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Hypolipidemic and Antiatherogenic Effects of Aqueous Extract of Libyan Propolis in Lead Acetate Intoxicated Male Albino Mice

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Abstract: ***Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis-associated conditions. Coronary artery disease is the epidemic of modern civilization in which dyslipidemia contributes significantly to its pathogenesis. Flavonoids and various phenolics are the most important pharmacologically active constituents in propolis capable of scavenging free radicals and thereby protecting lipids from being oxidized or destroyed during oxidative damage. The aim of this study was to investigate the hypolipidemic and antiatherogenic effects of aqueous extract of Libyan propolis in lead acetate intoxicated male albino mice. In this study, Thirty two adult male albino were used for this study and divided into four groups. The first group was control group, the 2nd was the propolis group orally received propolis (200 mg/kg body wt), the 3rd was the experimental and received lead acetate (500 mg /kg diet), the 4th one co-administered lead acetate (500 mg/kg diet) with propolis (200 mg/kg body wt)daily for 30 days. Blood samples were obtained for assessment of serum cholesterol, triglycerides, HDL, LDL, parameters. In lead treated animals, the serum cholesterol, triglycerides, HDLc, LDLc, VLDL, Castelli's Risk Index I (TC/HDLc), Castelli's Risk Index II (LDLc/HDLc), Atherogenic Coefficient {(TC-HDLc)/HDLc } and Atherogenic Index of Plasma{ (AIP)= log(TG/HDLC)} parameters were increased and serum HDLc was decreased. Co-administration of propolis significantly improved of lipids profile parameters the ratios based on these parameters. Serum cholesterol, triglycerides, non HDLc, LDLc, VLDL, Castelli's Risk Index I (TC/HDLc), Castelli's Risk Index II (LDLc/HDLc), Atherogenic Coefficient {(TC- HDLc)/HDLc } and Atherogenic Index of Plasma{ (AIP)= log(TG/HDLC)} parameters were significantly declined and serum HDLc was elevated. It can be concluded that, the lead had adverse effects on serum lipids profile parameters and the ratios based on these parameters. Propolis showed hypolipidemic and antiatherogenic effects in lead acetate intoxicated male albino mice. So, the populations of high risk to lead should be advised to take propolis.***

Keywords: Antiatherogenic, Male albino mice, Hypolipidemic effect, Lead acetate, Libyan propolis.

1. Introduction

Heavy metals like lead, cadmium etc. have very long half life and are severely toxic at a very low dose [1**].** Lead is a natural stable element and is bioaccumulative in nature. It is an environmental poison of significance to the grazing livestock and a potential public health hazard, as it is excreted in milk [2]. It represents an exclusive case (among cumulative metal contaminants) because of its ubiquitous presence in the environment and easy recognition of its major sources, which give rise to environmental pollution [3]. It has been used in medicines, paintings, pipes, ammunition and in more recent times in alloys for welding storage materials for chemical reagents [4]. It is an environmental pollutant that causes damage to biological systems [5]**.** Several reports have indicated that lead can cause neurological, histopathological, hematological, gastro-intestinal, reproductive, circulatory and immunological pathologies, all of them related to the dose and the duration of time of lead exposure [6 - 9]. It also produces high blood pressure that increases the risk of heart attack [10]. Toxicities due to lead exposure have been attributed to the ability of lead to induce oxidative stress through the generation of reactive oxygen species [11]. Elevation of total cholesterol, triglycerides and lipoproteins such as (LDL, VLDL) levels and reduction in HDL - CHOL level were recorded in serum of lead intoxicated rats [5 & 12].

Hyperlipidemia is a condition which characterized by abnormal elevation of lipid such as (triglyceride and cholesterol) and lipoproteins such as (LDL, VLDL) levels in the blood [13]. Scientific evidence indicates that oxidation of low density lipoprotein (LDL), which carry cholesterol in the blood stream plays an important role in the development of atherosclerosis, the underlying disorder leading to heart attacks and ischemic strokes [14 & 15]. Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis-associated conditions, such as coronary heart disease (CHD) [15], ischemic cerebrovascular disease, and peripheral vascular disease [16]. Coronary artery disease is the epidemic of modern civilization in which dyslipidemia contributes significantly to its pathogenesis [17]. The basic pathogenesis of atherosclerosis involves an insult to the endothelial and smooth muscle cells of the arterial wall by various harmful factors such as viral infection, mechanical damage and dislipidemia, especially abnormal oxidized low density lipoproteins.[16]**.** It is important to reduce excessive cholesterol and LDL-cholesterol oxidation to low levels, which represent adequate mechanisms for maintenance of normal body functions [18].

Several experimental studies in various laboratories are underway, to study the prophylactic effect of various natural antioxidant compounds against toxic metals. Herbs are generally considered safe and proved to be effective against various human ailments and their medicinal uses have been

gradually increasing in developed countries [19]. Natural antioxidants strengthen the endogenous antioxidants defenses from reactive oxygen species and restore the optimal balance by neutralizing the reactive species [20].

Propolis is resinous natural product collected from cracks in the bark of trees and leaf buds which are enriched with salivary enzymes of honey bees. It has more than 180 compounds including polyphenols, flavonoids, phenolic acids and its esters [21-23]. Melatonin and caffeic acid phenethyl ester are compounds of hony bee propolis, that were recently found to be potent free radical scavengers and antioxidants [24]. Many flavonoids are known to be antioxidants, and several of these, such as quercetin which has been identified as constituents of propolis have been shown to be inhibitors of low density lipoprotein oxidation [25]. It is believed that propolis exerts a therapeutic or preventive effect in inflammation, heart disease, and even diabetes mellitus and cancer and there have been several reports indicating various biological activities of propolis and its constituents, such as anticancer [26 & 27], antioxidant, anti-inflammatory and antibiotic activities [28]. The actual ingredients in individual propolis products may differ significantly, according to a number of variables including the type of bees that produced the propolis, time of the year and the geographic location of the hives [29]. Flavonoids and various phenolics are the most important pharmacologically active constituents in propolis capable of scavenging free radicals and thereby protecting lipids from being oxidized or destroyed during oxidative damage [30]. The evidence reporting the hypolipidemic and anti-atherogenic effects of propolis in lead acetate intoxicated male albino mice are hardly found. So, the present work aimed to evaluate hypolipidemic and antiatherogenic effects of propolis in lead acetate intoxicated male albino mice.

2. Materials and Methods

2.1. Chemicals

Lead acetate was purchased from Sigma Chemical Co., USA. Lead acetate was given in diet as 500 mg/kg diet daily [31] for 30 days. Propolis samples were collected from different localities of Surman city, west Libya. Aqueous propolis extract was prepared according to the method of El-khayat et *al.* [32]. Briefly, propolis was kept dry and freezed (-40°C) until used. Propolis samples were mixed with distilled water, heated gently and filtered through Whatman No:1 filter paper. The choice of the dose of propolis was based on the results of the previous studies, where the antioxidant effect of this agent was confirmed. Propolis was freshly prepared and administered to animals orally by gavage at a dose of 200 mg/kg body wt [33] once daily for 30 days.

2.2. Animals

Thirty two adult male albino mice (*Mus musculus*) weighting 25-30 g were used for this study. The animals were obtained from animal house unit in the Faculty of Pharmacy, Tripoli University, Libya. The animals were housed in plastic cages measuring about (29×15×12) cm, with about four mice per cage. Floors of cages were covered with soft crushed wood shaving; all cages were washed two times per week with 70% alcohol throughout the period of the study. The animals were provided with tape water ad libitum and fed with the standard commercial chow. The animals were kept in the animal house of Faculty of Science , Alejelat, Zawia University in an air conditioned room with an optimum temperature of 25±2 °C, humidity (60-70%) and light/dark condition (12/12). The animal procedures were performed in accordance with Guide Lines for Ethical Conduct in the Care and Use of Animals.

2.3 Experimental Design

After one week of acclimation, the animals were randomized and divided into four groups (8 albino mice for each) as follow:
Group I (control group): provided with tape water and fed with normal diet.
Group II (propolis group): The animals received propolis (200 mg/kg body wt/day) orally by gavage daily for 30 days.
Group III (lead acetate treated group): The animals received 500 mg lead acetate/kg diet daily for 30 days.
Group IV (lead acetate/propolis co-administered): The animals received 500 mg lead acetate/kg diet concurrently with propolis (200 mg/kg body wt/day) orally by gavage daily for 30 days.
At the end of the experimentation and 24 hours after the last dose, all animals were sacrificed under light ether anesthesia, then rapidly dissected and subjected to the following examinations:

2.4 Biochemical Analysis

Blood samples were drown by cardiac puncture. The sample was collected in clean dry tube and centrifuged at 3000 rpm for 15 minutes then, serum was separated and kept in a deep freezer at -20◦C until biochemical measurements were carried out. Total cholesterol concentration was estimated according to Allain *et al.* [34], triglycerides concentration also by the method of Fossati and Prencipe [35] and HDL-cholesterol by Burstein et al. [36]. VLDL-cholesterol and LDL-cholesterol concentrations were estimated by using the Friedewald equation [37]. The atherogenic ratios were calculated as follows: Castelli's Risk Index (CRI-I) = TC/HDLc, Castelli's Risk Index (CRI-II) = LDLc/HDLc, Atherogenic Coefficient (AC)=(TC– HDLc) /HDLc and Atherogenic Index of Plasma (AIP)= log TG/HDLc.

2.5 Statistical Analysis

The values were presented as means ± SD of different groups. Differences between the mean values were estimated using one way ANOVA. The results were considered statistically significant when $p < 0.05$.

3. Results

Lipid profile parameters in serum of the different groups are shown in Table 1. Mice that received lead acetate (500 mg/kg diet) daily for 30 days had significantly ($p<0.05$), increased the serum cholesterol, triglycerides, non HDLc, LDLc and

VLDL concentrations. Co-administration of lead acetate with propolis were significantly ($p<0.05$) prevented the changes recorded in serum cholesterol, triglycerides, non HDLc, LDLc and VLDL concentrations as compared with control group(Fig. 1, 2, 4 & 5). On the other hand, serum HDL cholesterol concentration of lead acetate treated mice was significantly ($p<0.05$) decreased as compared to the control mice (Fig. 3). Co-administration of lead acetate with propolis were significantly ($p<0.05$) prevented the changes recorded in serum HDLc concentration as compared with control group.

Table 2 showed the means and standard deviations for Castelli's Risk Index I (TC/HDLc), Castelli's Risk Index II (LDLc/HDLc), Atherogenic Coefficient{(TC-HDLc) /HDLc} and Atherogenic Index of Plasma{(AIP)= log(TG/HDLC)} in control group, propolis group, lead acetate treated group and albino mice group co-administrated of lead acetate with propolis. These ratios were elevated in lead acetate treated male albino mice group compared with the control group with statistically significant differences ($p<0.05$). Co-administration of lead acetate with propolis were declined these ratios with statistically significant differences ($p<0.05$), when compared with lead acetate group (Figs. 6, 7, 8, 9 & 10)

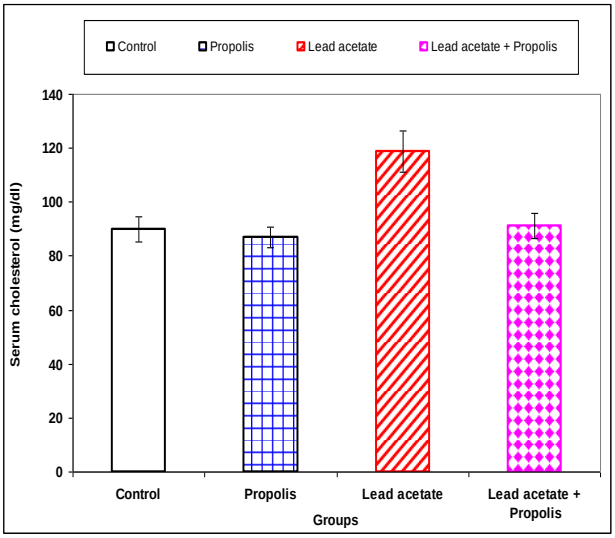

Figure 1: Serum cholesterol concentration in different groups.

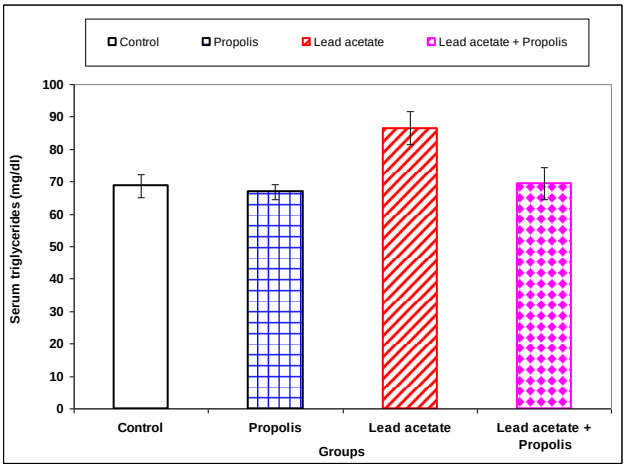

Figure 2: Serum triglycerides concentration in different groups.

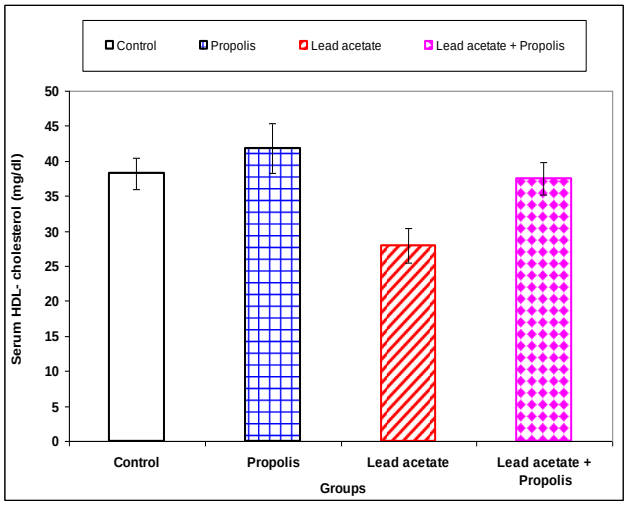

Figure 3: Serum HDL-cholesterol concentration in different groups.

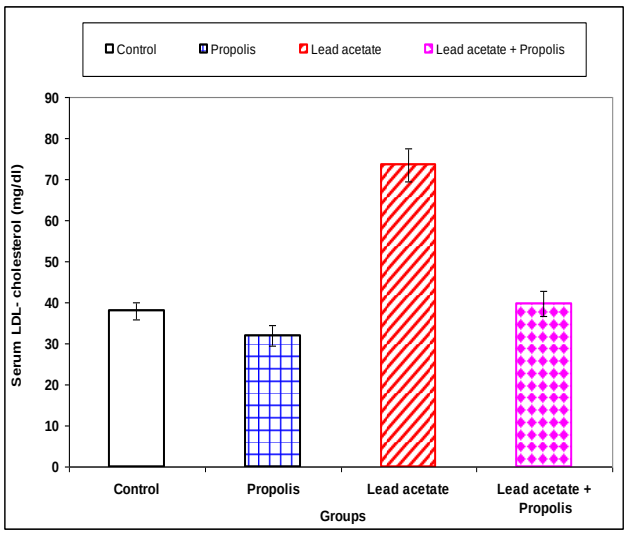

Figure 4: Serum LDL-cholesterol concentration in different groups.

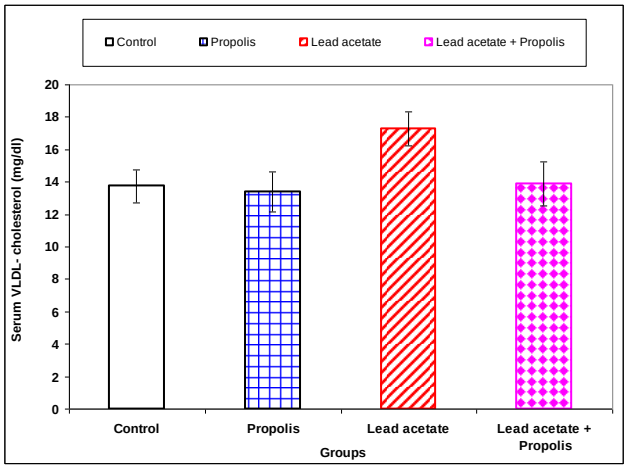

Figure 5: Serum VLDL-cholesterol concentration in different groups.

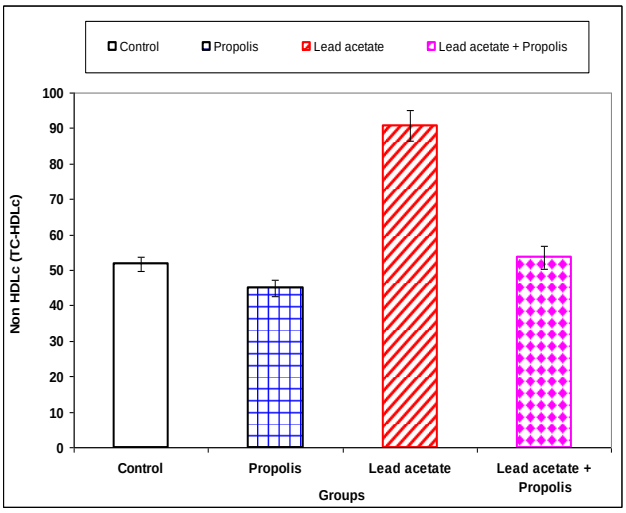

Figure 6: Serum non HDL-cholesterol(TC-HDLc) concentration in different groups.

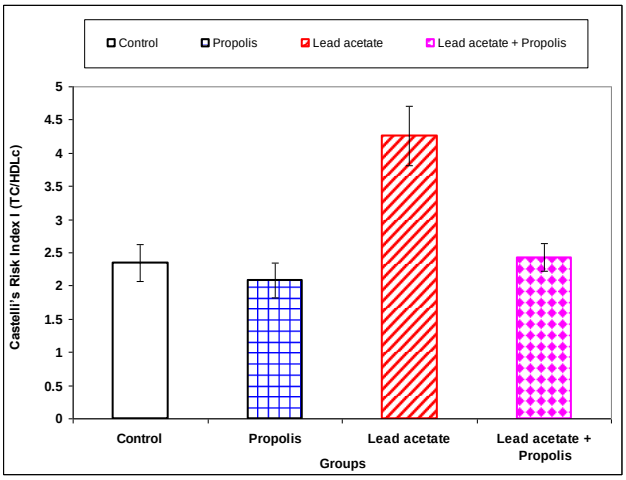

Figure 7: Cardiac Risk Ratio (Castelli's Risk Index I) TC/HDLC (LDLc/HDLc) n different animals groups

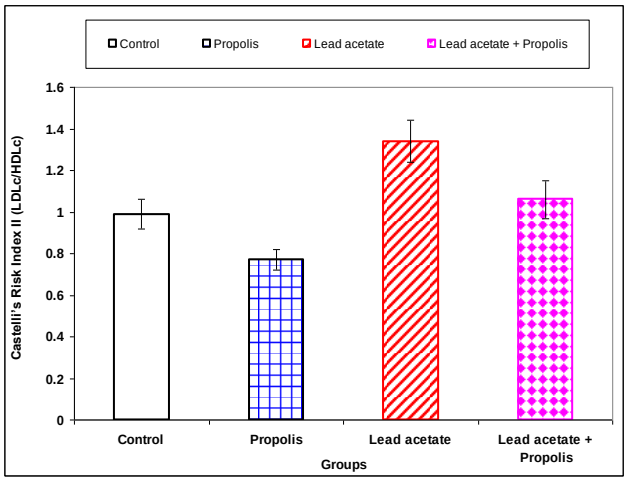

Figure 8: Castelli's Risk Index II in different animals groups.

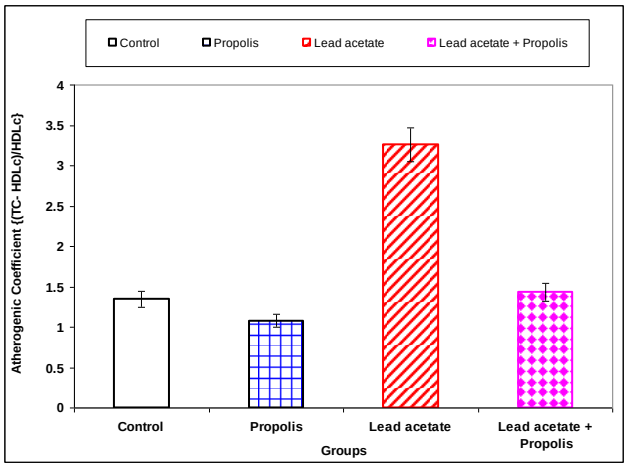

Figure 9: Atherogenic Coefficient {(TC- HDLc)/HDLc} in log(TG/HDLC) in different animals groups

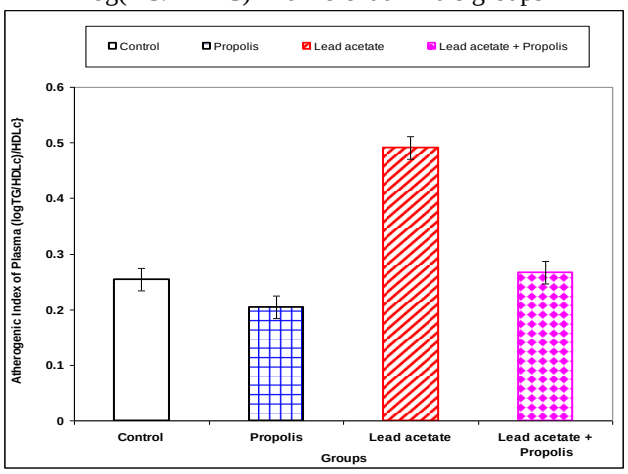

Figure 10: Atherogenic Index of Plasma(AIP)= different animals groups

Table 1: Effect of aqueous extract of propolis on lipid profile parameters of lead acetate treated male albino mice in different groups.

Parameters	Groups			
	Control	Propolis	Lead acetate	Lead acetate + Propolis
	Mean + SD	Mean + SD	Mean + SD	Mean + SD
Serum cholesterol (TC, mg/dl)	89.90 ±4.78	86.80 ±3.86	118.70 ±7.52[a]	91.12 ±4.71[b]
Serum triglycerides (TG, mg/dl)	68.70 ±3.49	66.90 ±2.38	86.50 ±5.13[a]	69.40 ±4.88[b]
Serum HDL- cholesterol (mg/dl)	38.20 ±2.28	41.80 ±3.60	27.90 ±2.47[a]	37.50 ±2.37[b]
Serum LDL- cholesterol (mg/dl)	37.96 ±2.01	31.96 ±2.40	73.50 ±4.10[a]	39.74 ±3.11[b]
Serum VLDL- cholesterol (mg/dl)	13.74±1.03	13.38±1.22	17.3±1.06[a]	13.88±1.37[b]
Non HDLc (TC-HDLc) (mg/dl)	51.7 ±2.00	45.00 ±2.30	90.80 ±4.30[a]	53.62 ±3.20[b]

a: Significant differences as compared with control group ($P < 0.05$) .
b: Significant differences as compared with lead acetate treated group ($P < 0.05$).
All data are mean of 6 individuals.

Table 2: Effect of aqueous extract of propolis on the ratios based on lipid profile parameters of lead acetate treated male albino mice in different groups

Parameters	Groups			
	Control	Propolis	Lead acetate	Lead acetate + Propolis
	Mean + SD	Mean + SD	Mean + SD	Mean + SD
Cardiac Risk Ratio (Castelli's Risk Index I) TC/HDLC	2.35±0.21	2.08±0.11	4.2±0.30[a]	2.43±0.20[b]
Castelli's Risk Index II (LDLc/HDLc)	0.99 ±0.07	0.77 ±0.05	1.34 ±0.10[a]	1.06 ±0.09[b]
Atherogenic Index of Plasma(AIP)= log(TG/HDLC)	0.254±0.019	0.204±0.018	0.491±0.022[a]	0.267±0.021[b]
Atherogenic Coefficient {(TC- HDLc)/HDLc}	1.35 ±0.10	1.08 ±0.08	3.26 ±0.21[a]	1.43 ±0.11[b]

a : Significant differences as compared with control group ($P < 0.05$) .
b : Significant differences as compared with lead acetate treated group ($P < 0.05$).
All data are mean of 6 individuals.

4. Discussion

The present data indicated that cholesterol, triglycerides, LDLc and VLDL concentrations were significantly increased by lead acetate treatment, while HDL-c concentration was decreased in the serum. Several studies have shown that lead exposure induces alterations in serum lipid profiles [5, 12 & 38-40]. These results run parallel to those reported by Ghosh *et al.*, [38] who found that treatment of rats with lead acetate at a dose of 15 mg / kg body weight intraperitoneally (i.p) for a period of seven consecutive days caused alterations in the total cholesterol, triglyceride, HDLc, LDLc. Also, There was significant increase ($p<0.05$) in the serum total cholesterol, LDL CHOL and triglycerides in oral treated albino rats with lead acetate(740mg/kg body weight) daily for 28 days group compared to the normal control group [5]. Lowering levels of high density lipoprotein (HDL) was a contrary effect because high HDL levels have been shown to bear an inverse correlation with risks for atherosclerosis [41].

Cholesterol is an essential part of every cell in the body. It is necessary for formation of new cells and for older cells to repair themselves after injury. It is also used by the adrenal glands in the synthesis of some hormone, such as cortisol, by the testicles to form testosterone, and by the ovaries to form estrogen and progesterone [42]. The high cholesterol level in plasma may be due to increased uptake of exogenous cholesterol and subsequent deposition and decreased cholesterol catabolism as evidenced by a reduction in bile acid production and turnover of bile acids. The metabolism of free and ester cholesterol are impaired in liver, spleen and thymus tissue and the rate of turnover was specifically decreased in all tissues of hyperlipidemic rats [15]. Lead nitrate-mediated development of hypercholesterolemia involves the activation of cholesterol biosynthetic enzymes (i.e. 3-hydroxy- 3methyglutaryl-CoA reductase, farnesyl diphosphate synthase, and squalene synthase, CYP51) and the simultaneous suppression of cholesterol-catabolic enzymes such as 7a-hydroxylase [43]. Increase in LDL, VLDL levels are increase the risk of cardiovascular diseases [44 & 45].

Oxidative stress, specifically the oxidation of low density lipoprotein (LDL), has long been suspected of having a critical role in the development of atherosclerosis, in consequence of which antioxidants have been expected to have potential as antiatherogenic agents. Such agents would be able, in theory, to inhibit the oxidative modification of LDL that leads to the accumulation of cholesterol in atherosclerotic lesions [46 & 47].

Results of the present study have shown that Castelli's Risk Index I (TC/HDLc), Castelli's Risk Index II (LDLc/HDLc), Atherogenic Coefficient{(TC-HDLc) /HDLc} and Atherogenic Index of Plasma{(AIP)=log(TG/HDLC)} were elevated in lead acetate treated male albino mice group compared with the control group with statistically significant differences ($p<0.05$). These results run parallel to those reported by Ghosh *et al.*, [38] who reported that **t**reatment of rats with lead acetate at a dose of 15 mg/kg body weight intraperitoneally (i.p) for a period of seven consecutive days caused alterations in the Cardiac Risk Ratio (Castelli's Risk Index I) TC/HDLC and Castelli's Risk Index II (LDLc/HDLc). Bhardwaj et al. [17] reported that lipid ratios like Atherogenic Index of Plasma, Castelli risk index and

Atherogenic coefficient could be used for identifying individuals at higher risk of cardiovascular disease in Indian population in the clinical setting especially when the absolute values of individual lipoproteins seem normal and in individuals with elevated triglycerides concentrations. Thus, the use of these indexes should be encouraged to complement the existing profile of tests for identifying high risk individuals for Coronary Artery Disease (CAD) and effective drug management.

Results of the present study which have shown that co-administration of propolis with lead acetate induced significant reduction in serum cholesterol, triglycerides, LDLc and VLDL concentrations and elevation in serum HDL- cholesterol. These results are in concordant with those of Maimuna *et al*.,[5] who, reported that the deleterious effects caused by lead intoxication were prevented in albino rats given lead concomitantly with aqueous extract of *Capsicum annuum L*. fruits, suggesting that the extract offered protection against lead-induced organ damage in albino rats. Co-administration of propolis to chlorpyrifos treated rats restored serum total cholesterol, triglycerides and LDL-cholesterol parameters to normal levels [48]. Also, Abdel-Wahab [49] reported that pretreatment with melatonin in AlCl3-treated rats alleviated the elevation of total cholesterol and triglycerides in the plasma and restored their values toward the normal value of the control group. This anti-hyperlipidimic effect of melatonin may be primarily attributed to its antioxidant activity and the protection of cellular membrane integrity from Al-induced oxidative damage [50]. Another possible mechanisms for the effect of melatonin on lipid profile may be its action on the gastrointestinal tract and the inhibition of cholesterol and triglycerides uptake, the augmentation of endogenous cholesterol clearance mechanisms through increasing the activity of cholesterol degrading enzymes and/or its effect on thyroid hormones which in turn affect lipid metabolism [51 & 52].

Treatment of male albino mice with lead acetate plus propolis decreased triglyceride level compared to the male albino mice treated with lead acetate only. Similar results were obtained by Cetin *et al*., [53] who found that treatment of rats with propetamphos plus propolis decreased triglyceride levels compared to the rats treated with propetamphos. This suggests that propolis can modulate lipid metabolism. Fuliang *et al*., [54] reported propolis to cause decrease in triglyceride level when administered to rats with diabetes mellitus. In addition, Kolankaya *et al.* , [55] reported that propolis caused a decrease in triglyceride level of rats treated with alcohol.

Oral ethanolic extracts of propolis (EEP) caused a significant decrease in plasma levels of total cholesterol, triacylglycerol, LDL-cholesterol and VLDL-cholesterol and significant increase in HDL-cholestrol in rabbits fed cholesterol diet. The data suggest that EEP may be protective against atherosclerosis and cardiovascular disease, particularly because they also decreased plasma LDL-cholesterol level [56]. Flavonoids supplementation significantly increased HDL-cholestrol and HDL-cholesterol/ total-cholesterol ratio [57]. The favorable lipid profile indicates a possible antiatherogenic property of the flavonoids [58]. Bok *et al*.,[18] suggest that flavonoids reduce cholesterol biosynthesis by means of inhibition of hepatic 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase and acyl CoA: cholesterol o-acyltransferase (ACAT). Reduced ACAT activity may lead to lower availability of cholesterol ester for VLDL cholesterol packing, thereby resulting in a reduction of VLDL-cholesterol secretion from the liver, as suggested by Carr *et al.*, [59]. Diets containing flavonoids reduced the VLDL [60].

Increases of HDL have cardioprotective effect and it was proved by various studies. [44 & 45]. The increase in HDL-C observed in the present study, might be due to stimulation of pre-β HDL-C and reverse cholesterol transport as demonstrated by previous studies [15 & 61]. High HDL-C levels could potential contribute to its anti-atherogenic properties, including its capacity to inhibit LDL oxidation and protect endothelial cells from the cytotoxic effects of oxidized LDL [62]**.** The ethanol extract of propolis resulted in decreased serum levels of total cholesterol, triacylglycerol, LDL-cholesterol, VLDL-cholesterol of fasting rats; and to increased serum levels of HDL-cholesterol. This suggests that propolis can modulate the metabolism of blood lipid [54].

In the present study, co-administration of lead acetate with propolis were reduced Castelli's Risk Index I (TC/HDLc), Castelli's Risk Index II (LDLc/HDLc), Atherogenic Coefficient{(TC-HDLc) /HDLc} and Atherogenic Index of Plasma{(AIP)=log(TG/HDLC)}with statistically significant differences ($p<0.05$), when compared with lead acetate group.

In our study hypolipidemic and antiatherogenic effects of aqueous extract of propolis may be due to the antioxidant actions of the extract. Some antioxidant compounds identified in propolis include ferulic acid, quercetin and caffeic acid [63]. Some propolis is made bioactive by the presence of prenylated compounds [64]. Russo *et al*., [65] studied a propolis and determined the antioxidant properties that are conferred by galangin, caffeic acid, ferulic acid, *p*-cumaric and CAPE. The antioxidant activities of propolis are related to its ability to scavenge singlet oxygen, superoxide anions, proxy radicals, hydroxyl radicals and peroxynitrite [66]. The primary mechanism of the effect of propolis may involve the scavenging of free radicals that cause lipid peroxidation. The other mechanism may comprise the inhibition of xanthine oxidase, which is known to cause free radicals to be generated [67].

5. Conclusion

From the previous discussion, It can be concluded that, the lead had adverse effects on lipid profile parameters and the ratios based on these parameters. Aqueous extract of Libyan propolis showed hypolipidemic and anti-atherogenic effects in lead acetate intoxicated male albino mice. So, the populations of high risk to lead should be advised to take

propolis. Further studies are necessary to elucidate exact mechanism of hypolipidemic and anti-atherogenic effects and potential usefulness of propolis as a hypolipidemic and antiatherogenic agent against heavy metals toxicity in clinical trials.

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