Journal of Nutrition & Food Sciences
ISSN: 2155-9600
Hassan Abd et al., J Nutr Food Sci 2017, 7:4
DOI: 10.4172/2155-9600.1000619
Research Article
OMICS International

Effect of Saudi Propolis on Hepatitis Male Rats

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Received date: July 03, 2017; **Accepted date:** July 22, 2017; **Published date:** July 29, 2017

Abstract

This study was conducted to evaluate the benefits of the Saudi gum (propolis) by reduction of the toxic substances in rats that target the liver and affect its performance. The chemical components of propolis were identified. The study included 42 Albino male rat of a healthy weight ranged from 255-287 g, and they were divided into six equal groups. The first group was fed the standard diet (negative control group), while the other 35 rats were injected with carbon tetrachloride under the skin (1.5 ml/ kg) in order to infect them with acute hepatitis. After 24 h, the groups of infected rats were divided and the second group (positive control group) was also fed a standard meal, while the other groups infected which were the third, fourth, fifth and sixth were fed on a diet with access to a standard concentration of Saudi bee gum of 200, 300, 400 and 500 mg/kg, respectively, through the mouth for 4 consecutive weeks. The results showed that propolis contains 41 compounds and out of these 17 compounds have been identified. Volatile oil was in proportion of 20.37%, aliphatic acids in 16.87%, and esters in 15.48% and alcohols in 13.98%. The results showed a significant improvement in the biochemical parameters in hepatitis rats which were treated with propolis. Results also showed that propolis increased the activity of antioxidant enzymes in the liver of hepatitis rats treated with propolis. The study concluded that propolis plays an effective role in protecting the liver from damage and inflammation that can be caused by the components of antioxidation and inflammation.

Keywords: Propolis; Hepatitis; Antioxidant

Introduction

Liver is considered as one of the most important and the largest glandular vital organs of the human body, and it leads to many vital functions [1]. Liver function depends on the manufacturing ability of the liver, such as albumin and total protein, functions that rely on the integrity of the liver cells called liver enzymes which are present within the cells of the liver. These functions likewise rely on the extractive capacity of the liver such as alkaline phosphatase and Bilirubin [2].

Hepatitis threatens the lives of millions of people around the world with an estimated number of sufferers around the world by about 2 billion people according to the World Health Organization statistics [3]. The Hepatitis disease represents an epidemic problem where statistics showed up that until 2007 the number of cases of hepatitis disease in the Kingdom of Saudi Arabia reached 8852 of which about 61% were Hepatitis (A) between the age group 5-14 years, and Hepatitis (B) 65% for the age group 15-44 years, while the prevalence of Hepatitis (C) was 65% of the age group that exceed 45 years [4].

The honeybee gum (propolis) is a bee product, and it is a tonic antiseptic substance and a natural antibiotic that works to strengthen the body's immune system and to help in disease resistance and thus maintain the vitality of the body and the safety of its organs. Honeybee gum helps to resist aging, heart disease, liver disease, skin, stomach, intestine and colon cancer [5,6]. Honeybee gum was used around the world for thousands of years in folk medicine as an anti-microbial and anti-ulcer and tumors. And it raises the immunity of a healthy body since it contains more than 300 compounds such as phenols, amino acids, inorganic compounds, steroids and the substances Sequiterpene quinines and Coumarins [7,8].

Study of Bhadauria et al. [9], indicated that mice injected with carbon tetrachloride (1.5 ml/kg) caused a poisoned liver cells and an increased in the concentration of malondialdehyde (an indicator of fat oxidation), and the break out of enzyme lactic dehydrogenase, gamma glutamyl trans betadase (an indicator of cell toxicity); and there had been a significant improvement in these variables that occurred after the rats intake of honeybee gum at concentrations of 200 mg/kg body weight per day for a month.

Jasprica et al. [5] mentioned that the honeybee gum improved the hepatic effects as a result of liver damage in experimental rats treated with paracetamol pills. The study concluded that honeybee gum and its components of Flavonoid reduce free radicals like superoxide and hydroxyl.

Study of El Fiky [10] showed an improvement of the level of blood glucose in the group that fed on honeybee gum was added to the meal by 30%. The study results also indicated an improvement of liver function in the groups fed on honeybee gum 20%.

The importance of this research emerged in order to study the benefit of the Saudi honeybee gum in the reduction of toxic substances that target the liver and affect its performance of which can be monitored during the biochemical factors.

Materials and Methods

This research was conducted at the King Fahd Medical Research Center, King Abdul Aziz University in Jeddah. The samples of honeybee gum were purchased from the Saudi market produced by the

Herbs factory, Madinah, license 783/r. The components of honeybee gum has been identified using a chromatographic separation gaseous device Model 6980 produced by Palo Alto company (California, USA) according to the method of Hegazi and Abd El Hady [11].

A total number of 42 apparently healthy albino male, weighted from 255 to 287 g were used. The food and water had been provided to the point of satiety ad libitum. After the rats adapted to the new environmental conditions they were divided into six groups (7 rats each). The first group (negative control group) was fed a standard meal, while others 35 rats were injected with carbon tetrachloride subcutaneously (1.5 mL/kg) in order to infect the rats with acute liver hepatitis. After 24 h, the rats were divided into second group which have been fed with a standard meal (positive control group), while the rest of the rats were divided into four experimental groups that have been fed with a standard meal of crude Saudi honeybee gum by concentrations of 200, 300, 400 and 500 mg/kg of body weight, respectively, through the mouth for four consecutive weeks. The treatment period ended. The animals were slaughtered after fasting 8 hours, and some of the biochemical parameters in the blood serum and the liver were evaluated. The study was performed according to animal care ethics recommended by the University Committee.

Biochemical parameters in the blood serum have included the estimation of urea and creatinine according to the method of Neumann and Ziegenhorn [12]; Bartels [13] respectively. The level of cholesterol and triglycerides were estimated, and the high and low density lipoprotein levels according to the method of Boehringer–Mannheim [14]; Lang and Schettler [15]; Fruchart [16]; Weinsier and Morgan [17]; respectively. The activity of each of the enzyme aspartate amino transferase and alanine amino transferase was estimated according to the methods of Bergmeyer et al. [18]. The glucose and total bilirubin was estimated according to the method of Trinder [19]; Parviainen [20], respectively. Antioxidant activity in the liver was estimated that included the compounds Malondialdehyde (MDA) by the method of Esterbauer and Cheeseman [21]. The enzymatic activity was estimated for Glutathione-S-transferase, catalase (GST), Catalase enzyme (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH), all according to the method of Habig et al. [22]; Aebi [23]; Arthur and Boyne [24] and Barjade et al. [25], respectively. All results obtained are expressed as means ± standard deviation (S.D.). Differences between group means were calculated by one-way Analysis of Variance (ANOVA) and a post-hoc Duncan test used by SPSS/PC computer program [26]. Results were considered significant at $p<0.05$.

Results and Discussion

The results of Table 1 and Figure 1 indicated that the gas chromatographic separation device GC-MS was used for the separation of Saudi propolis sample components. The results showed that the honeybee gum contains 41 compounds; it has been identified, including 17 components. The proportion of volatile oils was 20.37%, 16.87% aliphatic acids, and esters 15.48% and 13.98% alcohols. The volatile oils were used to represent the proportion of high ratio. It was separated into eight components of which were a compound Cadinene present by 5.39%, a high ratio, and the least was Alpha-Guaiene compound by 0.88%. These results agreed with the study of Sobhi et al. [26] which compared the composition of Egyptian and Dubai, UAE honeybee gum samples.

The results showed that blood glucose level decreased in the experimental groups that have been infected and treated with honeybee gum compared to positive control (Table 2).

Component	%	Component	%
Volatile oils	0.88	Aliphatic acids	-
Alpha-Guaiene	-	Palmitic acids	16.87
Alpha-Longipinene	0.91	-	-
AR-Curcumene	4.53	-	-
Cadinene	5.39	-	-
Cyercene	2.48	-	-
Beta cadinene	1.77	-	-
Valencene	1.78	-	-
Germacrene	2.63	-	-
Total	20.37	Total	16.87
Esters	-	Alcohols	
Methyl Palmitate	3.64	Guaiol	1.51
Ethyl Palmitate	5.16	Veridiforol	5.04
Methyl Stearate	2.37	Eudesmol	5.8
Ethyl Stearate	4.31	Levomenol	1.63
Total	15.48	Total	13.98

Table 1: Chemical composition of Saudi honeybee gum.

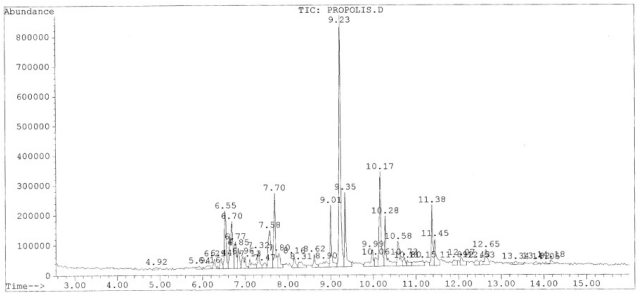

Figure 1: Chromatographic separation device for separating gaseous sample of Saudi honeybee gum.

The increase in the blood glucose level to an imbalance in the metabolism of carbohydrates was due to the increased destruction of liver glycogen and this is because of the increased activity of the hormones glucagon, adrenaline with low activity of insulin. And it also causes oxidative stress imbalance in the composition of the pancreatic islets of Langerhans with the formation of amyloid proteins that inhibit insulin release into the circulation and destroyed beta cells to secrete insulin [27,28]. The results were similar with the results of El Sayed et al. [28].

Table 2 also shows high concentration of bilirubin in the blood serum of the hepatitis infected rats compared to rats that fed on

standard meal and decreased in the treated groups. Significant differences emerged (P<0.05) between rats infected with hepatitis, which is not treated on the one hand and between the rats that were treated with honeybee gum at different doses on the other hand. It may increase the total bilirubin in the blood plasma as a result of the liver's lack of ability to extract or increase the production of bilirubin due to breakdown of red blood cells [29,30].

Parameter Groups	Glucose	Bilirubin	Urea	Creatinine
Negative control	70.72 ± 5.19^{d}	0.15 ± 0.03^{b}	33.04 ± 2.06^{b}	0.66 ± 0.041^{b}
Positive control	116.01 ± 7.18^{a}	0.26 ± 0.02^{a}	48.03 ± 4.67^{a}	0.86 ± 0.05^{a}
200 mg/kg	93.70 ± 4.36^{b}	0.16 ± 0.01^{b}	36.03 ± 5.30^{b}	0.65 ± 0.12^{b}
300 mg/kg	86.76 ± 7.23^{c}	0.15 ± 0.02^{b}	34.23 ± 4.90^{b}	0.64 ± 0.03^{b}
400 mg/kg	87.01 ± 5.66^{c}	0.16 ± 0.91^{b}	32.00 ± 4.39^{b}	0.64 ± 0.03^{b}
500 mg/kg	85.77 ± 4.71^{c}	0.17 ± 0.02^{b}	31.40 ± 5.36^{b}	0.64 ± 0.05^{b}
Values are expressed as means ± SD; $^{a-d}$ Represent the significant differences from control at (P<0.05)				

Table 2: Level of glucose, urea bilirubin and creatinine (mg/100 ml) in the rat's serum.

And this study is consistent with Newairy et al. [30], which showed an increase in glucose and bilirubin concentration in the blood plasma of rats exposed to aluminum chloride, but the intake of honeybee gum (50 mg/kg body weight) led to an improved glucose values and bilirubin once again.

Table 2 illustrates the average level of urea in the blood serum of the experimented rats of the control groups. It led to the treatment of rats infected with hepatitis with honeybee gum in different doses to improve the level of urea and approximated the normal values.

The high level of both ammonia and creatinine in the blood serum may be due to infection of hepatitis so that one of the most important functions of the liver is to get rid of ammonia, and subsequently nitrogen from the blood by turning it into urea to come out through the urine. When cirrhosis of the liver cells occurs, active cells turn into fibrous cells. If the blood does not circulate naturally in the liver vessels around the fibrous tissue, and it changes the course of the vessels carrying ammonia for disposal in the liver to reach the brain toxicity and fainting occurs [31,32].

The results of this study was in agreement with the study of Bhadauria et al. [32], which showed that the treatment with honeybee gum resulted in improvement of the level of urea, uric acid. Also, results of El Fiky [10] showed a significant decrease in the concentration of each of the urea and creatinine in diabetic rats fed with honey or its products. As well as the results agreed with Newairy et al. [30] which indicated that treatment of rats with honeybee gum resulted in a reduction of urea and creatinine.

It was noted that there were significant differences between the positive control group, and all the affected groups of hepatitis, which have been treated with various doses of honeybee gum and corresponded with the results of low-density lipoproteins, cholesterol and triglycerides (Table 3).

Increase the levels of cholesterol and triglycerides, low level of density lipoproteins, as well as the low level of high density lipoproteins may be due to a density disorder that infects the liver as a result of hepatitis which causes deficiency in the performance of its vital functions. Fulianga et al. [33] explained that the intake of Chinese honeybee gum orally led to lower total cholesterol levels, triglycerides, and low and very low density lipoproteins in the blood serum.

Alves et al. [34] noted that the effect of honeybee gum that occurred in a significant decrease of cholesterol in the blood was the result of the direct effect on the liver or indirect effect over the thyroid hormones, as these hormones affect the reactions of the metabolism of fat.

Parameter Groups	Cholesterol	Triglycerides	High density lipoproteins	Low-density lipoproteins
Negative control	59.98 ± 6.25^{b}	36.25 ± 2.36^{b}	21.21 ± 0.90^{a}	9.50 ± 1.29^{b}
Positive control	82.87 ± 5.38^{a}	51.72 ± 5.97^{a}	17.67 ± 1.47^{b}	13.25 ± 1.21^{a}
200 mg/kg	57.42 ± 3.32^{b}	39.75 ± 3.96^{b}	19.88 ± 1.51^{a}	8.19 ± 0.9^{b}
300 mg/kg	57.59 ± 3.84^{b}	41.16 ± 4.10^{b}	20.05 ± 0.75^{a}	8.12 ± 1.32^{b}
400 mg/kg	59.93 ± 5.19^{b}	37.13 ± 0.85^{b}	21.65 ± 2.12^{a}	8.73 ± 2.03^{b}
500 mg/kg	59.42 ± 5.87^{b}	36.44 ± 1.67^{b}	21.54 ± 2.46^{a}	8.40 ± 1.21^{b}
Values are expressed as means ± SD; ^{a,b}Represents the significant differences from control at (P<0.05)				

Table 3: Level of cholesterol, triglycerides, and lipoproteins high and low density (mg/100 ml) in the rat's serum.

Also, the results of this study agreed with the study of El Sayed et al. [28], who explained that the treatment of diabetic rats with daily oral dose of honey bee glue of 200 mg/kg body weight for 5 weeks led to improvement their lipid profile.

The results (Table 4) showed an increase in enzyme activity of aspartate aminotransferase in the serum of the control group of rats. And the enzyme activity decreased as a result of the treatment of rats infected with hepatitis with honeybee gum of different doses (P<0.05). Enzyme activity of alanine amino transferase also decreased as a result

of the treatment of rats infected with hepatitis with the honeybee gum and the differences have been significant (P<0.05). Both of the enzymes aspartate aminotransferase and alanine aminotransferase resides in large quantities in the liver, but when the liver is diseased the liver functions differ and disorder occurs in the manufacture of these enzymes that changes the permeability of the liver membrane, which leads to the release of these enzymes to the plasma of the infected liver cells [35,36].

The results of this study have agreed with Bhadauria et al. [32], who pointed out a significant increase in the activity of an aspartate and alanine aminotransferase in serum of hepatitis rats that have been treated with the honeybee gum at a dose of 200-400 mg/kg. It was concluded that the honeybee gum was better than drug therapy in liver protection.

The results (Table 5) showed the average values of thiobutyric acid which expresses substance of malondialdehyde levels in the liver. Thiobutyric acid value has increased in the group of hepatitis infected rats and also in the infected group which was treated with honeybee gum at a dose of 200 mg/kg. Then thiobutyric acid value began to decrease significantly (P<0.05) compared to the positive control group as a result of increasing the dose of honeybee gum to 300 400 500 mg/kg.

Parameter Groups	Aspartate aminotransferase IU/liter	Alanine aminotransferase IU/liter
Negative control	126.00 ± 17.26^{b}	47.71 ± 8.13^{b}
Positive control	149.57 ± 3.55^{a}	79.86 ± 6.64^{a}
200 mg/kg	106.14 ± 25.37^{cd}	50.86 ± 12.07^{b}
300 mg/kg	113.29 ± 17.43^{bc}	47.71 ± 13.02^{b}
400 mg/kg	101.00 ± 7.83^{cd}	50.14 ± 5.69^{b}
500 mg/kg	96.00 ± 7.34^{d}	50.14 ± 7.64^{b}
Values are expressed as means ± SD; $^{a-d}$Represents the significant differences from control at (P<0.05)		

Table 4: Activity of aspartate and alanine aminotransferases in the rat's serum.

The results showed the increased activity of the enzyme superoxide dismutase by the increase of dose of honeybee gum. Statistical analysis showed that there were significant differences between the experimental groups (Table 5).

The enzyme activity of catalase decreased in positive control compared to negative control. And then the enzyme activity returned to rise again when the rats had intake of honeybee gum at doses of 200, 300, 400 and 500 mg/kg (Table 5). The activity of the enzyme glutathione-S-transferase has also decreased in positive control compared to negative control. The enzyme activity has improved and approached the value of the negative control group when hepatitis infected rats had intake of honey bee gum.

Increased activity of the enzyme glutathione peroxidase resulted with the treatment of hepatitis infected rats with the honeybee gum compared to the hepatitis infected rats. Results of statistical analysis showed that there were significant differences between the positive control groups on the one hand and the experimental groups on the other. And there was no significant difference between the negative control group and the three groups with higher dose of honeybee gum treatment (Table 5).

Parameter Groups	Thiobutyric acid nmol/g wet liver	Superoxide dismutase mg/g wet liver	Catalase mg/g wet liver	Glutathione-S-transferase mg/g wet liver	Glutathione peroxidase mg/g wet liver
Negative control	271.15 ± 19.93^{c}	3.831 ± 17.17^{a}	1.68 ± 0.02^{a}	5.67 ± 0.20^{a}	41.34 ± 4.87^{a}
Positive control	318.16 ± 13.3^{a}	167.86 ± 16.29^{c}	1.40 ± 0.04^{d}	3.85 ± 0.30^{d}	18.62 ± 5.10^{c}
200 mg/kg	320.32 ± 11.55^{a}	276.62 ± 19.42^{b}	1.53 ± 0.02^{c}	4.57 ± 0.09^{c}	32.94 ± 5.27^{b}
300 mg/kg	299.32 ± 13.97^{b}	291.48 ± 10.26a^{ab}	1.65 ± 0.03^{ab}	5.23 ± 0.22^{b}	35.63 ± 4.33^{b}
400 mg/kg	289.41 ± 26.48^{b}	303.27 ± 22.47^{a}	1.63 ± 0.07^{b}	5.53 ± 0.28^{a}	37.99 ± 5.13^{ab}
500 mg/kg	292.89 ± 6.83^{b}	318.96 ± 17.59^{a}	1.63 ± 0.02^{b}	5.53 ± 0.03^{a}	40.35 ± 8.05^{a}
Values are expressed as means ± SD; $^{a-d}$Represents the significant differences from control at P<0.05					

Table 5: Level of thiobutyric acid and antioxidant enzymes in the rat's liver.

As a result of Seven et al. [36], the activities of SOD, CAT, and GSH increased depending on the cellular defense mechanisms under oxidative stress in accordance with the increased MDA level. Vitamin C (500 mg/kg diet) and propolis (1 g/kg diet) decreased the SOD activity and have shown a tendency to reduce CAT and GSH levels. Propolis used as an antioxidant in broilers exposed to lead showed similar antioxidant effects as vitamin C in the case of oxidative stress. The decrease in the thiobutyric acid concentration and the increase in each of the activity of the enzyme glutathione-S-transferase, catalase, and superoxide dismutase, glutathione peroxidase may be due to the active ingredients in the honeybee gum that work to attack the free radicals formed within the body of hepatitis infected rats [37]. This study agreed with the results of Kanbur et al. [6]; El Sayed et al. [28].

Conclusion

It is concluded from this study that oxidative stress, which is caused by an imbalance in the production of free radicals and the body's defense mechanisms to fight them, may lead to chronic diseases. It may also be caused by oxidative damage defect in the liver functions. The results of this study have shown that honeybee gum is loaded with medicinal value of natural compound because it contains many effective materials. The treatment with honeybee gum resulted in reduced activity of free radicals and increased the activity of enzymes superoxide dismutase and glutathione-S-transferase and catalase in the tissues of the body. The study recommends the need to maintain liver health and attention to proper diet for Hepatitis patients which has an impact on speedy recovery. Encourage the intake of honeybee gum (propolis) because it contains antioxidants such as vitamins and minerals, where it works on the treatment of liver infected disease, and has a role as an antifungal, antivirus and anti-AIDS and protect the living cells from the free radicals in which infection occurs. These

results encourage a new natural product in future to treat patients with acute hepatitis.

References

1. Alqasoumi S (2007) Isolation and chemical structure elucidation of hepatoprotective constituents from plants used in traditional medicine in Saudi Arabia. Department of Pharmacognosy. College of Pharmacy, King Saud University, Saudi Arabia.
2. Wahibi A, Abdullah S (2000) Medical tests and pathological significance. Electronic Printing Press, Riyadh, Saudi Arabia.
3. Lavanchy D (2004) Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat 11: 97-107.
4. The Ministry of Health (2007) Statistical yearbook. Kingdom of Saudi Arabia.
5. Jasprica D, Mornar A, Debeljak Z, Smolcic-Bubalo A, Medic-Saric M, et al. (2007) *In vivo* study of propolis supplementation effects on antioxidative status and red blood cells. J Ethnopharmacol 110: 548-554.
6. Kanbur M, Eraslana G, Silicib S (2008) Antioxidant effect of propolis against exposure to propetamphos in rats. Ecotoxicol Environ Saf 72: 909-915.
7. Khalil M (2006) Biological activity of bee propolis in health and disease. Asian Pac J Cancer Prev 7: 22-31.
8. Al Naggara Y, Tanc Y, Rutherfordc C, Connore W, Griebele P, et al. (2016) Effects of treatments with Apivar and Thymovar on V. destructor populations, virus infections and indoor winter survival of Canadian honey bee (*Apis mellifera* L.) colonies. J Apic Res 54: 548-554.
9. Bhadauria M, Nirala SK, Shukla S (2007) Duration-dependent hepatoprotective effects of propolis extract against carbon tetrachloride-induced acute liver damage in rats. J Adv Ther 24: 1136-1145.
10. El Fiky N (2008) Biological study of bees honey impacts on the immunity of experimental animals. Minufiya University, Egypt.
11. Hegazi A, Abd El Hady F (2002) Egyptian propolis: 3. Antioxidant, antimicrobial activities and chemical composition of propolis from reclaimed lands. Z Naturforsch 57: 395-402.
12. Neumann U, Ziegenhorn J (1977) Kinetic enzymatic method for automated determination of HDL cholesterol in plasma. Scand J Clin Lab Invest.
13. Bartels H (1972) Determination of serum Creatinine. Clin Chim Acta 37: 193.
14. Mannheim B (1984) Keeping atherosclerosis in checks disorders of lipid metabolism. Boehringer-Mannheim West Germany.
15. Lang P, Schettler G (1985) Arteriosclerosis. Grundlagendiagnostik-Theraoi, German Physician Publisher Gm bH, Koln.
16. Fruchart JC (1982) Simultaneous measurement of plasma apolipoproteins A-I and B by electro immunoassay. Rev Fr Des Laboratories 103: 107.
17. Weinsier RL, Morgan SL (1993) Fundamentals of clinical nutrition. Virginia: Gilbert Perrin Mosby Year Book, NY, USA.
18. Bergmeyer H, Hrder M, Rej J (1986) IFCC recommendation. J Clin Chem Clin Biochem 24: 497
19. Trinder P (1969) Cited from chmory enzymatic glucose reagent set (colorimetric). Ann Clin Biochem 6: 24.
20. Parviainen M (1997) A modification of the diazo coupling method (Malloy-Evelyn) for the determination of serum total bilirubin. Scand J Clin Lab Invest 57: 275-280.
21. Esterbauer H, Cheesman K (1990) Determination of aldehydic lipid peroxidation products: Malonaldehyde and 4-hydrooxynonenal. Methods Enzymol 186: 407-421.
22. Habig W, Pabst M, Jakoby W (1974) Glutathione-S-Transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 249: 7130-7139.
23. Aebi HS (1984) Catalase. In: Bergmeyer HU (eds.) Methods in enzymatic analysis. Weinhiem, Verlag Chemie, Germany 3: 278-282.
24. Arthur J, Boyne R (1985) Superoxide dismutase assay. Life Sci 36: 1569-1575.
25. Barjade G, Gil P, Lopeztorres M (1988) Physiologic significance of catalase and glutathion peroxidase *in vivo* peroxidation in selected tissues of the toad *Discoglossus pietus* (amphibian) during actimation to normobaric hyperoxics. J Comp Physiol B 158: 583-590.
26. Sobhi A, Saeed A, Khan Ahmad I, Ali H (2006) Chemical composition of Egyptian and UAE propolis. Pak J Pharm Sci 19: 58-61.
27. Hayden M (2002) Islet myeloid, metabolic syndrome and the natural progressive history of type 2 diabetes mellitus. J Orthod Pract 3: 126-138.
28. El Sayed M, Osama M, Hamdy A, Ahmed M (2009) Potential antidiabetic and hypolipidemic effects of propolis extract in streptozotocin-induced diabetic rats. Pak J Pharm Sci 22: 168-174.
29. El-Sharakym A, Newairy A, Badreldeen M, Eweda S, Sheweita S (2007) Protective role of selenium against renal toxicity induced by cadmium in rats. Toxicology 235: 185-193.
30. Newairy A, Salama A, Hussien H, Yousef M (2009) Propolis alleviates aluminium-induced lipid peroxidation and biochemical parameters in male rats. Food Chem Toxicol 47: 1093-1098.
31. Tina M, John M, Sandt S (2008) Diverse Viewpoints and choices for your hepatitis C journey. Hepatitis C Choices (4th edn.) Caring Ambassadors Program.
32. Bhadauria M, Kumar S, Shukla S (2008) Multiple treatment of propolis extract ameliorates carbon tetrachloride induced liver injury in rats. Food Chem Toxicol 46: 2703-2712.
33. Fulianga H, Hepburnb H, Xuana H, Chenc M, Dayad S, et al. (2005) Effects of propolis on blood glucose, blood lipid and free radicals in rats with diabetes mellitus. Pharmacol Res 51: 147-152.
34. Alves M, Mesquita F, Sakaguti M, Tardivo A (2008) Hypocholesterolemic effect of propolis caffeic acids. Braz J Med Plants 10: 100-105.
35. Gaskill C, Miller L, Mattoon J, Hoffmann W, Burton S, et al. (2005) Liver histopathology and liver serum alanine aminotransferase and alkaline phosphatase activities in epileptic dogs receiving phenobarbital. Vet Pathol 42: 147-160.
36. Seven I, Aksu T, Seven P (2010) The effects of propolis on biochemical parameters and activity of antioxidant enzymes in broilers exposed to lead-induced oxidative stress. Asian-Aust J Anim Sci 23: 1482-1489.
37. Marquele F, Di Mambroa V, Georgetti, S, Casagrande R, Valim Y, et al. (2005) Assessment of the antioxidant activities of Brazilian extracts of propolis alone and in topical pharmaceutical formulations. J Pharm Biomed Anal 39: 455-462.