International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Sodium Nitrite Induced Biochemical Alterations in the Blood Serum and its Amelioration by Aqueous Extract of Libyan Propolis in Guinea Pigs

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Abstract: ***The most essential aspects of food chemistry are the additives and preservatives. The addition of sodium nitrite 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. These free radicals, known to cause oxidative stress, that could be harmful to different organs including liver and kidney. Flavonoids and various phenolics are the most important pharmacologically active constituents in propolis capable of scavenging free radicals. The aim of this study was to evaluate the effect of sodium nitrite administration on some biochemical parameters in the blood serum and to explore the ability of aqueous extract of Libyan propolis as a natural source of antioxidants to minimize the harmful effects of sodium nitrite in male 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 30 days. Blood samples were obtained for assessment of serum biochemical (glucose, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and γ- glutamyltransferase activities, total proteins albumin, and globulin concentrations, A/G ratio, urea, uric acid, creatinine concentrations, sodium ion, and potassium ion concentrations) parameters. In sodium nitrite treated animals, the serum glucose, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and γ- glutamyl transferase activities, urea, uric acid, creatinine, sodium ion, and potassium ion concentrations parameters were increased and serum total proteins albumin, and globulin concentrations, and A/G ratio, were decreased. Co-administration of propolis significantly improved of all biochemical parameters. It can be concluded that, sodium nitrite had adverse effects on some biochemical parameters 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 serum biochemical alterations and potential usefulness of aqueous extract of Libyan propolis as a protective agent against sodium nitrite induced biochemical toxicity in clinical trials.***

Keywords: Hepato-renal dysfunction, Libyan propolis, Male Guinea pig, Serum glucose, Serum proteins, Sodium Nitrite

1. Introduction

The most essential aspects of food chemistry are the additives and preservatives. Additives have been used for many years to preserve, flavor, blend, thicken and color foods, and have played an important and essential role in reducing serious nutritional deficiencies. Additives help to assure the availability of wholesome, appetizing and affordable foods that meet consumer's demands from season to season [1]. 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 [2 &3]. 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 [4]. Nitrates and nitrites are precursors in the formation of N-nitroso compounds, a class of genotoxic compounds consisting of nitrosamines and nitrosamides [5]. N-nitroso compounds are known to cause congenital malformations in animal models, and the role of these compounds in adverse pregnancy outcomes warrants further exploration [6]. Humans are exposed to N-nitroso compounds from exogenous sources and through endogenous formation. Dietary sources of nitrosamines include cured meats, beer, and smoked fish; these foods may contain preformed nitrosamines as the result of cooking and/or preservation methods [2 & 7].

Sodium nitrite, with the chemical formula $NaNO_2$, is a white to slightly yellowish crystalline powder. The addition of NaNO2 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 [8]. Sodium nitrite has been reported to have adverse health effects due to increased oxidative stress that could be harmful to different organs including the liver [3]. The reactive nitrogen species that are produced by exposure to nitrite have many toxic effects including hepatotoxicity, nephrotoxicity and dysregulation of inflammatory responses and tissue injury [9].

Several experimental studies in various laboratories are underway, to study the prophylactic effect of various natural antioxidant compounds against toxic metals. Natural antioxidants strengthen the endogenous antioxidants defenses from reactive oxygen species and restore the optimal balance by neutralizing the reactive species [10]. 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.

Its 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[11].

Previous reviews [12 &13] have covered the knowledge about the chemical composition and botanical origin of propolis throughout 20th century. Until 2000,over 300 chemical components belonging to the flavonoids, terpenes, and phenolics have been identified in propolis. Some representative chemical compounds are summarized in Figure 1.

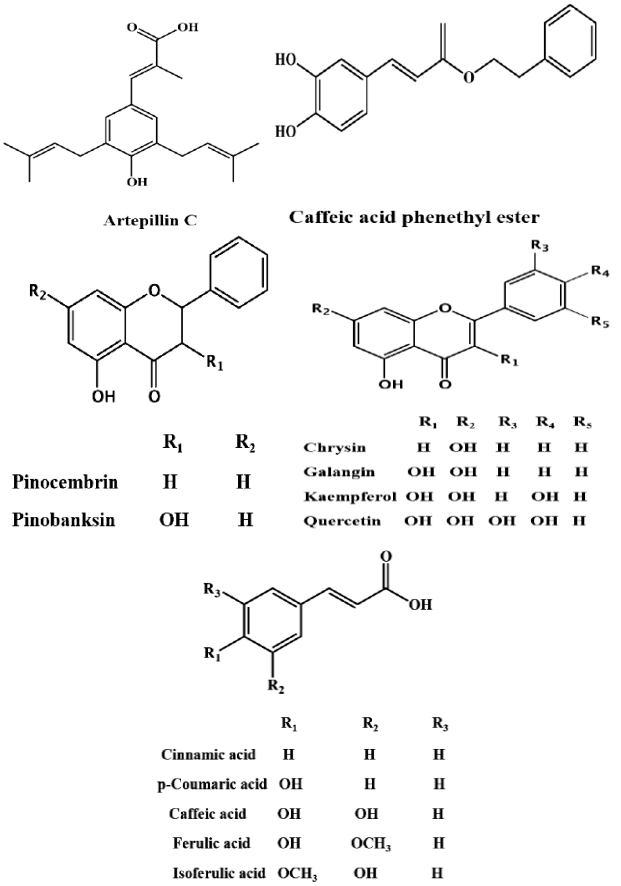


Figure 1: Representative chemical components in propolis.

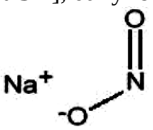
Recently, propolis has been used for upper respiratory tract infections, common cold, flu-like infections, as dermatological preparations in wound healing, treatment of burns, acne, herpes simplex and genitalis, and neurodermatitis, as mouthwashes and toothpastes to prevent caries and treat gingivitis 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 [11, 14 & 15].

Propolis possesses several biological properties, such as antibacterial [16], antifungal [17], antiviral [18], antiprotozoan [19], antitumour [20], anti-inflammatory [21], local-anesthetic [22], antioxidant [23], immuno-stimulating [24], cytotoxic [25], nephroprotective [26], hepato-protective [27], hypolipidemic and anti-atherogenic [28]. Melatonin and caffeic acid phenethyl ester are compounds of hony bee propolis, that were recently found to be potent free radical scavengers and antioxidants [28]. 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 [30]. The evidence reporting the amelioration by aqueous extract of propolis in sodium nitrite induced biochemical alterations in the blood of Guinea pigs are hardly found. So, the present work aimed to evaluate ameliorating effect by aqueous extract of libyan propolis in sodium nitrite induced biochemical alterations in the blood of 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 [3 & 31], daily for 35 days.



Chemical structure of sodium nitrite

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 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/day) 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/day) 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. 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. The activities of Alanine aminotransferase(ALT), aspartate aminotransferase (AST) were determined in serum according to the methods described by Reitman and Frankel [34]. Serum alkaline phosphatase (ALP) activity was determined according to Kind et al. [35]. Serum γ-GT activity was determined according to the method of Szas [36].

Serum glucose was determined using Trinder method [37]. Serum total proteins concentration was determined according to Biuret method explained by Weichselbaum [38]. Serum albumin concentration was determined according the method of Doumas *et al*. [39]. Serum globulin concentration was determined according to the formula:

Globulin = total protein–albumin.

The ratio of serum albumin/ globulin was determined as albumin / globulin level. Serum urea measurement was based upon the cleavage of urea with urease [40]. Serum uric acid was determined [41]. Serum creatinine was measured without protein precipitation [42]. Serum electrolytes will estimate according to the method of [43]. Using Chiron diagnostics kits.

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

Biochemical parameters in serum 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 ($p<0.05$), increased the serum glucose, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and γ- glutamyltransferase activities, urea, uric acid, creatinine, sodium ion, and potassium ion concentrations parameters. Co-administration of sodium nitrite with propolis were significantly ($p<0.05$) prevented the changes recorded in serum glucose, liver function serum enzymes activities, and serum kidney function parameters as compared with control group (Fig. 1- 5 & 10-13). On the other hand, serum total proteins, albumin, and globulin concentrations, and A/G ratio of sodium nitrite treated Guinea pigs were significantly ($p<0.05$) decreased as compared to the control Guinea pigs (Fig. 6-9). Co-administration of sodium nitrite with propolis were significantly ($p<0.05$) prevented the changes recorded in serum total proteins, albumin, and globulin concentrations, and A/G ratio as compared with control group.

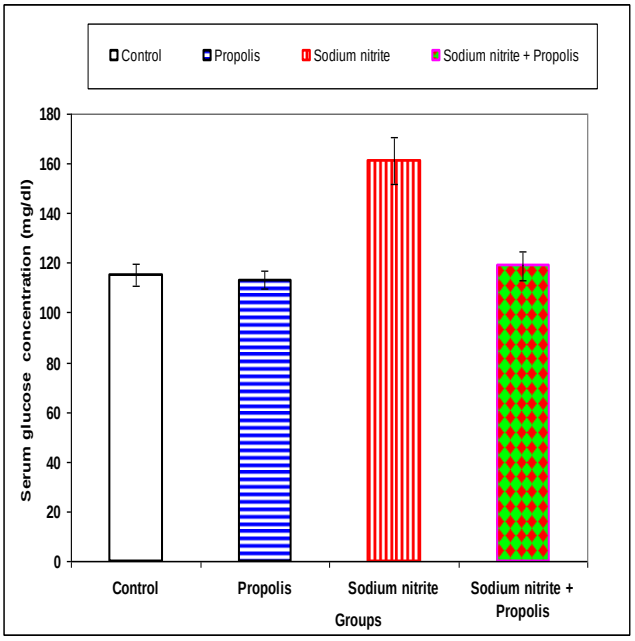


Figure 1: Serum glucose concentration in different animals groups

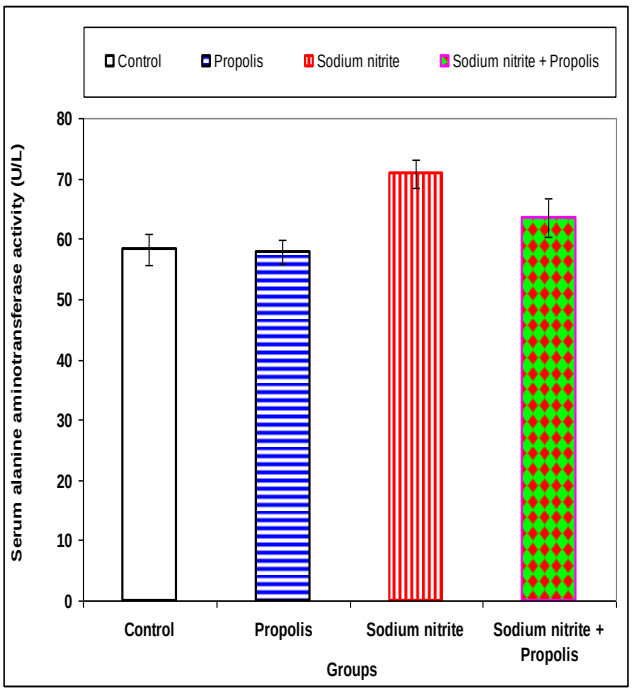


Figure 2: Serum alanine aminotransferase activity in different animals groups

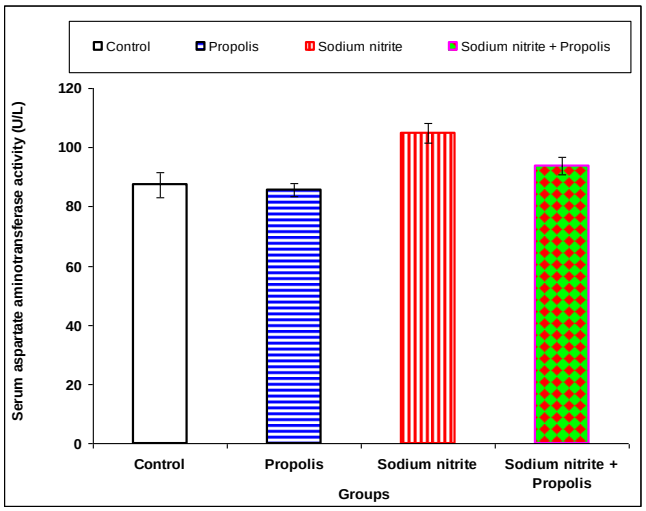


Figure 3: Serum aspartate aminotransferase activity in different animals groups

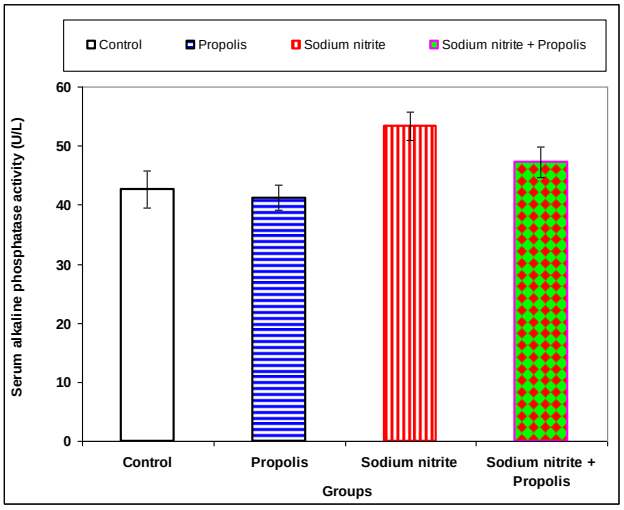


Figure 4: Serum alkaline phoasphatase activity in different animals groups

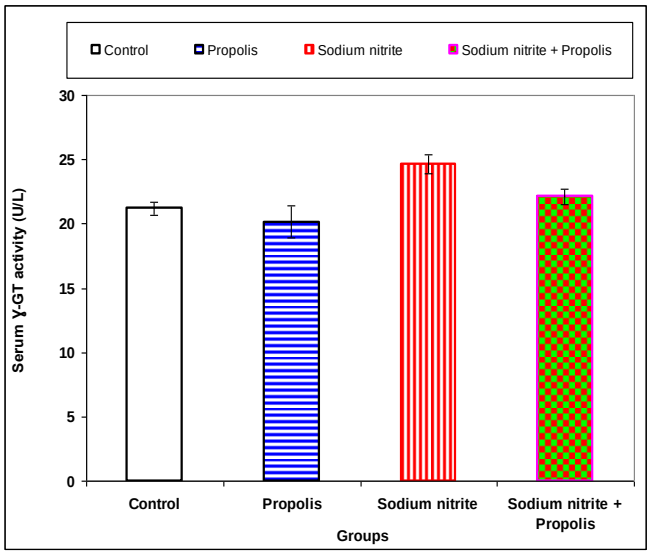


Figure 5: Serum γ-glutamyltransferase activity in different animals groups.

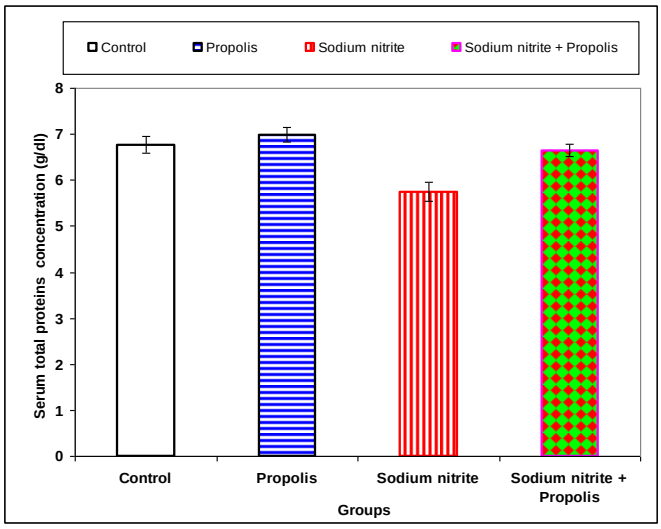


Figure 6: Serum total proteins concentration in different animals groups

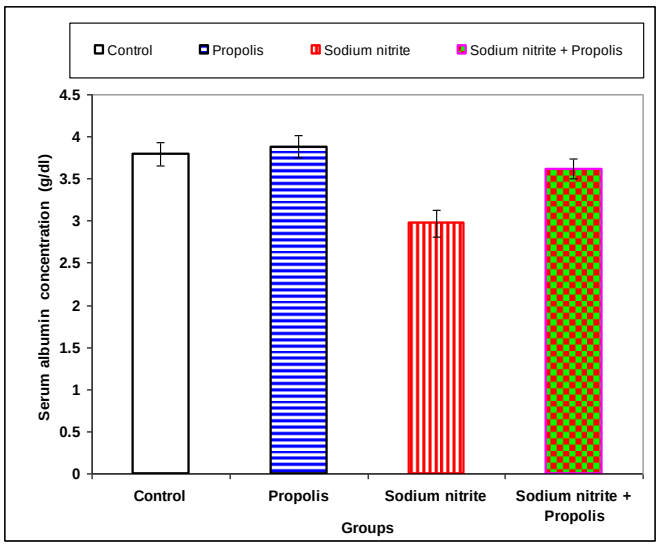


Figure 7: Serum albumin concentration in different animals groups

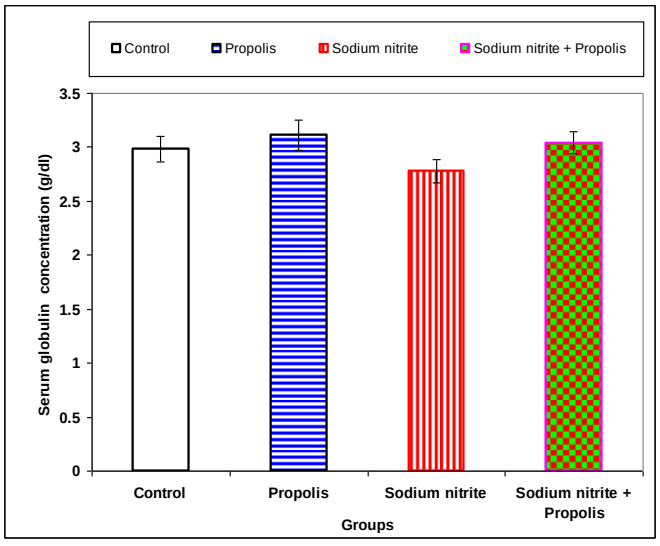


Figure 8: Serum globulin concentration in animals different groups**.**

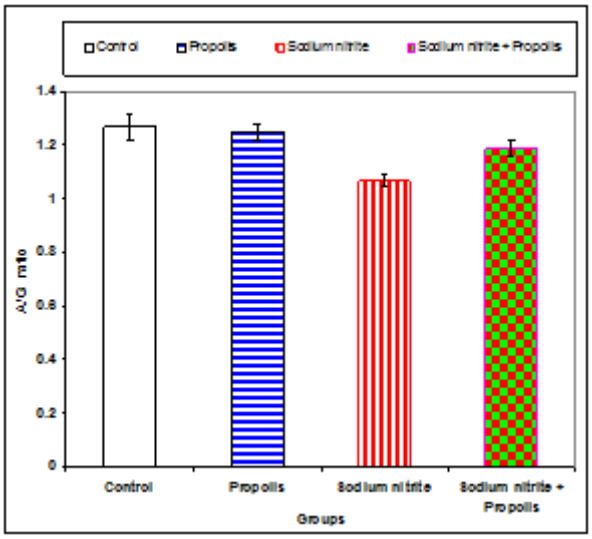


Figure 9: Serum A/G ratio in different animals groups

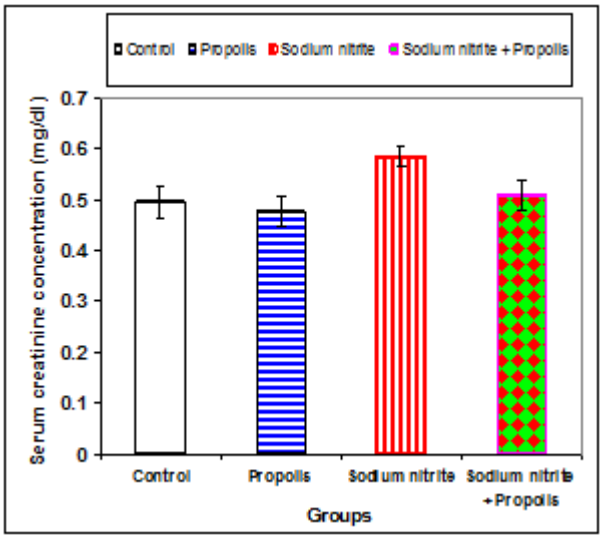


Figure 12: Serum creatinine concentration in different animals groups

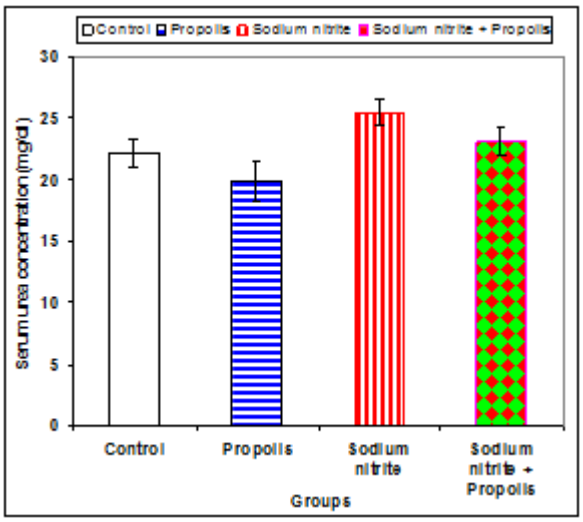


Figure 10: Serum urea concentration in different animals groups

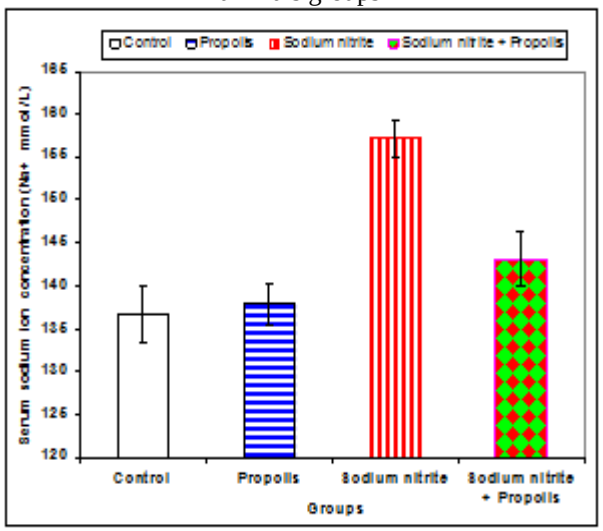


Figure 13: Serum sodium ion concentration in different animals groups

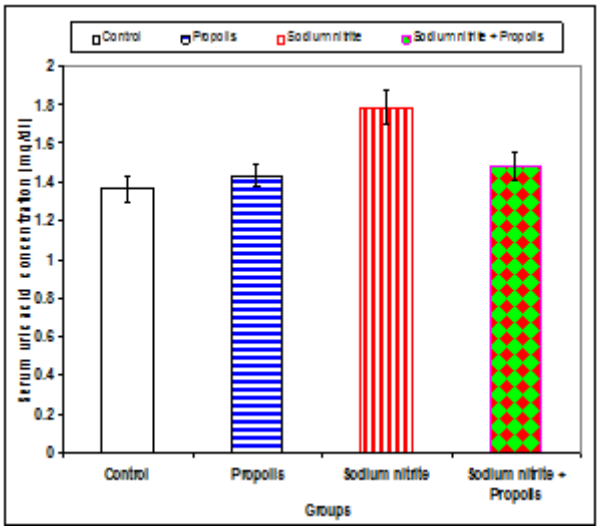


Figure 11: Serum uric acid concentration in different animals groups

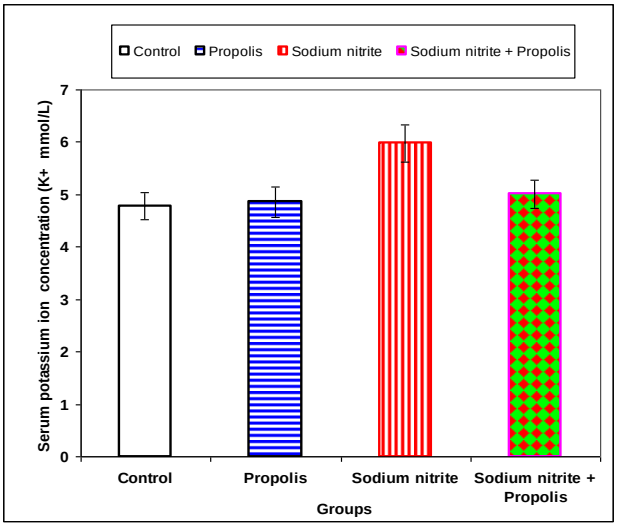


Figure 14: Serum potassium ion concentration in different animals groups.

Table 1: Effect of aqueous extract of propolis on serum biochemical parameters in different Guinea pigs groups

Parameters	Groups			
	Control	Propolis	Sodium nitrite	Sodium nitrite+ Propolis
	Mean + SD	Mean + SD	Mean + SD	Mean + SD
Glucose concentration (mg/dl)	115.2±4.46	113.1±3.65	161.1±9.32[a]	118.9±5.68[b]
ALT (U/L)	58.3±2.6	57.8±2.01	70.79±2.31[a]	63.47±3.15[b]
AST (U/L)	87.4±4.23	85.6±2.15	104.9±3.18[a]	93.7±2.94[b]
ALP (U/L)	42.69±3.11	41.22±2.16	53.36±2.36[a]	47.21±2.61[b]
Ɣ-GT (U/L)	21.22±0.51	20.15±1.23	24.63±0.76[a]	22.13±0.59[b]
Total proteins concentration (g / dl)	6.77±0.19	6.99±0.15	5.75±0.21[a]	6.65±0.14[b]
Albumin concentration (g / dl)	3.79±0.14	3.88±0.13	2.97±0.16[a]	3.62±0.12[b]
Globulin concentration (g / dl)	2.98±0.12	3.11±0.14	2.78±0.11[a]	3.04±0.1[b]
A/G ratio	1.27±0.048	1.25±0.029	1.07±0.025[a]	1.19±0.033[b]
Urea concentration (mg/dl)	22.13±1.13	19.87±1.69	27.45±1.05[a]	23.1±1.19[b]
Uric acid concentration (mg/dl)	1.36±0.07	1.43±0.06	1.78±0.09[a]	1.48±0.07[b]
Creatinine concentration (mg/dl)	0.497±0.03	0.478±0.03	0.557±0.02[a]	0.511±0.03[b]
Sodium ion concentration (Na^+ mmol/L)	136.7±3.26	137.9±2.4	157.21±2.15[a]	143.1±3.22[b]
Potassium ion concentration (K^+ mmol/L)	4.78±0.26	4.86±0.29	5.98±0.35[a]	5.01±0.27[b]

ALT*: alanine aminotransferase activity*, A ST: *aspartate aminotransferase activity, ALP: alkaline phoasphatase activity, Ɣ-GT: Ɣ-glutamyltransferase activity. 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

Sodium nitrite and other food additives may react with amines of foods in the stomach and produce nitrosamines and free radicals. Such products may increase lipid peroxidation, which can be harmful to different organs including the liver and kidney [44].

In this study, the sodium nitrite had adverse effects on the serum glucose, total proteins, albumin, activities of liver enzymes and kidney functions parameters in the serum. Results of the present study have shown that serum glucose concentration was elevated in sodium nitrite treated male Guinea pigs group compared with the control group with statistically significant differences ($p<0.05$). This is in agreement with many authors who reported the toxicity of sodium nitrite on the serum glucose [31& 45]. Increase in serum glucose of the sodium nitrite treated group suggests nitrite stimulation of gluconeogenesis [46], and glucose shift from tissue to blood or an impairment of glucose mobilization[31& 45]. Furthermore, nitroso-compounds can alter the antioxidant system causing disturbance in the metabolic processes leading to hyperglycemia [47].

The present study, revealed that treatment of Guinea pigs with sodium nitrite induced a significant increase in the activities of the serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and γ- glutamyl-transferase. Similar findings in the liver enzymes (AST, ALT, ALP) coincided by Sherif , and Al-Gayyar,[3], Hassan et al.,[31], Kalantari and Salehi [48], Enovwo, [45], Ibrahim et al.,[49], Salama et al [50]. The increase in the activity of AST, ALT and ALP enzymes in the serum of sodium nitrite treated group, could be attributed to the toxic effect of nitroso-compounds formed in the acidic environment of the stomach causing severe hepatic necrosis [31, 45 & 48], or it may be due to anaemia and methaemoglobinemia which induced hypoxic injury to centrilobular hepatocytes that consequently cause enzyme leakage [51]. Sodium nitrite caused oxidative damage to cell membrane, liver tissue damage and inhibition of oxidative stress, resulting in the increased activity of liver enzymes and reduced albumin levels [50]. High levels of AST, ALT, ALP and GGT may be due to the escape of these enzymes from the liver cytosol into the blood stream and liver disfunction and defect in the biosynthesis of these enzymes with change in the permeability of liver membrane takes places [52].

The present data indicated that, treatment of Guinea pigs with sodium nitrite caused a significant decreased in serum total proteins, albumin, and globulin concentrations, and A/G ratio as compared to the control. Similar observations in serum total proteins, albumin were reported by Hassan et al. [31], Enovwo [45]. Eremin and Yocharina [53],who suggest that a stimulation of the thyroid and adrenal glands by sodium nitrite can lead to a blockade in protein synthesis, fast breakdown, increased rate of free amino acids and decreased protein turnover. In addition, nitrite interactions result into nitric oxide release, which can inhibit total protein synthesis [54]. Sodium nitrite caused oxidative damage to cell membrane, liver tissue damage and inhibition of oxidative stress, resulting in reduced albumin levels [50].

This study shows that, 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 urea, uric acid, and creatinine concentrations suggesting an impairment of kidney function. This is in agreement with many authors who reported the toxicity of sodium nitrite on the serum urea, uric acid, and creatinine concentrations [31, 45 & 55]. These effects could be attributed to changes in the threshold of tubular re-absorption, renal blood flow and glomerular filtration rate [45 & 55].

The present study, revealed that treatment of Guinea pigs with sodium nitrite induced a significant elevations in the serum sodium ion, and potassium ion concentrations. These results agreed with that observed by [45 & 56].

Co-administration of sodium nitrite with propolis were significantly ($p<0.05$) prevented the changes recorded in serum glucose, liver function serum enzymes activities, and serum kidney function parameters as compared with control group. This is in agreement with many authors [52, 57- 59]. Talas et al.,[52] found that decreases in levels of glucose, creatinine, urea, AST, ALT, ALP and GGT activities in blood plasma of rats treated with Nω-Nitro-L-arginine methyl ester + propolis group compared with Nω-Nitro-L-arginine methyl ester group. Therefore propolis protected the status of cellular biomolecules towards normal by improving the cellular metabolism and reversed the hepatic necrosis and renal tubular damage [52 & 60]. Ramadan et al., [59] reported that, Injection of galactosamine/ lipopolysaccharide to rats induced hepatic damage that was manifested by a significant increase in the activities of aminotransferases, alkaline phosphatase, lactate dehydrogenase in serum. Oral dosing of propolis before or once immediately after intoxication reversed these altered parameters near to normal values. These results suggest that propolis could afford significant protection and therapy in alleviation of hepatotoxicity. Abdel Malak et al.,[61] reported that i.p. administration of CCl4 induced severe hepatic injury. There is a significant increase in serum ALT, and AST levels accompanied with a significant decrease in total protein were found in rats treated with CCl_4. The treatment with propolis resulted in a significant improvement in all evaluated parameters. Authors concluded that propolis protect rats against the severe CCl4-induced hepatic toxic effects. These results suggest that the protective activity of propolis may have been related to their antioxidant properties. Rats administrated with sodium fluoride alone 1 g/kg diet for 42 days showed that significant increase alkaline phosphatase activity, urea, creatinine, sodium and potassium levels in serum. And significant decrease total protein level in serum as compared to control group. Whereas administration of propolis powder in diet 0.2% with sodium fluoride led to significant decrease ALP activity, urea, creatinine, sodium and potassium levels in serum. The propolis enhanced total protein level in serum as compared to sodium fluoride group alone [57]. Azab et al., [58] reported that the serum levels of urea, creatinine, and uric acid were elevated in guinea pigs treated with gentamicin at a dose of 100 mg/kg body wt for 10 days. Co-administration of Propolis with gentamicin led to significant decrease the serum urea, creatinine and uric acid. This is perhaps due to the antioxidant actions of the propolis extract. Some antioxidant compounds identified in propolis include ferulic acid, quercetin and caffeic acid [62]. Some propolis is made bioactive by the presence of prenylated compounds [63]. Russo *et al*., [64] 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 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. A direct relationship has been found between the phenolic content and antioxidant capacity of plants [65). 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

The present study, concluded that, sodium nitrite had adverse effects on some biochemical parameters 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 serum biochemical alterations and potential usefulness of aqueous extract of Libyan propolis as a protective agent against sodium nitrite induced biochemical toxicity in clinical trials.

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