Erciyes Med J 2020; 42(2): 132–8 • DOI: 10.14744/etd.2020.83097
ORIGINAL ARTICLE – OPEN ACCESS

The Protective Role of Poplar Propolis Against Alcohol-Induced Biochemical and Histological Changes in Liver and Testes Tissues of Rats

Numan Baydilli[1], Emre Can Akınsal[1], Züleyha Doğanyiğit[2], Oğuz Ekmekçioğlu[1], Sibel Silici[3]

ABSTRACT

Objective: This study evaluated the biochemical and histopathological effects of propolis oil extract on liver and testicular tissue in rats subjected to alcohol toxicity.

Materials and Methods: Sixty Wistar albino rats were randomly assigned into 6 groups: Control, Ethanol (EtOH: 20% ethanol) Propolis-1 (PR1: 100 mg/kgbw) Propolis-2 (PR2: 200 mg/kgbw) EtOH+PR1, and EtOH+PR2. Rats were administered ethanol and propolis by gastric tube for 15 days. Hematological, biochemical, and histopathological (liver and testes) parameters were examined. In addition, sperm concentration and motility were determined.

Results: Increased deterioration of complete blood count parameters was statistically significant in the EtOH group when compared to the control group. Propolis was preventative for some effects of EtOH. The most pronounced preventive effect of propolis on ethanol-induced damage was observed in nucleated red blood cells (NRBC) and mean corpuscular hemoglobin concentration (MCHC) parameters ($p<0.01$). Cholesterol and triglyceride levels were found to be highest in the EtOH group, and propolis was found to reduce these effects of ethanol ($p<0.01$). While the highest value for the testosterone hormone was seen in the PR2 group, propolis was found to decrease the hormone level decline caused by ethanol especially in the EtOH+PR2 group. Although it was not statistically significant, giving rats ethanol showed reduced sperm motility and epididymal sperm concentration, whereas co-administering propolis and ethanol showed an increase in these values.

Conclusion: It was found that ethanol caused negative effects on the biochemical parameters studied, and histopathological assessments also showed negative effects on liver and testicular tissue. Propolis (200 mg/kgbw), on the other hand, was found to have a mitigating effect on these values.

Keywords: Ethanol, alcohol, propolis, liver, testes, semen

Cite this article as: Baydilli N, Akınsal EC, Doğanyiğit Z, Ekmekçioğlu O, Silici S. The Protective Role of Poplar Propolis Against Alcohol-Induced Biochemical and Histological Changes in Liver and Testes Tissues of Rats. Erciyes Med J 2020; 42(2): 132-8.

[1]Department of Urology, Erciyes University Faculty of Medicine, Kayseri, Turkey
[2]Department of Histology and Embryology, Yozgat Bozok University Faculty of Medicine, Yozgat, Turkey
[3]Department of Agricultural Biotechnology, Erciyes University Seyrani Faculty of Agricultural, Kayseri, Turkey

Submitted 25.06.2019

Accepted 09.01.2020

Available Online Date 06.02.2020

Correspondence Numan Baydilli, Department of Urology, Erciyes University Faculty of Medicine, Kayseri, Turkey
Phone: +90 352 207 66 66/ 21125
e-mail: dr_numan38@hotmail.com

INTRODUCTION

Excess use of ethanol negatively affects human health, which is recognized as a serious worldwide problem. Ethanol plays a direct role in the production of reactive oxygen species and reactive nitrogen species, createing an environment prone to oxidative stress. Alcohol can increase malondialdehyde (MDA) levels by reducing antioxidant activity (1). Studies have shown that alcohol causes cirrhosis of the liver, cardiomyopathy, myopathy, and acute and chronic pancreatitis (2, 3). Among health problems caused by ethanol, its adverse effects on the liver and the reproductive system are some of the most severe. It has been shown that as alcohol dehydrogenase activity increases, serum testosterone levels decrease and also that hematologic parameters in testicular interstitial tissues deteriorate in rats that are induced to chronically consume alcohol (4, 5). In recent years, researchers have sought health support products with beneficial biological activity, especially antioxidant activity, to eliminate or at least mitigate the harmful effects of alcohol. One of the natural products whose antioxidant activity is well defined in the literature and about which there are many research studies is propolis (6).

Some plants protect leaves, flowers, fruits and buds from cold and harmful environmental factors by producing a strong, antimicrobial, waterproof and heat-insulating resin compound. This resinous substance is collected by honeybees (Apis mellifera L.), mixed with wax and saliva and transported to the hive, then is referred to as propolis. The chemical structure of certain propolis types, such as the European poplar propolis, and Brazil green and red propolis, have been well-described and standardized. Many beneficial biological activities of propolis, such as antioxidant, antimicrobial, anti-inflammatory, anticarcinogenogenic, and immunostimulant activities, are due to its phenolic compounds (7). As propolis is not produced by food and pharmaceutical industries, it is generally extracted using ethyl alcohol. However, for religious reasons, alcohol intolerance, resinous taste of alcohol, and limited use in children, researchers have sought to find new solvents for extracting phenolic compounds. One such solvent is olive oil, which has more than 200 different chemical compounds, including sterols, triterpenic alcohols, carotenoids and phenols. Phenolic compounds found in extra-virgin olive oil include both lipophilic and

hydrophilic phenols. Dissolving propolis in vegetable oils with appropriate methods can be beneficial to health as it can be used in multiple industries as well as providing additional benefits directly from the solvent. It has been scientifically proven that propolis, which is extracted from different vegetable oils, has biological activity, specifically antimicrobial, anti-tumor, anxiolytic and antidepressant activity (7). Scarce data exists describing protective effects of propolis extracted using olive oil against liver and testis damage in the literature. The aim of study was to determine the biochemical and histopathological effects of poplar-type propolis, which was obtained in the form of two different doses of olive oil extracted propolis on liver and testicular tissues.

MATERIALS and METHODS

Chemical Materials

Olive oil extracted propolis was purchased from Nutral Therapy Company in Kayseri, Turkey. Ethanol (20%) was purchased from Merck, USA.

HPLC Analysis of Propolis

The analyses of the propolis used in this study was investigated with an Agilent 1100 HPLC system equipped with a photodiode array detector and an ion-trap mass spectrometer detector (Agilent Technologies, Germany). The mobile phase of the method was methanol (A) and 0.5% v/v acetic acid in water (B). The extrication profile was: 10% A in B: 0 min, 60% A in B: 28 min, and 10% A in B: 30 min. A volume of 10 µL of sample was administered on to the column operating at room warmth at a flow rate of 1 mL/min. UV detection was performed at 290nm. UV chromatograms were inscribed at 280 and 360 nm with a bandwidth of 8nm. The eluted components were determined on the basis of the retention time by comparison with the retention time of the reference standard.

Total Phenolic Content, Antioxidant Activity, Free Radical Scavenging Activity

Total phenolic content was analyzed using the method proposed by Fuentes et al. (8). The total antioxidant efficiency of the oil extract of propolis was evaluated using the phosphomolybdenum method as defined by Prieto et al. (9). The effect of 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging was evaluated using the method of Gyamfi et al. with light modification (10).

Animals and Experimental Design

The study was approved by the Animal Care and Use Committee at the Erciyes University Faculty of Medicine (ID=16/078). Experimental procedures were performed according to the Guide to the Care and Use of Laboratory Animals. Sixty Wistar albino rats (250–260 g) were randomly divided into 6 equal groups. The groups were as follows: in the Ethanol (EtOH) group, 10 ml of 20% ethanol was administered for 15 days. In the Propolis groups, propolis was administered at doses of 100 mg/kg (PR1) and 200 mg/kg (PR2) for 15 days. In the EtOH plus Propolis groups, 10ml of 20% ethanol and Propolis was administered at doses of 100 mg/kg (EtOH+PR1) and 200 mg/kg (EtOH+PR2) for 15 days. In the Control group, 10 mg/kg of 0.9% saline was administered for 15 days. The designated substances were administered once a day by gastric tube.

Sample Collection

At the conclusion of the study, blood samples were taken from rats after 6 hours of fasting. Light ether anesthesia was administered prior to taking blood samples. Blood samples were collected from each animal by heart cannulation. Blood samples were centrifuged for 10 minutes at 3000 g. Testes and the liver tissue was collected, and one testis from each animal was fixed in 10% formalin for histopathological examination. Prior to biochemical analysis, specimens (serum, liver, testis) were stored at -20°C.

Biochemical Studies

Complete blood count was performed using a Sysmex analyzer (Sysmex Co., Kobe, Japan). Biochemical parameters, including glucose, triglyceride, total cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were analyzed using a Cobas 8000 series analyzer (Roche Diagnostics, Mannheim, Germany) using the spectrophotometer technique. Total testosterone was analyzed by a Cobas 8000 series analyzer (Roche Diagnostics, Mannheim, Germany) using the electrochemiluminescence immunoassay (ECLIA) technique. The standard spectrophotometric method described by Ohkawa et al. was used to evaluate lipid peroxide reaction levels with thiobarbituric acid (TBA) (11).

Epididymal Sperm Concentration and Motility

Epididymal sperm concentration was determined using the method described by Yokoi et al. (12). The epididymis was triturated with scissors in 5 mL of 0.9% saline and processed using a rocker for ten minutes, then incubated for two minutes at room temperature. The resulting supernatant was diluted 1:100 with sodium bicarbonate (5 g), 100 mL of 25 mg of eosin, and 1 mL of 35% formalin. Sperm count was determined using a hemocytometer. 10 µL of diluted sperm suspension was counted at 200 × magnification by light microscopy. Sperm motility was determined according to the method described by Sonmez et al. (13). Fluid collected from the caudal epididymis by pipette was diluted with Tris buffer solution to 2mL and the percentage of motility in a preheated system (35°C) was counted at 400 × magnification. Motility estimations were taken from three different areas of each sample and the average was reported as the final motility score. The method described by Evans and Mawwell (14) was used to identify the percentage of morphologically abnormal sperm. A total of 300 sperm were counted on each slide by light microscopy at 400x magnification from slides prepared according to this method.

Histopathological Examination

Liver and testicular tissue samples were fixed in 10% formaldehyde solution for use in histological examinations. The tissue as then embedded in paraffin, applying stages of routine tissue monitoring. 5–6-µm sections of the paraffin blocks were placed on flat slides. The prepared slides were then incubated for a predetermined time using standard histological methods. Paraffin was then removed using xylene, and the slides were diluted using a graded alcohol series in order to visualize the general histological structure stained with hematoxylin eosin. Histopathological testicular assessment was made in 50 areas according to Johnsen score (15). The liver was scored in 50 areas, taking into consideration the following criteria: sinusoidal dilatation, the presence of necrotic areas, and cellular radiation line age. These criteria were graded as follows:

Table 1. Heamatologic parameters of experimental groups

	Control (n=10)	EtOH (n=10)	PR1 (n=10)	PR2 (n=10)	EtOH+PR1 (n=10)	EtOH+PR2 (n=10)
WBC (10^3/mm^3)	7.32±1.5^{a}	11.08±2.0^{b}	9.66±2.7^{ab}	6.90±2.4^{a}	10.63±1.7^{b}	10.35±2.0^{b}
HB (g/dL)	15.18±1.2	14.77±1.5	15.27±0.4	15.56±0.5	14.88±0.9	15.40±0.5
Neutrophil (%)	17.03±2.3	18.15±2.9	17.12±7.0	16.24±3.2	17.56±2.2	16.0±3.4
Lymphocyte (%)	82.74±3.3	80.50±1.9	81.37±6.9	81.45±2.3	78.83±3.6	81.6±3.9
RBC (10^6/mm^3)	8.69±0.3^{ab}	8.14±0.7^{a}	8.52±0.4^{a}	9.18±0.3^{b}	8.43±0.2^{a}	8.28±0.6^{a}
PLT (10^3/mm^3)	738.8±33.5^{ab}	486.17±16.5^{a}	800.5±14.7^{ab}	948.2±25.7^{b}	516.0±22.4^{a}	628.0±34.5^{ab}
NRBC (10^3/mm^3)	0.10±0.07^{ab}	0.04±0.02^{a}	0.17±0.1^{abc}	0.27±0.1^{c}	0.11±0.04^{abc}	0.23±0.2^{bc}
HCT (%)	47.82±1.44	45.92±4.25	48.7±1.93	50.87±3.94	46.38±3.32	48.27±1.7
MCV(fL)	57.95±1.47^{c}	55.04±1.19^{a}	57.22±1.44^{bc}	57.28±1.35^{bc}	56.03±1.23^{ab}	56.38±1.06^{abc}
MCHC (g/dL)	32.22±0.52^{b}	30.53±0.67^{a}	32.15±0.59^{b}	32.10±0.57^{b}	31.65±0.67^{b}	31.98±0.70^{b}
MCH (pg)	17.72±0.^{22}	18.08±0.38	18.13±0.32	17.98±0.37	18.10±0.42	17.68±0.52
PCT (%)	0.55±0.02^{ab}	0.36±0.01^{a}	0.71±0.02^{b}	0.57±0.01^{ab}	0.38±0.01^{a}	0.44±0.02^{a}

PR1: Propolis 100 mg/kgbw; PR2: Propolis 200 mg/kgbw; EtOH: Ethanol; WBC: white blood cells; HB: Hemoglobin; RBC: Red blood cells; PLT: Platelet; NRBC: Nucleated red blood cells; HCT: Hematocrit; MCV: Mean corpuscular volume; MCHC: Mean corpuscular hemoglobin concentration; MCH: Mean corpuscular hemoglobin, PCT: platecrit. Data is presented as mean±standard deviation. No significant differences were seen betweengroups with the same letter (a–b) ($p<0.01$)

0=no damage, 1=little damage, 2=moderate damage, 3=serious damage. These investigations were performed using an Olympus BX 51 microscope.

Statistical Analysis

To determine the data normality, the Shapiro-Wilk's test was used, and to assess the variance homogeneity, the Levene test was used. The one-way ANOVA test was used to determine the differences between groups for continuous variables. The Tukey test was used for post-hoc analyses. Descriptive statistics were reported as mean and standard deviation. A P value less than 0.01 was considered statistically significant. A SPSS statistical package (version 22.0; SPSS, Inc., Chicago, IL, USA), was used for data analysis.

RESULTS

The phenolic compounds obtained in the chemical analysis of olive oil extracted propolis with HPLC are caffeic acid 65.92 ppm, p-coumaric acid 49.25 ppm, ferulic acid 52.4 ppm, and caffeic acid phenethylester (CAPE) 558.03 ppm. Total phenolic content for propolis in a concentration of 25% used in this study was determined to be 1621.93±26.10 mg GAE/100 g. The antioxidant activity and antiradical activity were measured as 23.75±0.38 mg AAE/g and 81.85±0.14%, respectively.

With regard to complete blood count parameters, significant differences were seen between the treatment groups and the control group (Table 1). According to hematologic parameters, No statistically significant difference was seen in the WBC value among the control, PR1 and PR2 groups. The WBC value was found to be significantly higher in all groups treated with ethanol (EtOH, EtOH+PR1 and EtOH+PR2) when compared to the control group. Although an improvement seen in the groups receiving propolis with ethanol (EtOH+PR1 and EtOH+PR2), the differences between these groups were not statistically significant. There also were no statistically significant differences between the groups with regard to hemoglobin, neutrophil, lymphocyte, hematocrit or MCH values (Table 1).

Significant differences were found with regard to the RBC value when the intervention groups were compared to the control group. The lowest RBC value was found in the EtOH group, whereas the highest RBC value was detected in the PR2 group; this difference was found to be statistically significant. Significant differences were also found between the PLT values of the groups. The lowest mean platelet value among the groups was found in the EtOH group, whereas the highest mean platelet value was seen in the PR2 group. It can be said that the PR2 dose mitigated the decrease in PLT value attributed to ethanol (Table 1).

With regard to NRBC value, the highest value was observed in the PR2 group and the lowest in the EtOH group. With regard to MCHC value, the highest value was observed in the PR1 group and the lowest in the EtOH group. The ethanol-induced NRBC value decrease was seen to improve with the administration of propolis, especially at a dose of PR2 ($p<0.01$). With regard to MCV value, the lowest MCV value was found in the EtOH group, and propolis was seen to improve the MCV value in all groups receiving propolis. Similarly, with regard to PCT, the lowest value was found in the ethanol group, while the highest was found in the PR2 group (Table 1).

When assessed in terms of biochemical parameters, although not statistically significant, the highest glucose value was observed in the EtOH group, whereas the lowest value was seen in the PR2 group. When the effect of ethanol on blood lipids was assessed, the highest levels of cholesterol and triglycerides were in the EtOH group, and propolis was found to reduce the effects of ethanol ($p<0.01$). It was found that while MDA levels increased with ethanol administration, the value decreased statistically significantly in the EtOH+PR1 and EtOH+PR2 groups ($p<0.01$). AST, ALT and ALP activities were examined to determine the effects of ethanol on liver enzymes. The difference between groups with

Table 2. Biochemical parameters of experimental groups

	Control (n=10)	EtOH (n=10)	PR1 (n=10)	PR2 (n=10)	EtOH+PR1 (n=10)	EtOH+PR2 (n=10)
Glucose (mg/dL)	246.20±75.80	280.33±66.61	245.66±44.5	209.33±19.8	245.60±30.45	242.0±28.4
Cholesterol (mg/dL)	55.0±9.66^{a}	67.6±12.7^{b}	52.67±5.81^{a}	54.83±7.25^{a}	59.0±5.25^{ab}	51.33±7.57^{a}
Triglyceride (mg/dL)	90.40±27.8^{a}	132.33±31.18^{b}	96.67±28.9^{a}	96.33±21.9^{a}	93.8±24.5^{a}	70.66±16.1^{a}
MDA (nmol/g protein)	0.95±0.008^{a}	2.44±0.15^{c}	0.90±0.08^{a}	0.92±0.11^{a}	1.17±0.09^{b}	1.23±0.10^{b}
AST (UI/L)	115.83±5.19^{ab}	227.0±2.43^{c}	114.0±8.54^{a}	96.67±9.01^{a}	159.33±2.6^{b}	127.0±3.7^{ab}
ALT (UI/L)	46.33±6.0^{a}	77.25±8.9^{b}	45.25±5.7^{a}	49.60±4.2^{a}	60.67±1.4^{ab}	40.67±4.7^{a}
ALP (UI/L)	121.0±19.0^{a}	201.0±18.6^{b}	121.33±12.3^{a}	117.0±15.2^{a}	146.25±16.7^{a}	128.33±18.1^{a}
Testosterone (ng/dL)	458.0±30.4^{bc}	70.0±6.4^{a}	348.3±24.2^{bc}	527.7±38.8^{c}	175.52±23.5^{ab}	256.33±28.5^{bc}

PR1: Propolis 100 mg/kg bw; PR2: Propolis 200 mg/kg bw; EtOH: Ethanol; MDA: Malondialdehyde; AST: Aspartate aminotransferase; ALT: Alanine amino transferase; ALP: Alkaline phosphatase. Data is presented as mean±standard deviation. No significant differences were seen between groups with the same letter (a–b) ($p<0.01$)

Table 3. Sperm characteristics of groups

	Control (n=10)	EtOH (n=10)	PR1 (n=10)	PR2 (n=10)	EtOH+PR1 (n=10)	EtOH+PR2 (n=10)
Epididymal sperm concentration (million/g)	253.33±61.10	226.66±45.02	280.00±45.61	282.00±84.85	240.0±68.11	270.00±61.64
Spermmotility (%)	27.00±6.56	19.10±6.26	35.62±19.98	38.48±10.78	20.73±16.06	24.67±10.39

PR1: Propolis 100 mg/kg bw; PR2: Propolis 200 mg/kg bw; EtOH: Ethanol. Data is presented as mean±standard deviation

Table 4. Results of histopathological assessment of testicular and liver tissues

	Control (n=10)	EtOH (n=10)	PR1 (n=10)	PR2 (n=10)	EtOH+PR1 (n=10)	EtOH+PR2 (n=10)
JTBS Testis Score	9.73±0.44^{a}	8.31±0.76^{b}	9.22±0.38^{a}	9.44±0.78^{a}	9.55±0.63^{a}	9.79±0.49^{a}
Seminiferous tubule diameter (µm)	311.73±37.73^{a}	264.29±27.36^{b}	273.28±42.27^{b}	271.66±53.37^{b}	273.39±27.02^{b}	317.96±55.12^{a}
Liver score	0.03±0.18^{a}	2.14±1.04^{b}	0.32±0.61^{a}	0.21±0.49^{a}	0.14±0.35^{a}	0.17±0.38^{a}

PR1: Propolis 100 mg/kg bw; PR2: Propolis 200 mg/kg bw; EtOH: Ethanol; JTBS: Johnson tubular biopsy score. Data is presented as mean±standard deviation. No significant differences were seen between groups with the same letter (a–b) ($p<0.01$)

regard to these 3 enzyme activities was found to be statistically significant ($p<0.01$). The activity of all 3 enzymes increased significantly in the ethanol group when compared with the control group. Especially in the EtOH+PR2 group, the increase in enzyme activity caused by ethanol was found to be normalized (Table 2).

The effect of ethanol on testosterone was also found to be significantly different between the groups ($p<0.01$). While the highest testosterone value was seen in the PR2 group, propolis was found to mitigate the decline in testosterone level caused by ethanol, especially in the EtOH+PR2 group (Table 2).

Although not statistically significant, rats administered ethanol showed reduced sperm motility and epididymal sperm concentration, whereas co-administering propolis and ethanol showed increased values (Table 3). Histological examination of the testicles revealed healthy, normal testicular structure in the control group. Deterioration of seminiferous tubule structure and decrease in spermatogenic lineage were both significantly different in the EtOH group compared to the control group. The diameter of the seminiferous tubules was significantly decreased in the intervention groups compared to the control. The testicular tissue in the PR1 and PR2 groups showed an appearance that was more similar to that seen in the control group. A significant improvement in tissue quality was observed in the EtOH+PR1 and EtOH+PR2 groups when compared with the EtOH group (Table 4, Fig. 1).

The highest liver function test values were observed in the EtOH group. Pyknotic cells were seen in the EtOH group, thought due to sinusoidal dilatation and the emergence of necrotic areas. Histological structure similar to that in the control group was observed in the PR1, PR2, EtOH+PR1 and EtOH+PR2 groups (Table 4, Fig. 2).

DISCUSSION

The main source of propolis in terrestrial regions around the world is from resinous secretions of trees, especially the black poplar (Populus nigra L). The propolis from poplar, common in Europe, typically contains "poplar bud" phenolics,known as ferulic acid, caffeic acid and their esters (16). According to the analysis results of the olive oil extracted propolis used in our study, it was confirmed that propolis contained caffeic acid, p-Coumaric acid, fer-

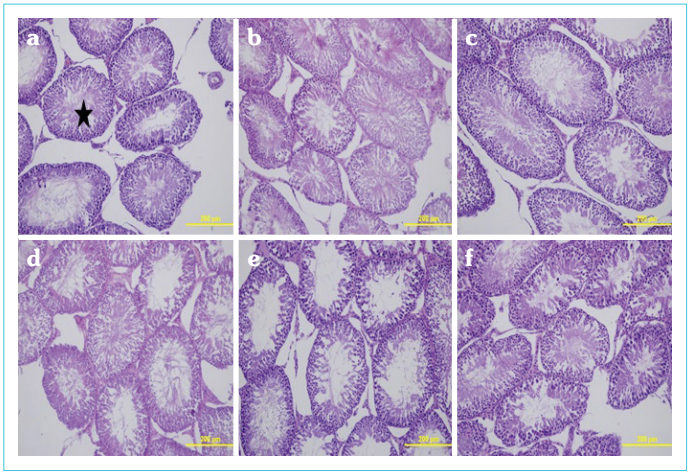

Figure 1. A: Control group—the normal seminiferous tubular structure is illustrated by the star; B: Ethanol group—the reduction and deterioration in the spermatogenic line; C: PR1 Group; D: PR2Group; E: EtOH+PR1 Group; F: EtOH+PR2 Group

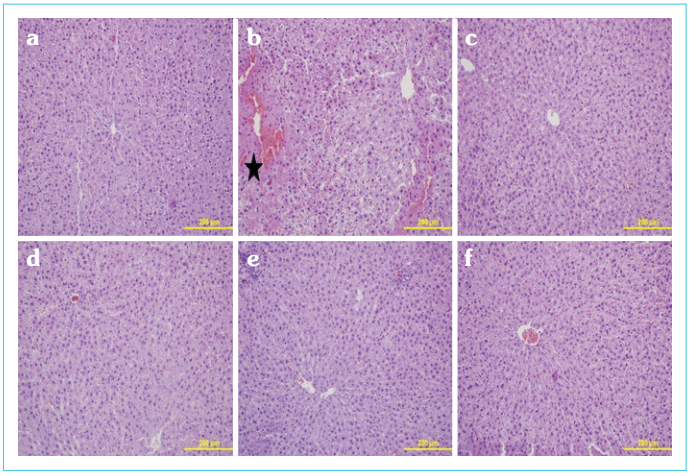

Figure 2. A: Control group—the normal liver structure is shown; B: Ethanol group—the necrotic area is illustrated by the star, C: PR1 Group; D: PR2 Group; E: EtOH+PR1 Group; F: EtOH+PR2 Group

ulic acid and phenethyl ester of caffeic acid, as in poplar propolis. Propolis extracts differ according to the solvent used for extraction. Researchers have begun to test extraction with water and vegetable oil in research in order to obtain compounds that are otherwise obtained with alcohol extraction in order to avoid the disadvantages of alcohol extraction. Olive oil extracted propolis was used instead of ethanol extracted propolis for the purposes of this study.

Pujirahayu et al. stated that the flavonoid content of propolis extract collected by honeybees (Trigona sp) prepared with different solvents such as propylene glycol, ethanol, water and olive oil was determined as 0.55%, 0.33%, 0.22% and 0.20%, respectively. They also stated that using olive oil as a solvent produces similar flavonoid content to ethanol and water (17). Kubiliene et al. reported that caffeic, trans-p-coumaric and ferulic acid were detected in all propolis extracts prepared using water, ethanol and olive oil. In addition, both ethanolic and non-ethanolic extracts have been reported to exhibit high antiradical activity (18). Russo et al. stated that propolis containing CAPE showed more active free radical repellent activity than non-cape-containing propolis. Therefore, they postulated that CAPE has a major role in the antioxidant activity of propolis (19). We could not provide a reference since we could not find a publication with a CAPE value comparable to the propolis used in our study in the literature.

With ethanol exposure, levels of adenosine tri phosphate and 2,3-diphosphoglycerate in erythrocytes decrease and cell deformation occurs due to increased oxidative stress. Consequently, increased hemolysis reduces hemoglobin levels and increases anemia (20). In this study, ethanol was found to have adverse effects on hematologic parameters. Although the difference was not statistically significant, co-administration of propolis with ethanol was seen to mitigate the negative effects of ethanol on WBC, RBC, and HCT values. Our findings indicate that the toxic effects of ethanol on hemoglobin were reduced by caffeic acid, and anemia was prevented by significantly increased hemoglobin levels in the blood. Active metabolites of caffeic acid (ferulic acid and isoferulic acid) may protect the erythrocyte membrane from deformation and hemolysis by preventing peroxidation induced by ethanol (21).

Ethanol is an agent that is well-known to cause liver damage. A measurable sign of hepatic damage is cellular enzymes euted into the plasma. An increase in serum levels of the liver enzymes AST, ALT, ALP and gamma glutamyl transferase in rats administered alcohol was observed, indicating hepatocyte damage and/or necrosis (3). Kolankaya et al. gave 200 mg/kg bw Turkish chestnut propolis to rats for 15 days. They observed a significant decrease in cholesterol and triglyceride levels in the group given propolis with alcohol and did not detect any changes in the level of glucose. ALP enzyme activity, which increased in the alcohol group declined in the propolis+alcohol group, but no changes in the AST level was observed (22). Pari et al. reported that caffeic acid used in their study appeared to protect the constructive stability of the cell membrane, normalizing serum ALT, ALP, AST and GGT (23). Treatments with ferulic acid have also been reported to significantly reduce alcohol-induced toxicity in rats (24). Certain adverse effects of ethanol were demonstrated by changes in biochemical parameters in our study (Table 2). Mani et al. reported that when rats were given 1 mg/kg/day propolis for 90 or 150 days, no changes in liver enzyme activities and serum lipid levels were seen (25). In our study, it was found that propolis had a normalizing effect on blood lipids, liver enzymes and testosterone levels in terms of ethanol-induced adversities. It was observed that the dose of propolis that best reduced ethanol toxicity was in the EtOH+PR2 group.

Ethanolic extract of Chilean propolis was investigated on human spermatozoa; propolis was shown to protect from damage caused by hydrogen peroxide (26). Moreover, propolis extract was shown to reduce the formation of TBARS (Thio barbituric acid reactive substances) and the release of LDH (lactate dehydrogenase) by preserving the sperm membrane from harmful effects of oxidative attacks. Researchers have interpreted the results they obtained to report that the protective influence of propolis on human spermatozoa is associated with its antioxidant capacity, and that propolis can be a protective agent against male infertility. Russo et al. also added propolis extract to cells subjected to benzo[a]pyrene to en-

sure that sperm is protected from the harmful effects of this mutagenic substance and exogenous reactive oxygen species. They reported that intracellular oxidants were significantly decreased, resulting in reduction in sperm DNA damage (26). These results are especially important because it has been assumed that oxidation in sperm DNA is not necessarily related to reduced cell mobility or viability and fertilization can still occur in a cell containing damaged DNA (27). Antioxidant activity has been attributed to compounds such as hydroxycinnamic acid, galangin, p-cumaric acid, caffeic acid and CAPE, which are found in the Chilean propolis extract; the antioxidant activity of hydroxycinnamic acids (ferulic, caffeic and p-Coumaric acid) and galangin has been demonstrated in numerous studies in the literature (28, 29). It has also been reported that CAPE plays a major role in this regard. Propolis extract with CAPE has shown to have more effective antioxidant activity than propolis extract without CAPE and greater antioxidant activity than galangin (28). Furthermore, it has been shown that CAPE prevents cisplatin-induced testis tissue damage in rats (30). In our study, ethanol-induced testicular damage was demonstrated histopathologically. It was seen that propolis can be protective against this damage and that the PR2 dose, in particular, was statistically significantly superior. In rats administered ethanol, the reduction in epididymal sperm concentration and sperm motility indicates testicular damage. The fact that the ethanol-induced deterioration was not statistically significantly improved with propolis administration may be related to the propolis dosage or the duration of intervention.

In conclusion, according to the data obtained during this research study, ethanol can cause adverse effects on hematological and biochemical parameters in rats, and also

changes in histopathological findings. The olive oil extracted propolis was found to have a mitigating effect on changes caused by ethanol, especially in 200 mg/kg doses. It is an important finding that products such as propolis, which are natural and without known adverse effects, show protective effects. However, in this study, ethanol was administered for only 15 days. It will be important to establish whether these effects change with increased dose and duration, and to determine the mechanism of healing action from propolis.

Ethics Committee Approval: The study was approved by the Animal Care and Use Committee at the Erciyes University Faculty of Medicine (date: 18.05.2016, number: 16/078).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – SS, NB; Design – SS, NB, ZD; Supervision – OE, SS, ECA; Resource – NB, ZD; Materials – SS, ZD; Data Collection and/or Processing – NB, ZD; Analysis and/or Interpretation – ECA, OE, SS; Literature Search – NB, SS, ECA; Writing – NB, SS, ZD; Critical Reviews – OE, SS, ECA.

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

Financial Disclosure: This project (TCD-2018-7366) was supported by the Scientific Research Directorate at Erciyes University, Kayseri, Turkey.

REFERENCES

1. Baliño P, Romero-Cano R, Sánchez-Andrés JV, Valls V, Aragón CG, Muriach M. Effects of Acute Ethanol Administration on Brain Oxidative Status: The Role of Acetaldehyde. Alcohol Clin Exp Res 2019; 43(8): 1672–81. [CrossRef]
2. Polsky S, Akturk HK. Alcohol Consumption, Diabetes Risk, and Cardiovascular Disease Within Diabetes. Curr Diab Rep 2017; 17(12): 136. [CrossRef]
3. Hosseini N, Shor J, Szabo G. Alcoholic Hepatitis: A Review. Alcohol Alcohol 2019; 54(4): 408–16. [CrossRef]
4. Abarikwu SO, Duru QC, Chinonso OV, Njoku RC. Antioxidant enzymes activity, lipid peroxidation, oxidative damage in the testis and epididymis, and steroidogenesis in rats after co-exposure to atrazine and ethanol. Andrologia 2016; 48(5): 548–57. [CrossRef]
5. Edenberg HJ, McClintick JN. Alcohol Dehydrogenases, Aldehyde Dehydrogenases, and Alcohol Use Disorders: A Critical Review. Alcohol Clin Exp Res 2018; 42(12): 2281–97. [CrossRef]
6. Kocot J, Kiełczykowska M, Luchowska-Kocot D, Kurzepa J, Musik I. Antioxidant Potential of Propolis, Bee Pollen, and Royal Jelly: Possible Medical Application. Oxid Med Cell Longev 2018; 2018: 7074209.
7. Pasupuleti VR, Sammugam L, Ramesh N, Gan SH. Honey, Propolis, and Royal Jelly: A Comprehensive Review of Their Biological Actions and Health Benefits. Oxid Med Cell Longev 2017; 2017: 1259510.
8. Fuentes E, Báez ME, Bravo M, Cid C, Labra F. Determination of Total Phenolic Content in Olive Oil Samples by UV–visible Spectrometry and Multivariate Calibration. Food Analytical Methods 2012; 5(6): 1311–9. [CrossRef]
9. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 1999; 269(2): 337–41. [CrossRef]
10. Gyamfi MA, Yonamine M, Aniya Y. Free-radical scavenging action of medicinal herbs from Ghana: Thonningia sanguinea on experimentally-induced liver injuries. Gen Pharmacol 1999; 32(6): 661–7. [CrossRef]
11. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95(2): 351–8. [CrossRef]
12. Yokoi K, Uthus EO, Nielsen FH. Nickel deficiency diminishes sperm quantity and movement in rats. Biol Trace Elem Res 2003; 93(1-3):141–54. [CrossRef]
13. Sönmez M, Türk G, Yüce A. The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats. Theriogenology 2005; 63(7): 2063–72. [CrossRef]
14. Evans G, Maxwell WMC. Salamon's Artificial Insemination of Sheep and Goats. London; Butterworth-Heinemann Ltd: 1987.
15. Johnsen SG. Testicular biopsy score count--a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. Hormones 1970; 1(1): 2–25. [CrossRef]
16. Catchpole O, Mitchell K, Bloor S, Davis P, Suddes A. Antiproliferative activity of New Zealand propolis and phenolic compounds vs human colorectal adenocarcinoma cells. Fitoterapia 2015; 106: 167–74.
17. Pujirahayu N, Ritonga H, Uslinawaty Z. Properties and flavonoids content in propolis of some extraction method of raw propolis. Int J Pharm Pharm Sci 2014; 6(6): 338–40.
18. Kubiliene L, Laugaliene V, Pavilonis A, Maruska A, Majiene D, Barcauskaite K, et al. Alternative preparation of propolis extracts: comparison of their composition and biological activities. BMC Complement Altern Med 2015; 15: 156. [CrossRef]
19. Russo A, Longo R, Vanella A. Antioxidant activity of propolis: role of caffeic acid phenethyl ester and galangin. Fitoterapia 2002; 73(Suppl 1): S21–9. [CrossRef]
20. Yokoyama A, Yokoyama T, Brooks PJ, Mizukami T, Matsui T, Kimura M, et al. Macrocytosis, macrocytic anemia, and genetic polymorphisms of alcohol dehydrogenase-1B and aldehyde dehydrogenase-2 in Japanese alcoholic men. Alcohol Clin Exp Res 2014; 38(5): 1237–46.
21. Colina JR, Suwalsky M, Manrique-Moreno M, Petit K, Aguilar LF,

Jemiola-Rzeminska M, et al. An in vitro study of the protective effect of caffeic acid on human erythrocytes. Arch Biochem Biophys 2019; 662: 75–82. [CrossRef]
22. Kolankaya D, Selmanoğlu G, Sorkun K, Salih B. Protective effects of Turkish propolis on alcohol-induced serum lipid changes and liver injury in male rats. Food Chemistry 2002; 78(2): 213–7. [CrossRef]
23. Pari L, Karthikesan K. Protective role of caffeic acid against alcohol-induced biochemical changes in rats. Fundam Clin Pharmacol 2007; 21(4): 355–61. [CrossRef]
24. Li C, Li L, Yang CF, Zhong YJ, Wu D, Shi L, et al. Hepatoprotective effects of Methyl ferulic acid on alcohol-induced liver oxidative injury in mice by inhibiting the NOX4/ROS-MAPK pathway. Biochem Biophys Res Commun 2017; 493(1): 277–85. [CrossRef]
25. Mani F, Damasceno HC, Novelli EL, Martins EA, Sforcin JM. Propolis: Effect of different concentrations, extracts and intake period on seric biochemical variables. J Ethnopharmacol 2006; 105(1-2): 95–8. [CrossRef]
26. Russo A, Troncoso N, Sanchez F, Garbarino JA, Vanella A. Propolis protects human spermatozoa from DNA damage caused by benzo[a] pyrene and exogenous reactive oxygen species. Life Sci 2006; 78(13): 1401–6. [CrossRef]
27. Potts RJ, Notarianni LJ, Jefferies TM. Seminal plasma reduces exogenous oxidative damage to human sperm, determined by the measurement of DNA strand breaks and lipid peroxidation. Mutat Res 2000; 447(2): 249–56. [CrossRef]
28. Choi MJ, Lee EJ, Park JS, Kim SN, Park EM, Kim HS. Anti-inflammatory mechanism of galangin in lipopolysaccharide-stimulated microglia: Critical role of PPAR-γ signaling pathway. Biochem Pharmacol 2017; 144: 120–31. [CrossRef]
29. Valenzuela-Barra G, Castro C, Figueroa C, Barriga A, Silva X, de Las Heras B, et al. Anti-inflammatory activity and phenolic profile of propolis from two locations in Región Metropolitana de Santiago, Chile. J Ethnopharmacol 2015; 168: 37–44. [CrossRef]
30. Mercantepe T, Unal D, Tümkaya L, Yazici ZA. Protective effects of amifostine, curcumin and caffeic acid phenethyl ester against cisplatin-induced testis tissue damage in rats. Exp Ther Med 2018; 15(4): 3404–12. [CrossRef]