Chron Precis Med Res 2022; 3(3): 107-114
DOI: 10.5281/zenodo.7193089

ORIGINAL ARTICLE
ORİJİNAL ARAŞTIRMA

The Hepatic Antioxidant Defense Efficacy of IDA Propolis in Aflatoxin B1 Administered Male Rats and Related Histopathological Findings

Aflatoksin B1 Uygulanan Erkek Sıçanlarda İDA Propolisin Hepatik Antioksidan Savunma Etkinliği ve Histopatolojik Bulgularının Araştırılması

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ABSTRACT

Aim: Natural products such as honey, pollen, propolis and royal jelly are generally friendly to the human liver and all other body cells, with a high antioxidant capacity. The present study aims at observing the damages caused by Aflatoxin B1 on rat liver and determining the beneficial effects of propolis on liver tissue.

Material and Method: The duration of the study was determined as 60 days and 45 male rats of Wistar albino breed with an average weight of 250-350 grams suitable for the working conditions were included in the study. Experimental animals were randomly and equally divided into 3 groups (The Control Group, The Aflatoxin B1 group, The Aflatoxin B1 + IDA Propolis group). The rats liver tissue and blood samples were taken and placed in separate sterile vials. These liver tissues and blood samples were sent to the our hospital Pathology and Biochemistry Department for histopathological examination and for biochemical blood analysis to be performed.

Result: The Gamma glutamyl transferase (GGT), Alanine Aminotransferase (ALT) and Alkaline phosphatase (ALP) levels of groups that were administered Aflatoxin B1 and Aflatoxin B1 + Propolis were higher than those of the control group. Administering Aflatoxin B1 alone increased Aspartate Aminotransferase (AST) levels whereas administering Aflatoxin B1 + Propolis decreased AST levels. The Total Bilirubin, Direct Bilirubin and c-reaktive protein (CRP) levels of groups that were administered Aflatoxin B1 and Aflatoxin B1 + Propolis were higher than those of the control group. The Total Bilirubin, Direct Bilirubin and CRP levels of rats that were administered Aflatoxin B1 were higher than those of rats that were administered Aflatoxin B1 + Propolis. Administering Aflatoxin B1 and Aflatoxin B1 + Propolis have increased the malondialdehyde (MDA) levels but decreased the catalase (CAT) levels, and this decrease is statistically significant (p<.0001). Administering Aflatoxin B1 + Propolis has resulted in a similar level of glutathione peroxidase.(GPx) with the control group. However, administering Aflatoxin B1 alone has resulted in a GPx level that is lower than both other groups. Administering Aflatoxin B1 and Aflatoxin B1 + Propolis have caused a statistically significant difference in the total antioxidant (TAS), total oxidant (TOS) and superoxide dismutase (SOD) levels (p<.0001).

Conclusion: The results of this study indicate that bee pollen is an important source of flavonoids, which can be considered as natural antioxidants. It is also reported that bee products prevent lipid peroxidation and scavenge a number of free oxygen radicals that are known oxidants and carcinogenic agents.

Keywords: Aflatoxin B1, histopathology, hepatic antioxidant defense, IDA propolis, Wistar albino (Rat).

ÖZ

Giriş: Bal, polen, propolis ve arı sütü gibi doğal ürünler insan karaciğer ve diğer tüm vücut hücreleri ile genellikle dost olup yüksek antioksidan kapasiteye sahip maddelerdir. Mevcut çalışma, Aflatoksin B1'in sıçan karaciğerinde oluşturduğu zararlar ve propolisin karaciğer dokusu üzerindeki yararlı etkilerini gözlemlemeyi amaçlamaktadır.

Gereç ve Yöntem: Çalışma süresi 60 gün olarak belirlenmiş ve çalışma şartlarına uygun ortalama ağırlığı 250-350 gram Wistar albino cinsi 45 adet erkek rat çalışmaya alınmıştır. Deney hayvanları rastgele ve eşit sayıda 3 gruba (Kontrol Grubu, Aflatoksin B1 grubu, Aflatoksin B1 + IDA Propolis grubu).ayrılmıştır. Ratların karaciğer doku ve kan örnekleri alınarak ayrı steril flakonlara yerleştirilmiştir. Karaciğer dokuları ve kan örnekleri histopatolojik inceleme ve biyokimyasal kan analizi için hastanemiz Patoloji ve Biyokimya laboratuvarına gönderildi.

Bulgular: Aflatoksin B1ve Aflatoksin B1+Propolis uygulanan gruplarda gama glutamil transferaz (GGT), alanin aminotransaminaz (ALT) ve alkalen fosfotaz (ALP) düzeylerinin kontrol grubuna göre yüksek olduğu görülmektedir. Tek başına AflatoksinB1 uygulamasının aspartat aminotransaminaz (AST) düzeyini yükselttiği, Aflatoksin B1+Propolis uygulamasının ise AST düzeyini düşürdüğü görülmektedir. Aflatoksin B1 uygulanan ratlar Aflatoksin B1+Propolis uygulanan ratlardan daha yüksek Total Bilirubin, Direk Bilirubin ve c-reaktif protein (CRP) düzeyine sahiptir. Aflatoksin B1 ve Aflatoksin B1+Propolis uygulaması malondialdehit (MDA) seviyesini arttırmakta ve katalaz (CAT) seviyesini ise düşürmektedir ve bu düşüş istatistiksel olarak önemlidir (P<,0001). Aflatoksin B1+Propolis uygulaması glutatyon peroksidaz (GPx) bakımından kontrol grubu ile benzer bir düzeye sahiptir. Aflatoksin B1 uygulaması ise her iki gruptan daha düşük bir GPx seviyesine neden olmuştur. Aflatoksin B1 ve Aflatoksin B1+Propolis uygulaması total antioksidan (TAS), total oksidan (TOS) ve süperoksit dismutaz (SOD) değerlerinde istatistiksel anlamada önemli bir farklılığa neden olmuştur (P<,0001).

Sonuç: Bu çalışmanın sonuçları, arı poleninin doğal antioksidanlar olarak kabul edilebilecek önemli bir flavonoid kaynağı olduğunu göstermektedir. Arı ürünlerinin lipid peroksidasyonunu önlediği ve oksidan ve kanserojen ajan olarak bilinen bir dizi serbest oksijen radikali temizlediği de bildirilmektedir.

Anahtar Kelimeler: Aflatoksin B1, Histopatoloji, Hepatik antioksidan savunma, IDA Propolis Wistar albino (Rat).

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Başvuru Tarihi/Received: 08.06.2022
Kabul Tarihi/Accepted: 27.07.2022

INTRODUCTION

Natural products such as honey, pollen, propolis and royal jelly are generally friendly to the human liver and all other body cells, with a high antioxidant capacity (18). Apitherapeutic bee products not only contain high levels of vitamin A (beta-carotene) and vitamin C (ascorbic acid) but they also constitute a source of phenolic acids, flavonoids and anthocyanins as well as Vitamin B (B1-B2) and elements such as Na, K, Ca, Mg, Fe, Cu, Zn, Se, F and CI with coenzyme function, which are used as a metabolic regulator and are required to be obtained externally. It is reported in the literature that the bee pollen phenolic extract has an anti-allergic effect on rats, the ethanolic extract of honeybee collected pollen in the Mexican flora inhibit lipid peroxidation and that bee products prevent lipid peroxidation and scavenge a number of free oxygen radicals that are known oxidants and carcinogenic agents (2-21-22). Apiculture products are known to be effective in the prevention of numerous diseases and treatment of the liver disease in particular. Products such as honey, pollen, propolis and royal jelly have a high antioxidant effect on the human liver and all other body cells. These products, which are collected and concentrated by honeybees in the nature, contain elevated levels of carbohydrates, vitamins, coenzymes, polyphenols, aromatic compounds and phytosterols. Honey, pollen, propolis and other bee products that are composed of nectars and pollens collected by honeybees from plants contain elevated levels of polyphenolic compounds.The liver is a vital organ of the human body (15). It is exposed to toxic substances and drugs when performing its anatomical, physiological, and biochemical functions. A major function of the liver is detoxification.

Consequently, various chemical agents and drugs escalate liver damage, impairing its histopathological structure (1). Thus, acute and chronic hepatitis and liver cirrhosis have become very common nowadays. Therefore, the treatment method using natural products has gained prominence in the prevention of liver damage. Consequently, animal tests are conducted with a view to preventing hepatitis using natural extracts. Research studies involving apiculture products have proven to be effective in the recent years, most of which is focused on using pollen, propolis, honey and royal jelly to prevent hepatitis (2-21-22). The present study aims at observing the damages caused by Aflatoxin B1 on rat liver and determining the beneficial effects of propolis on liver tissue.

MATERIAL AND METHOD

The study was carried out with the permission of Çanakkale Onsekiz Mart University Animal Research Ethics Committee (Decision No: 2018/11-08). The rats were housed in separate cages in a room with a 12 hour light-dark cycle and fresh air supply, throughout the experiment. They were provided with rat food and water ad libitum save for the 8 hour anesthesia period. The duration of the research study was 60 days, where 45 male Wistar albino rats meeting the requirements of the study and weighing between 250-350 grams on the average were examined. The test animals were randomly divided into (n=20) 3 groups of equal size.

The Groups

1. The Control Group: The rats in this group were fed a standard diet for 2 months and then sacrificed on day 60 (Rats in this group were fed a standard diet for 2 months and then liver and blood samples were taken on day 60). Liver tissue and blood samples were taken from all rats and placed in separate sterile vials. These liver tissue and blood samples were sent to the Medical Pathology and Medical Biochemistry Department of our university to be dissected for histopathological examination and for biochemical analysis to be performed.

2. The Aflatoxin B1 group: The rats in this group were fed a standard diet for 2 months and Aflatoxin B1 was added to their drinking water at the rate of 0-04 mg/kg. (Rats were fed with a standard diet for 2 months and added 0-04 mg / kg Aflatoxin B1 to 1 liter of drinking water every day. has been added) sacrificed on day 60 and liver tissue and blood samples were taken from them.

3. The Aflatoxin B1+IDA Propolis group: The rats in this group were fed a standard diet for 2 months, where Aflatoxin B1 was added to their drinking water at the rate of 0- 04 mg/kg and IDA Propolis was added to their drinking water at the rate of 2-7.3 g per3.liter. (In addition, 2-7.3 g of İDA Propolis was added to 1 liter of drinking water every day for 2 months. These rats were then). These rats were then sacrificed on day 60 and liver tissue and blood samples were taken from them.

Postoperative Procedures

An abdominal centerline incision was performed while the rats were anesthetized with Ketamine hydrochloride (35 mg/kg, Alfamine 10%, Egevet/Turkey) and Xylazine (15 mg/kg, Alfazyne 2%, Egevet/Turkey) (12) and liver tissue and blood samples were taken and placed in separate sterile vials. These liver tissue and blood samples were sent to the Medical Pathology and Medical Biochemistry Department of xxxxxxxxxxxxxx University) to be dissected for histopathological examination and for biochemical blood analysis to be performed.

Histopathological Examination

The histopathological examination was performed by a pathologist blinded to the groups. All liver tissue

samples taken from the groups were fixated in 10% formaldehyde solution. The tissues were rinsed in tap water overnight and embedded in paraffin after running a series of routine histological techniques. Subsequently, 5 µm thick sections of paraffin blocks were placed on microscope slides. The tissue samples were stained with Hematoxylin-Eosin (H&E). All sections were examined and photographed by a ZEISS Primo Star light microscope.

Blood Sample Collection and Biochemical Analyses

The rats were sacrificed at the end of the experimental procedures and blood and liver tissue samples were taken from them. Blood samples were collected in vials containing anticoagulants (EDTA) and centrifuged at 3,000 RPM and +4 °C for 10 minutes to segregate the plasma. The hemolysate was prepared by washing the erythrocytes with physiological saline solution (0.9% NaCl) three times. The hemolysate and liver tissue samples were stored at -80 °C until the biochemical analyses were performed. EDTA blood samples were centrifuged to remove their plasma, and the MDA levels of removed plasma were examined. These diluted blood samples were further diluted with 50 mm phosphate buffer (pH: 7.0) using a dilution factor of 1:100 to determine catalase activity in the hemolysate. Centrifuged blood samples were placed in silicone tubes to segregate the serum. The aspartat aminotransaminaz (AST), Alanine Aminotransferase (ALT), Alkaline phosphatase (ALP), Gamma glutamyl transferase (GGT) total bilirubin and direct bilirubin levels were determined using photometric, colorimetric, and enzymatic methods. The blood samples were transferred to biochemical tubes to be used in further studies. The blood samples were centrifuged at 3,000 RPM for 5 minutes to examine total oxidant (TOS), total antioxidant (TAS), superoxide dismutase (SOD) and glutathione peroxidase (GPx) levels. The serum c-reaktive protein (CRP) level was measured using the enzyme-linked immunosorbent assay (ELISA) test. The statistical package for the social sciences (SPSS) analysis method, where study groups are treated as fixed factors, was used for statistical analysis. The groups were compared with each other by using the Duncan test in post-hoc analyses.

RESULTS

The following results were obtained upon examination of the blood samples and histopathological sections of tissue liver. Biochemical Parameter Findings: following results were obtained upon examination of the blood samples and Administering Aflatoxin B1 and Aflatoxin B1 + Propolis have caused statistical differences in the GGT, AST, ALT and ALP levels of blood (p<.0001). The GGT, ALT and ALP levels of groups that were administered Aflatoxin B1 and Aflatoxin B1 + Propolis were higher than those of the control group. Administering Aflatoxin B1 alone increased AST levels whereas administering Aflatoxin B1 + Propolis decreased AST levels. Propolis extract has been shown to decrease the level of AST enzyme in rats (30). AST, ALT, LDH and ALP activities have also increased as diagnostic indicators of liver injury (22). The study titled "Antioxidant Effects of Herbal Extracts on Rats Contaminated with Aflatoxin" has reported an increase in serum AST, ALP, cholesterol, total bilirubin, LDH and urea levels (16). The GGT, ALT and ALP levels of groups that were administered Aflatoxin B1 and Aflatoxin B1 + Propolis were higher than those of the control group.

Table 1: The Biochemical Parameters of GGT, AST, ALT and ALP in Aflatoxin B1 and Propolis Administered Rats

Blood	GGT (U/L)	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	1.36^{c}	153.30^{c}	60.16^{b}	136.5^{c}
Aflatoxin B1	1.68^{a}	181.35^{a}	85.10^{a}	153.2^{a}
Aflatoxin B1+Propolis	1.42^{b}	135.17^{b}	63.60^{b}	140.1^{b}

*The mean values marked by different superscript letters in a column differ significantly (P<.0001). **Gamma glutamyl transferase (GGT). Aspartat aminotransaminaz (AST), Alanine Aminotransferase (ALT), Alkaline phosphatase (ALP).

Administering Aflatoxin B1 and Aflatoxin B1 + Propolis have caused statistical differences in the Total Bilirubin, Direct Bilirubin and CRP levels (p<.0001). The Total Bilirubin, Direct Bilirubin and CRP levels of groups that were administered Aflatoxin B1 and Aflatoxin B1 + Propolis were higher than those of the control group. The Total Bilirubin, Direct Bilirubin and CRP levels of rats that were administered Aflatoxin B1 were higher than those of rats that were administered Aflatoxin B1 + Propolis. Bilirubin, Direct Bilirubin and CRP levels of groups that were administered Aflatoxin B1 and Aflatoxin B1 + Propolis were higher than those of the control group. The Total Bilirubin, Direct Bilirubin and CRP levels of rats that were administered Aflatoxin B1 were higher than those of rats that were administered Aflatoxin B1 + Propolis.

Table 2: The Biochemical Parameters of Total Bilirubin, Direct Bilirubin and CRP in Aflatoxin B1 and Propolis Administered Rats

Blood	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)	CRP (µg/ml)
Control	0.26^{c}	0.08^{c}	3.32^{c}
Aflatoxin B1	0.34^{a}	0.11^{a}	5.08^{a}
Aflatoxin B1+Propolis	0.30^{b}	0.10^{b}	4.12^{b}

*The mean values marked by different superscript letters in a column differ significantly (P<,0001). *c-reaktive protein (CRP)

Administering Aflatoxin B1 and Aflatoxin B1 + Propolis have increased the MDA levels but decreased the CAT levels, and this decrease is statistically significant (p<.0001). Administering Aflatoxin B1 causes the MDA levels to be higher and the CAT levels to be lower than

administering Aflatoxin B1 + Propolis. Administering Aflatoxin B1 + Propolis has resulted in a similar level of GPx with the control group. However, administering Aflatoxin B1 alone has resulted in a GPx level that is lower than both other groups.

Table 3: The Biochemical Parameters of MDA , CAT and GPx in Aflatoxin B1 and Propolis Administered Rats

Blood	MDA (nmol/ml)	CAT (k/gHb)	GPx (U/mg Hb)
Control	7.05^{c}	59.18^{a}	0.29^{b}
Aflatoxin B1	10.08^{a}	46.20^{c}	0.24^{a}
Aflatoxin B1+Propolis	8.03^{b}	54.26^{b}	0.27^{b}

*The mean values marked by different superscript letters in a column differ significantly (P<.0001). **Malondialdehyde (MDA), catalase (CAT), glutathione peroxidase.(GPx)

Administering Aflatoxin B1 and Aflatoxin B1 + Propolis have caused a statistically significant difference in the TAS, TOS and SOD levels (p<.0001). Administering Aflatoxin B1 has caused the TAS levels to increase in comparison to the control group. On the contrary, administering Aflatoxin B1 + Propolis has caused the TAS levels to decrease in comparison to the control group. The TOS levels are higher in the Aflatoxin B1 and Aflatoxin B1 + Propolis groups compared to the control group. Administering Aflatoxin B1 + Propolis has resulted in lower TOS levels in comparison to the Aflatoxin B1 group. Administering Aflatoxin B1 + Propolis has resulted in a similar level of SOD with the control group. However, administering Aflatoxin B1 alone has resulted in a SOD level that is higher than the control group. Histopathological Findings: Normal histological liver structures were observed in the liver tissue sections of the rats in the control group (**Figure 1**). Liver tissues of the rats in the Aflatoxin B1 administered group exhibited sinusoidal dilatation (S), Kupffer cell proliferation (KC) and congestion of the central vein (CV) (**Figure 2a**), inflammatory cell infiltration in the portal area (**Figure 2b**), haloes around congested hepatocyte nuclei (perinuclear halo, PH) (**Figure 2c**) and porto-portal fibrosis (**Figure 2d**). The liver tissues of the rats in the Propolis administered group demonstrated comparable properties with the control group (**Figure 3**). As a result, liver tissues of rats that are exposed to Aflatoxin B1 demonstrated significant injuries whereas liver tissues of rats that are given propolis extracts demonstrated comparable properties with the control group.

Table 4: The Biochemical Parameters of TAS, TOS and SOD in Aflatoxin B1 and Propolis Administered Rats

Blood	TAS (Mmol Trolox eq/L)	TOS (µmol H2O2 eq/L)	SOD (U/mg protein)
Control	1.01^{c}	7.05^{c}	7.45^{b}
Aflatoxin B1	1.15^{a}	11.90^{a}	8.54^{c}
Aflatoxin B1+Propolis	0.81^{b}	9.15^{b}	7.10^{b}

*The mean values marked by different superscript letters in a column differ significantly (P<,0001). *Total antioxidant (TAS),total oxidant (TOS), superoxide dismutase (SOD).

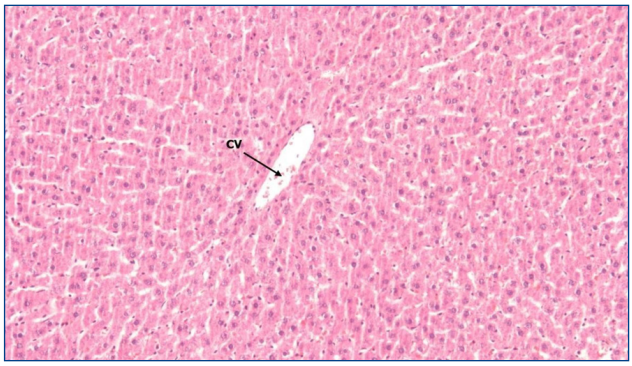

Figure 1 (Control Group): Normal liver histology and central vein (CV) structures were observed microscopically (HEX100).

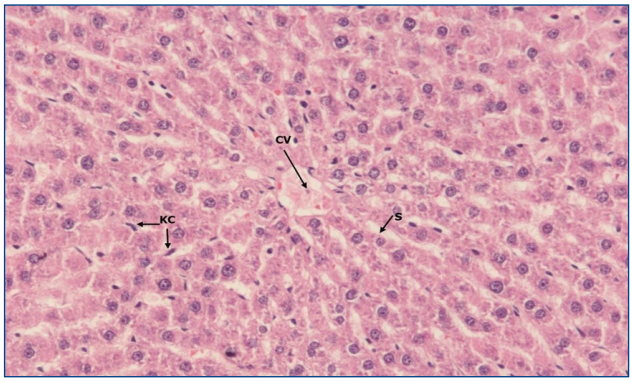

Figure 2 (Aflatoxin Group): Congestion of the central vein (CV), Kupffer cell increase (KC) and sinusoidal dilatation (S) were observed microscopically (HEX200).

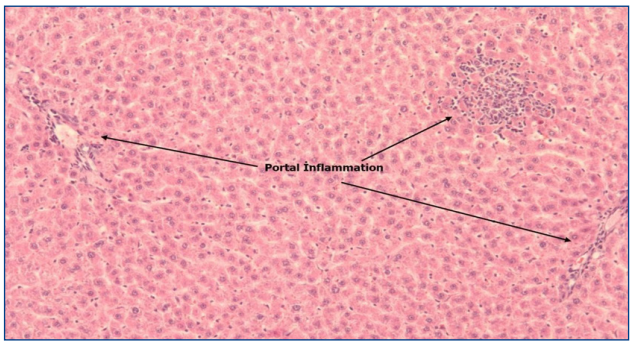

Figure 2b (Aflatoxin Group): All portal areas of the section demonstrated inflammatory cell infiltration in varying degrees of severity microscopically (HEX100).

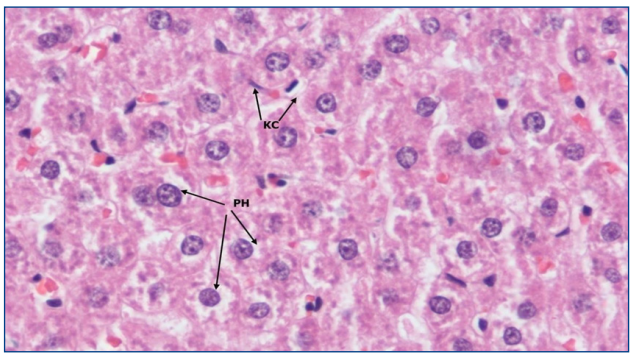

Figure 2c (Aflatoxin Group): Congested liver cells, perinuclear haloes (PH) and Kupffer cell proliferation (KC) were observed microscopically Haloes were observed around congested hepatocyte nuclei (perinuclear halo, PH). (HEX400).

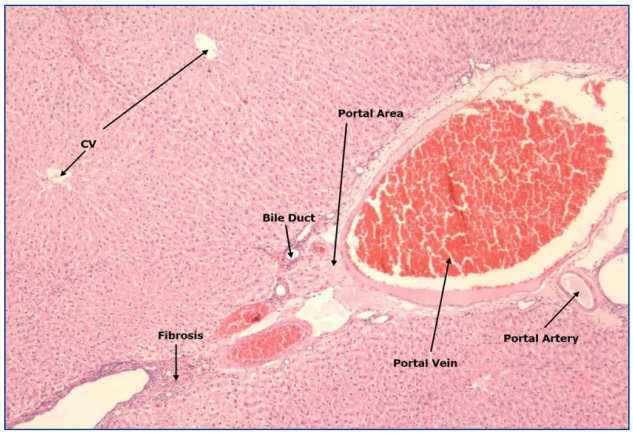

Figure 2d (Aflatoxin Group): Porto-portal fibrosis, dilatation of the portal area and portal vein, proliferation of the biliary duct and congestion of the portal artery and veins in the portal area were observed microscopically (HEX40).

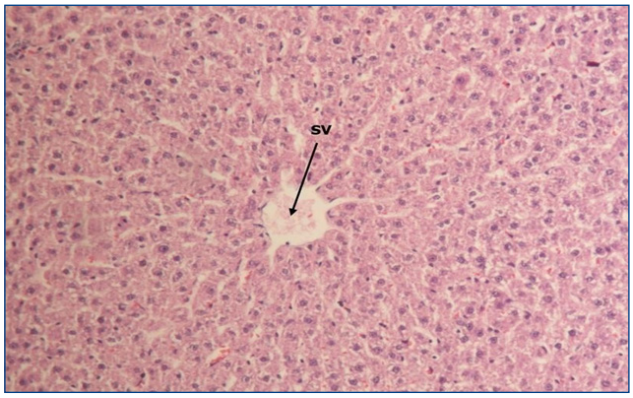

Figure 3 (Propolis Group): Normal liver tissue histology, similar to that of the control group, was observed in the Propolis administered group microscopically (HEX40).

DISCUSSION

The study titled "Antimicrobial activity and pollen composition of honey samples collected from different provinces in Turkey" has reported that "Honey proved to be more effective on bacteria than antibiotics and these natural products contain carbohydrates, vitamins, coenzymes, polyphenols, aromatic compounds and phytosterols, which are collected and concentrated by honeybees in the nature, as well as numerous terpenes and terpenoids, aliphatic compounds and volatile compounds such as fatty acids, the functions of which are not fully known (18). It is also reported that methanolic extracts of pollen and propolis demonstrate antibacterial activity (6). The study titled "Anti- allergic effect of bee pollen phenolic extract and myricetin in ovalbumin-sensitized mice" reported that "The bee pollen is used in folk medicine to alleviate allergic reactions. The bee pollen phenolic extract (BPPE) consists in phenolic compounds (flavonoids) from plants picked by Apis mellifera bee and myricetin is one of the compounds that is present in this the extract, The results of this study support the hypothesis that myricetin is one of the flavonoids of BPPE responsible for the anti-allergic effect and a potential tool to treat allergies (16).

The study titled "Antioxidant Activity of Polyphenolic Extract of Honeybee-collected Pollen" reported that "The antioxidant capacity related to the phenolic composition of honeybee- collected pollen extract acts as an inhibitor of lipid peroxidation on mouse hepatic microsomal preparations in an in vitro biological system (2). The results of this study indicate that bee pollen is an important source of flavonoids, which can be considered as natural antioxidants. Pollen extracts have demonstrated antioxidant activity related to the flavonol concentration in both the in vitro-biological system and the in vivo system but it was reported that a high concentration of flavonols in the extract of pollen can also have a pro-oxidant effect. It is also reported that bee products prevent lipid peroxidation and scavenge a number of free oxygen radicals that are known oxidants and carcinogenic agents (22-23). In another study, the effect of pesticides (Carbaryl) on the oxidative stress markers in rats was examined, demonstrating he ameliorative effect of bee pollen (11). Antioxidants are defense molecules that are brought in by diet from herbal sources, protecting the organism against oxidative degeneration. The bee pollen is promoted as a health food with a wide range of nutritional and therapeutic properties, however it is reported that antioxidants that are brought in by diet or generated by the organism cannot be stored in vivo. They are ingested and egested on a regular basis. Antioxidants such as Vitamin A and E function in hydrophobic environments. On the other hand, many compounds such as ascorbic acid (Vitamin C) and phenolic substances (gallic acid, catechin, quercetin, caffeic acid, cinnamic acid, ferulic acid, coumaric acid, resveratrol, rutin, kaempferol, naringin, apigenin and luteolin) are effective in hydrophilic environments. It is reported that all antioxidants are responsible for absorbing the impact of radical agents with their protective properties to protect all other biomolecules against all kinds of oxidative damage (23).

The importance of honey and its products for human health is emphasized. It is aimed to show the harm that aflatoxin B1 will cause in humans on rats. It has been shown that propolis reduces the negative effects of Aflatoxin B1 on the liver and blood tissue.

AST and GGT parameters were examined in a study conducted on 34 bovines which were given Aflatoxin B1, and it was reported that GGT activity has increased significantly after Aflatoxin B1 was administered but AST levels were not altered considerably (14,17). Varying amounts of Aflatoxin B1 was added artificially to the fodder of dairy ewes in a 14 day study. It was reported that the levels of blood parameters AST, GGT, ALP and

LDH have increased significantly (7). The study titled "Effect of Ascorbic Acid Supplement on Hematological Parameters and some enzyme activities of Male Rabbits Exposed to Aflatoxin B1" has reported that plasma AST, ALT and LDH levels have increased significantly and plasma.

AST, ALT, LDH and ALP activities have also increased as diagnostic indicators of liver injury (22). The study titled "Antioxidant Effects of Herbal Extracts on Rats Contaminated with Aflatoxin" has reported an increase in serum AST, ALP, cholesterol, total bilirubin, LDH and urea levels (16). It was reported that, biochemical changes were observed prior to the emergence of clinical symptoms in aflatoxin contamination, resulting from the damage sustained by the cells and tissues depending on the level of toxins received and the duration of exposure, the levels of serum ALP, GGT, LDH, ALT, AST and serum bilirubin were elevated, and the levels of serum protein, non-protein nitrogen, urea, hemoglobin and coagulation factor have decreased significantly (5,29).

The biochemical parameters obtained under the present study are generally in accord with the literature, where administering Aflatoxin B1 and Aflatoxin B1 + Propolis have caused statistical differences in the GGT, AST, ALT and ALP levels of blood. The GGT, ALT and ALP levels of groups that were administered Aflatoxin B1 and Aflatoxin B1 + Propolis were higher than those of the control group. Administering Aflatoxin B1 alone increased AST levels whereas administering Aflatoxin B1 + Propolis decreased AST levels.

The study titled "Bioactive Properties of Apitherapeutic bee Products (honey, pollen, propolis and royal jelly) and their Roles in the Prevention of Liver Damage" has examined the antioxidant properties of propolis and its role in the prevention of liver damage. The roles played by ALT and AST enzymes, and enzymes such as MDA, SOD and CAT in the formation of liver damage as well as the changes in the histopathological tests of liver tissue were examined. It was observed that the antioxidant activities of the bee products used in the study have varied depending on their total phenolic compound content and that propolis had the highest antioxidant capacity amongst these bee products (20). It was reported that most types of the CAT enzyme are capable of increasing the rate of free radical formation in certain body organs and thus antioxidant enzymes play a vital role both in preserving the stability of the cells and eradication of free radicals (8).

Table 3 demonstrates that Administering Aflatoxin B1 and Aflatoxin B1 + Propolis have increased the MDA levels but decreased the CAT levels, and this decrease is statistically significant. Administering Aflatoxin B1 causes the MDA levels to be higher and the CAT levels to be lower than administering Aflatoxin B1 + Propolis. Administering Aflatoxin B1 + Propolis has resulted in a similar level of GPx with the control group. However, administering Aflatoxin B1 alone has resulted in a GPx level that is lower than both other groups. When we compare the effect of Aflatoxin B1 on CAT activity with the control group in the present study, we see that CAT activity has significantly decreased statistically. Our findings are in line with the literature. The liver is the sole organ in the human body that carries out all biosynthesis and regulation activities including but not limited to regulating blood sugar, storing minerals, generating bile acid and cholesterol, and regulating blood coagulation factors (13-19-21). Hepatocytes, which are referred to as the liver cells, are the most functional cells in the human body (10-25-26).

We have observed inflammatory cell infiltration in the portal area and haloes around congested hepatocyte nuclei (perinuclear halo, PH) on rats in the Aflatoxin B1 administered group. The liver tissues of the rats in the Aflatoxin B1 administered group also exhibited sinusoidal dilatation (S), Kupffer cell proliferation (KC), congestion of the central vein (CV) and inflammatory cell infiltration in the portal area. Fibrotic tissue is formed in the liver in response to direct toxic damage causing inflamation. These fibrous connective tissues conjugate in various areas of the liver (portal-portal-, portal-central, central-central) in time. This is referred to as bridging fibrosis. In contrast to all other recoverable lesions, fibrosis in particular and hepatic damage in general are not recoverable, resulting in cirrhosis (4).

We have observed sinusoidal dilatation (S), Kupffer cell proliferation (KC) and congestion of the central vein (CV), inflammatory cell infiltration in the portal area, haloes around congested hepatocyte nuclei (perinuclear halo, PH) and porto-portal fibrosis in the liver tissues of rats in the Aflatoxin B1 administered group under the present study, confirming the findings of Armbrust et.al.

The liver is the largest organ and gland in the human body, located between the gastrointestinal system and portal circulation, and peripheric organs and systematic circulation, receiving blood supply both from the hepatic artery and from the hepatic portal vein. The liver is confronted with all drugs, toxic substances and microbic agents that are received physiologically or biochemically, orally, or parentally and is exposed to their harmful effects. The liver detoxifies harmful substances that ingress the human body or responses to the damage caused by them with its regenerative capabilities on an ongoing basis. There are numerous causes that damage the liver, some of which are commonly observed (1-9- 27).

Most harmful chemicals damage the liver by inhibiting protein synthesis indirectly whereas others damage the liver by inhibiting protein synthesis directly. For

example, the fungal toxin called alpha- Amanitin stops all kinds of protein synthesis by directly inhibiting the RNA polymerase II enzyme (25). Liver damage will be escalated if the antioxidant/oxidant balance is disturbed in favor of oxidation and mitigated if it is disturbed in favor of antioxidation. Consequently, all antioxidants that are brought in by diet will be effective in protecting liver cells and the rest of the somatic system against oxidation. An increase in liver enzyme levels is considered as a diagnostic indicator of liver damage (3). These enzymes are released to the circulatory system as in the case of liver damage exhibiting hepatocellular lesions and parenchymal cell necrosis[28]. Aflatoxins are the strongest known carcinogens of the liver (24).

This effect has been demonstrated in many animal groups. For instance, up to 100% of female and male rats develop liver cancer within 80 weeks and 70 weeks respectively upon intoxication with 15 ppb of aflatoxin in their feed, even only once. Aflatoxins also cause kidney tumors, colonic mucinous adenocarcinoma, sarcoma and fibrosarcoma (14). Histopathological effects of aflatoxins are particularly seen in liver and biliary duct cells. These effects are manifested in the form of biliary duct cell hyperplasia, nucleus dilatation, nucleus inclusions and hepatocyte dilatation (12). In the present study, we have observed proliferation of biliary duct cells and congestion of portal arteries in the portal area in the rats that were administered Aflatoxin B1, confirming the findings of Hastings et.al (13).

CONCLUSIONS

The findings of the present study, supported by the literature, indicate that propolis, the bee product with the highest apitherapeutic effect, prevents liver injury by blocking oxidative damage, and accelerates treatment with its antibacterial, antiviral, anti-inflammatory and anti- tumoral activities. Consequently, it is considered that both healthy individuals and hepatitis patients will be healthier by increasing propolis intake in their diets. Therefore, it is recommended to use extracts containing propolis on a regular basis with a view to protecting the liver against oxidative damage and enhancing the immune system. Propolis is a natural food product capable of performing numerous phytobiological activivties including but not limited to wound therapy, treatment of upper respiratory tract infections and preventing carcinogenesis.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Çanakkale Onsekiz Mart University Animal Research Ethics Committee (Decision No: 2018/11-08).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: This study was supported by Çanakkale Onsekiz Mart University Scientific Research Projects Coordination Unit (BAP) (Project Number: BAP-2810).

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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