

Histopathological Examination of the Effects of Oral Consumption of Various Doses of Propolis in Mice Liver

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Abstract Introduction: Considering the widespread application of biological substances, such as Propolis, it is essential to identify the importance of its liver metabolism, its effects of the liver and safe doses. The aim of this study was to determine the histopathological effects of oral administration of Propolis on the liver in mice. **Methods and materials:** This study consisted of five experimental groups of male mice (25-30 g), including control, placebo and 3 treatment groups. Male mice (25-30 g). Each of the groups receiving Propolis extract at one of the 2000, 4000 and 8000 mg/kg concentrations. Mice were gavage fed with the alcoholic extract of Iranian Propolis once daily for 14 days. After induction of a deep anesthesia, the liver was removed and examined for histopathological changes. All the data were analyzed using SPSS software ($P < 0.05$). **Results:** The results of this study indicated that chronic administration of various doses of routine Propolis induced different histopathological changes in a dose-dependent manner. These changes initiated around the central venous; furthermore, their severity was higher in this part of the liver compare to the other areas. **Conclusion:** Based on the results of this study, it is essential to carefully monitor the dosage and consumption duration of Propolis. Moreover, periodical examination of liver function is essential during chronic consumption of Propolis.

Keywords: Propolis, mice, liver, histopathology

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1. Introduction

The liver plays a vital role in the excretion of the metabolites of most drugs, hormones, toxins and foreign compounds; therefore, it is considered to be the most important target organ in toxicity. Hepatotoxic agents can cause liver damage by affecting major subcellular structures and intracellular organelles [1].

The released intracellular materials from the necrotic cells, resulted from liver damage, are able to activate immune cells, therefore mainly triggers the development of inflammation, necrosis and further damages as the result of free radicals production [2].

Acute and chronic liver tissue injuries, based on their severity, cause reversible or irreversible changes or hepatocytes death. In histopathological examination of the liver, these lesions can be investigated as follows: central venous dilatation, steatosis, cholestasis, increase in the number of Kupffer cells and bile ducts, congestion of liver sinusoids, feathery degeneration of liver parenchymal cells, periportal interface hepatitis, confluent necrosis, apoptosis and focal necroinflammation, portal and sinusoidal inflammation [3].

Propolis is produced by honey bees by mixing the enzymes in the saliva with various parts of the plant, such as sap of the flower, stem, bud, leaf, and even the tree bark and exudate gums [4]. Propolis contains about 50% gum or plant resin, 30% wax, 10% essential fatty acids, 5% pollen and 5% other organic compounds, vitamins and minerals, such as Ag, Na, Hg, Cu, Mn, Fe, Ca, vanadium (V), silica (Si), and vitamin B-complex, C and E groups. The amount and type of the chemical composition of Propolis vary depending on the harvest time, location and production method [4].

Various studies have demonstrated that Propolis has a variety antibacterial, anti-fungal, anti-parasitic, antioxidant, anti-inflammatory and anti-tumour effects and boosts the body immune system [5,6].

Propolis has a wide range of applications, including being used to treat anemia, respiratory and digestive tract infections, eczema, infectious diseases of mucous membranes and skin lesions, wound healing (specially burn wounds), prevent tooth decay as well as treatment of certain cancers. Furthermore, it boosts and improves the immune system [5,7,8,9].

Several studies have reported that Propolis plays a protective role in liver. It prevents the development of

fatty liver and its related complications, such as cirrhosis, by reducing the total serum cholesterol, triglyceride, LDL and HDL levels [10,11].

Also Propolis decreases the levels of proinflammatory cytokines, such as IL6 and α TNF. Therefore, it reduces inflammation-induced liver damages by its anti-inflammatory effects. [12]

Furthermore, Propolis reduces CRP serum level which is considered as a proinflammatory indicator [13].

Propolis eliminates free radicals from the body and protects the liver against their damages by its antioxidant effect [14].

Oral and topical administration of Propolis causes stomatitis and allergic dermatitis [15].

Other side effects of Propolis include acute renal failure, cytotoxicity and genotoxicity [16,17,18].

Considering the widespread desire to use biological compounds as dietary supplements or complementary remedy in traditional medicine, understanding their metabolism in the liver seems to be essential. Few studies have been conducted to investigate the effects of common dosages of Propolis on liver or other internal organs, on the other hand, most of these studies have examined the protective effect of Propolis on the liver in the presence of liver damaging substances. The hepatotoxicity of common dosages of Propolis, when administered alone, is yet to be fully clarified.

Propolis is a complex natural compound which consists of a wide variety of chemicals and plays various structural and biological roles. Therefore, its harmlessness must assuredly be investigated and confirmed. Moreover, due to the lack of quality control of Propolis, its application may be harmful to humans [19].

Also, the metabolism of Propolis and products are challenging. The complex of cytochrome P-450 isoenzymes of hepatocytes involve in the metabolism functional food including Propolis. They are accountable for detoxification of these compounds. On the other hand however, super family of cytochrome P-450 isoenzymes cause hepatotoxicity through the production of active intermediate metabolites. Also results of Chang et al. study shown Propolis was able to inhibit some of the cytochrome P450 isoenzymes [20].

In order to benefit from the advantageous effects of Propolis, a comprehensive examination of its beneficial and toxic effects is necessary. Since the histopathological effects of Propolis in liver damage have not been fully investigated in the previous studies, the aim of this study was to evaluate the histopathological effects of three different doses of Propolis on the liver of male BALB/c mice.

2. Materials and Methods

2.1. Animals

This experimental study was carried out during April 2017 at the Qazvin University of Medical Sciences (QUMS) using 50 male mice, (Razi Company-Karaj-Iran) weighing 30-25 g. All the animals were kept in clean standard cages with a 12/12 h light-dark cycle, controlled temperature (22 ± 2 °C) and free access to food and water. All experiments were carried out in accordance

with the approved guidelines and the ethical code of IR.QUMS.REC.1396.158 from the Ethics Committee of QUMS.

2.2. Extraction Method

The applied Propolis was harvested from beehive located in the mountainous area of northern Qazvin. The alcoholic extract was prepared according to the method presented by Moreno et al. The Propolis was first shredded into small pieces and 25 g of the shredded Propolis was mixed with 100 ml ethanol 80% (Merck, Germany). This mixture was then horizontally shaken (150 rpm) in dark for 48 hrs. at room temperature, then passed through filter paper grade 4 twice and the alcohol was removed using a rotary evaporator. Finally, obtained pure alcoholic extract was weighted and stored in a dark glass container at refrigerator. Prior to oral administration, appropriate amounts of dried extract of Propolis was dissolved in propylene glycol considering the intended dosage [21].

2.3. Experimental Design

First, the median lethal dose (LD50) of Propolis alcoholic extract was estimated [22,23].

The mice were randomly divided into five groups of ten; 1. Control group, 2. Placebo group: Propolis solvent, 3. three experimental groups, each of which received one of the following concentrations of the extract: 2000, 4000 and 8000 mg/kg body weight. The mice were administered with 1 ml of the extract or solvent via nasogastric intubation, once daily for a period of 14 days [24].

2.4. Histopathological Studies

On the last day of treatment, the mice were anesthetized using Ketamine (60 mg/kg) and xylazine (6 mg/kg), followed by laparotomy in order to remove the liver which was then fixed using formalin 10%. The paraffin-embedded tissue blocks were then serially sectioned at a thickness of 3 μ m using a Leica microtome (Leica/Germany). Hematoxylin and Eosin staining was used stain the tissue in order to investigate the structure of the liver and morphology of the hepatocytes. The histopathological evaluation criteria included: 1. central venous dilatation, 2. accumulation of triglyceride within hepatocytes (steatosis), 3. bilirubin deposition in the liver cells (cholestasis), 4. increase in the number of Kupffer cells, 5. increase in the number of bile ducts, 6. hyperaemia of liver sinusoids, 7. feathery degeneration, 8. lymphocytic infiltration in the adjacent hepatic parenchyma accompanied with the destruction of hepatocytes along edges of periportal area (periportal interface hepatitis), 9. death of large groups of hepatocytes (Confluent necrosis) including focal, zone 3 necrosis, zone 3 necrosis with portal central bridging, 10. apoptosis and focal necroinflammation, 11. portal inflammation and 12. sinusoid inflammation. The respective slide to each of the liver sample was carefully examined and the predominant histopathological patterns were reported and taken into consideration base on their severity. The grading of the changes was mild, moderate and severe [3]. Reticulin staining was used for confirmation

of hepatocytes necrosis especially confluent form, based on reticulin collapse areas. The exclusion criteria included the death or morbidity of the mice.

2.5. Statistical Analyses

All the obtained data were analyzed using SPSS V.20. software. One-way ANOVA, Tukey test and Fisher's Exact test were applied for statistical analyses. (P values <0.05)

3. Results

In the control group, no pathologic changes were observed. (Table 1 and Figure 1). However, mild hyperaemia of central venous, mild dilation and hyperaemia of liver sinusoids, mild increase in the number of Kupffer cells and mild portal inflammation were detected in the liver of the mice in the placebo group (Table 1 and Figure 2).

The experimental group that received the extract at a concentration of 2000 mg/kg represented moderate central venous dilatation, moderate hyperaemia of liver sinusoids, mild steatosis, mild increase in the number of Kupffer cells and bile ducts, mild confluent necrosis, mild to moderate apoptosis and focal necroinflammation, and a mild portal inflammation (infiltration lymphocytes, plasma cells and eosinophils) (especially Zone 3). Furthermore, mild to moderate cholestasis, feathery degeneration and apoptosis and focal necroinflammation were observed in the liver samples of this group after 14 days of treatment (Table 1 and Figure 3).

The experimental group that was treated with 4000 mg/kg of the extract showed moderate to severe central venous dilatation and hyperaemia of liver sinusoids, as well as mild steatosis, periportal interface hepatitis, confluent necrosis, portal and sinusoidal inflammation, (especially Zone 3). Hence, moderate feathery degeneration and apoptosis and focal necroinflammation were observed in this group (Table 1 and Figure 4).

The pathologic changes that were detected in the liver of the mice that received 8000 mg/kg of the extract included moderate central venous dilatation and hyperaemia of liver sinusoids and steatosis, as well as mild increase in the number of Kupffer cells and bile ducts, confluent necrosis, apoptosis and focal necroinflammation

and portal, sinusoidal inflammation, (especially Zone 3). Moreover, moderate to severe feathery degeneration and apoptosis and focal necroinflammation were detected samples obtained from this group (Table 1 and Figure 5).

According to the results of the Fisher's Exact test, the differences in the pathologic changes of the control and the experimental groups as well as the experimental groups treated with different doses of the extract were significant ($P= 0.00$).

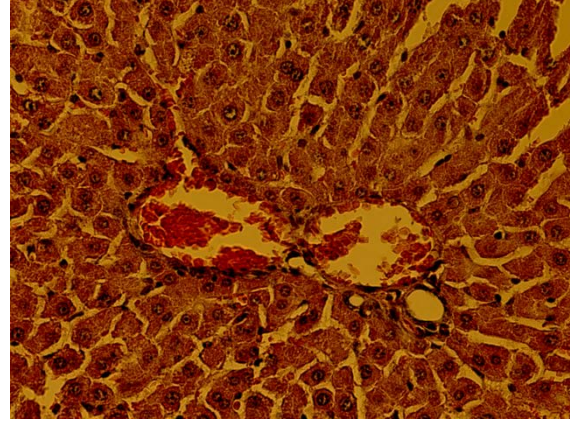


Figure 1. The histopathologic findings of control group. Liver lobule including hepatocytes and associated with portal tract. Magnification x400, Hematoxylin and Eosin

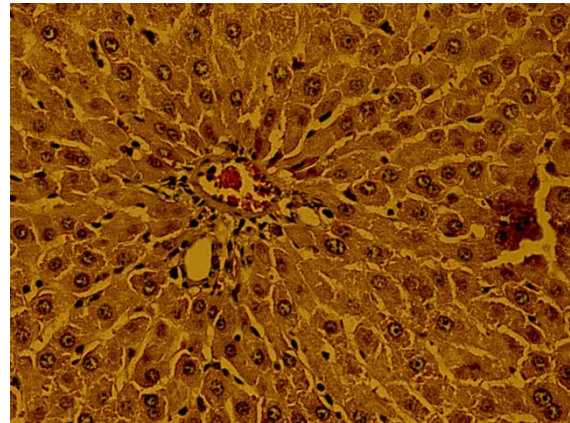


Figure 2. The different histopathologic changes of mice liver, sham group. Hyperemia(M), increase of Kupffer cells(M).(Mild:M.). Magnification x400, Hematoxylin and Eosin

Table 1. Liver histopathologic findings of oral administration of chronic doses of propolis in examined groups

Groups	Control	Sham	Propolis 2000 mg/ kg	Propolis 4000 mg/ kg	Propolis 8000 mg/ kg	P value
Histopathologic findings						
Central vein dilation	Negative	Mild	Moderate	Moderate to severe	Moderate	0.00
Hyperemia	Negative	Mild	Moderate	Moderate to severe	Moderate	0.00
Steatosis	Negative	Negative	Mild	Mild	Moderate	0.00
Cholestasis	Negative	Negative	Mild to moderate	Mild to moderate	Moderate	0.00
Increase of Kupffer	Negative	Mild	Mild	Mild to moderate	Mild	0.00
Ductular reaction	Negative	Negative	Mild	Mild to moderate	Mild	0.00
Feathery change	Negative	Negative	Mild to moderate	moderate	Moderate to severe	0.00
Periportal interface hepatitis	Negative	Negative	Mild	Mild	Mild	0.00
Confluent necrosis	Negative	Negative	Mild	Mild	Mild	0.00
Apoptosis and focal necroinflammation	Negative	Negative	Mild to moderate	Moderate	Moderate to severe	0.00
Portal inflammation	Negative	Mild	Mild	Mild	Mild	0.00
Sinusoidal inflammatory cells infiltrating (L,P,E)	Negative	Negative	Mild	Mild	Mild	0.00

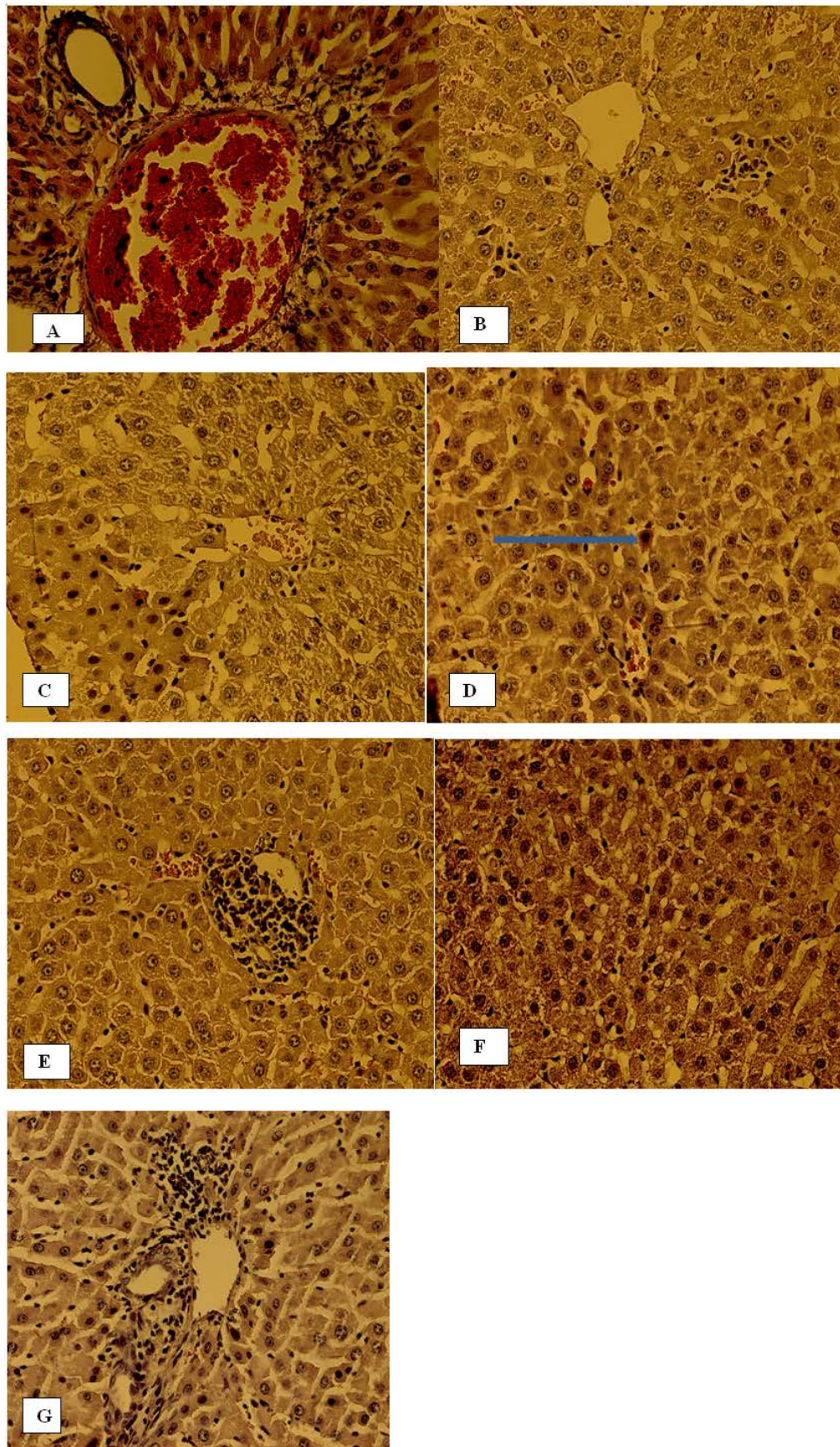


Figure 3. The different histopathologic changes of mice liver received propolis extract at concentrations of 2000 mg/ kg. **A:** Hyperemia(Mo), ductular reaction (M). **B:** Central vein dilation(Mo), hyperemia(Mo), increase of Kupffer cells(M), sinusoidal inflammatory cells infiltrating (M), cholestasis (M to Mo), focal necroinflammation(M to Mo). **C:** Central vein dilation(Mo), hyperemia, cholestasis(M to Mo), feathery change (M to Mo). **D:** Apoptosis (M to Mo)(arrow), hyperemia(Mo), cholestasis(M to Mo), feathery change (M to Mo). **E:** Hyperemia (Mo), cholestasis(M to Mo), feathery change (M to Mo), increase of Kupffer cells(M), sinusoidal inflammatory cells infiltrating (M), portal inflammation(M). **F:** Cholestasis(M to Mo), feathery change(M to Mo), steatosis (M). **G:** Portal inflammation (M), Periportal interface hepatitis (M), increase of Kupffer cells(M), sinusoidal inflammatory cells infiltrating (M). (Mild:M, Moderate:Mo,Mild to moderate: M to Mo). Magnification x400, Hematoxylin and Eosin

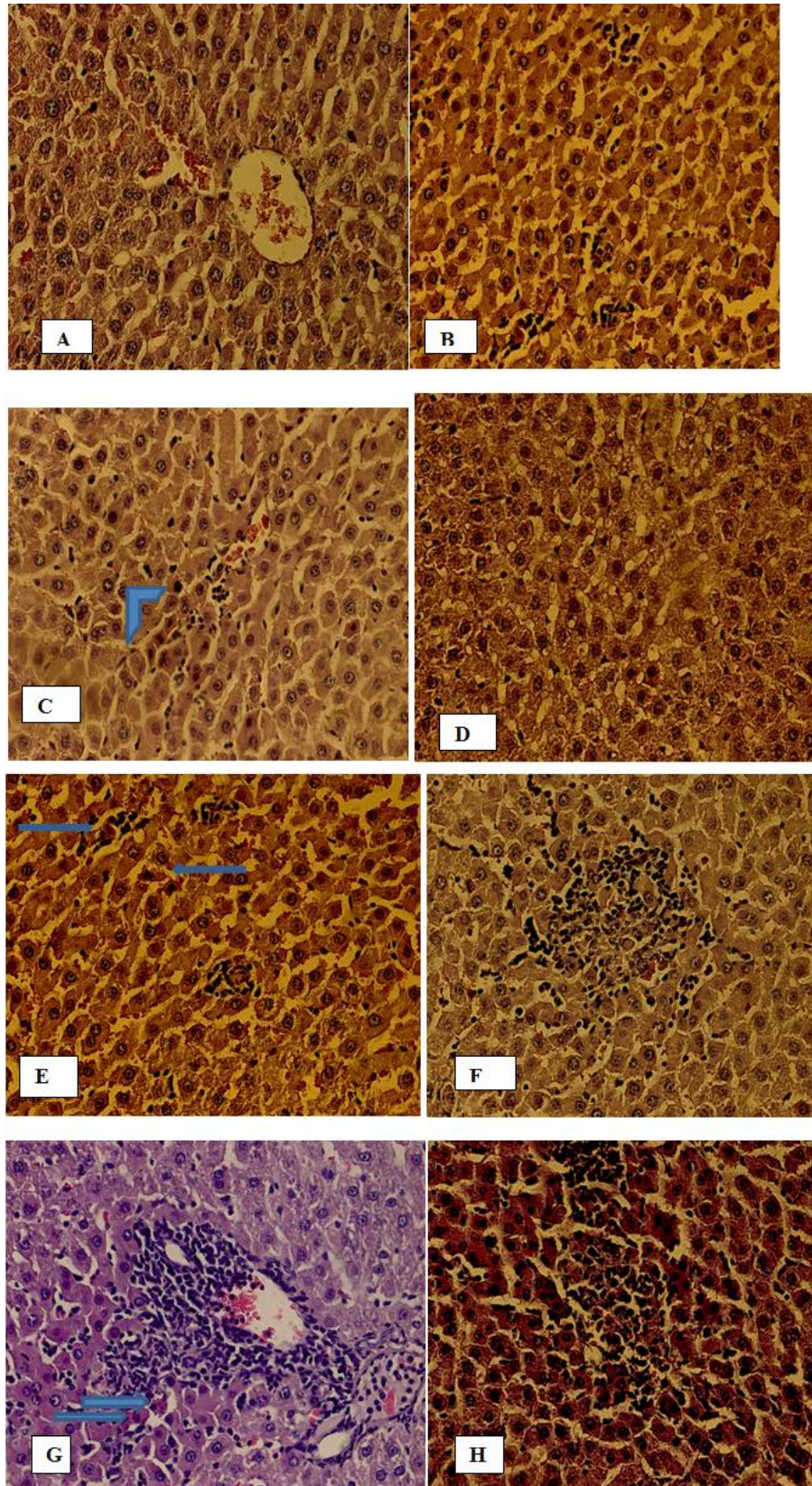


Figure 4A-H. The different histopathologic changes of mice liver received propolis extract at concentrations of 4000 mg/ kg. **A:** Hyperemia(Mo), central vein dilation(Mo), cholestasis(M to Mo), feathery change(Mo). **B:** Hyperemia(Mo to Se), increase of Kupffer cells(M to Mo), sinusoidal inflammatory cells infiltrating(M), cholestasis(M to Mo), multiple focal necroinflammation(Mo). **C:** Hyperemia(Mo), cholestasis(M to Mo), feathery change(Mo), multiple apoptosis(Mo) (arrow), increase of Kupffer cells(M to Mo), sinusoidal inflammatory cells infiltrating(M). **D:** Hyperemia(Mo), cholestasis(M to Mo), feathery change(Mo), increase of Kupffer cells(M to Mo), steatosis(M). **E:** Hyperemia(Mo), cholestasis(M to Mo), feathery change(Mo), multiple focal necroinflammation(Mo) and apoptosis(arrows). **F:** Hyperemia(Mo), cholestasis(M to Mo), feathery change(Mo), increase of Kupffer cells(M to Mo), sinusoidal inflammatory cells infiltrating(M), focal necroinflammation(Mo). **G:** Hyperemia(Mo), cholestasis(M to Mo), feathery change(Mo), increase of Kupffer cells(M to Mo), sinusoidal inflammatory cells infiltrating(M), periportal interface hepatitis(M), multiple apoptosis(Mo)(arrows). **H:** Hyperemia(Mo), cholestasis(M to Mo), feathery change(Mo), increase of Kupffer cells(M to Mo), sinusoidal inflammatory cells infiltrating(M), focal necroinflammation(Mo).

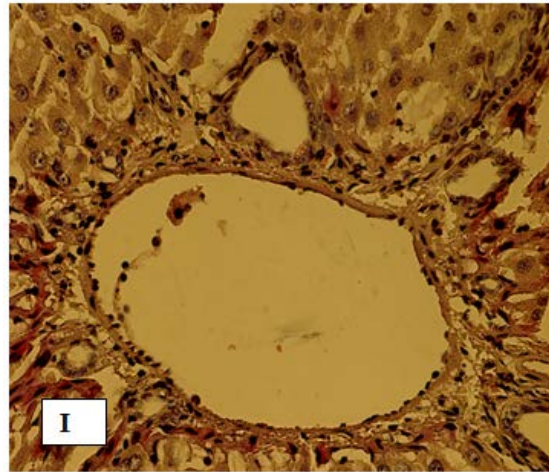


Figure 4I. The different histopathologic changes of mice liver received propolis extract at concentrations of 4000 mg/ kg. **I:** Ductular reaction (M to Mo). (Mild:M, Moderate: Mo, Mild to moderate: M to Mo, Moderate to Severe: Mo to Se). Magnification x400, Hematoxylin and Eosin

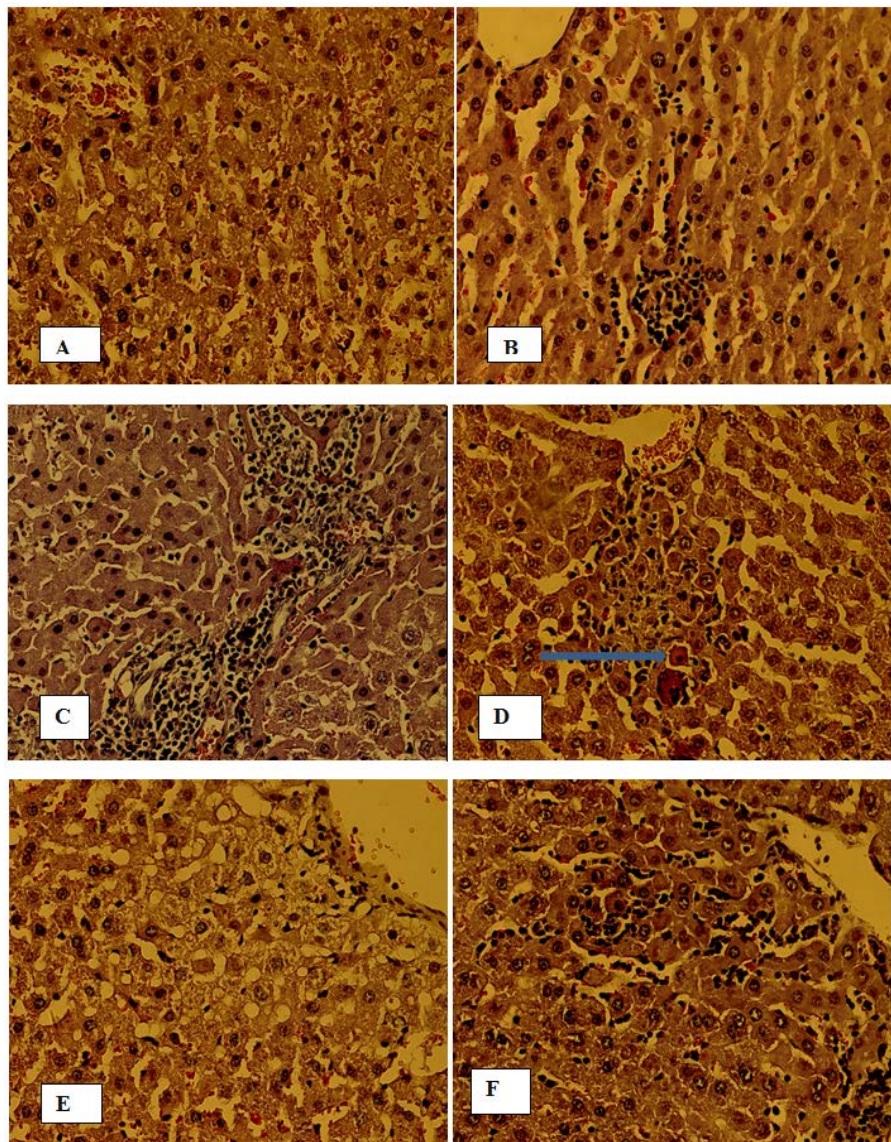


Figure 5 A-F. The different histopathologic changes of mice liver received propolis extract at concentrations of 8000 mg/ kg. **A:** Hyperemia(Mo), cholestasis(Mo), feathery change(Mo). **B:** Hyperemia(Mo), cholestasis(Mo), feathery change(Mo), increase of Kupffer cells(M), sinusoidal inflammatory cells infiltrating(M), focal necroinflammation(Mo). **C:** Cholestasis(Mo), feathery change(Mo), periportal interface hepatitis(M), confluent necrosis(M), Portal inflammation(M). **D:** Hyperemia(Mo), cholestasis(Mo), feathery change(Mo), increase of Kupffer cells(M), apoptosis (Mo) (arrow), focal necroinflammation(Mo). **E:** Hyperemia(Mo), cholestasis(Mo), feathery change(Mo), steatosis(Mo). **F:** Hyperemia(Mo), cholestasis(Mo), feathery change(Mo),), increase of Kupffer cells(M), sinusoidal inflammatory cells infiltrating(M), focal necroinflammation(Mo)

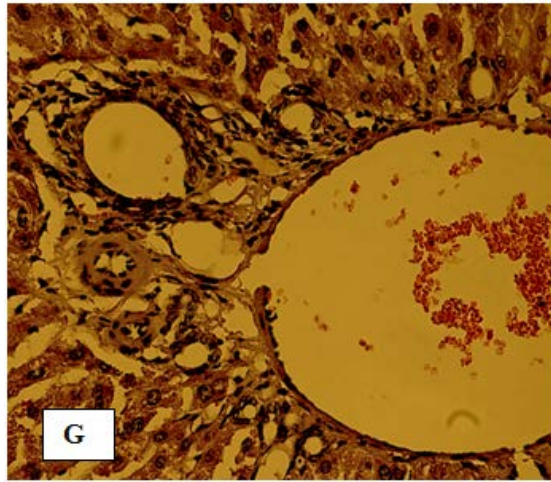


Figure 5 G. The different histopathologic changes of mice liver received propolis extract at concentrations of 8000 mg/ kg. **G:** Hyperemia(Mo), cholestasis(Mo), feathery change(Mo), ductular reaction(M). (Mild:M, Moderate:Mo). Magnification x400, Hematoxylin and Eosin

4. Discussion

This study consisted of five groups, including control, placebo, and three experimental groups which were treated with one of the 2000, 4000 and 8000 mg/kg concentrations of the Propolis extract. The histopathological changes of the hepatocytes, vasculatures, portal tracts and bile ducts were then investigated [5]. The most common observed pathologic changes included moderate central venous dilatation, hyperaemia of liver sinusoids, steatosis and cholestasis, as well as mild increase in the number of Kupffer cells and bile ducts, periportal interface hepatitis, confluent necrosis, portal and sinusoidal inflammation, (especially Zone 3). Moreover, moderate to severe feathery degeneration, apoptosis and focal necroinflammation were observed in the experimental group treated with 8000 mg/kg of the extract.

Propolis has been studied as a biological compound that is consumed as a supplement and a complementary remedy in traditional medicine. Therefore, the determining its safe and toxic dosages has been the subject of discussion of various studies [25].

Hepatoprotective properties of Propolis have been widely studied. Combined oral administration of Propolis in mercuric chloride poisoning inhibits lipid peroxidation and formation of oxidized glutathione. Also, it increases glutathione levels and the activity of the antioxidant enzymes of the liver shifts to their normal levels. Furthermore, the release of liver enzymes in the blood stream becomes normal [26].

Moreover, oral administration of Propolis (200 mg/kg) in carbon tetrachloride poisoning regulates the protective activity of the antioxidant compounds [27].

However, similar to other biological substances and dietary supplements, application of high doses of this compound can be toxic. The results of the present study showed that chronic administration of Propolis at 2000 mg/kg and higher doses could induce various histopathological damages to the liver, and the severity of these complications was almost dose-dependent. Hence, the findings of this study indicated that the histopathologic changes initiated in the central vein and the severity of damages in this area was higher than other parts. These results were consistent with findings of Ramadan et al.

who have demonstrated that oral administration of Propolis alcoholic extract at doses lower than 5000 mg/kg did not incuse acute poisoning. They have also reported that the oral LD50 of this compound is higher than 5000 mg/kg. Furthermore, Arvouet-Grand has reported that the oral LD50 of Propolis alcoholic extract is higher than 7.34 g/kg. Therefore, administration of this compound at doses lower than 5000 mg/kg should be safe [23,28].

Mohammadzadeh et al. have demonstrated that oral administration of hydroalcoholic solution of Propolis extract at doses of 4.5, 9, 13 and 20 g/kg does not induce toxic effects. These findings are not consistent with the results of the present study, Ramadan et al. and Arvouet-Grand [23,28,29].

Albeit, Mohammadzadeh at al. have studied rat and the present study examined mice and it should be taken into consideration that liver metabolic pathways vary in different species. Various studies have demonstrated the anatomical, physiological and liver metabolic pathways similarities between mice and human. Therefore, mice can be a more suitable model to predict, evaluate and examine the importance of hepatotoxic effect of chronic consumption of Propolis compared to rat [30,31].

The reason some of the findings of this study are inconsistent with those reported by other researchers might be the geographic collection site of Propolis since it alters the chemical composition of Propolis due to the following factors: phytogeographical characteristics and vegetation of the collection site, diversity of climatic conditions, ambient temperature, altitude, presence of air and soil pollutants. Also preparation methods, differences in dosage and storage duration of Propolis, as well as compliance with the available protocols (Quality Assurance) for the preparation of the final product, which is the Propolis extract.

Ramadan et al. did not find any histopathological changes at doses lower than 5000 mg/kg. In other words, the effect of Feed Conversion Ratio (FCR) in doses lower than 5000 mg/kg was very small [23].

The results of this study were not consistent with those reported in most of studies conducted to investigate the hepatoprotective effects of Propolis [11,12,13,14,32].

The reason for these contradictions seems to be the difference in the dosage and consumption duration of

Propolis. In addition, in most of studies, Propolis has been used in combination with a harmful substance and it reduced the toxic effects of that substance on liver. However, our study investigated the histopathological changes induced by Propolis, alone, when orally administered on a daily bases for two weeks.

High doses of Propolis or its metabolites as well as its chronic consumption can cause liver damage. Studying the toxic effects of various substances on the liver have indicated that the intermediated metabolites of many edibles can lead to reversible and irreversible changes in the liver and promote hepatocytes apoptosis via activation of cytochrome P450 enzymes or caspase cascade pathway. Therefore, depending on the toxicity level of the consumed compound, hepatocytes death appears as apoptosis or focal necroinflammation. Caspases are a family of cysteine proteases that play an important role in apoptosis as well as its associated biochemical and morphological changes. On the other hand, cytochrome C, which is always present in the mitochondrial intramembrane space, is released into cytosol and, following induction by intermediate metabolites, triggers apoptosis by many chemotherapeutic stimuli and DNA damaging stimuli [13,33,34,35].

As illustrated in Figure 1 and Table 1, severity of Propolis-induced damages can be evaluated in a dose-dependent manner as limited (apoptosis and focal necroinflammation) or wider (confluent necrosis).

Removal of apoptotic cells occurs via immunological processes, that beside inducing activation of the liver reticuloendothelial cells, (the Kupffer cells) induce their proliferation, via various mediators and cytokines, and portal, sinusoidal inflammation. The rate of inflammation is dependent on the severity of hepatotoxicity [36].

As the dosage of Propolis increased (Figure 3, Figure 4, Figure 5 and Table 1), inflammatory responses to cellular damages, including central venous dilatation, hyperaemia of liver sinusoids, increase in the number of Kupffer cells and portal, sinusoidal inflammation (especially Zone 3), were significantly enhanced ($P=0.00$).

Cholestasis is one of the major causes of liver damage which is induced by biliary compounds. Cholestasis can be directly induced by Propolis or by its metabolites at high doses or by autoimmune responses to its combinations. These changes initially appears as feathery changes (Figure 3, Figure 4, Figure 5 and Table 1) [37].

Steatosis and lipid accumulation within hepatocytes are known as one of the liver damages and reversible changes associated with medicine consumption. Propolis, at high doses, might be responsible for the modification of fatty acid metabolism. This leads to the accumulation of triacylglycerol within the hepatocytes. The amount of fatty acid in the liver depends on the balance between the lipid catabolism and anabolism processes. Therefore, in some cases, steatosis is accompanied with liver inflammation and hepatocytes death (Figure 3, Figure 4, Figure 5 and Table 1) [35,36,37].

The metabolism of many exogenous materials occurs in hepatocytes, in two phases. The phase I liver metabolism requires a catalytic element. At this stage, dehydrogenases, monooxygenases, and esterases play critical roles; however, the most common catalyst is the family of cytochrome P450 enzymes that are found in humans and rodents [38].

The cytochrome P450 enzymes selectively activate oxygen and therefore catalyze a series of reactions, including hydroxidation, oxidation, dealkylation and redox. These reactions involve the addition of a functional group, such as -OH, to a compound. Therefore, a conjugation occurs during phase II metabolism. These compounds have a negative effect on the activity of the cytochrome P450 enzymes and eliminate them in severe conditions [39].

About 60% of the liver is constituted of hepatocytes and 40% is composed of endothelial, bile ducts, Kupffer and Ito cells. The closest area to the portal triad is called Periportal zone I. Hepatocytes are basically subdivided in this area. As they age, they approach the central venous toward an area known as the Centrilobular zone III [38]. Hepatocytes in this area are less protected against reactive oxygen species and are fed with blood that contains less oxygen and nutrients. Therefore, the rate of biotransformation is higher in Zone III and hepatocytes of this site are more susceptible to damage [40]. In this study, the rate of Propolis-induced hepatocytes damage in this area was higher and in a dose-dependent manner (Figure 3, Figure 4, Figure 5 and Table 1).

The prediction of the toxic effect of Propolis on the liver is difficult. Therefore, further studies, consisting of larger number of samples and various doses of this compound, are required to understand the mechanism of pathological effects of Propolis. Application of biochemical markers, simultaneous with histopathological examination, may determine the precise manner and quality of the beneficial or harmful effects of Propolis on the liver.

Application of Propolis is expanding due to its beneficial effects. Although most of the related studies have reported the protective effects of Propolis on the liver, the results of the present study indicated that Propolis might induce hepatotoxic effects. Therefore, further studies are required to investigate various doses and consumption duration of Propolis besides periodical examination of liver function.

Declarations of Interest

The authors declare no financial relationship with any organization regarding this research.

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References

- [1] Baruah MJ, Baruah PK, Borthakur MK. A Study of Histopathological Examination of Alcohol Induced Hepato-Toxicity in Albino Rats and Protective Effect of Cuminum cyminum. *International Journal of Science and Research (IJSR)*. 2016; 5(10): 693-699.
- [2] Weiskirchen R, Weiskirchen S, Tacke F. Recent advances in understanding liver fibrosis: bridging basic science and

- individualized treatment concepts. *F1000Research* 2018, 7(F1000 Faculty Rev): 921 Last updated: 15 AUG 2018.
- [3] Ishak KBaptista A, Callea F, De Groote J, Gudat F, and et al. Histopathological grading and staging of chronic hepatitis. *J Hepatol.* 1995; 22: 696-699.
- [4] Bankova V, Popova M, Trusheva B. Propolis volatile compounds: chemical diversity and biological activity: a review. *Chemistry Central Journal.* 2014; 8(1): 28.
- [5] Campos JF, dos Santos UP, Macorini LF, de Melo AM, Balestieri JB, Paredes-Gamero EJ, Cardoso CA, de Picoli Souza K, dos Santos EL. Antimicrobial, antioxidant and cytotoxic activities of propolis from *Melipona orbignyi* (Hymenoptera, Apidae). *Food and Chemical Toxicology.* 2014 Mar 1; 65: 374-80.
- [6] Cavendish RL, de Souza Santos J, Neto RB, Paixão AO, Oliveira JV, de Araujo ED, e Silva AA, Thomazzi SM, Cardoso JC, Gomes MZ. Antinociceptive and anti-inflammatory effects of Brazilian red propolis extract and formononetin in rodents. *Journal of ethnopharmacology.* 2015 Sep 15; 173: 127-33.
- [7] Ali BM, Ghoname NF, Hodeib AA, Elbadawy MA. Significance of topical propolis in the treatment of facial acne vulgaris. *Egyptian Journal of Dermatology and Venerology.* 2015 Jan 1; 35(1): 29.
- [8] Olczyk P, Komosinska-Vashev K, Wisowski G, Mencner L, Stojko J, Kozma EM. Propolis modulates fibronectin expression in the matrix of thermal injury. *BioMed research international.* 2014; 2014. Article ID 748101
- [9] Ivana K, Ivan M, Vatroslav S, Albina D, Jasna J, Maja M. The Effects of Propolis and Bee Pollen Supplementation on Biochemical Blood Parameters of Broilers. *Acta Veterinaria-Beograd* 2018, 68 (2), 190-200.
- [10] Li H, Liu Y, Zhang X, Xu Q, Zhang Y, Xue C and Guo C. Medium-chain fatty acids decrease serum cholesterol via reduction of intestinal bile acid reabsorption in C57BL/6J mice. *Nutrition & Metabolism* (2018) 15: 37.
- [11] Mujica V, Orrego R, Pérez J, Romero P, Paz Ovalle P, Zúñiga-Hernández J, et al. The Role of Propolis in Oxidative Stress and Lipid Metabolism: A Randomized Controlled Trial. *Evid Based Complement Alternat Med.* 2017; 2017: 4272940.
- [12] Yuan X, Wang Y, Shi B, Zhao Y. Effect of propolis on preserving human periodontal ligament cells and regulating pro-inflammatory cytokines. *Dental traumatology.* 2018, Volume 34, issue4: 245-253.
- [13] Rao Pasupuleti V, Sammugam L, Ramesh N, and Hua Gan S. Honey, Propolis, and Royal Jelly: A Comprehensive Review of Their Biological Actions and Health Benefits. *Oxid Med Cell Longev.* 2017; 2017: 1259510.
- [14] Wali AF, Avula B, Ali Z, Khan IA, Mushtaq A, Rehman MU, Akbar S, Masoodi MH. Antioxidant, hepatoprotective potential and chemical profiling of propolis ethanolic extract from Kashmir Himalaya region using UHPLC-DAD-QToF-MS. *BioMed research international.* 2015; 2015: 393462.
- [15] Martínez Pallás I, Conejero Del Mazo C, Prieto Torres L. Allergic contact stomatitis caused by propolis candies. *Contact dermatitis.* 2018, 78(6): 418-419.
- [16] Nauffal M, Gabardi S. Nephrotoxicity of Natural Products. *Blood Purif* 2016; 41: 123-129.
- [17] Regueira-Neto MDS, Tintino SR, Rolón M, Coronal C, Vega MC, de Queiroz Balbino V, de Melo Coutinho HD. Antitrypanosomal, antileishmanial and cytotoxic activities of Brazilian red propolis and plant resin of *Dalbergia ecastaphyllum* (L) Taub. *Food Chem Toxicol.* 2018 Sep; 119: 215-221.
- [18] Cruz M, Antunes P, Paulo L, Ferreira AM, Cunha A, Almeida-Aguiar C and Oliveira R. Antioxidant and dual dose-dependent antigenotoxic and genotoxic properties of an ethanol extract of propolis. *RSC Adv.* 2016, 6, 49806-49816.
- [19] Sforcin JM. Biological Properties and Therapeutic Applications of Propolis. *Phytother. Res.* 30: 894-905 (2016).
- [20] Ryu CS, Oh SJ, Oh JM, Lee J-Y, Lee SY, Chae J-w, et al. Inhibition of cytochrome P450 by propolis in human liver microsomes. 2016; 32(3): 207.
- [21] Moreno MI, Isla MI, Sampietro AR, Vattuone MA. Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. *Journal of ethnopharmacology.* 2000 Jul 1; 71(1-2): 109-14.
- [22] Lorke D. A new approach to practical acute toxicity testing. *Archives of toxicology.* 1983 Dec 1; 54(4): 275-87.
- [23] Ramadan A, Soliman G, Mahmoud SS, Nofal SM, Abdel-Rahman RF. Evaluation of the safety and antioxidant activities of *Crocus sativus* and Propolis ethanolic extracts. *Journal of Saudi Chemical Society.* 2012 Jan 1; 16(1): 13-21.
- [24] Gheibi N, Shahbazi J, Zarmohammadi Z, Alipoor Heydari M, Kakaie E, Sofiabadi M. Effect of Oral Administration of Ethanolic Extracts of Propolis on Passive Avoidance Learning and Memory in Adult Male Mice. *Journal of Ardabil University of Medical Sciences.* 2017; 17(1): 104-112.
- [25] Wagh VD. Propolis: a wonder bees product and its pharmacological potentials. *Advances in pharmacological sciences.* 2013; 2013. Article ID 308249.
- [26] Scandinarì Manzollì E, Mara Serpeloni J, Grotto D, Kennup Bastos J, Maria Greggì Antunes L, Barbosa F, I and Rafael Mazzaron Barcelos G. Protective Effects of the Flavonoid Chrysin against Methylmercury-Induced Genotoxicity and Alterations of Antioxidant Status, In Vivo Oxid Med Cell Longev. 2015; 2015: 602360.
- [27] Bhadauria M. Propolis prevents hepatorenal injury induced by chronic exposure to carbon tetrachloride. *Evidence-Based Complementary and Alternative Medicine.* 2012; 2012. Article ID 235358.
- [28] Arvouet-Grand A, Lejeune B, Bastide P, Pourrat A, Privat AM, Legret P. Propolis extract. I. Acute toxicity and determination of acute primary cutaneous irritation index. *Journal de pharmacie de Belgique.* 1993; 48(3):165-70.
- [29] Mohammadzadeh S, Shariatpanahi M, Hamedì M, Ahmadvaniha R, Samadi N, Ostad SN. Chemical composition, oral toxicity and antimicrobial activity of Iranian propolis. *Food chemistry.* 2007 Jan 1; 103(4): 1097-103.
- [30] Charlton M, Krishnan A, Viker K, Sanderson S, Cazanave S, McConico A, Masuoko H, Gores G. Fast food diet mouse: novel small animal model of NASH with ballooning, progressive fibrosis, and high physiological fidelity to the human condition. *Am J Physiol Gastrointest Liver Physiol.* 2011 Aug 11; 301(5): G825-34.
- [31] McGill MR, Williams CD, Xie Y, Ramachandran A, Jaeschke H. Acetaminophen-induced liver injury in rats and mice: comparison of protein adducts, mitochondrial dysfunction, and oxidative stress in the mechanism of toxicity. *Toxicol Appl Pharmacol.* 2012 Nov 1; 264(3): 387-94.
- [32] Najmi AK, Pillai KK, Pal SN, Akhtar M, Aqil M, Sharma M. Effect of l-ornithine l-aspartate against thioacetamide-induced hepatic damage in rats. *Indian J Pharmacol.* 2010 Dec; 42(6): 384.
- [33] McArthur K, T. Kile B. Apoptotic Caspases: Multiple or Mistaken Identities? *Trends in Cell Biology,* June 2018, Vol. 28, No. 6:475-493
- [34] Paschkowsky S, Hsiao JM, Young JC, Munter LM. The discovery of proteases and intramembrane proteolysis. *Biochem Cell Biol.* 2018 Aug 13:1-5.
- [35] Campbell KJ, Tait SWG. Targeting BCL-2 regulated apoptosis in cancer. *Open Biol.* 2018 May; 8(5): 180002.
- [36] Padda, M.S., M. Sanchez, A.J. Akhtar, and J.L. Boyer. Drug-induced cholestasis. *Hepatology* (Baltimore, Md.). 2011; 53: 1377-1387.
- [37] Guy CD, Suzuki A, Zdanowicz M, Abdelmalek MF, Burchette J, Unalp A, Diehl AM. Hedgehog pathway activation parallels histologic severity of injury and fibrosis in human nonalcoholic fatty liver disease. *Hepatology.* 2012 Jun 1; 55(6): 1711-21. Epub 2012 Apr 18.
- [38] Danielson PB. The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. *Current drug metabolism.* 2002 Dec 1; 3(6): 561-97.
- [39] Jeffery, E.H.. *Biochemical Basis of Toxicity.* In Handbook of Toxicologic Pathology. W.M. Haschek and C.G. Rousseaux, editors. Academic Press Inc., London 1991. 49-70.
- [40] J. Felmler D, Grün D, F. Baumert T. Zooming in on liver zonation. *Hepatology* 2018, volume 67, issue 2: 784-787.

