 a, *	6.98 ↑
vitamin C (300 mg/kg)	0.4 1± 0.02	0.33 ± 0.02 b, †	19.5 ↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg)	0.39 ± 0.01	0.34 ± 0.03 b, †	12.8 ↓
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg)	0.40 ± 0.03	0.33 ± 0.06 b, †	17.5 ↓
Atorvastatin (1 mg/kg b.w)	0.42 ± 0.02	0.35 ± 0.07 b, †	16.7 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.

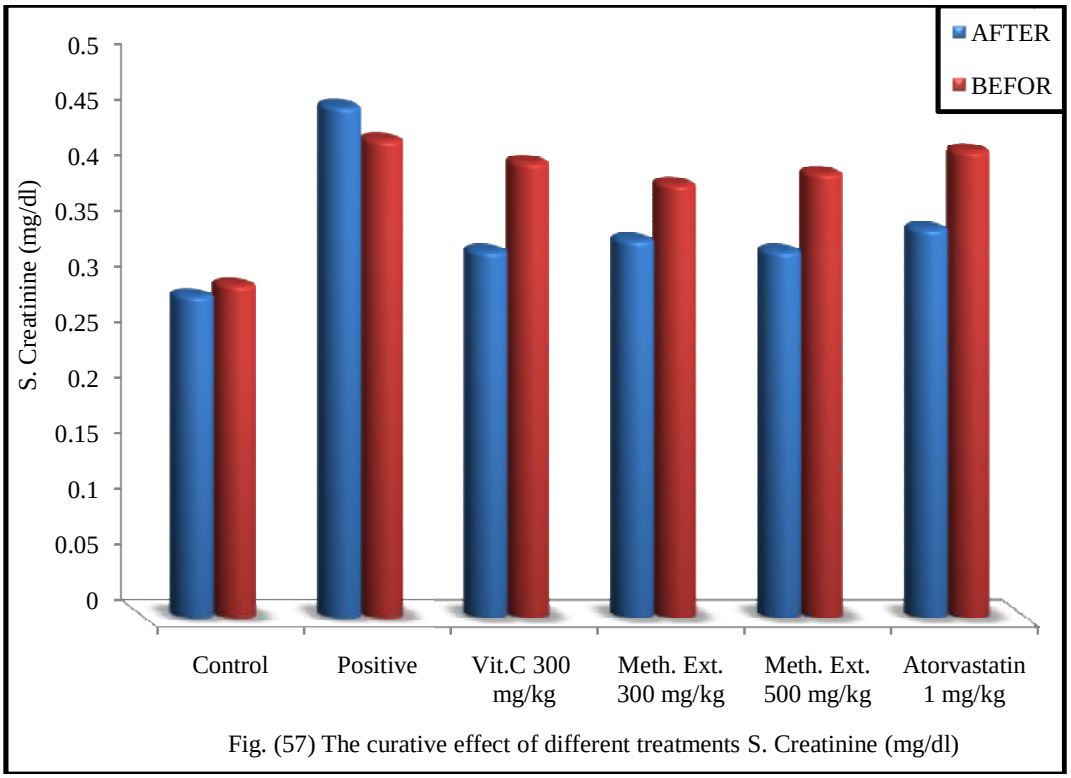


Fig. (57) The curative effect of different treatments S. Creatinine (mg/dl)

Table (44): The curative effect of different treatments on plasma SOD (u/mol ± S.D) and % variation from the corresponding control after induction of hypercholesterolemia as well as during 8 weeks of treatment in male albino rats.

Animal groups	Before treatment	After treatment	% var.
Control	7.36 ± 1.7	7.39 ± 2 †	0.41 ↑
positive control	4.30 ± 1.9	3.98 ± 0.7 a, *	7.4 ↓
vitamin C (300 mg/kg)	4.44 ± 1	5.20 ± 0.31 b, †	17.1 ↑
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg)	4.22 ± 0.86	5.10 ± 0.17 b, †	20.8 ↑
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg)	4.34 ± 0.32	5.56 ± 0.12 b, †	28.1 ↑
Atorvastatin (1 mg/kg b.w)	4.40 ± 1.3	5.33 ± 0.19 b, †	21.1 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.

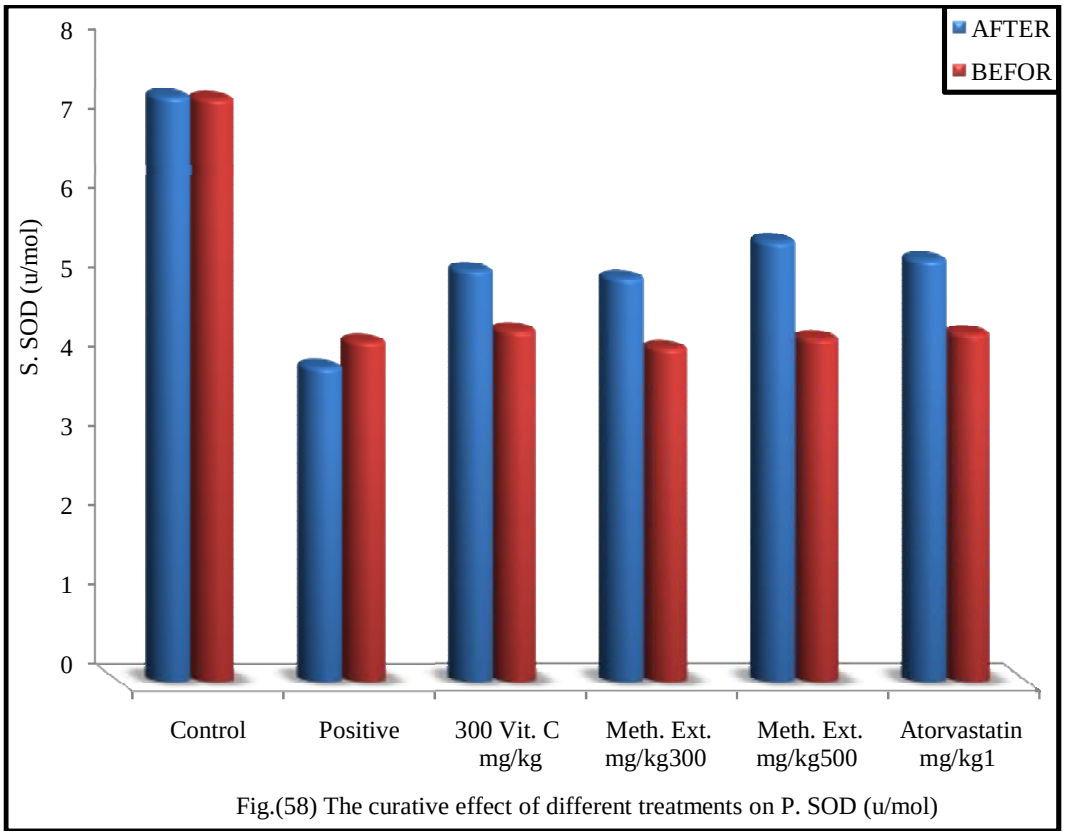


Fig.(58) The curative effect of different treatments on P. SOD (u/mol)

Table (45): The curative effect of different treatments on plasma MDA (n mol/ml ± S.D) and % variation from the corresponding control after induction of hypercholesterolemia as well as during 8 weeks of treatment in male albino rats.

Animal groups	Before treatment	After treatment	% var.
Control	15.8 ± 4	15.6 ± 2 †	1.3 ↓
positive control	25.6 ± 2	24.9 ± 1.78 a, *	2.7 ↓
vitamin C (500 mg/kg)	24 ± 3	18.4 ± 2 b, †	23.3 ↓
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg)	24.3 ± 1.3	17.7 ± 3 b, †	27.2 ↓
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg)	24.6 ± 3	17.2 ± 2.2 b, †	30.1 ↓
Atorvastatin (1 mg/kg b.w)	25.2 ± 2	16.1 ± 3 b, †	36.1 ↓

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.

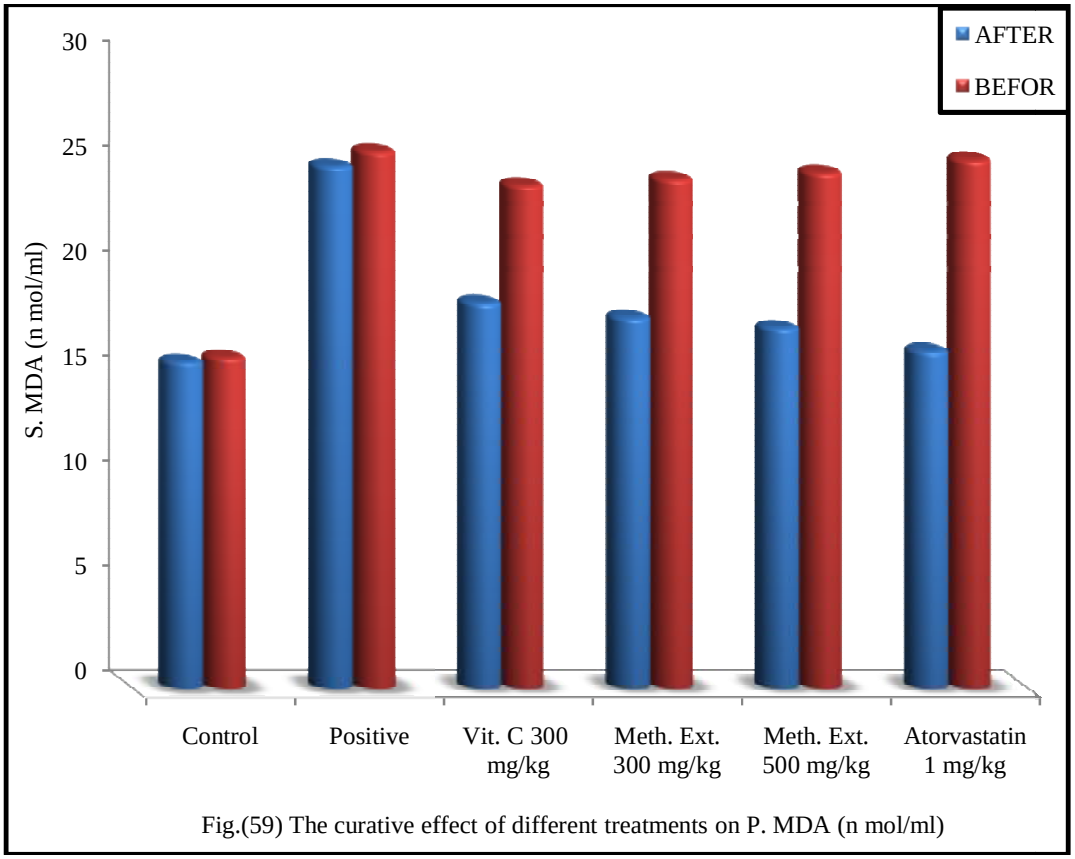


Fig.(59) The curative effect of different treatments on P. MDA (n mol/ml)

Table (46): The curative effect of different treatments on plasma GR (u/l of NADPH oxidized/min mg protein-± S.D) and % variation from the corresponding control after induction of hypercholesterolemia as well as during 8 weeks of treatment in male albino rats.

Animal groups	Before treatment	After treatment	% var.
Control	25.29 ± 4	25.30 ± 3 †	0.03 ↑
positive control	14.11 ± 3	13.15 ± 2 a, *	6.8 ↓
vitamin C (300 µg/ml.)	13.11 ± 3	18.20 ± 2 b, †	38.8 ↑
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg)	13.12 ± 2	19.20 ± 4 b, †	46.3 ↑
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg)	12.7 ± 2	18.73 ± 1 b, †	74.5 ↑
Atorvastatin (1 mg/kg b.w)	13.10 ± 3	19.17 ± 2 b, †	46.3 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.

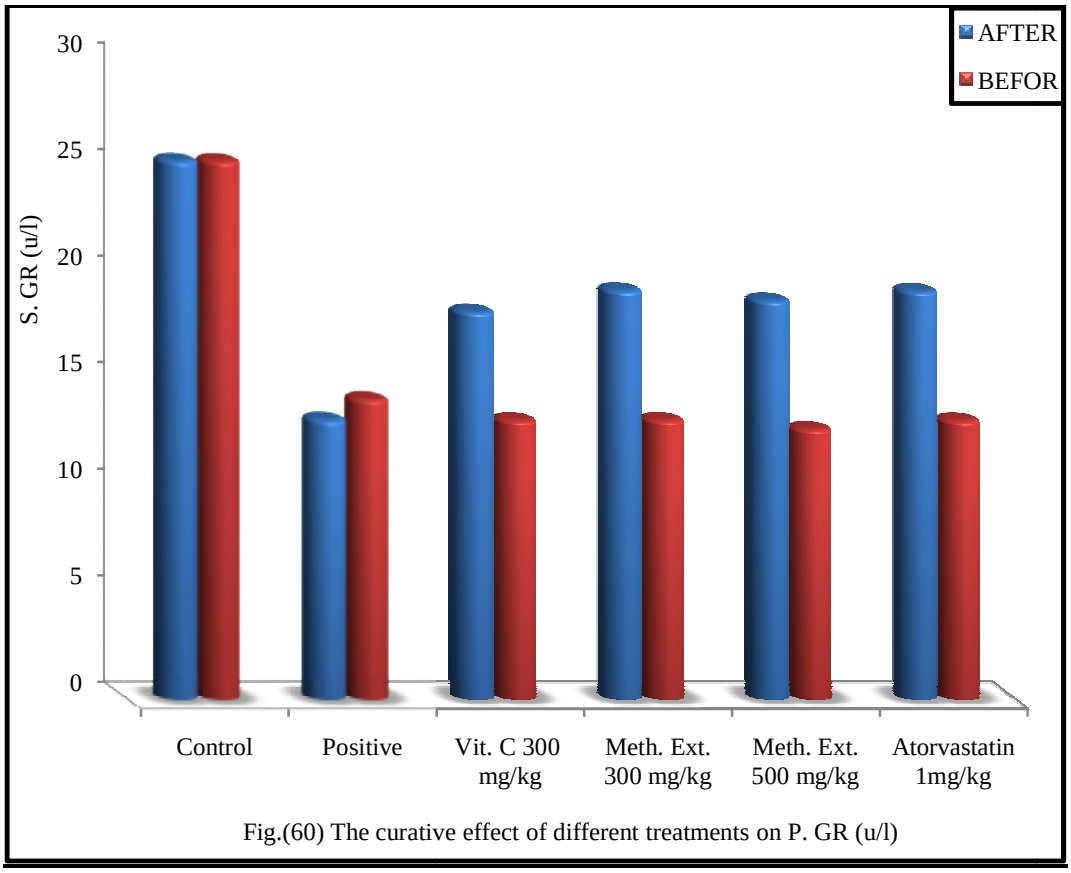


Fig.(60) The curative effect of different treatments on P. GR (u/l)

Table (47): The curative effect of different treatments on plasma GP_X (mu/ml of GSH consumed/min mg protein ± S.D) and % variation from the corresponding control after induction of hypercholesterolemia as well as during 8 weeks of treatment in male albino rats.

Animal groups	Before treatment	After treatment	% var.
Control	34 ± 2	33.81 ± 2 †	0.6 ↓
positive control	25.64 ± 3	24.71 ± 3 a, †	3.6 ↓
vitamin C (300 µg/ml.)	24.66 ± 2	27.9 ± 4 b, †	13.1 ↑
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg)	24.63 ± 4	27.78 ± 2 b, †	12.8 ↑
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg)	23.67 ± 1.8	28.81 ± 2 b, †	21.7 ↑
Atorvastatin (1 mg/kg b.w)	25.63 ± 3	30 ± 3 b, †	17.4 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.

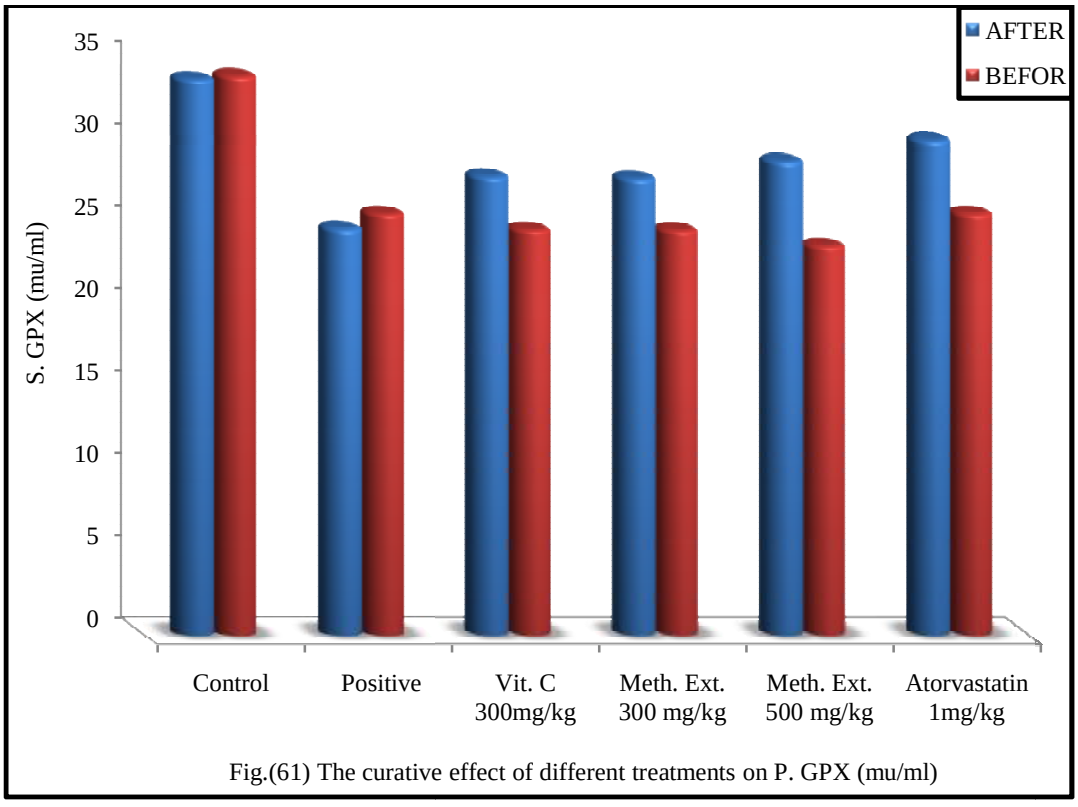


Fig.(61) The curative effect of different treatments on P. GPX (mu/ml)

Table (48): The curative effect of different treatments on plasma CAT (u/l ± S.D) and % variation from the corresponding control after induction of hypercholesterolemia as well as during 8 weeks of treatment in male albino rats.

Animal groups	Before treatment	After Treatment	% var.
Control	53.7 ± 4	53.2 ± 2.93 †	0.9 ↓
positive control	37.9 ± 2	36.4 ± 6.87 a, *	3.9 ↓
vitamin C (300 mg/kg)	36.0 ± 3	43.0 ± 4 b, †	19.4 ↑
Meth. Ext. of Arbutus pavarii leaves (300 mg/kg)	35.9 ± 5	44.3 ± 3 b, †	23.4 ↑
Meth. Ext. of Arbutus pavarii leaves (500 mg/kg)	35.6 ± 3	43.7 ± 6 b, †	22.8 ↑
Atorvastatin (1 mg/kg b.w)	35.9 ± 2	42.7 ± 3 b, †	18.9 ↑

† Nonsignificant difference from the corresponding control at P > 0.1; * Significant difference at P < 0.05; ** highly sig. difference at P < 0.01; *** Very highly sig. difference at P < 0.001; ↓ Decrease; ↑ Increase; a compared with control group; b compared with positive group.

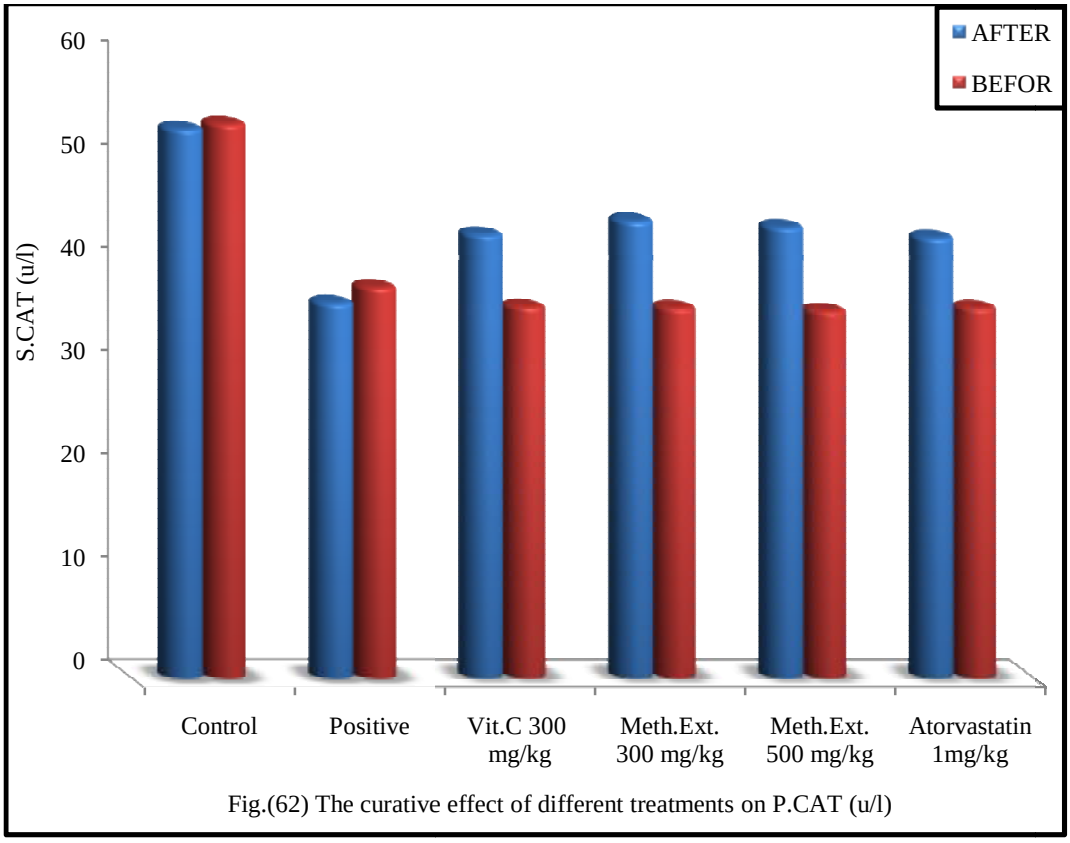


Fig.(62) The curative effect of different treatments on P.CAT (u/l)

Histopathological studies

Liver tissue

In control group of animals**,** the parenchyma of liver in all animals showed normal pattern regarding to size, shape, arrangement and staining characters. Also, the portal tract and control area were normal (Fig. 63).

In all examined animals of the (+ve) control group, histopathological changes of liver tissue revealed diffuse vacuolar degenerative changes of hepatocytes vary from mild to marked in severity. Ballooning of hepatocytes together with focal lymphocytic cells aggregates in portal areas were seen in Fig.(64).

The hepatocytes distortion was ameliorated where near normal appearance of hepatocytes in methanolic extract of *Arbutus pavarii* leaves at (300 and 500) µg/ml in pretreated livers, also at the 300 mg/kg of vitamin C as illustrated in (figs. 65, 66, 67). In addition to Atorvastatin at 1mg/kg which illustrated in fig. (68)

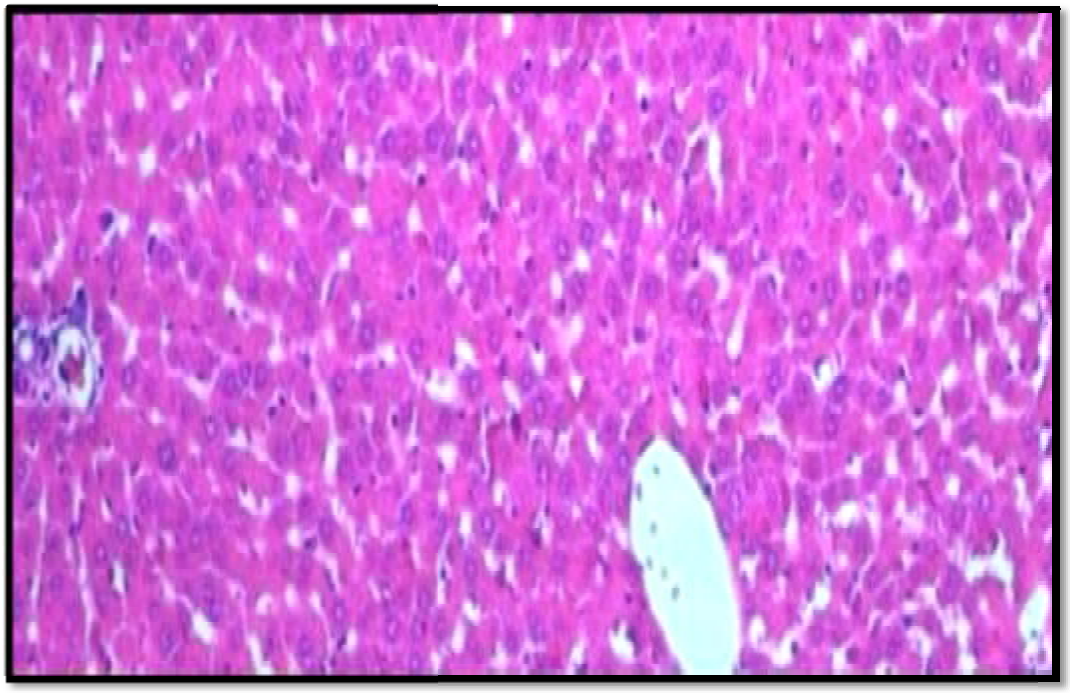


Fig. (63): control group of animals**,** the parenchyma of liver in all animals showed normal pattern regarding to size, shape.

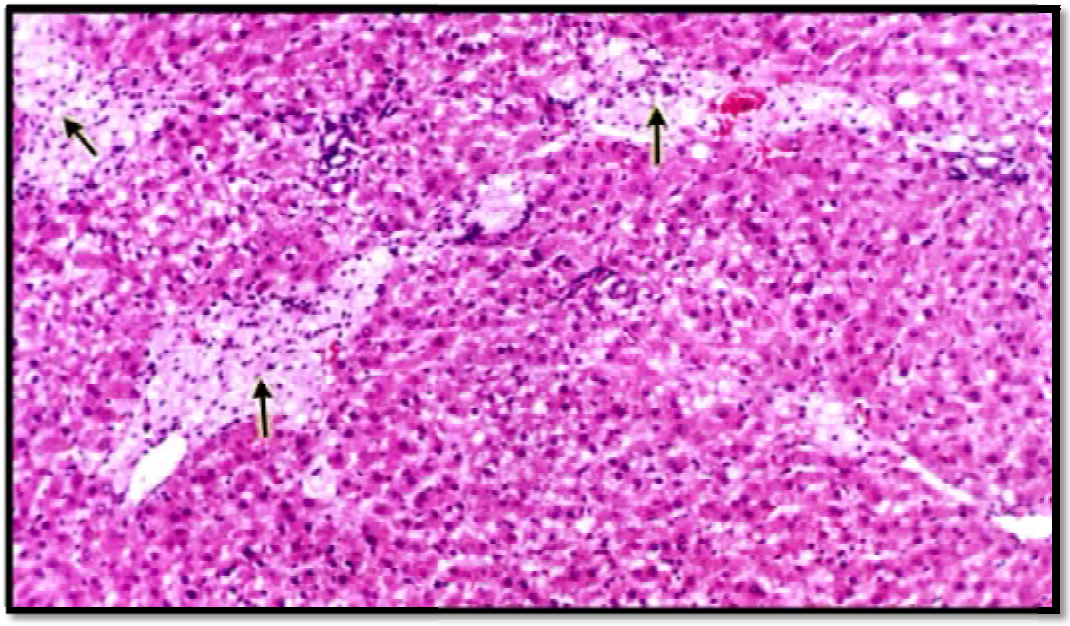


Fig. (64): Representing the liver of positive control rat, and showing small nests (arrow) of reticulated faintly stained cells scattered through the liver tissues.

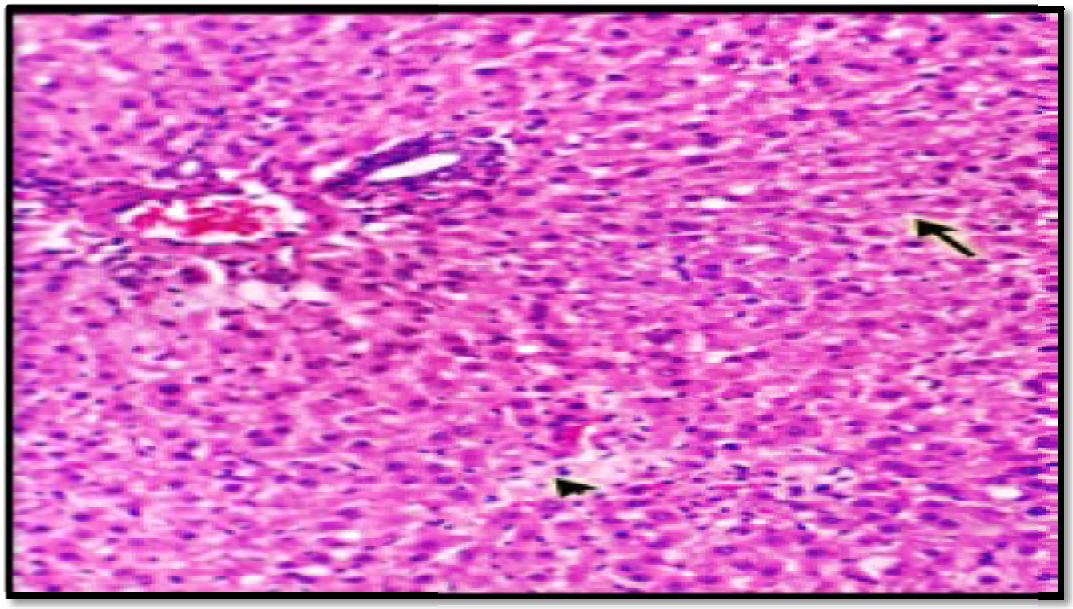


Fig. (65): The liver of methanolic extract *Arbutus pavarii* leaves treated (300mg/kg. b.w.), showing hepatocytes vacuolated (arrow) and nodular cells (arrow head) of faintly.

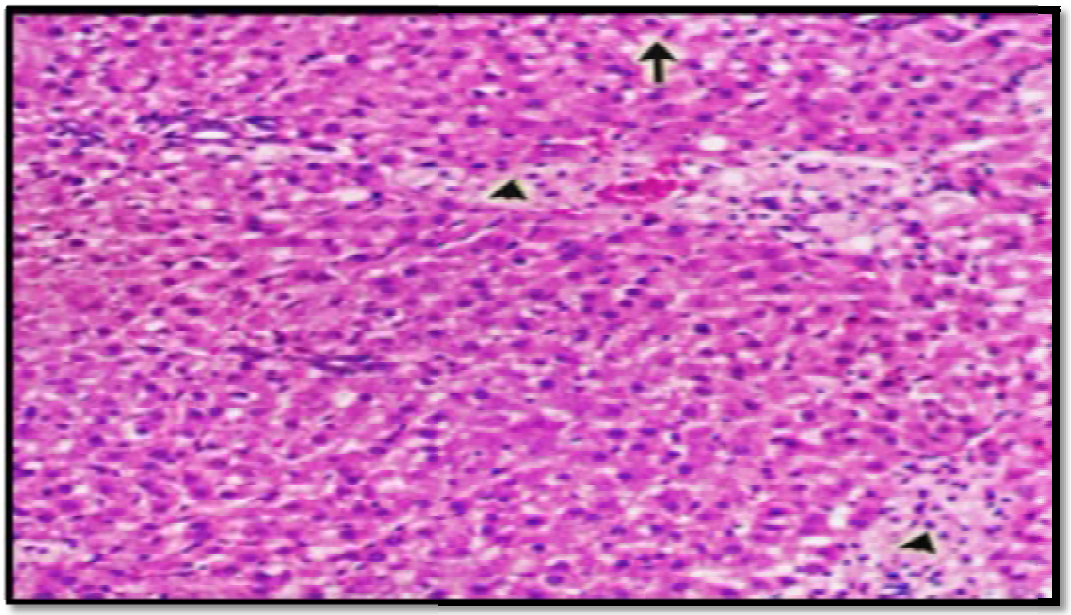


Fig. (66): The liver of methanolic extract *Arbutus pavarii* leaves treated (500mg/kg. b.w.), showing hepatocytes vacuolated (arrow) and nodular cells (arrow head) of faintly.

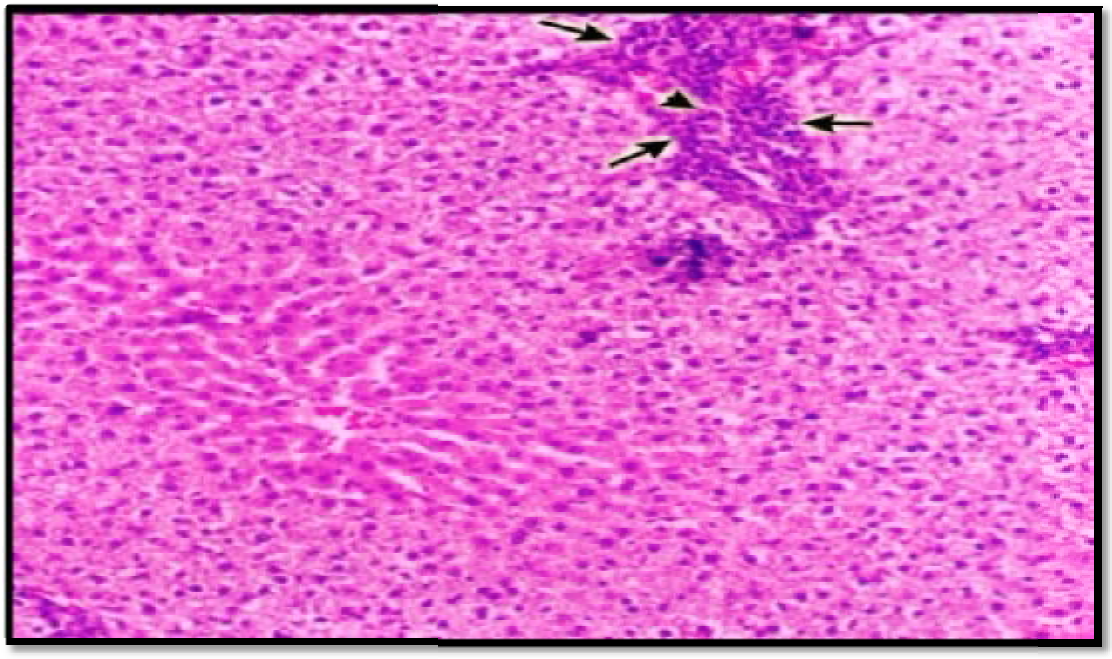


Fig. (67): The liver section of vitamin C treated (300mg/kg. b.w.), with mild increase in lymphocytic aggregates (arrow) in portal areas.

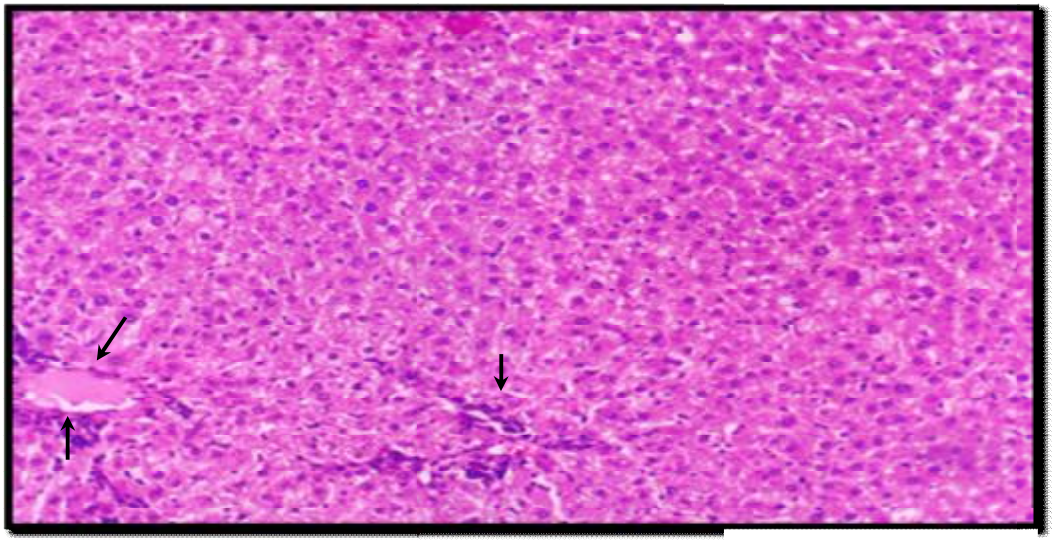


Fig. (68): Atorvastatin treated (1 mg/kg.bw) for8 weeks, showing dilated and congested Blood vessels (arrow) and number of mast cells (arrow head).

DISCUSSION

1. Antioxidant activity of Arbutus Pavarii leaves.

Phytochemicals are natural bioactive compounds found in plants and their parts, such as vegetables, fruits, medicinal plants, aromatic plants, leaves, flowers and roots, which work with nutrients and fibres to act as a defense system against disease or, more accurately, to protect against disease. Phytochemicals are divided into two groups, which are primary and secondary constituents, according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while secondary constituents comprise alkaloids, terpenoids, flavonoids, saponins, tannins and so on. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds **[119]**.

For several years, many researchers have been searching for powerful but nontoxic antioxidants from natural sources, especially edible or medicinal plants. Such natural antioxidants could prevent the formation of reactive species-related disorders in human beings without the use of synthetic compounds, which may be carcinogenic and harmful **[120]**.

1.1.Total phenolic content (TPC)

Dissociation of a phenolic proton leads to form a phenolate anion, which is capable of reducing Folin-Ciocalteu reagent. This supports the notion that the reaction occurs through electron transfer mechanism. The blue compounds formed between phenolate and Folin-Ciocalteu reagent are independent of the structure of phenolic compounds, therefore ruling out the possibility of coordination complexes formed between the metal center and the phenolic compounds **[121]**.

The intensity of color increase with increasing the concentration of MEAPL which consider as an indicator for increasing the total phenolic content and finally reflect its antioxidant activity. The results expressed according to pyrogallol as a phenolic compound in figure (8) where found each 100 µg/ml of MEAPL equivalent to

63 µg/ml of pyrogallol. The *Arbutus Pavarii* leaves may have some antioxidant activities and health benefits related to its phenolic content.

The results obtained in the present study indicate that the MEAPL exhibit antioxidant activity which may be attributed to the presence of polyphenolic and other Phytochemicals constituents. The MEAPL could be potential sources of natural antioxidant that could have great importance as therapeutic agents in preventing various disorders like hypercholesterolemia specially oxidation of LDL which play an important role in pathogenesis of atherosclerosis and oxidative stress related degenerative diseases **[122]**.

1.2. Total Flavonoid content (TFC)

The principle of aluminum chloride colorimetric method is that aluminum chloride forms acid stable complexes with the C_4 keto group and either the C_3 or C_5 hydroxyl group of flavones and flavonols. In addition, aluminum chloride forms acid labile complexes with the orthodihydroxyl groups in the A or B ring of flavonoids. Which has the C_5 hydroxyl group and the orthodihydroxyl groups in B ring formed a complex that showed a strong absorption at 415 nm. In compromise, therefore, the wavelength 415 nm was chosen for absorbance measurement **[123]**.

Based on the absorbance values of the various concentrations, and compared with the standard solutions of quercetin equivalents, results obtained in this study figure (11) revealed that the level of flavonoids in 100µg/ml of MEAPL equivalents to 66µg/ml of quercetin. Flavonoids are polyphenolic substances present in most plants with a rich structural diversity.

Antioxidant capacity assays have mainly classified flavonoids as scavenging by electron transfer (ET) or by hydrogen atom transfer (HAT). ROS-scavenging potential of flavonoids has been related to the stability of their radical species, and the stability of flavonoid radicals is expected to increase by extended conjugation **[124]**.

1.3. Reducing power assay

The intensity of the color increase with increasing of the amount of MEAPL and measured at 700 nm by uv-visible spectrophotometer. Which reflects its reducing capacity, the results expressed according to ascorbic acid as standard in figure(12)

where each 100 µg/ml of MEAPL equivalents to 94.5 µg/ml of ascorbic acid. Reducing power values was reported that the reducing properties are generally associated with the presence of reducers, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. Accordingly, MEAPL might contain higher amount of reducers, which could react with free radicals to stabilize and block radical chain reactions **[125]**. So we can suggest that there are relationship between the amount of total phenolic content and reducing power.

1.4. DPPH$^{\bullet}$ radical scavenging

DPPH$^{\bullet}$ is a free radical stable at room temperature which produces a purple solution in methanol. Where the absorbance measured at 517 nm. The purple color of DPPH$^{\bullet}$ changed to yellow methanolic solution which induce decrease in the absorbance of DPPH$^{\bullet}$ when reacts with the MEAPL. A lower absorbance at 517 nm indicates a radical scavenging activity of the DPPH$^{\bullet}$ radical by MEAPL and finally can give us clear evidence for its antioxidant activity. The percent of radical scavenging activity (RSA) calculated from the following equation.

% RSA = [Abs. of Control – Abs. of Sample / Abs. of Control] x 100

According to the above equation the percent of DPPH radical scavenging activity by MEAPL ranged from 39% at 100 µg/ml to 85% at 500 µg/ml and ranged from 45% at 100 µg/ml to 84% at 500 µg/ml of ascorbic acid which used as standard antioxidant as elucidated in figure (14).

The role of antioxidant is its interaction with oxidative free radicals. The essence of DPPH$^{\bullet}$ assay is that the antioxidant reacts with the stable free radical 1, 1-diphenyl-2-picrylhydrazyl (deep violet color) and converts it to 1, 1-diphenyl-2-picrylhydrazine with a yellow color. The degree of discoloration indicates the scavenging potential of the sample as an antioxidant **[126]**.

2. Induction of hypercholesterolemia

Induction of hypercholesterolemia was performed using cholesterol: cholic acid mixture at a ratio 3: 1 **[127]**. In addition, saturated fats (10%) and sucrose (50%) were added to the diet. Bile acid (cholic acid) was used to overcome the difficulty of cholesterol absorption.

After the induction of hypercholesterolemia, the level of serum HDL-C, and the plasma SOD,CAT,GP_X and GR will decrease also the level of serum total proteins and albumin decreased. These findings proved the efficiency of the modification employed in inducing hypercholesterolemia.

The decreased levels of total albumin may be due to reduction in protein intake from the intestine as a result of a high calorie lipid diet **[128]**.

The increased in serum albumin may be related to dehydration due to decrease water intake or possible nutritional effects **[129]**. highly significant elevations were indicated in the level of serum total cholesterol, risk ratio, triglycerides, LDL-C, VLDL-C, G-GT, MDA also ALT, AST, LDH, and ALP. While slight elevations were indicated in the level of serum urea, and creatinine after 8 weeks from the induction of hypercholesterolemia.

Elevations indicated in serum lipid profile seem to be logic and runs parallel with the excess of saturated fat and sugar available in the diet. Elevations in the total lipids were also indicated after the induction of experimental hypercholesterolemia **[5]**.

Also The increase indicated in the level of cholesterol runs parallel with reductions indicated in HDL-C may be an important because it is stimulate the removal of cholesterol from the peripheral cells back to the liver for excretion. On the contrary, raised level of HDL-C was associated with reduced risk of atherosclerosis, since high density lipoprotein in serum is thought to facilitate the translocation of excess cholesterol from the peripheral tissue to liver for further catabolism **[130]**.

Cholesterol-enriched diet resulted in an increase in serum total cholesterol and

LDL cholesterol **[128]**.

The results of serum AST, ALT and LDH activities in the controls and experimental groups. There were significant increases in the plasma AST, ALT and LDH activities of hypercholesterolemic rats as compared to normal control rats **[131]**.

Hypercholesterolemia is a major risk for coronary artery diseases. In the development of atherosclerosis, ROS are produced by endothelial cells, and macrophages oxidize LDL in the subendothelial space **[132]**. Important role in process of atherosclerosis plays oxidative stress, especially oxidative modification of LDL cholesterol. Oxidative stress is an imbalance between pro-oxidatively acting compounds and antioxidants in favour of pro-oxidants causing the damage of important biomolecules. It can directly evoke development of a disease or worsen or complication its progress. Oxidative stress participates in pathogenesis of atherogenesis through oxidative damage to lipids in the process of lipoperoxidation **[133]**.

decrease in the superoxide dismutase (SOD) and the catalase (CAT) levels in the high cholesterol fed animals is again attributed to increased oxidative stress on cholesterol feeding in these animals **[134]**.

Lipid peroxidation is initiated by free radical attack on membrane polyunsaturated fatty acids leading to their transformation and fragmentation to alkenes and reactive aldehyde compounds. Evaluation of the effect of high cholesterol diet in experimental rats showed increase in lipid peroxidation (TBARS) in animals fed with high cholesterol compared to the normal group. The observed is consistent with several clinical and experimental studies which have shown that hypercholesterolemia leads to increased lipid peroxidation **[135]**.

3. Evaluation of methanolic extract of *Arbutus Pavarii* leaves:

In hypercholesterolemia, one of the mechanisms that might be activated and might hinder coronary vascular function is a shift in scavenging activity and redox status, a state known as increased oxidative stress. Numerous studies show that a close relationship exists between high blood cholesterol and atherosclerosis, it has also been suggested that this relationship may be dependent on enhanced oxidative stress. Studies

have also shown that cholesterol rich diet increases the formation of peroxynitrile, a toxic reaction product of superoxide and nitric oxide in the rat **[136]**.

Rats fed on hypercholesterolemia diet developed hypercholesterolemia mark by significant increase in serum total lipids (TL), triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and decrease in high density lipoprotein cholesterol (HDL-C) compared with normal control rats. Also the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gama glutamyl transferase (G-GT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and malondialdehyde (MDA). The level of total protein and albumin decreased in addition to antioxidant enzymes glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) also decreased when compared with normal control rats.

The previous results concerning the effect of methanolic extract of *Arbutus Pavarii* leaves on protective and curative effect against hypercholesterolemia. It has an amelioretic effect against incidence of hypercholesterolemia.

The decrease of TL, , TC, LDL-C, VLDL-C and TG and increase of HDL-C in rats fed on a high-cholesterol diet when orally treated by 500 mg/kg of *Arbutus pavarii* leaves in prophylactic and curative groups. This significant effect of methanolic extract of *Arbutus Pavarii* leaves is due to its contents of very important substances which in general act as antioxidant substances e.g. flavonoids, phenolic compounds **[20]**.

Flavonoids decreased LDL- Cholesterol and increased HDL-Cholesterol. High density lipoprotein may hasten the removal of cholesterol from peripheral tissue to the liver for catabolism and excretion. Also, the increase of HDL concentration could protect LDL against oxidation *in vivo* **[137]**.

In our study, it was observed that, enzymes such as AST, ALT, G-GT, LDH and ALP were released into blood as a result of hypercholesterolemia. Their increase in the serum activities of these enzymes was directly proportional to the degree of cellular damage. These values decreased by the effect of methanolic extract of *Arbutus pavarii* leaves at 500 mg/kg when compared with positive group.

The present study revealed lower levels of total protein and albumin in rats on high cholesterol diet when compared to animals with normal diet. The decreased levels of total protein and albumin may be due to reduction in protein intake from the intestine as a result of a high calorie lipid diet, an indication of diminished synthetic function of the liver resulting probably from hepatocellular damage or stress resulting from the increased metabolic need for tissue repair and free radical neutralization occasioned by the high fat diet. However, co-treatment with extracts of *Arbutus pavarii* leaves significantly restored the protein levels to near control levels **[138]**.

The measurement of thiobarbituric acid (TBARS) is commonly used to monitor lipid peroxidation and indirectly, oxidative stress in vitro and *in vivo* **[139]**.

Treated of HCD-fed rats with methanolic extract of *Arbutus pavarii* leaves at 500 mg/kg reduced the TBARS concentration. The ability of methanolic extract to inhibit the process of lipid peroxidation in vivo may be due to the free radical scavenging activities of its phytochemical components.

Previous study demonstrated that a decrease in lipid peroxidation lead to the reduction of atherosclerosis caused by hypercholesterolemia **[140]**.

It had been mentioned that high-cholesterol diet might cause the generation of ROS, and the biological effects of ROS were controlled *in vivo* by enzymatic defense mechanisms. As an index for the redox status after four weeks with different treatments, the antioxidant capacities in serum was determined **[141]**.

Our results showed that high-cholesterol diet might lead to reduction in antioxidant enzymes include SOD, CAT, GR and GPx when compared with normal control group.

whereas orally treated with methanolic extract of *Arbutus pavarii* leaves at 500 mg/kg could increase the serum antioxidant capacity in rats. In the enzymatic defense mechanism, SOD, CAT, GR and GPx are regarded as four primary antioxidant enzymes since they play important role in scavenging free radical *in vivo.*

SOD catalyzes dismutation of superoxide anions into hydrogen peroxide, which was converted to water by both CAT and GPx. Nutrient antioxidants, included in the dietary antioxidants, are chain breaking antioxidants, which work with enzyme antioxidants, to regular the ROS within physiological limits **[142]**.

Phytochemicals, especially the phenolic compounds and flavonoids in methanolic extract of *Arbutus pavarii* leaves, have been proposed as the major bioactive compounds increasing antioxidant potential *in vivo,* treatment of hypercholesterolemic rats.

serum of blood urea and creatinine levels were increased in groups treated with cholesterol: cholic acid mixture, demonstrating that deterioration of the renal function, in comparison with those of the control. cholesterol: cholic acid mixture administration resulted in significant increase in the serum marker like blood urea and serum creatinine. makes it a candidate for its role in the pathogenesis induced renal disorder.

Ferulic Acid, like many phenols exhibits antioxidant effect in response to free radicals by donating hydrogen from its phenolic hydroxyl group. It has been revealed that the antioxidant capacity of phenolic acid In addition, the reactive oxygen species scavenging effect of ferulic acid has been reported to be similar to that of superoxide dismutase **[143]**. Ferulic acid inhibited growth of colon cancer cells *in vitro,* further *in vivo* test confirmed the inhibitory effect on carcinogenesis of colon cancer in rats **[144]**.

This significant effect of methanolic extract of *Arbutus Pavarii* leaves is due to its contents of very important substances which in general act as antioxidant substances e.g. triterpenes and flavonoids, phenolic compounds, amyrin, lupeol, arbutin, catechin, isoquercitrin, myricetin and ferulic acid **[20]**.

Rats fed on hypercholesterolemia-induced diet developed hypercholesterolemia mark by increase in serum total lipids (TL), triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C),and decrease the level high density lipoprotein cholesterol (HDL-C) compared with normal control rats. Supplementation of vitamin C with Atorvastatin falls in total lipids, triglycerides, total cholesterol, LDL-C and insignificant alteration in HDL-C compared with hypercholesterolemic group. It must be noticed that, the effect of Atorvastatin is more effective than vitamin C for

lowering lipids profile. The levels of total lipids, total cholesterol, triglycerides, LDL-C were decreased But HDL-C level was increased.

The observed effect may be due to of decreased cholesterogenesis and fatty acid synthesis. Also lowering of total cholesterol, triglycerides, LDL-cholesterol and raise in HDL cholesterol is a very desirable biochemical state for prevention of atherosclerosis and ischaemic conditions **[145]**.

Atorvastatin belongs to 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-COA) reductase inhibitors, potent inhibitors of cholesterol biosynthesis that are used extensively to treated hypercholesterolemia **[35]**.

The present study which indicated that, the vitamin C and Atorvastatin have more lowering effect of lipid profile. Which may prevent of the increase in the factors causing coronary heart diseases (CHD) and cardiovascular diseases (CVD), so it may prevent of atherosclerosis.

The levels of AST, ALT, G-GT, LDH and ALP activities in serum of hypercholesterolemic rats (positive group) were increased as compared to normal control rats.

Treatment by vitamin C (300 mg/kg) and Atorvastatin (1mg/kg) reduced the serum levels of liver enzymes toward the normal value this clearly indicates that the vitamin C has stabilizes the plasma membrane as well as helped in healing of the hepatic tissue damage, resulting in lower levels of ALT, AST, ALP, G-GT, LDH, and ALP than the hypercholesterolemic rats in both prophylactic and curative groups. The activity of vitamin C and Atorvastatin improved the decrease in total protein and albumin activity by increase their levels in serum especially when compared with positive groups.

In the current study, antioxidant enzymes (SOD, CAT, GPx and GR) activities decreased in rats fed a cholesterol-rich diet compared to those in control group. The decrease in the activities of these enzymes could be attributed to the excessive utilization of these enzymes in inactivating the free radicals generated due to the high cholesterol diet **[42]**.

This suggests that ROS may already have exerted their cytotoxic effects include damage of polyunsaturated fatty acids in cell membrane leading to formation of malondialdehyde.

This study demonstrated that the elevated concentrations of MDA, an end product of polyunsaturated fatty acid peroxidation, had present in hypercholesterolemic group. Supplementation of hypercholesterolemic rats with vitamin C and Atorvastatin showed decrease in MDA level.

Vitamin C and Atorvastatin administration to HCD-fed rats increased the levels of SOD, GAT, GPx and GR and decreased the serum of MDA, in compared with positive group.

Results of the present study suggests vitamin C's ameliorating effects to be likely mediated via inhibition of free radicals generation and/or free radical scavenging activity. Were we can notice these effects in prophylactic and curative groups when compared to "positive group". Atorvastatin possesses antioxidant properties by reducing lipid peroxidation and ROS production. Atorvastatin reduces the susceptibility of lipoproteins to oxidation both in vitro and in vivo i.e. they decrease the LDL oxidation **[35]**. The curative action of Atorvastatin is associated with its antioxidant property, as it possibly acts as a free radical scavenger, an inhibitor of lipid peroxidation.

Summary and conclusion

Herbal medicines are widely used all over the world. They are often perceived as being natural and therefore harmless. Many herbal remedies individually or in combination with different formulations such as leaf, powder, pastes, decoction, infusion, etc. had been recommended to treat various diseases. Many, if not most of medicinal plants contain flavonoids, such compounds had been associated with several beneficial effects such as antioxidants which consider to be fundamental property important for life.

1. The prophylactic effect of different treatments against hypercholesterolemia

To study the protective effect of the methanolic extract of *Arbutus pavarii* leaves against hypercholesterolemia, a total of 30 rats were used and the experiment lasted for 8 weeks. Animals were divided into five groups (6 rats each) as follows:

Group 1: Rats were fed on the standard synthetic diet and served as negative control (- ve) for 8 week.

Group 2: Rats were daily attained to the hypercholesterolemia diet (H.C.D) and served as positive control group (+ ve).

Group 3: Rats were daily administered vitamin C at a dose of 300 mg/Kg b.w. (oral).

Group 4: Rats were daily administered methanolic extract of leaves of *Arbutus pavarii* at a dose of 300 mg/kg b.w. (oral)

Group 5: Rats were daily administered methanolic extract of leaves of *Arbutus pavarii* at a dose of 500 mg/kg b.w. (oral).

2. The curative effect of different treatments on hypercholesterolemic rats

In this experiment, a total of 36 rats were used. six rats were fed on the standard synthetic diet and served as negative control (- ve) "**group1**". The other rats were subjected to the induction of experimental hypercholesterolemia for 8 weeks as described before and treated for 8 weeks

The hypercholesterolemia rats (30 rats) were divided randomly into equal 5 sub-groups (6 rats each) as follows:

Group 2: Rats were served as hypercholesterolemic animals (+ ve).

Group 3: Rats were daily received vitamin C at a dose of 300 mg/kg b.w.(oral).

Group 4: Rats were daily received methanolic extract of leaves of *Arbutus pavarii* at a dose of 300 mg/kg b.w. (oral).

Group 5: Rats were daily received methanolic extract of leaves of *Arbutus pavarii* at a dose of 500 mg/kg b.w. (oral).

Group 6: Rats were daily received 1mg/kg b.w. of Atorvastatin as a standard hypolipaemic agent (oral).

Experimental induction of hypercholesterolemia was induced ″using cholesterol and cholic acid″ in order to study the effect of methanolic extract of *Arbutus pavarii* leaves against hypercholesterolemia. Atorvastatin was used as a reference hypercholesterolemia agent.

In the first and second experiments, blood samples were collected before treatment and then after 4 and 8 weeks. Also blood samples were collected before and after induction of hypercholesterolemia and then after 4 weeks from the administration of the different treatments.

The different biochemical parameters were carried out (Blood urea, serum creatinine, serum total lipids, serum total cholesterol, serum HDL-Cholesterol, serum triglycerides, serum transaminases, serum alkaline phosphatase, serum lactate dehydrogenase, serum total protein and serum albumin) as well as the histopathological studies.

The results obtained revealed that methanolic extract of *Arbutus pavarii* leaves against hypercholesterolemia has a prophylactic and curative effect against hypercholesterolemia compared with the reference standard hypercholesterolemia agent ″Atorvastatin″.

In general, to use these plant extracts as safe prophylactic and curative agents, more studies should be carried out to know all the active / inactive components and their mechanism of actions weither synergesic or antagonist using different doses from these extracts and another types of experimental animals for a long period in order to assessment the biological activity of these herbs as well as their side effects.

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الملخص العربى

نظرا للاستعمالات الشائعة للنباتات الطبية في علاج العديد من الأمراض مثل السرطان – الفشل الكبدي – الفشل الكلوي- ارتفاع دهون الدم وتصلب الشرايين. وذالك لاحتواء هذه النباتات على قيمه غذائية عالية واحتوائها أيضا على العديد من المركبات الكيميائية ذات الفاعلية المضادة للأكسدة ولكونها مصادر طبيعية فإن ذلك يبشر بإمكانية استخدامها في علاج العديد من الأمراض والوقاية منها كبدائل للأدوية المخلقة كيميائياً.

وتهدف هذه الدراسة لتحديد الفاعلية المضادة للأكسدة لمستخلص الميثانول لأوراق نبات الشمـــــارى وكذالك لمعرفة التأثير المضاد لارتفاع نسبة الكوليستيرول في الدم.

حيث تم تحديد الفاعلية المضادة للأكسدة من خلال تحديد المحتوي الكلي للمركبات الفينولية وكذالك الفلافونويد ومن خلال تثبط الظذر الحر لمركب (1, 1-diphenyl-1,2-picryl hydrazyl radical DPPH•) بالإضافة إلي القوة الاختزالية.

وقد أجريت الدراسات البيولوجية على فئران التجارب البيضاء حيث تم تقسيمها إلي مجموعتين رئيسيتين:

أولاً : دراسة التأثير الواقي للمستخلص على مستوى الكوليستيرول في الدم. استمرت التجربة لمدة 8 أسابيع آخذت فيها عينات الدم قبل بدء التجربة ثم بعد أربعة أسابيع وثمانية أسابيع. استخدم في هذه التجربة (30) فأر على خمس مجموعات رئيسية (ستة فئران في كل مجموعة).

المجموعة الأولى : استخدمت كمجموعة ضابطة تغذت على كل الغذاء العادي.

المجموعة الثانية : استخدمت كمجموعة ضابطة أيضا لكنها تغذت على الغذاء المحتوى على الكوليسترول وحمض الكوليك.

المجموعة الثالثة : عملت بمستخلص أوراق الشماري 300 ملجم/كجم و تغذت على الغذاء المحتوى على الكوليسترول وحمض الكوليك.

المجموعة الرابعة : عملت بمستخلص أوراق الشماري 500 ملجم/كجم و تغذت على الغذاء المحتوى على الكوليسترول وحمض الكوليك.

المجموعة الخامسة : عملت بفيتامين سي 300 ملجم/كجم و تغذت على الغذاء المحتوى على الكوليسترول وحمض الكوليك.

ثانياً : دراسة التأثير العلاجي للمستخلص على مستوى الكوليستيرول في الدم. في هذه التجربة استخدم ستة وثلاثون فأراً واستمرت لمدة ستة عشر أسبوعاً. استخدم ستة فئران كمجموعة ضابطة تغذت على الغذاء الطبيعي بينما تغذى باقي الفئران (ثلاثون) على الغذاء المحتوى على الكوليستيرول وحمض الكوليك (1:3) بجرعة تعادل ½ جم من هذا الخليط لكل كجم من وزن الفأر. واستمر تغذية الفئران على هذه العليقة لمدة 8 أسابيع لأحداث مستوى عالي من الكوليستيرول في الدم. بعد ذلك قسمت الفئران إلى خمس مجموعات متساوية.

المجموعة الثانية : استخدمت كمجموعة ضابطة أيضا لكنها تغذت على الغذاء المحتوى على الكوليسترول وحمض الكوليك.

المجموعة الثالثة : تم إعطاؤها مستخلص الميثانول لأوراق نبات الشماري بجرعة 300 ملجم/كجم من وزن الفأر.

المجموعة الرابعة : تم إعطاؤها مستخلص الميثانول لأوراق نبات الشماري بجرعة 500 ملجم/كجم من وزن الفأر.

المجموعة الخامسة : تم إعطاؤها فيتامين سي بجرعة 300 ملجم/كجم من وزن الفأر.

المجموعة السادسة : تم إعطاؤها اتور- فاستاتين بجرعة قدرها 1 ملجم/كجم من وزن الفأر.

بعد أخذ عينات الدم ثم فصل المصل وتركه في الثلاجة لحين تحليل المكونات : الدهون وظائف الكبد وظائف الكلى.

كذلك ثم اخذ عينات من أنسجة الكبد بعد نهاية التجارب لعمل الدراسات الهستوباثولوجية.

أوضحت النتائج المتحصل عليها أن مستخلص الميثانول لأوراق نبات الشماري لديه فاعلية بيولوجيه على خفض مستوى الكوليستيرول في الدم نظرا لمحتواها من مضادات الأكسدة كما أكد ذلك الدراسات الهستوباثولوجية.

وننصح بعدم التسرع في استخدام هذه الأعشاب فى علاج ارتفاع الكوليستيرول في الدم إلا بعد عمل المزيد من الدراسات لمعرفة ميكانيكية عمل المواد الفعالة بها واستخدام نوعيات مختلفة من حيوانات التجارب ولفترات أطول حتى يمكن تقييمها ومعرفة أثارها الجانبية لتحديد إمكانية استخدامها كعلاج للإنسان من عدمه.