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Effect of methanolic extract of *Arbutus Pavarii* Leaves as an antioxidant in the treatment of hypercholesterolemia in albino rats

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Abstract

The aim of this study was to investigate the antioxidant activity and hypocholesterolemic effect of methanolic extract of *Arbutus pavarii* leaves (MEAPL). The hypercholesterolemia induced by cholesterol and cholic acid at 3:1 ratio in male albino rats. It was administered orally once a *day*, for 60 days. The effect of (MEAPL) against hypercholesterolemia in male rats has been evaluated by various types of lipoproteins, but the two most abundant are Low-density Lipoprotein (LDL) and High-density Lipoprotein (HDL). Total cholesterol (TC), triglycerides (TG), in addition to liver marker enzymes such as "ALT, AST, G-GT, LDH, ALP and proteins. Antioxidant enzymes activity (SOD, GR, GPx and CAT), and lipid peroxidation marker MDA were measured 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. The level of MDA was increased in HFD rats compared with control rats while antioxidant enzymes SOD, GR, GPx and CAT activity were decreased. The histopathological studies also supported the prophylactic effects of (MEAPL). The results obtained revealed that methanolic extract of *Arbutus pavarii* leaves (MEAPL) has a prophylactic effect against hypercholesterolemia compared with the reference standard hypercholesterolemia agent ˝Atorvastatin˝ and standard antioxidant vitamin C.

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1. Introduction

Abnormal lipid metabolism is a main cause of dyslipidemia, which is a major risk factor for cardiovascular disease, obesity and overall mortality **[1]**. 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. It is well known that diet plays an important role in the control of cholesterol homeostasis **[2]**. Although humans synthesize cholesterol to maintain minimum levels for biological functioning, diet also is known to play a role in serum cholesterol levels. 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 **[3]**.

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 **[4]**. It was reported that hypercholesterolemia may be responsible for oxidative modification of LDL with excess production of free radicals and lipid peroxidation products **[5]**. The current hypothesis suggests oxidative stress as an underlying mechanism by which dyslipidemia, particularly hypercholesterolemia, induces tissue damage or provokes several human diseases **[6]**. 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. **[5, 7]**. 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 **[8]**. 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 **[9]**.

In recent years, the usage of herbal drugs for the treatment of diseases has increased all over the world. The herbal drugs are believed to be harmless and free from serious adverse reactions, as they are obtained from nature, and are easily available. Also, the limited therapeutic options and disappointing therapeutic success of modern medicine has increased the usage of alternative medicine including herbal preparations **[10]**. 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. 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 **[11]**.

Arbutus pavarii Pamp. (Ericaceae) is one of the endemic species in El-Jabel El-Akhdar. As it described in the Libyan flora **[12]**. The methanolic extract of the leaves of *Arbutus pavarii* were subjected to flash chromatography to yield α-amyrin. In addition to catechin, quercitin-3-o-rhamnoside, isoquercitrin, myricetin, ferulic acid and arbutin **[13]**. According to its constitute specially catechins which have been reported to be associated with antioxidative action in biological systems, acting as scavengers of free radicals **[14]**. 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 **[15]**.

2. Materials and Methods

Chemicals

Commercial kits to estimate antioxidant enzymes were from Biodignostic company, liver function tests were carried out in Benghazi medical center.

***Arbutus pavarii* Pamp. (Ericaceae)**

The leaves of Arbutus pavarii, were collected from Al-Jabal Al Akhdar 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

Animals:

Twenty eight healthy adult male albino rats weighing between 110-120 g were used for this study. The animals were kept in polypropylene cages and maintained at 24 ± 3ºC and a constant light-dark schedule (12 hours light and 12 hours dark cycle). The animals were allowed free access standard commercial rat chow (pellet form, in the sack, Benghazi Animal Feed Company, Benghazi, Libya) and water ad libitum. The animal experiment was approved by research committee of Benghazi University, Libya and performed according to the guidelines laid by National Institutes of Health (NIH).

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**[16]**.

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).

Sample collection and biochemical assays:

The blood samples obtained were collected into plain sample tubes and centrifuged at 1000 rev/min. for 5 minutes to separate serum. Serum was carefully withdrawn and kept in eppendrof tubes for the determination of the biochemical parameters.

Assessment of serum marker enzymes: The activities of Serum Alanine Transaminase (ALT). Aspartate Transaminase (AST). Lactate Dehydrogenase (LDH). G-Glutamyl transferase (GGT). Serum total protein (T. Protein). Serum albumin (ALB). Serum total bilirubin (T. BIL). Alkaline phosphatase (ALP). Were all assayed using standard Diagnostic kits at Benghazi medical center.

Assessment of antioxidant enzymes: The activities of Glutathione reductase (GR), Glutathione peroxidase (GPx). Catalase (CAT), Superoxide dismutase (SOD), Malondialdehyde (MDA). Where assayed in research laboratory of biochemistry.

Histological assessment: 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 Annoon medical laboratory. After which photographs were taken at Annoon medical laboratory, Benghazi.

Statistical analysis: Resulting data were represented as mean ± SD. Statistical data was analysed by T test, between control vs all treated groups. A probability level of less than 5% ($p<0.05$) was considered significant.

3. Results

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 (93%, 174.1%, 425%, 467.3%, 129.6 and 129.6%), respectively, but the level of HDL-C. decreased by 49.3%. Pretreatment the rats with methanolic extract of *Arbutus Pavarii* leaves at 500 mg/kg lead to decreases by (47.3%, 57.8%, 77.4%, 81.9%, 59.7 and 59.7%), in T. lipid, T. Chol ., T. Chol./HDL-C., LDL.C., VLDL-C. and T.G., respectively, but the level of HDL-C. increased by 91.1%, when compared with positive group. As illustrated in table (1)

Antioxidant enzymes in prophylactic group:

After the exposure 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 (46.7%, 48.8%, 33.4%, and 23.9%), respectively but the MDA level shows significant increase by 56.25% in table (2). Pretreatment of the rats with methanolic extract of *Arbutus Pavarii* leaves at 500 mg/kg increase the activity of these enzymes SOD, GR, GPx and CAT by (65.7%, 61.4%, 40.8%, and 11.75%), respectively and significant decrease in MDA by 32% when compared with the hypercholesterolemic diet treated group. As illustrated in table (2)

Table 1: Effect of different treatments on lipid profile during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Parameters	S.T. lipid (mg/dl)		S. T. cholesterol (mg/dl)		S.HDL- Chol. (mg/dl)		S. LDL (mg/dl)		S. Triglyceride (mg/dl)	
	zero	8-weeks	zero	8-weeks	zero	8-weeks	zero	8-weeks	zero	8-weeks
Control	273 ± 15	301 ± 16 †	62.5 ± 10	54.9 ± 13 †	34.6 ± 8	35.3 ± 5 †	18 ± 8	19.14 ± 8 †	52.0 ±12	52.3 ± 8 †
Positive control	268 ± 13	581 ± 23 a, ***	60.8 ± 13	150.5 ± 9 a, ***	33.7 ± 6	17.9 ± 8 a, **	16.6 ± 5	108.6 ± 10 a, ***	54.2 ± 10	120 ± 13 a, ***

vitamin C (300 mg/Kg.b.w)	266 ± 20	340 ± 21 [b, ***]	63.8 ± 17	82.9 ±13 [b, ***]	34.1 ± 9	30.0 ± 9 [b, *]	19.7 ± 7	42.42 ± 13 [b, ***]	50.1± 15	52.4 ± 15 [b, ***]
Meth. Ext. of Arbutus pavarii leaves (300 mg/Kg.b.w)	272 ± 17	315 ± 20 [b, ***]	66.8 ± 11	79.3 ± 15 [b, ***]	34.8 ± 7	32 ± 8 [b, *]	20.8 ± 7	35.8 ± 8 [b, ***]	55.8 ± 11	57.6 ± 10 [b, ***]
Meth. Ext. of Arbutus pavarii leaves (500 mg/Kg.b.w)	269 ± 18	306 ± 17 [b, ***]	65.0 ± 12	63.5 ±13 [b, ***]	36.9 ± 9	34.2 ± 9 [b, *]	17.6 ± 9	19.61 ±7 [b, ***]	53.7 ± 16	48.4 ± 11 [b, ***]

Table 2: Effect of different treatments on antioxidant enzymes during the induction of hypercholesterolemia for 8 weeks in male albino rats.

Parameters	**P. SOD (u/mol)**		**P. MDA (n mol/ml)**		**P. GR (u/l)**		**P. GP_X (mu/ml)**		**P. CAT (u/l)**	
	zero	**8-weeks**	**zero**	**8-weeks**	**zero**	**8-weeks**	**zero**	**8-weeks**	**zero**	**8-weeks**
Control	8.23 ± 2	8.16 ± 1.5 [†]	15 ± 4	16 ± 0.38[†]	25.3 ± 4	25.8 ± 4 [†]	35.5 ± 5	35.3 ± 4 †	50.6 ± 9	50.6 ± 5[†]
Positive control	8.42 ± 1.7	4.35 ± 0.7 [a, **]	16 ± 3	25 ± 6.8 [a, *]	25.3 ± 2	13.2 ± 3 [a, †]	35.2 ± 4	23.5 ± 2 [a, †]	50.3 ± 6	38.5 ± 6 [a, *]
vitamin C (300 mg/Kg.b.w)	8.36 ± 1	7.18 ±1.9 [b, **]	15 ± 2	17 ± 5 [b,†]	26.3 ± 5	21.3 ± 2 [b, †]	34.3 ± 2	32.2 ± 2 [b, †]	49.8 ± 8	41.5 ± 8 [b, †]
Meth. Ext. of Arbutus pavarii leaves (300 mg/Kg.b.w)	8.29 ±2	7.07 ±2 [b, **]	14 ± 3	18 ± 3 [b,†]	24.8 ± 3	22.2 ± 3 [b, †]	33.3 ± 3	31.2 ±5 [b, †]	48.9 ± 7	42.2 ± 8 [b, †]
Meth. Ext. of Arbutus pavarii leaves (500 mg/Kg.b.w)	8.32 ± 2	7.21 ±1.6 [b, **]	15 ± 2	17 ± 4 [b,†]	25.3 ± 4	21.3 ± 2 [b, †]	35.2 ± 4	33.1 ± 4 [b, †]	50.4 ± 9	43 ± 7 [b, †]

† 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

Histopathology:

A photomicrographs of liver Sections showing effect of various treatments: **(1)** control group of animals**,** the parenchyma of liver in all animals showed normal pattern regarding to size, shape. **(2)** The liver of positive control rat, showing marked vacuolated hepatocytes (arrow) and aggregates of mononuclear inflammatory cells (arrow head) in portal areas. **(3)** The liver section of methanolic extract *Arbutus pavarii* leaves (300 mg/kg. b.w.), with mild increase in lymphocytic aggregates (arrow) in portal areas. **(4)** The liver section of methanolic extract *Arbutus pavarii's* leaves treated (500 mg/kg. b.w.) with mild increase in lymphocytic aggregate (arrow) in portal areas. **(5)** The liver section of vitamin C treated (300 mg/kg. b.w.), with mild increase in lymphocytic aggregates (arrow) in portal areas Stain.

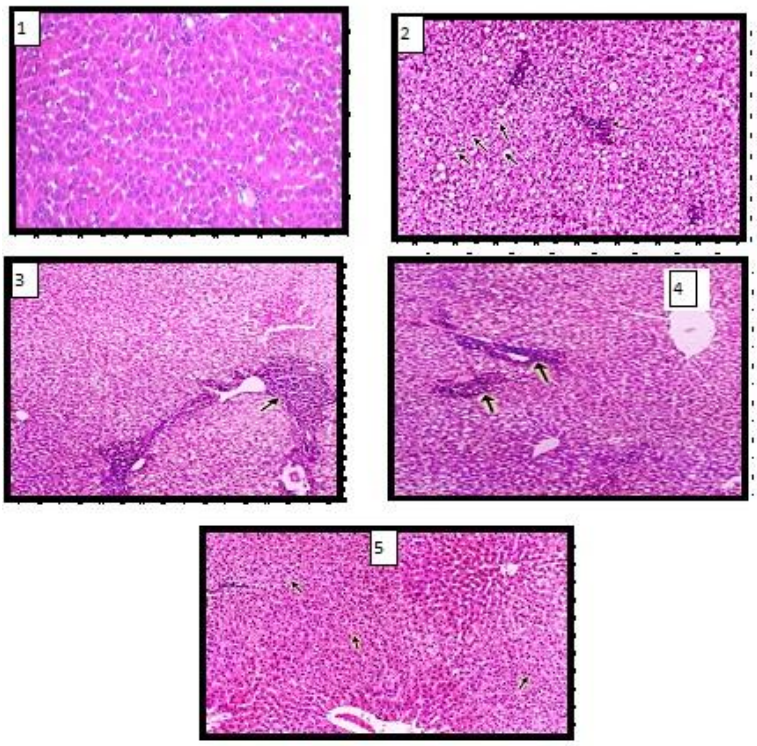

Figure 1

4. Discussion

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. **[17]**. Rats fed on hypercholesterolemia diet developed hypercholesterolemia mark by significant increase in serum total lipids (T. Lipid), triglycerides (T.G), total cholesterol (T. Chol.), low density lipoprotein cholesterol (LDL-C), and decrease in high density lipoprotein cholesterol (HDL-C) compared with normal control rats.

Also the activity of antioxidant enzymes glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) also decreased when compared with normal control rats and elevation in lipid peroxidation indicator malondialdehyde (MDA). The measurement of thiobarbituric acid (TBARS) is commonly used to monitor lipid peroxidation and indirectly, oxidative stress *in vitro* and *in vivo* **[18]**.

The decrease of T. Lipid, , T. Chol., LDL-C and T. G 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 group. 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 and 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* **[19]**. 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 **[20]**. 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 **[21]**. 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 [22].

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 **[13]**. 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. Ferulic Acid, like many phenols exhibits antioxidant effect in response to free radicals by donating hydrogen from its phenolic hydroxyl group. Supplementation of vitamin C 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 methanolic extract of *Arbutus Pavarii* leaves 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.

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 **[6]**. 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 (MDA). 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 showed decrease in MDA level, and increased the levels of SOD, GAT, GPx and GR, in compared with positive group. Results of the present study suggests that methanolic extract of *Arbutus pavarii* leaves and vitamin C ameliorating effects to be likely mediated via inhibition of free radicals generation and/or free radical scavenging activity. Were we can notice these effects when compared to "positive group".

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