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Influence of dietary supplementation of propolis on hematology, biochemistry and lipid profile of rats fed high cholesterol diet

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ABSTRACT

The objective of the present study was to monitor the hypolipidemic and hypocholesterolemic effects of propolis in rats fed high cholesterol diet. The rats (n=32) were divided into four equal groups. The rats of group 1 (control) were fed basal diet, whereas rats of group 2 were fed basal diet mixed with cholesterol (1%). The rats of group 3 and 4 were fed high cholesterol diet (1%) mixed with propolis powder 1 and 2%, respectively. Hematological parameters were comparable among all groups. Cholesterol, triacylglycerol and ALT activities were increased significantly in rat fed high cholesterol diet as compared to control. Inclusion of propolis in high cholesterol diets reduced these parameters in serum. Hematological and biochemical findings were supported by histopathological analysis of liver tissues. Conclusively, 1% propolis was found as safe and enough to induce beneficial hypolipidemic and hypocholesterolemic effects in serum of rats fed high cholesterol diet.

Keywords

Biochemistry, Blood, Lipoproteins, Serum, Physiology, Propolis

ARTICLE HISTORY

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INTRODUCTION

Hyperlipidemia is the most predisposing factor of atherosclerosis and chronic heart disease (Wang et al., 1997). Focal accumulation of cholesterol in intima of large and medium-sized arteries form the atheromas (Varshney and Sharma, 1996) resulted in narrowing of the arterial lumen, damage the underlying media and frequently become ulcerated and calcified, reducing the blood flow to the myocardium (Goldstein and Brown 1990). Without a doubt, success lowering of serum cholesterol reduces coronary artery disease. Chemical drugs are used for lowering cholesterol, however, higher price and undesirable side effects are the main disadvantages (Thomas, 2003). Currently, world attentions are directed to the traditional medicine (Elmahdi et al., 2014). Propolis is one of the hive products produced by the worker bees who apply the resin to seal any cracks and fissures in the hive and they line their front door with it to prevent contamination (Popova et al., 2013). They use it as an antiseptic in breeder cells and they mix propolis with wax to distribute a fine varnish over every inch of the hive to protect it (Burdock, 1998). The chemical consistency of propolis is highly dependent on the flora of the region from where it is collected (Marcucci, 1995; Burdock, 1998; Banskota et al., 2001). However, caffeic acid phenethyl ester is the active component of propolis (Song et al., 2002). Propolis contains 50-70% resins and 10% essential oils, coming from the trees, mixed with 30-50% wax for proper consistency and 5-10% pollen, acquired from being transported in the bees's pollen baskets. Propolis has been used extensively in folk medicine, due to its several pharmacological and biological properties such as antimicrobial, immunomodulatory, antiinflammatory, and antioxidant (Shawky, 1996; Amal, 1997; Abd El-Fattah et al., 1999; Nakamura et al., 2010; Vatansever et al., 2010). Parallel with the recent increasing interest in alternative/herbal medicine for the prevention and treatment of various illnesses including

hypercholesterolemia, therefore, this study was undertaken to evaluate the effect of feeding propolis with the diet on hematology, biochemistry and histopathology of rats.

MATERIALS AND METHODS

Plant, chemicals and kits: Diagnostic kits for serum glucose, albumin, total proteins, total cholesterol, triglyceride, high density lipoprotein cholesterol (HDL-c), alanine aminotransferase (ALT) and aspartate amino transferase (AST), urea, alkaline phosphatase (ALP), uric acid and creatinine were purchased from ELIPSE, United diagnostic industry, UDI, Dammam, Saudi Arabia). Pure cholesterol (Cat# C3045) was purchased from sigma-Aldrich, USA. Routine chemicals and solvents used in the study were of highest grade and commercially available. The propolis used in the present study was purchased from Wadi Al-Nahil, Trading and Marketing Company, Riyadh, Saudi Arabia.

Animals and treatment: A total of 32 albino rats (200-250 g) was obtained from Laboratory House of College of Veterinary Medicine and Animal Resources, King Faisal University, Al-Ahsa, Saudi Arabia. The rats were acclimated for 10 days before commencing the experiment. The rats were housed in standard cages (5 rats/cage), and were fed with standard laboratory diet and water *ad libitum*. The animals were housed in air-conditioned rooms at 21-23°C and 60-65% of relative humidity, and kept on a 12 h light/12 h dark cycle. All the rats received humane care in accordance with the Guidelines for Care and Use of Laboratory Animals, published by ethics of scientific research committee of King Faisal University, Saudi Arabia (#DSR 140106).

Induction of hypercholesterolemia: One gram of pure cholesterol powder was added to each 99g of basal diet (1%) except the control for induction of hypercholesterolemia according to Sharma (1984) and Pandya et al. (2006).

Experimental groups and protocol: Rats were fed on standard diet and divided randomly into 4 groups comprising 8 rats in each group.
Group (1): Rats fed on basal diet without any additives and served as a control group.
Group (2): Rats fed on cholesterol diet 1% (1 g/99g of basal diet) Sharma (1984).
Group (3): Rats fed on cholesterol diet mixed with propolis powder (1g/99g cholesterol diet; 1%).
Group (4): Rats fed on cholesterol diet and treated with propolis powder (2g/98g cholesterol diet; 2%).

Samples collection: Blood samples were collected after two weeks following treatment so as to confirm the induction of hypercholesterolemia. After completing the experiment, overnight fasted animals (the control and experimental animals) were sacrificed, and by cardiac puncture, blood samples (5 mL) were collected in plain and EDTA vacutainers. Whole blood was used for the determination of selected hematological parameters. Sera were harvested and stored at –20°C until the time of analysis of biochemical markers. Liver tissues were collected and cut in small pieces, and subsequently immersed in neutral buffered formalin for 24 h for histopathological examination.

Hematological analysis: Total erythrocytic count (TEC), and total leucocytic count (TLC) were determined by standard hematological techniques (Feldman et al., 2000). Hemoglobin percentage (Hb%) was assessed according to Drubkin (1947).

Biochemical analysis: Commercial diagnostic kits (United Diagnostic Industry, UDI, Dammam, Saudi Arabia) were used for determination of total proteins (EP56-660), Glucose (EP37L-660), Albumin (EP03-570), ALT (EP07-500), creatine kinase, AST (EP15-500), CK (EP28-310), Uric acid (EP61-620), BUN (EP20-420), creatinine (EP33K-660), cholesterol (EP24-660), TAG (EP59-660), Calcium (EP22-660), HDL-c (EP41HD), Phosphorus (EP46-660), Magnesium (EP50-660) and Chloride (EP27-500) using ELIPSE full automated chemistry analyzer (Rome, Italy). Biochemical constituents was calculated according to the manufacture instruction. Very low density lipoprotein cholesterol (VLDL-c) was calculated by division of TAG by 5 while LDL-c was calculated by subtracting the values of sum HDL-c and VLDL-c from total cholesterol value (Bauer, 1982).

Histopathological examination: The fixed liver tissues were processed routinely, embedded in paraffin, sectioned, deparaffinized and rehydrated using the standard techniques described by Bancroft and Gamble (2002). The effect of high cholesterol diet induced fatty degeneration was evaluated by assessing morphological changes in the liver sections visualized after staining with hematoxylin and eosin (H&E).

Statistical analysis: All data was presented as mean ± standard error of mean by using one way analysis of variance ANOVA. All tests were performed using

computer package of the statistical analysis system (SAS, 2002).

RESULTS AND DISCUSSION

World ethnobotanical information reported that a number of herbal medicines from vegetables and plants are used for controlling hyperlipidemia and related complications (Dahanukar et al., 2000). It has been postulated that, decrease in serum cholesterol by 1%, reduced the risk of chronic heart disease by 2% (Jain et al., 2007). The synthetic hypolipidemic drugs are expensive beside their side effect as liver and muscle toxicity, renal insufficiency and hyperthyroidism (Moosa et al., 2006) were also reported. Propolis (bee glue) is a resinous hive product, produced by honey bees from various plant sources. Propolis has been used from ancient time as antimicrobial, anti-inflammatory and antioxidant agent (Nakamura et al., 2010; Vatansever et al., 2010). Based on traditional claims, the current study has been undertaken to investigate the effect of propolis on hematological and biochemical parameters with special reference to lipid profile of rats fed high cholesterol diet.

Hematological analysis: The obtained results (**Table 1**) indicated that the mean values of TEC, TLC, and Hemoglobin showed non-significant variation in rat fed propolis 1% (11.6±1.01 012/L; 11.6±2.3 109/L; 12.6±0.8 g/dL) and that received 2% propolis (11.7±1.0; 11.8±2.3; 12.8±0.8) compare to high cholesterol fed rats (11.9±2.0; 11.4±0.7; 12.2±2.0), respectively and the control group (12.2±1.3; 12.6±0.4; 12.0±0.9), respectively. Both doses of propolis induced the same effect on hematological parameters without any significant difference. Similar to the present study, propolis did not affect the TEC and hemoglobin percentages in carp. In the contrary, propolis induced significant elevation of TEC and hemoglobin in Muscovy broiler ducks kept on ethanol extracted propolis at a rate of 2 g/Kg of diet (Abdel-Rahman and Mosaad, 2013).

Biochemical analysis: The effect of propolis on glucose, total proteins and lipid profiles of rats fed high cholesterol diet and treated with two doses of propolis (1 and 2%) are presented in **Table 2**. These findings indicated that, total cholesterol was increased in rat fed high cholesterol diet (33.4±0.2 mg/dL) compare to control groups (28.1±0.1). However, inclusion of propolis in high cholesterol diets (1 and 2%) reduced serum total cholesterol (30.5±0.1; 31.5±0.1), respectively compare to rats fed high cholesterol diet (33.4±0.2) and toward the normal control values (28.1±0.1). These findings indicated also that, Triacylglycerol was increased in rat fed high cholesterol diet (43.6±0.4 mg/dL) compare to control groups (40.9±1.6). However, inclusion of propolis in high cholesterol diets (1 and 2%) reduced serum triacylglycerol (35.7±2.2; 32.8±2.0), respectively compare to rats fed high cholesterol diet (43.6±0.4) and toward the normal control values (40.9±1.6). The values of total proteins, Albumin, Glucose, HDL-c, LDL-c and VLDL-c remained comparable to that of control groups. The non-significant differences in serum total proteins, albumin, globulins and glucose of all experimental groups agree with previous report (Denli et al., 2005) in Japanese quail fed Turkish propolis. The significant increase of total cholesterol and triglycerides in rats fed high cholesterol diet compared to control group observed in the current study come in accordance with previous works (Ebesunun et al., 2007; Rachh et al., 2012; Elmahdi et al., 2014; Althnaian, 2014) in rats fed on the same cholesterol diet. Similar to the present study, previous report (Ebesunun et al., 2007) demonstarated that, HDL-c, LDL-c and VLDL-c concentrations remained unchanged in serum of all experimental groups including the control animals. Authors attributed this insignificant changes of these parameters to the short duration of feeding. The most acceptable reason for the lack of significance in the plasma HDL-c could be attributed to the fact that propolis might be not interfere with HDL-c synthesis but its mode of action might be induced by inhibiting cholesterol biosynthesis; it may happen through the inhibition of HMG-CoA reductase, the rate-limiting enzyme that mediates the first step in cholesterol biosynthesis. This could also be speculated that longer time of feeding is needed to allow a notable significant changes in the mean plasma HDL-c in these groups of animal fed diet containing propolis. As evident from this study, propolis decreases plasma triglyceride level in the rats fed propolis containing diets. This might be exhibited through lipase stimulation. The present findings summarized in **Table 3** indicated that, ALT activity was increased in rat fed high cholesterol diet (27.5±0.6 IU/l) compare to control groups (23.7±0.4). However, inclusion of propolis in high cholesterol diets (1 and 2%) reduced serum ALT activity (21.8±1.5; 22.5±1.7), respectively as compare to rats fed high cholesterol diet (27.5±0.6) and toward the normal control values (23.7±0.4). The activities of AST

Table 1. Hematological parameters of rats fed high cholesterol diet and treated with two doses of propolis (1 and 2%).

Parameters	Groups			
	1	2	3	4
TEC (10^{12}/L)	12.2 ± 1.3	11.9 ± 2.0	11.6 ± 1.0	11.7 ± 1.0
TLC (10^{9}/L)	12.6 ± 0.4	11.4 ± 0.7	11.6 ± 2.3	11.8 ± 2.3
Hb (g/dL)	12.0 ± 0.9	12.2 ± 2.0	12.6 ± 0.8	12.8 ± 0.8

Each value represents the mean ± standard deviation of 8 rats.
TEC: total erythrocyte count
TLC: total leucocyte count
Hb: hemoglobin
Group 1: Rats fed basal diet and served as control group
Group 2: Rats fed basal diet mixed with cholesterol powder 1%
Group 3: Rats fed basal diet mixed with cholesterol powder 1% and 1% propolis
Group 4: Rats fed basal diet mixed with cholesterol powder 1% and 2% propolis.

Table 2. Glucose, protein and lipid profiles of rats fed high cholesterol diet and treated with two doses of propolis (1 and 2%).

Parameters	Groups			
	1	2	3	4
Glucose (mg/dL)	120.2 ± 3.0	130 ± 5.0	130.3 ± 5.0	133.7 ± 4.0
Total proteins (g/dL)	4.7 ± 0.5	4.5 ± 0.4	4.5 ± 0.4	4.0 ± 0.4
Albumin (g/dL)	3.4 ± 0.3	3.5 ± 0.5	3.4 ± 0.3	3.6 ± 0.3
Globulins (g/dL)	1.3 ± 0.1	1.0 ± 0.2	1.1 ± 0.1	1.2 ± 0.4
Triglycerides (mg/dL)	40.9 ± 1.6	43.6 ± 0.4*	35.7 ± 2.2**	32.8 ± 2.0**
Total cholesterol (mg/dL)	28.1 ± 0.1	33.4 ± 0.2*	30.5 ± 0.1**	31.5 ± 0.1**
HDL-c (mg/dL)	13.9 ± 1.5	12.9 ± 1.6	11.5 ± 1.0	13.2 ± 1.5
LDL-c (mg/dL)	8.5 ± 1,3	11.2 ± 1.5	12.7 ± 1.2	14.0± 2.0
VLDL-c (mg/dL)	8.2 ± 1.2	8.3 ± 1.1	7.0 ± 1.1	7.0 ± 1.0

Each value represents the mean ± standard deviation of 8 rats.
HDL-c: High density lipoprotein cholesterol
LDL-c: Low density lipoprotein cholesterol
VLDL-c: Very low density lipoprotein cholesterol
*Mean values are significantly (p<0.05) different compare to the control (group I). **Mean values are significantly (p<0.05) different compare to cholesterol treated rats (group II). Group 1: Rats fed basal diet and served as control group. Group 2: Rats fed basal diet mixed with cholesterol powder 1%. Group 3: Rats fed basal diet mixed with cholesterol powder 1% and 1% propolis. Group 4: Rats fed basal diet mixed with cholesterol powder 1% and 2% propolis.

Table 3. Liver function Biomarkers of rats fed high cholesterol diet and with two doses of propolis (1 and 2%).

Parameters	Group			
	1	2	3	4
ALT (IU/L)	23.7 ± 0.4	27.5 ± 0.6*	21.8 ± 1.5*	22.5 ± 1.7 *
AST (IU/L)	71.4 ± 5.1	64.4 ± 4.9	74.5 ± 4.0	77.7 ± 5.0
CK (IU/L)	510.4 ± 10.1	493 ± 11.2	271 ± 10.1	270.8 ± 10.1

Each value represents the mean ± standard deviation of 8 rats.
ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; ACP: Acid phosphatase; CK: Creatine kinase
*Mean values are significantly (p<0.05) different compare to the control (group I). **Mean values are significantly (p<0.05) different compare to cholesterol treated rats (group II).
Group 1: Rats fed basal diet and served as control group
Group 2: Rats fed basal diet mixed with cholesterol powder 1%
Group 3: Rats fed basal diet mixed with cholesterol powder 1% and 1% propolis seed powder
Group 4: Rats fed basal diet mixed with cholesterol powder 1% and 2% propolis seed powder.

Table 4. Kidney markers of rats fed high cholesterol diet and treated with two doses of propolis (1 and 2%).

Parameters	Groups			
	1	2	3	4
BUN (mg/dL)	8.0 ± 1.3	7.0 ± 1.2	8.0 ± 1.2	8.4 ± 1.1
Creatinine (mg/dL)	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Uric acid (mg/dL)	1.0 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1

Each value represents the mean ± standard deviation of 8 rats.
BUN: Blood urea nitrogen
Group 1: Rats fed basal diet and served as control group
Group 2: Rats fed basal diet mixed with cholesterol powder 1%
Group 3: Rats fed basal diet mixed with cholesterol powder 1% and 1% propolis Group 4: Rats fed basal diet mixed with cholesterol powder 1% and 2% propolis.

Table 5. Electrolytes profile of rats fed high cholesterol diet and treated with two doses of propolis (1 and 2%).

Parameters	Groups			
	1	2	3	4
Calcium (mg/dL)	5.1 ± 0.5	5.5 ± 0.4	5.9 ± 0.4	5.7 ± 0.4
Phosphorus (mg/dL)	2.3 ± 0.5	2.8 ± 0.5	2.8 ± 0.5	3.0 ± 0.5
Magnesium (mg/dL)	4.0 ± 0.3	4.4 ± 0.2	3.9 ± 0.2	4.6 ± 0.3
Chloride (mEq/L)	44.4 ± 5.0	53.9 ± 6.0	52.0 ± 4.0	50.6 ± 3.0

Each value represents the mean ± standard deviation of 8 rats.
Group 1: Rats fed basal diet and served as control group
Group 2: Rats fed basal diet mixed with cholesterol powder 1%
Group 3: Rats fed basal diet mixed with cholesterol powder 1% and 1% propolis Group 4: Rats fed basal diet mixed with cholesterol powder 1% and 2% propolis.

and CK remained comparable to that of control groups. Without a doubt, biochemical changes and alterations in enzymes activities induced a stress on liver function. In the present study, there was an increase in ALT activity of rats fed high cholesterol diet compared to the control. The supplementation of propolis in diet caused a reduction in these enzyme activity compared to cholesterol fed rats. Based on this result, the present study can argue that propolis may have hepatoprotective effect. The data tabulated in **Table 4** indicated that, kidney functions were not disturbed in all experimental groups as reflected on the estimated values of BUN, Uric acid, creatinine. Similar report (Denli et al., 2005) recorded the same effect of Turkish propolis in Japanese quail. Electrolytes concentrations (calcium, phosphorus, magnesium and Chloride) were not changed in all experimental groups compared to the control (**Table 5**). In the contrary, previous study (Petruska et al., 2012) reported that, the addition of propolis to the feeding mixture for broiler chickens caused significant (p<0.05) decrease of serum phosphorus and magnesium in all experimental groups in comparison with the control group. Additionally, administration of propolis caused increase of phosphorus and magnesium level in bones of rats (Haro et al., 2000). They claimed this decrease to increases absorption of phosphorus and magnesium from the blood to the bone and thus decreased the level of these elements in the blood. The insignificant difference of chlorides and calcium content come in accordance with previous work (Petruska et al., 2012) in chickens.

Interestingly, both doses of propolis induced the same biochemical changes without any significant differences. This might be indicated that inclusion of propolis 1% on high cholesterol diet is quiet enough to induce the hypolipidemic and hypocholesterolemic effects in rats and no needs for higher doses.

Histopathological examination: Liver of the control rats showed central veins surrounded by polygonal cells arranged in regular cords separated from each other by sinusoids (**Figure 1a**). Liver of the cholesterol treated rat showed varying degrees of vacuolar degeneration and differences in size, shape, staining affinity. The majority of vacuolar degeneration was fatty degeneration which extended to outward appearance of fatty cysts (**Figure 1b**). The resulting irregularity of the liver cell plates is termed lobular disarray. Liver of the rats received high cholesterol diet and treated with propolis powder 1% showed complete

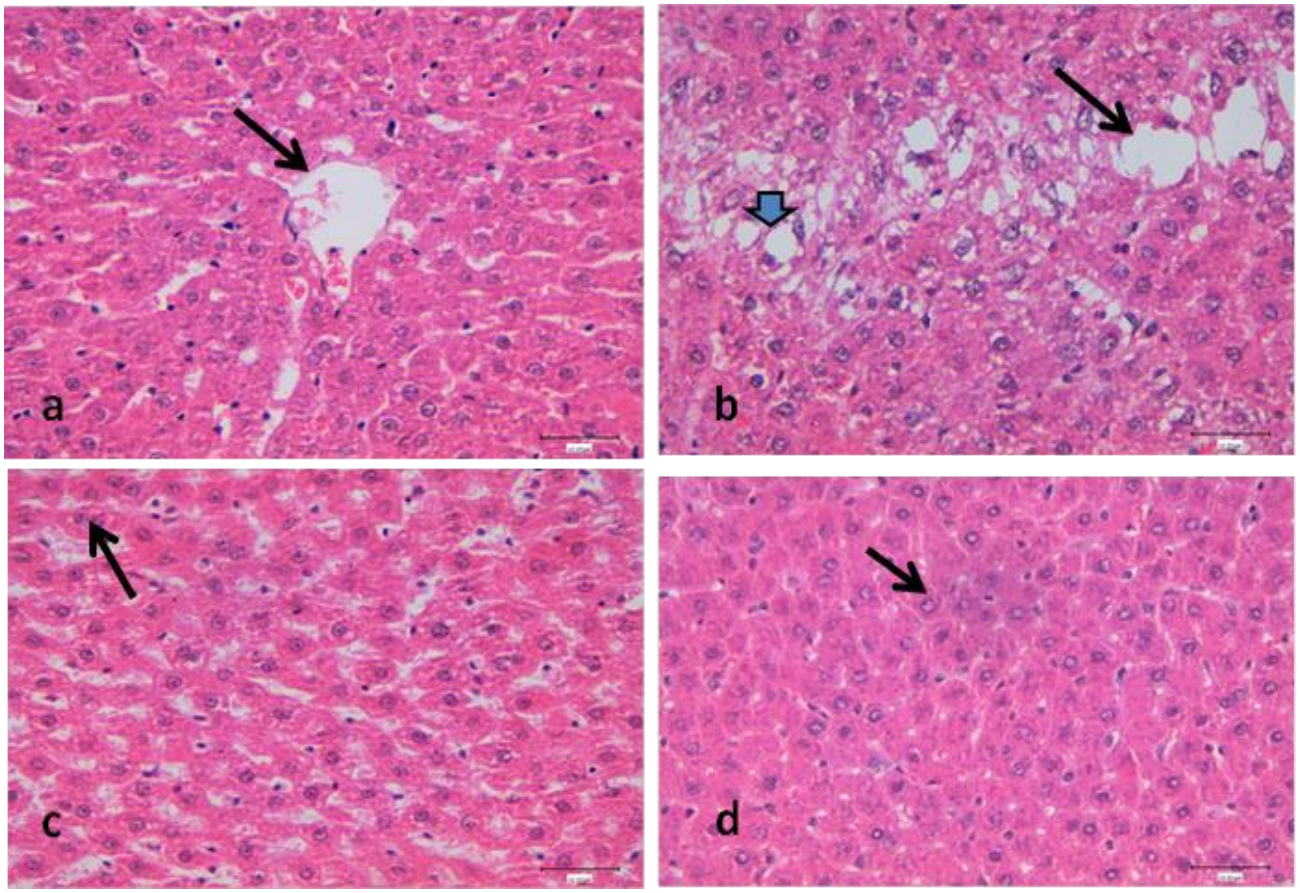

Figure 1. Histopathological image of rats liver fed high cholesterol diet and treated with two doses of propolis (1 and 2%). 1a. Liver of control rats showing normal central vein and regular hepatic cords (arrow). HE bar = 40 µm. 1b. Liver of cholesterol treated rats showing fatty degeneration (arrowhead) and fatty cysts (arrow). HE bar = 40 µm. 1c. Liver of Propolis 1% treated rats showing complete regenerating hepatocytes with binuclear cells (arrow). HE bar = 40 µm. 1d. Liver of Propolis 2% treated rats showing some degree of recovery and regenerating hepatocytes with normal cell plates (arrow). HE bar = 40 µm.

degree of recovery, in which the hepatocytes display signs of regeneration and became binuclear. Concomitantly, the liver cell plates restored its normal architecture (**Figure 1c**). Liver of the rats received high cholesterol diet and treated with propolis powder 2% showed some degree of recovery, in which the hepatocytes display signs of regeneration and the liver cell plates restored its normal architecture.

This histopathological image supported the biochemical findings as discussed above. The current histopathological findings (**Figure 1**) regarding the effect of high cholesterol diet on liver tissues come in accordance with previous work (Korish and Arafah, 2013; Elmahdi et al., 2014; Althnaian, 2014) in human. To the author knowledge, the current study demonstrated the first histopathological report regarding the protective effect of propolis on hepatic fatty changes induced by administration of high cholesterol diet in rats.

CONCLUSION

The present study demonstrated that propolis 1% is safe and quiet enough to induce beneficial hypolipidemic and hypocholesterolemic effects in serum of rats fed high cholesterol diet. The hypolipidemic and hypocholesterolemic effects of propolis could be investigated in birds and animals. In addition, future studies are recommended to investigate the mechanisms of action of propolis as hypolipidemic and hypocholesterolemic agent at molecular levels.

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