**American Journal of Bioscience and Bioengineering**
2015; 3(4): 22-32
Published online September 8, 2015 (http://www.sciencepublishinggroup.com/j/bio)
doi: 10.11648/j.bio.20150304.11
ISSN: 2328-5885 (Print); ISSN: 2328-5893 (Online)

# Haemato-Protective and Hypolipidemic Effects of Aqueous Extract of Libyan Propolis Against Sodium Nitrite Induced Haematotoxicity and Hyperlipidemia in Guinea Pigs

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## To cite this article:
Azab Elsayed Azab, Nuri Mohamed Lashkham, Mohamed Omar Albasha. Haemato-Protective and Hypolipidemic Effects of Aqueous Extract of Libyan Propolis Against Sodium Nitrite Induced Haematotoxicity and Hyperlipidemia in Guinea Pigs. *American Journal of Bioscience and Bioengineering*. Vol. 3, No. 4, 2015, pp. 22-32. doi: 10.11648/j.bio.20150304.11

**Abstract:** Flavonoids and various phenolics are the most important pharmacologically active constituents in propolis capable of scavenging free radicals. The present work aimed to evaluate the effectiveness of aqueous extract of Libyan propolis as a natural source of antioxidants to minimize the harmful effects of sodium nitrite induced haematotoxicity and hyperlipidemia in Guinea pigs. In this study, Twenty four adult male Guinea pigs 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 sodium nitrite orally at a dose of 80 mg/kg body weight, the 4th one co-administered sodium nitrite orally at a dose of 80 mg/kg body weight with propolis (200 mg/kg body wt) daily for 35 days. Blood samples were obtained for assessment of haematological parameters and serum lipids profile. In sodium nitrite treated animals, there were severe haematological changes and dyslipidemia. Haematologically, Guinea pigs that received sodium nitrite orally at a dose of 80 mg/kg body weight daily for 35 days had significantly ($p<0.05$) lower red blood cell count, hemoglobin content, haematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cell count, and platelets count than those in the control animals. On the other hand, mean corpuscular volume of sodium nitrite treated animals was significantly ($p<0.05$) elevated as compared to the control animals. The serum cholesterol, triglycerides, low density lipids cholesterol, very low density lipids cholesterol concentrations, and the atherogenic ratios based on lipid profile parameters (Castelli's risk index I, Castelli's risk index II, atherogenic coefficient and atherogenic index of plasma) were increased and serum high density lipids cholesterol concentration was decreased in sodium nitrite treated group. Co-administration of propolis significantly improved of all haematological and lipid profile parameters, and atherogenic ratios parameters. It can be concluded that, sodium nitrite had adverse effects on haematological, lipid profile parameters, and the atherogenic ratios parameters. Propolis supplementation showed a remarkable amelioration of these abnormalities in sodium nitrite treated male Guinea pigs. It is recommended that the use of sodium nitrite must be limited and use of propolis as antioxidant to prevent the toxic effect. Further studies are necessary to elucidate exact mechanism of protection of haematotoxicity, hyperlipidemia, atherogenic and potential usefulness of aqueous extract of Libyan propolis as a protective agent against sodium nitrite induced haematotoxicity, dyslipidemia and atherogenic in clinical trials.

**Keywords:** Haemato-Protective, Hypolipidemic, Anti-atherogenic, Libyan Propolis, Male Guinea Pig, Sodium Nitrite

## 1. Introduction

Nitrite salts are added to meats, poultry, and fish in minute quantities as a means of preservation; this has been a common practice for many centuries [1]. Nitrite in meat greatly delays the development of botulinum toxin, develops cured meat flavor and color, retards the development of rancidity during storage, inhibits the development of warmed-over flavor and preserves the flavors of spice and smoke [2].

The addition of $NaNO_2$ as a food additive, to our foods may react with amines of the foods in the stomach and produces nitrosamines or large numbers of free radicals. Such products may increase lipid peroxidation which can create many

harmful hazards to the different body organs [3]. Sodium nitrite has been reported to have adverse health effects due to increased oxidative stress that could be harmful to different organs [1]. The reactive nitrogen species that are produced by exposure to nitrite have many toxic effects including hepatotoxicity, nephrotoxicity and dysregulation of inflammatory responses, tissue injury [4], haematotoxicity [5, 6], and hyperlipidemic effects [1, 7].

Natural antioxidants strengthen the endogenous antioxidants defenses from reactive oxygen species and restore the optimal balance by neutralizing the reactive species [8]. The antioxidant activities of phenolics are related to a number of different mechanisms, such as free radical-scavenging, hydrogen-donation, singlet oxygen quenching, metal ion chelation, and acting as a substrate for radicals such as superoxide and hydroxyl [9]. Propolis is a wax-like resin produced by honeybees from substances collected from plants, which are mixed with beeswax and other compounds of bee metabolism. It's a mixture of balsams and resins, waxes, essential oils, pollen, and other substances which is used by bees in the construction, repair and protection of their hives, mainly due to its mechanical properties and antimicrobial activity [10].

Recently, propolis has been used for upper respiratory tract infections, common cold, flu-like infections, as dermatological preparations in wound healing, treatment of burns, acne, herpes simplex and genitalis, and neurodermatitis, as mouthwashes and toothpastes to prevent caries and treat gingivitis and stomatitis; in cosmetics; and in health foods and beverages not only to improve health and prevent diseases, but also as an ingredient in many dietary supplements and nutraceuticals [10, 11].

Propolis possesses several biological properties, such as antioxidant [12], immuno-stimulating [13], haemtoprotective [14] hepato-protective [15], hypolipidemic and anti-atherogenic [16]. Melatonin and caffeic acid phenethyl ester are compounds of honey bee propolis that were recently found to be potent free radical scavengers and antioxidants. 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 [17]. The evidence reporting the amelioration by aqueous extract of propolis in sodium nitrite induced haematotoxicity, hyperlipidemia, atherogenic effects in Guinea pigs are hardly found. So, the present work aimed to evaluate ameliorating effect by aqueous extract of Libyan propolis in sodium nitrite induced haematotoxicity, hyperlipidemia, atherogenic effects in guinea pigs.

# 2. Materials and Methods

### *2.1. Chemicals*

Sodium nitrite ($NaNo_2$) was purchased from Sigma Aldrich, St Louis, MO. It was applied as a freshly prepared solution and given by gavages at a dose of 80 mg/kg body weight as previously described [ 1&18], daily for 35 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.* [19]. 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 weight [20] once daily for 35 days.

### *2.2. Animals*

Twenty four adult male Guinea pigs (*Cavia porcellus*) weighting 450-600 gm were used for this study. The animals were obtained from animal house unit in the faculty of veterinary medicine, Tripoli University, Libya. The animals were housed in a room under standard conditions of ventilation, temperature (25 ± 2°C), humidity (60-70%) and light/dark condition (12/12). The animals were provided with tape water ad libitum and fed with the standard commercial chow. 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 (6 Guinea pigs 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) orally by gavage daily for 35 days.

Group III (Sodium nitrite treated group): The animals received sodium nitrite orally at a dose of 80 mg/kg body weight, daily for 35 days.

Group IV (Sodium nitrite/propolis co-administered): The animals received sodium nitrite orally at a dose of 80 mg/kg body weight followed after two hours by propolis (200 mg/kg body wt) orally by gavage daily for 35 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. Blood Sampling*

Blood samples were drawn by cardiac puncture. The first sample was collected in clean dry tube containing the anticoagulant substance EDTA (ethylene diamine tetra acetic acid) and used for the hematological studies. The second 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.

#### *2.4. 1. Haematological Parameters*

Red, white blood cells and blood platelets counts were done

by using the hemocytometer and hemoglobin content (Hb) was determined according to the method of Wong [21]. Hematocrite value (Hct) was estimated by using the heparinized capillary tubes. The mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC) were calculated according to Schalm [22] as the following equations: MCV = Hct / RBC's × 10, MCH = Hb / RBC's × 10 & MCHC = Hb / Hct) × 100.

#### *2.4.2. Biochemical Analysis*

Total cholesterol concentration was estimated according to Allain *et a*l. [23], triglycerides concentration also by the method of Fossati and Prencipe [24] and HDL cholesterol by Burstein *et al*., [25]. VLDL-cholesterol and LDL-cholesterol concentrations were estimated by using the Friedewald equation [26]. 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

Haematological parameters in blood of the different groups are shown in Table 1. Guinea pigs that received sodium nitrite orally at a dose of 80 mg/kg body weight, daily for 35 days had significantly ($p<0.05$) lower RBCs count, Hb, Ht, MCH, MCHC, WBCs counts, and platelets count, than those in the control animals (Fig. 1 - 3 & 5 -8). On the other hand, MCV, of sodium nitrite treated Guinea pigs was significantly ($p<0.05$) elevated as compared to the control Guinea pigs (Fig. 4). Co-administration of sodium nitrite with propolis were significantly ($p<0.05$) prevented the changes recorded in blood parameters as compared with control group.

Lipid profile parameters in serum of the different groups are shown in Table 2. Guinea pigs that received sodium nitrite orally at a dose of 80 mg/kg body weight, daily for 35 days had significantly ($p<0.05$), increased the serum cholesterol, triglycerides, non HDLc, LDLc and VLDLc concentrations. Co-administration of sodium nitrite with propolis were significantly ($p<0.05$) prevented the changes recorded in serum cholesterol, triglycerides, non HDLc, LDLc and VLDLc concentrations as compared with control group (Fig. 9, 10 & 12-14). On the other hand, serum HDL cholesterol concentration of sodium nitrite treated Guinea pigs was significantly ($p<0.05$) decreased as compared to the control animals (Fig. 11). Co-administration of sodium nitrite with propolis was significantly ($p<0.05$) prevented the changes recorded in serum HDLc concentration as compared with control group.

Table 3 showed the means and standard deviations for cardiac risk ratio {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, sodium nitrite treated group and Guinea pigs group co-administrated of sodium nitrite with propolis. These ratios were elevated in sodium nitrite treated Guinea pigs group compared with the control group with statistically significant differences ($p<0.05$). Co-administration of sodium nitrite with propolis were declined these ratios with statistically significant differences ($p<0.05$), when compared with sodium nitrite group (Figs. 13-18).

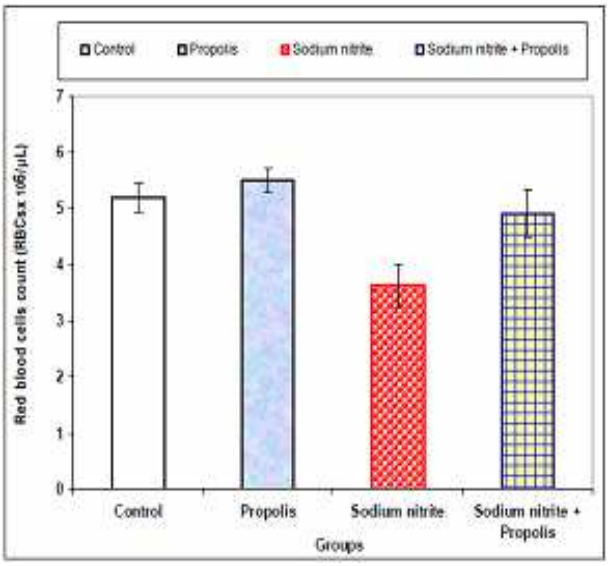


***Figure 1.*** *Red blood cells count in different animals groups.*

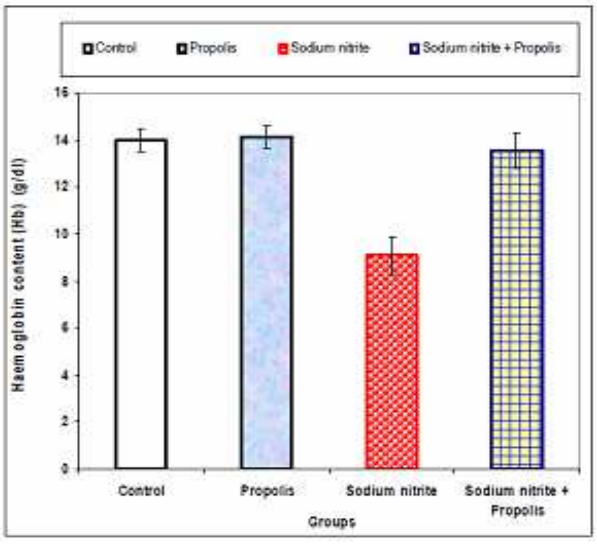


***Figure 2.*** *Haemoglobin content in different animals groups.*

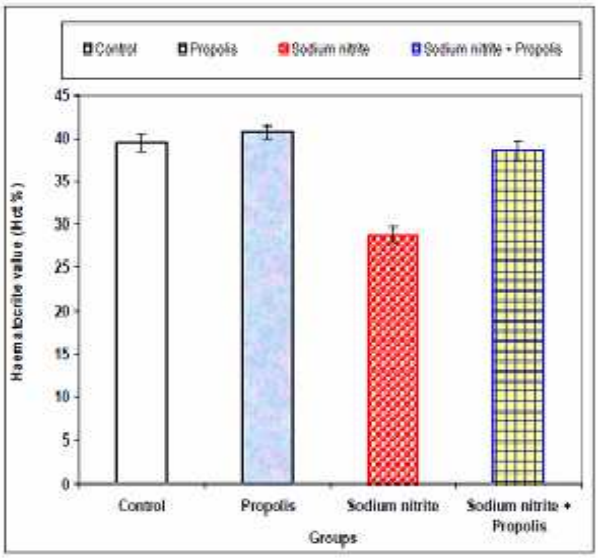


***Figure 3.*** *Haematocrite value in different animals groups.*

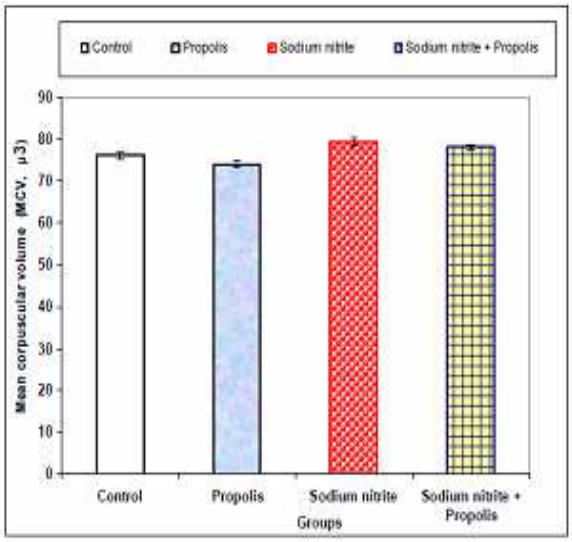


***Figure 4.*** *Mean corpuscular volume in different animals groups.*

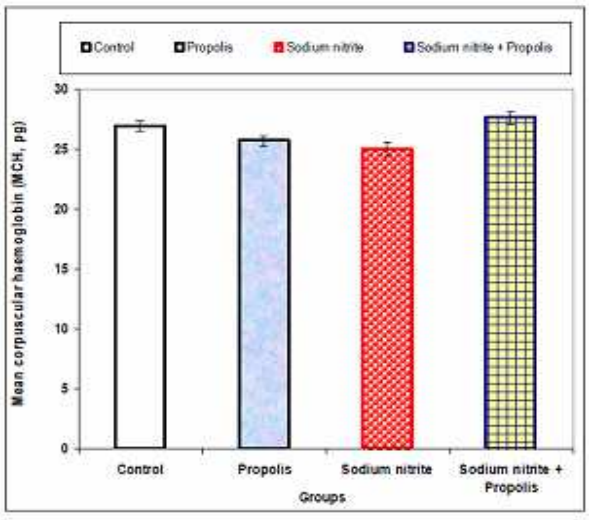


***Figure 5.*** *Mean corpuscular haemoglobin in different animals groups.*

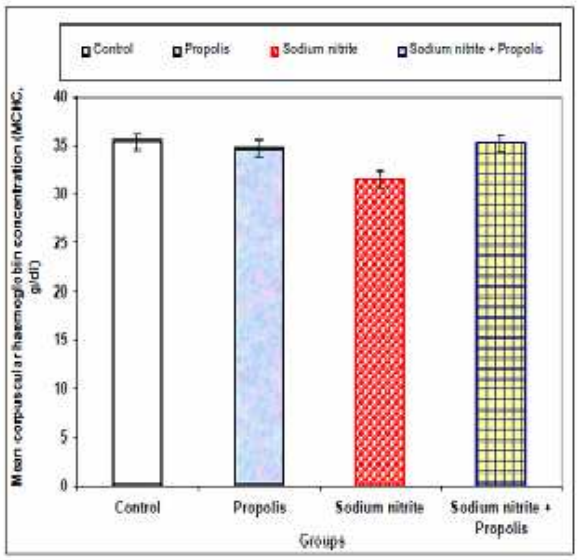


***Figure 6.*** *Mean corpuscular haemoglobin concentration in different animals groups.*

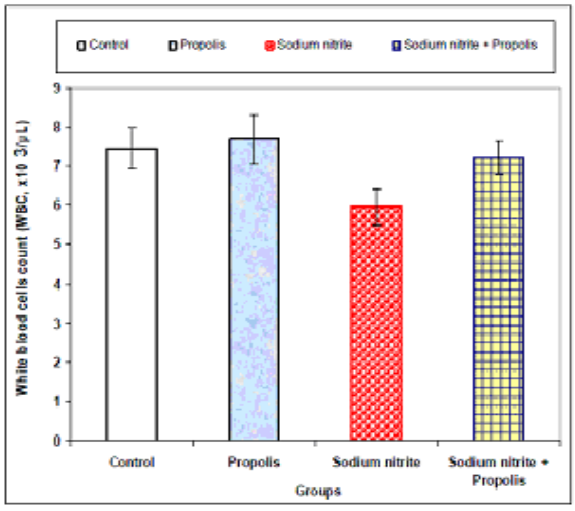


***Figure 7.*** *White blood cells count in different animals groups.*

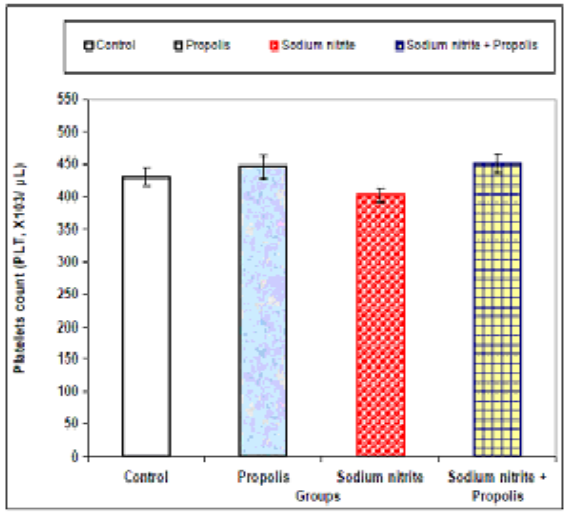


***Figure 8.*** *Platelets count in animals different groups.*

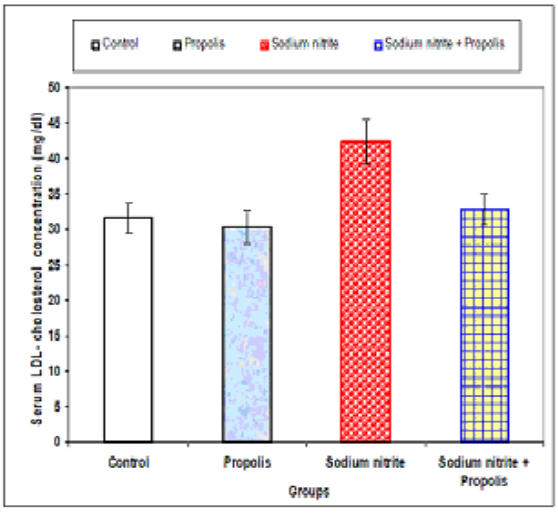


***Figure 9.*** *Serum cholesterol concentration in different animals groups.*

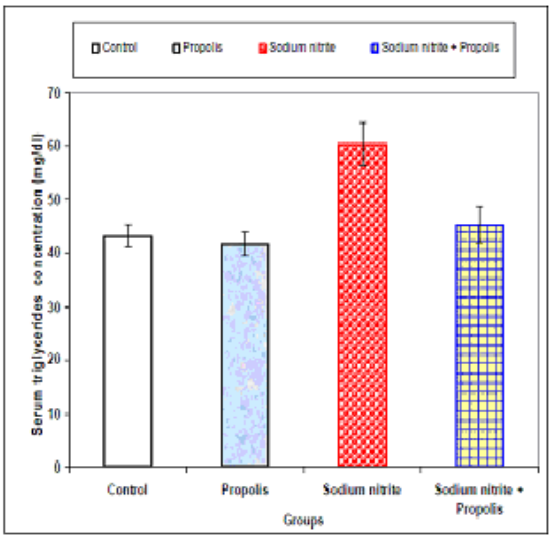


***Figure 10.*** *Serum triglycerides concentration in different groups.*

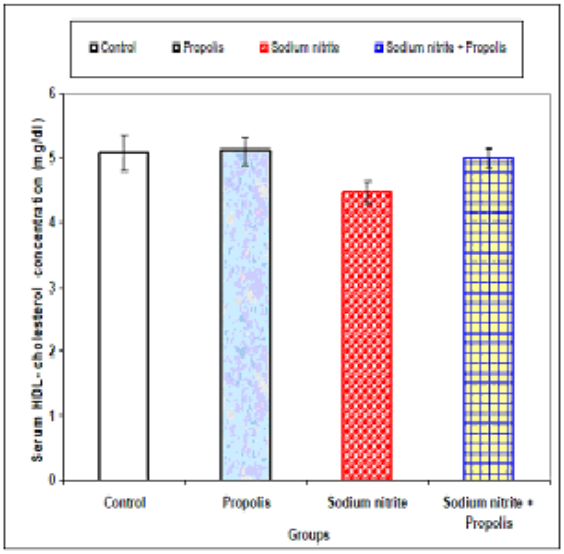


***Figure 11.*** *Serum HDL-cholesterol concentration in different animals groups.*

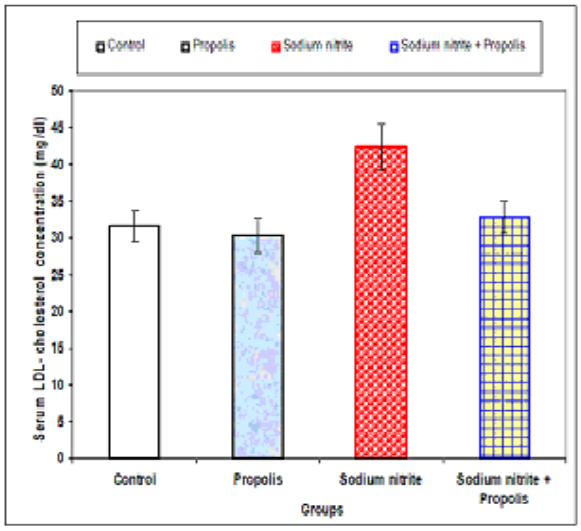


***Figure 12.*** *Serum LDL-cholesterol concentration in different groups.*

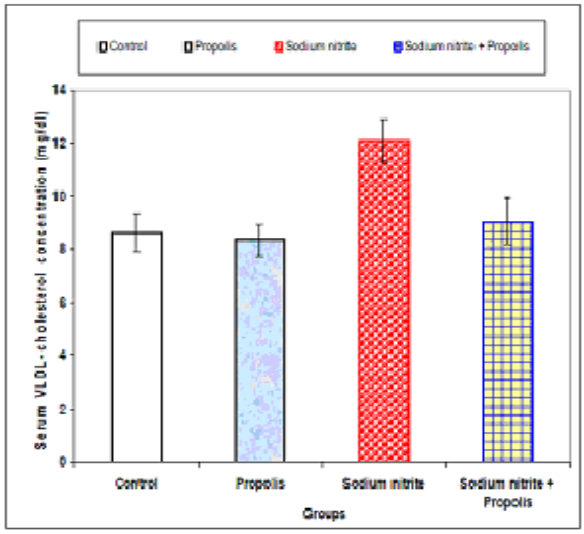


***Figure 13.*** *Serum VLDL-cholesterol concentration in different animals groups.*

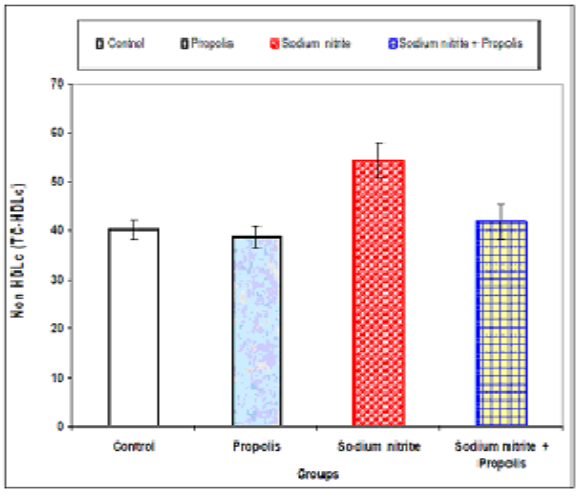


***Figure 14.*** *Serum non HDL-cholesterol( TC-HDLc) concentration in different animals groups.*

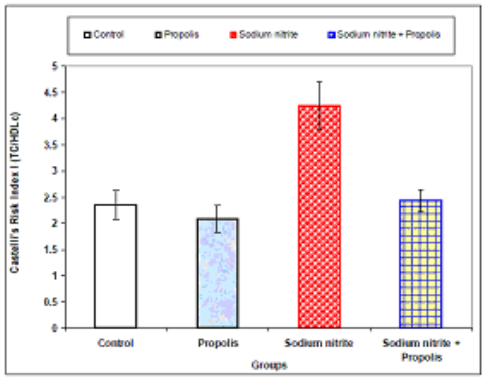


***Figure 15.*** *Cardiac Risk Ratio (Castelli's Risk Index I) in different animals groups.*

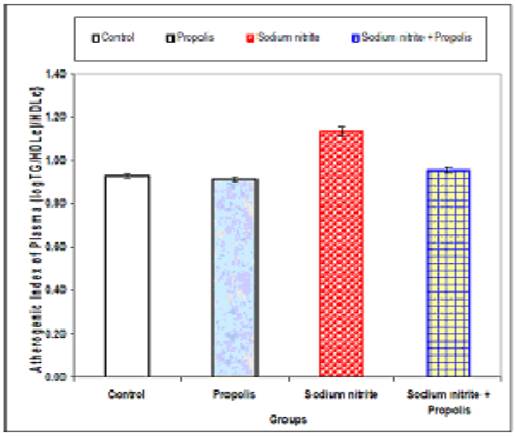


***Figure 17.*** *Atherogenic Index of Plasma(AIP) in different animals groups.*

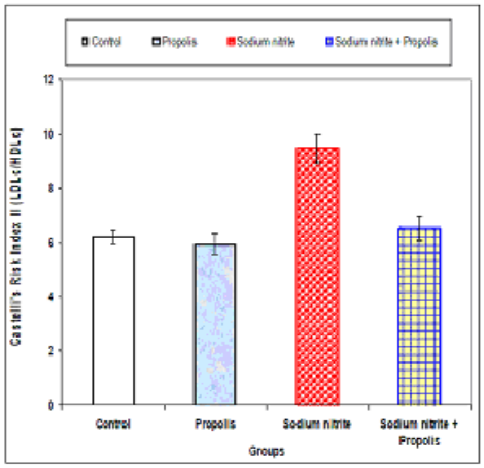


***Figure 16.*** *Castelli's Risk Index II in different animals groups.*

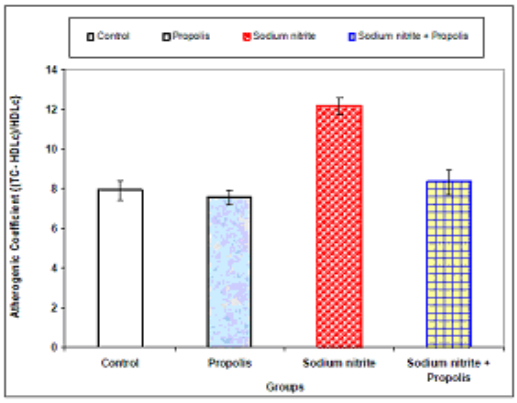


***Figure 18.*** *Atherogenic Coefficient in different animals groups.*

***Table 1.*** *Effect of aqueous extract of propolis on the haematological parameters in different Guinea pigs groups.*

| Parameters | Groups | | | |
|---|---|---|---|---|
| | **Control** | **Propolis** | **Sodium nitrite** | **Sodium nitrite + Propolis** |
| | **Mean + SD** | **Mean + SD** | **Mean + SD** | **Mean + SD** |
| *Red blood cell count* ( $x10^6$/ µl) | 5.19±0.26 | 5.51±0.22 | 3.63±0.37[a] | 4.92±0.42[b] |
| *Hemoglobin content* (g/dl) | 13.98±0.51 | 14.14±0.49 | 9.09±0.83[a] | 13.56±0.75[b] |
| *Haematocrit* (%) | 39.50±0.98 | 40.80±0.76 | 28.80±0.91$_a$ | 38.60±1.13[b] |
| *Mean corpuscular volume* ($\mu^3$) | 76.10±0.88 | 74.00±0.97 | 79.40±0.93[a] | 78.10±0.51[b] |
| *Mean corpuscular hemoglobin* (pg) | 26.90±0.45 | 25.70±0.43 | 25.00±0.59[a] | 27.60±0.51[b] |
| *Mean corpuscular hemoglobin concentration* (g/dl) | 35.40±0.83 | 34.70±0.89 | 31.50±0.91[a] | 35.30±0.79[b] |
| *White blood cell count* ($x10^3$/µl) | 7.45±0.54 | 7.69±0.62 | 5.97±0.46[a] | 7.22±0.42[b] |
| *Platelets count* ($X10^3$/µL) | 430±14.4 | 446±18.3 | 402±10.8[a] | 451±13.7[b] |

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

***Table 2.*** *Effect of aqueous extract of propolis on serum lipid profile parameters in different Guinea pigs groups.*

| Parameters | Groups | | | |
|---|---|---|---|---|
| | **Control** | **Propolis** | **Sodium nitrite** | **Sodium nitrite + Propolis** |
| | **Mean + SD** | **Mean + SD** | **Mean + SD** | **Mean + SD** |
| *Cholesterol concentration (mg/dl)* | 45.30±2.21 | 43.80±3.86 | 58.90±4.1[a] | 46.82±2.51[b] |
| *Triglycerides concentration (mg/dl)* | 43.26±2.2 | 41.78±2.38 | 60.56±4.13[a] | 45.32±3.36[b] |
| *High density lipids cholesterol concentration (mg/dl)* | 5.08±0.28 | 5.11±0.21 | 4.47±0.17[a] | 5.01±0.15[b] |
| *Low density lipids cholesterol concentration (mg/dl)* | 31.57±2.01 | 30.33±2.32 | 42.32±3.10[a] | 32.75±2.18[b] |
| *Very low density lipids cholesterol concentration (mg/dl)* | 8.65±0.73 | 8.36±0.62 | 12.11±0.81[a] | 9.06±0.87[b] |
| *Non HDLc (TC-HDLc) (mg/dl)* | 40.22±2.1 | 38.69±2.2 | 54.43±3.5[a] | 41.81±3.7[b] |

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

***Table 3.*** *Effect of aqueous extract of propolis on the ratios based on lipid profile parameters of in different Guinea pigs groups.*

| Parameters | Groups | | | |
|---|---|---|---|---|
| | Control | Propolis | Sodium nitrite | Sodium nitrite + Propolis |
| | Mean + SD | Mean + SD | Mean + SD | Mean + SD |
| Cardiac Risk Ratio (Castelli's risk index I) TC/HDLC | 2.35±0.28 | 2.08±0.26 | 4.25±0.45[a] | 2.43±0.21[b] |
| Castelli's risk index II (LDLc/HDLc) | 6.21±0.25 | 5.94±0.38 | 9.47±0.54[a] | 6.54±0.43[b] |
| Atherogenic index of plasma(AIP) log(TG/HDLC) | 0.93±0.031 | 0.91±0.028 | 1.13±0.041[a] | 0.96±0.023[b] |
| Atherogenic coefficient {(TC- HDLc)/HDLc} | 7.92±0.5 | 7.57±0.37 | 12.18±0.41[a] | 8.35±0.62[b] |

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

# 4. Discussion

The present study showed that treatment of male Guinea pigs with sodium nitrite were decreased red blood cell count, hemoglobin concentration, haematocrite, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and a significant increased mean corpuscular volume values as compared to the control Guinea pigs. Similar observations in erythrogram values were reported by Abu Aita and Mohammed [5] who found that a significant decreases in RBCs count, Hb, Ht, MCHC, and increase MCV values in rats treated with 30 mg/ kg body weight sodium nitrite for 2 months. Helal [6] reported that administration of both sodium nitrite and sunset yellow for one month to rats induced a decrease of WBCs count, RBCs count, haemoglobin content and haematocrite. It is known that nitrites convert the ferrous ion of haemoglobin to ferric ion both in vivo and vitro [27]. This can explain the reduction of haemoglobin level. In other words, administration of both nitrite and sunset yellow leads to haematopoietic tissue hypoxia resulting on the long term (one month) to a decrease of red blood cell production and hence to reduction of blood haemoglobin level [6].

Also, Ibrahim *et al.*, [28] reported that a significant decrease in Hb of rats taken high dose of sodium nitrite at ten weeks of experiment. The developed anaemia may be referred to the toxic effect of sodium nitrite on bone marrow, spleen and liver [5, 29]. Oxidative damage might be a more relevant cause of decreasing RBC, Hb, Hct which may be attributed to methaemoglobinaemia [28, 30] resulted from direct reaction of sodium nitrite on Hb which led to cell destruction [28, 31]. These changes may be attributed to haemolysis that resulted from MetHb formation. Furthermore, peroxynitrite ONOO-, a cytotoxic species (formed in vivo via the reaction between nitrite met Hb and hydrogen peroxide) and hypoxia (produced as result of methaemoglobinaemia) incriminated in high rate of hemolysis [28, 32]. Oxidative damage to haemoglobin resulting in Heinz body formation and/or methemoglobinaemia is a widely used indicator of oxidant damage in RBC [33, 34] resulting in important functional alterations, and both membrane and cytoplasmic structures are affected by such an oxidant attack [35]. Distorted RBC with increasing nitrite concentration leads to hypoxia since these are the component of blood for oxygen uptake and delivery [36].

The present study showed that administration of sodium nitrite to Guinea pigs had significantly lower WBCs counts, and platelets count compared to control group. These findings are similar to Abu Aita and Mohammed [5] who recorded that a significant decrease in the total leukocytic count of sodium nitrite administered rats in comparable to control group. The recorded leukopenia was associated with lymphopenia which reflects the immunosuppressive effect of sodium nitrite [5, 37]. Rats administrated sodium nitrite in drinking water for 14 weeks showed a significant decrease in blood platelets compared with controls [38].

The present data indicated that cholesterol, triglycerides, LDLc and VLDL concentrations were significantly increased by sodium nitrite treatment, while HDL-c concentration was decreased in the serum. Several studies have shown that sodium nitrite exposure induces alterations in serum lipid profiles [1, 7]. These results run parallel to those reported by Sherif, and Al-Gayyar,[1] who found that treatment of rats with sodium nitrite at a dose of 80 mg / kg body weight daily for 12 weeks were increased serum cholesterol, triglycerides, LDL and VLDL concentrations compared with control rats. Abu Aita and Mohammed [5] reported that significant hypercholesterolemia was recorded in sodium nitrite administered rats compared to control group. This elevation may be due to the mobilization of free fatty acids from the adipose tissue to the blood stream and increase level of acetyl CoA, leading to increase in the synthesis of cholesterol or due to peroxidation of cell membrane lipids [5, 39]. Lowering levels of high density lipoprotein was a contrary effect because high HDL levels have been shown to bear an inverse correlation with risks for atherosclerosis [40]. 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 [41]. 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 [42]. Increase in LDL, VLDL levels are increase the risk of cardiovascular diseases [43, 44]. Oxidative stress, specifically the oxidation of low density lipoprotein, 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 to inhibit the oxidative modification of LDL that leads to the accumulation of cholesterol in atherosclerotic lesions [45, 46].

Results of the present study have shown that Castelli's risk index I, Castelli's risk index II, atherogenic coefficient and atherogenic index of plasma were elevated in sodium nitrite treated Guinea pigs group compared with the control group with statistically significant differences ($p<0.05$). These findings are similar to Azab *et al*., [16] who recorded that elevations in Castelli's risk index I, Castelli's risk index II, atherogenic coefficient and atherogenic index of plasma in mice treated with lead acetate. Bhardwaj *et al*. [47] 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 and effective drug management.

The present study showed that administration of propolis alone to Guinea pigs did not cause any significant alteration in the haematological indices. These findings are similar to the data reported by Jasprica *et al*. [48] who recorded that administration of propolis did not cause any significant changes in haematological parameters such as blood cells, hemoglobin concentration and haematocrit in humans.

In our study, administration of propolis alone to Guinea pigs did not cause any significant alteration on the serum cholesterol, triglycerides, HDL-c, LDL-c, and VLDL-c levels. These results agreed with that observed by Azab *et al*., [16] who reported that administration of propolis alone did not cause any significant alteration on the serum cholesterol, triglycerides, HDL-c, LDL-c, and VLDL levels. These findings are similar to the data reported by Sforcin *et al.* [49], Mani *et al*. [50], and Gomaa *et al*., [51] who demonstrated that treatment with propolis did not cause any significant change in biochemical parameters such as triglyceride and total cholesterol in rats.

Co-administration of sodium nitrite with propolis were significantly prevented the changes recorded in erythrogram values which caused by treatment of Guinea pigs with sodium nitrite only as compared with control group. Similar results were recorded in rats concurrently administered sodium nitrite with marjoram oils. Marjoram oil contains essential oils that inhibit lipid peroxidation in the membranes of erythrocytes that resulted in increasing membrane resistance to spontaneous hemolysis, decreasing membrane microviscosity, maintenance of their integrity and functional activity [52]. Our results are in accordance with the findings that treatment of rats with propetamphos plus propolis increased total leukocyte count compared to rats treated with propetamphos [14]. The increase observed in total leukocyte count may indicate an activation of the animal's immune system. Previous studies have shown that propolis has anti-inflammatory and immunomodulatory activities [53, 54]. It has been also reported that propolis treatment increased proliferation of leucocyte precursors from pluripotent stem cell in mice [55].

Results of the present study which have shown that co-administration of propolis with sodium nitrite to male Guinea pigs induced significant reduction in serum cholesterol, triglycerides, LDL-c and VLDL-c concentrations and elevation in serum HDL- cholesterol. These results are in concordant with those of Azab *et al*., [16] who reported 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. Also, El-Nahrawy *et al*.,[56] reported that monosodium glutamate administration to male albino rats exerted significant elevation of the serum cholesterol, triglycerides, LDL, and VLDL levels. The protective group was first administered propolis alone for 4 weeks, and secondly received Monosodium glutamate in association with propolis for 4 weeks. In the protective group, propolis extract showed significant improvement in the previous fractions. Decrease in triglyceride and cholesterol levels following propolis intake may be related to the influence of propolis itself on lipid metabolism [56]. Antioxidants and flavonoids can act as inhibitors of lipid peroxidation by scavenging polyunsaturated fatty acids peroxy radicals and interrupting the chain reactions [57]. It is well-known that phenolic antioxidants can trap initiating radicals and/or propagating peroxy radicals to break the peroxidation chain reaction to protect the cells from oxidation damage [58]. Co-administration of propolis to chlorpyrifos treated rats restored serum total cholesterol, triglycerides and LDL-cholesterol parameters to normal levels [51]. Also, Cetin *et al*., [14] 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*., [59] reported propolis to cause decrease in triglyceride level when administered to rats with diabetes mellitus. In addition, Kolankaya *et al*., [60] 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-cholesterol 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 [61]. Flavonoids supplementation significantly increased HDL-cholesterol and HDL-cholesterol/ total-cholesterol ratio [62]. The favorable lipid profile indicates a possible antiatherogenic property of the flavonoids [63]. Bok *et al*.,[ 64] 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.*, [65]. Diets containing flavonoids reduced the VLDL [66].

Increases of HDL have cardio-protective effect and it was proved by various studies. [43, 44]. 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 [42, 67]. 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 [68]. 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 [59].

In the present study, co-administration of sodium nitrite with propolis were reduced Castelli's risk index I, Castelli's risk index II, atherogenic coefficient and atherogenic index of plasma with statistically significant differences ($p<0.05$), when compared with sodium nitrite treated group. This is in agreement with Azab *et al*.,[16] who recorded that a decreases in Castelli's risk index I, Castelli's risk index II, atherogenic coefficient and atherogenic index of plasma with statistically significant differences ($p<0.05$) in mice co-administrated of lead acetate with propolis , when compared with lead acetate treated 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 [69]. Some propolis is made bioactive by the presence of prenylated compounds [70]. Russo *et al*., [71] studied a propolis and determined the antioxidant properties that are conferred by galangin, caffeic acid, ferulic acid, pcumaric and CAPE. The antioxidant activities of propolis are related to its ability to scavenge singlet oxygen, superoxide anions, proxy radicals, hydroxyl radicals and peroxynitrite [72]. 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 [73].

## 5. Conclusion

The present study, concluded that, sodium nitrite had adverse effects on some haematological parameters, lipids profile, and atherogenic ratios in the blood serum. Propolis supplementation showed a remarkable amelioration of these abnormalities in sodium nitrite treated male Guinea pigs. It is recommended that the use of sodium nitrite must be limited and use of propolis as antioxidant to prevent the toxic effect. Further studies are necessary to elucidate exact mechanism of protection of haematotoxicity, hyperlipidemia, atherogenic and potential usefulness of aqueous extract of Libyan propolis as a protective agent against sodium nitrite induced haematotoxicity, dyslipidemia and atherogenic in clinical trials.

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