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Protective effects of Turkish propolis on alcohol-induced serum lipid changes and liver injury in male rats

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Abstract

The chemical content of Turkish *Castanea sativa* propolis is investigated, along with its protective effect against alcohol-induced oxidative stress. The ethanol-propolis extract, at dose of 200 mg/kg body weight/day, was given, by gavage, to male rats for 15 days. At the end of the treatment, serum lipid levels, activities of liver enzymes and other biochemical parameters were measured. HDL level decreased and LDL level increased in the alcohol group, while HDL level increased and LDL level decreased in the alcohol + propolis group. There were decreases in cholesterol and triglyceride levels in the alcohol + propolis group. Also, there were decreases in ALP and AST enzyme activities, but LDH enzyme activity increased in the propolis treatment group compared to the alcohol group. No toxic effects of Turkish *C. sativa* propolis were found, while it caused an increase in HDL level and a decrease in LDL level. We suggest that these effects are protective against degenerative diseases and against alcohol-induced oxidative stress via free radicals.

Keywords: *Castanea sativa*; Propolis; HDL; LDL; Liver enzymes

1. Introduction

Use of propolis by humans has a long history, predated only by the discovery of honey. Propolis contains 50–70% resins and 10% essential oils, coming from the trees, mixed with 30–50% wax for proper consistency and 5–10% pollen, acquired from being transported in the bees'pollen baskets (Schechter, 2000). The worker bees apply the resin to seal any cracks and fissures in the hive and they 'line their front door' with it to prevent contamination. They use it as an antiseptic in breeder cells, and they mix propolis with wax to distribute a fine varnish over every inch of the hive to protect it (Burdock, 1998). So far, 150 compounds have been identified from propolis (Greenaway, May, Scaysbrook, & Whatley, 1991). The main chemical classes found in propolis are flavanoids, phenolics, and various aromatic compounds. However, propolis contains many of the B-complex vitamins, important minerals and trace elements. But its bioflavanoid content is now receiving attention. Bioflavanoids are antioxidant molecules that play very important roles in the scavenging of free radicals, which are produced in degenerative heart diseases, atherosclerosis, aging and effects of toxic substances (e.g. ethyl alcohol) (Challem, 1994, 1996). At least 38 flavanoids have been found in propolis, including galangine, kaempferol, quercetin, pinocembrin, pinostobin, and pinobanksin (Schmitdt & Buchmann, 2000). Some of the phenolics include cinnamyl alcohol, cinnamic acid, vanillin, benzyl alcohol, benzoic acid, and caffeic and ferulic acids. The chemical composition of propolis is highly variable because of the broad range of plants visited by honey bees while collecting the substance. Important sources include poplars, alders and birches, chestnut, ash and various Pinus and Salix species. Variations in the bees-wax content of raw propolis also affect the chemical composition.

Propolis is relatively non-toxic, with a no-effect level (NOEL) in a 90 days' mouse study of 1400 mg/kg body weight/day (Burdock, 1998). Propolis has been shown to stimulate various enzyme systems, cell metabolism, circulation and collagen formation, as well as improve the healing of burn wounds. These effects have been shown to be the result of the presence of arginine in propolis. It was reported that propolis stimulated an immune response in mice (Ivanovka, Dimov, Pavlova, Bankova, & Popov, 1995; Young, 1987). It activates

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immune cells which produce cytokines. The results help to explain the anti-tumour effect produced by propolis.

Previously-published reports indicate that oxidised low-density lipoprotein (LDL) promotes heart disease more than native LDL. Simply reducing blood levels of cholesterol is not enough to reduce the risk of coronary heart disease (CHD). Further reduction of the risk of coronary heart disease could result from eating a diet high in vitamin E, beta-carotene, and flavanoids (Challem, 1994, 1995). These results indicate that the relationship between diet and cholesterol explains only part of the relationship between diet and CHD. Dietary factors that influence LDL oxidation and thrombotic (clot-causing) factors are also of great importance (Challem, 1996). A number of studies have shown that isoflavones (a flavanoid that is present in propolis) can reduce women's risk of CHD (Challem, 1997).

Although there is a report about their hepatoprotective and protective effects against atherosclerosis and alcohol, there is no reported protective action of propolis on blood cholesterol, LDL and HDL levels. In present study, we investigated effects on serum lipid profile and liver enzymes to determine the protective effect of Turkish *Castanea sativa* propolis against alcohol-induced oxidative stress in male rats.

2. Material and methods

2.1. Chemical analysis of Turkish C. sativa propolis

Propolis used in this study was obtained from The Civan Bee Farm in Bursa, Turkey. The extract of *C. sativa* propolis was prepared in 95% of ethanol. The content of propolis was determined by GC/MS (Greenaway et al., 1991). A gas chromatograph (GC 5890, Hewlett-Packard, Palo Alto, CA, USA), coupled with a mass detector (MS 572, Hewlett-Packard, Palo Alto, CA, USA) was used for the analysis of propolis in the ethanol extracts. The GC/MS system was equipped with HP-1 column (25 m × 0.2 mm and 0.02 μm of film thickness). The flow rate of helium mobile phase was set at 1.0 ml/min. The temperature program was set as follows: temperature was held for 1 min at 50 °C, then increased to 200 °C with a 15 °C/min heating ramp and then kept at 200 °C for 5 min. Finally, temperature was increased to 280 °C with a 25 °C/min heating ramp and the temperature was kept at 280 °C for 10 min.

2.2. Animals and experimental design

In the animal experiment, 30 male Wistar Swiss albino male rats (*Rattus rattus*) 2–3 months of age and weighing 185–210 g were used. The rats were obtained from the Experimental Animals Production Centre, Hacettepe University in Ankara, Turkey. The rats were divided randomly into three groups. Each group were housed in separate cages, and laboratory conditions were 22±2 °C and 65±5% relative humidity during the study. The first group served as a control. The second group received the 20% ethanol (alcohol-stress group) and the third group received ethanol and propolis (alcohol + propolis group), each having 10 animals. The rats were fed with standard rat diet and tap water. Propolis was prepared in 95% ethanol and then its alcohol degree was reduced to 20%. This ethanol-propolis extract, at a dose of 200 mg/kg body weight/day, was administered, by gavage, to male Swiss albino rats for 15 days, every day. The same volume of 20% alcohol, depending on body weight of rats, was given to rats in the alcohol-stress group for 15 days.

At the end of the experiment, blood samples were collected from hearts of rats and, after centrifugation at 3200 rpm for 20 min, serum was separated for analysis. Serum total triglyceride, cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), and serum enzymes, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactic dehydrogenase (LDH) and biochemical parameters (urea, uric acid, glucose, creatinine, total protein, total bilirubin and albumin) were measured using a Coulter STKS Counter model S Plus.

2.3. Statistical analysis

The results of biochemical analysis were analysed using one way analysis of variance (ANOVA), followed by Dunnett's test. The results of biochemical analysis were presented as the mean±standard deviation (S.D.). Comparisons were made between control and experimental groups. Values of $P \leqslant 0.05$ were regarded as statistically significant.

3. Results

3.1. Content of Turkish C. sativa propolis

Turkish *C. sativa* propolis was analysed by GC/MS and mainly contained flavanoids. The mass chromatogram of propolis (Fig. 1A) and mass spectrum of the main flavanone component of propolis that appeared at 15.93 min retantion time (Fig. 1B) are shown in Fig. 1. Content of Turkish *C. sativa* propolis is given in Table 1. Also, flavanoids content (31.8%) of Turkish *C. sativa* propolis is given in Table 2. These flavanoids are galangin, quercetin, kaempferol, apigenin, pinobanksin, pinocembrin and pinostobin.

3.2. Results of biochemical analysis

Enzyme activities in serum of rats given alcohol and alcohol + propolis are presented in Table 3. While a

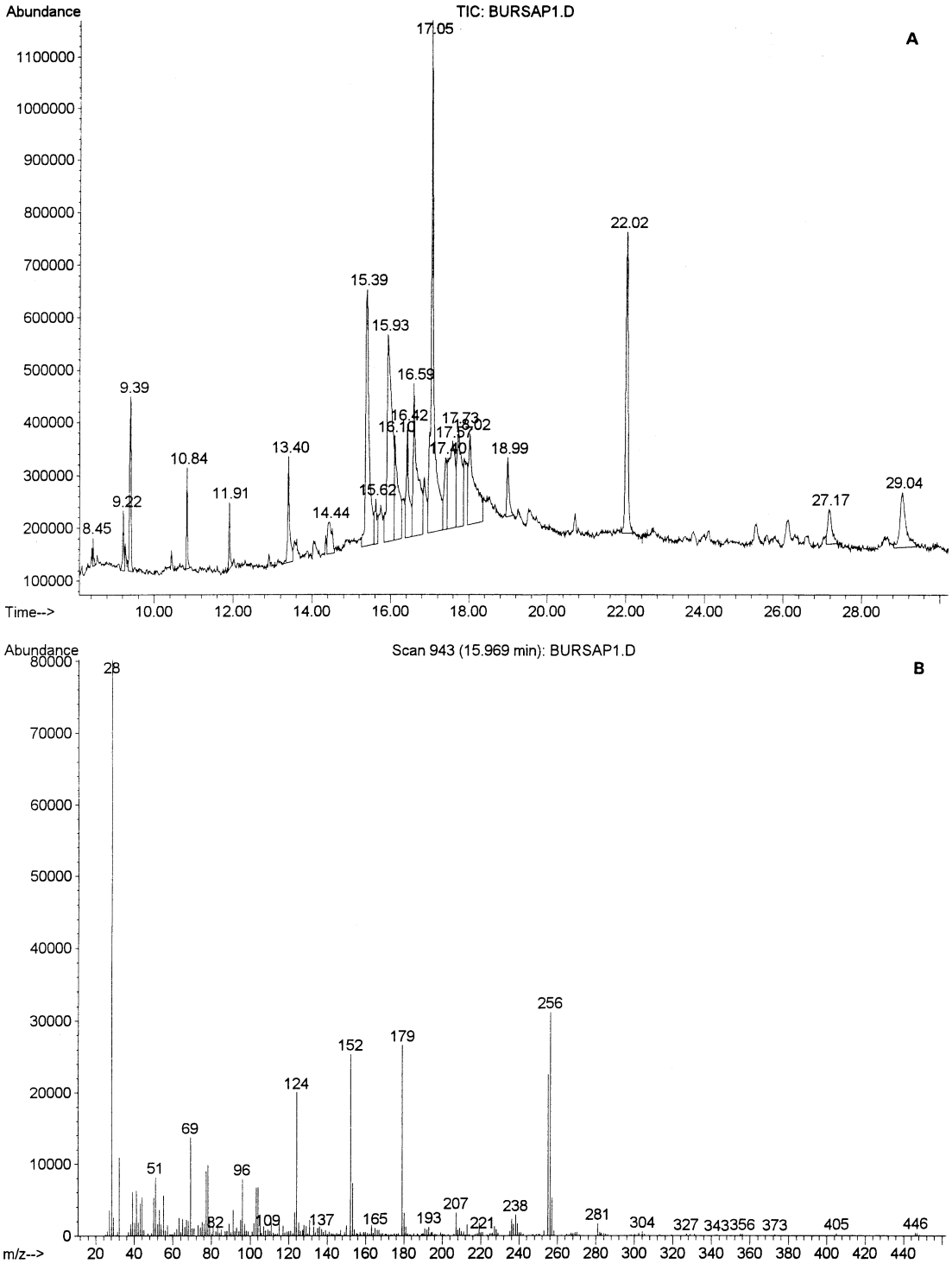

Fig. 1. Mass chromatogram of propolis (A) and mass spectrum of one flavone in sample (B).

significant increase was observed in serum AST activities of rats given alcohol, there were no changes in AST activities of rats given alcohol + propolis. Significant change was not determined in ALT activities of rats given alcohol and alcohol + propolis. A marked decrease in ALP activities of rats given alcohol + propolis was observed compared with control and alcohol groups. There was a significant decrease in lactate dehydrogenase (LDH) activities of rats given alcohol compared with control and alcohol + propolis groups.

Serum lipid levels of rats in all groups are presented in Table 4. HDL level was decreased and LDL level was increased in the alcohol group, while HDL level was increased and LDL level was significantly decreased in

Table 1
Chemical component of Turkish *Castenia sativa* propolis determined by GC/MS analysis

Chemical group	Content (%)
Aliphatic acids	2.22
Alcohols	1.71
Aromatic acids	1.52
Aromatic acid esters	13.1
Flavanones and flavones	31.8
Ketones	24.7
Terpenoids	4.50
Others	20.4

Table 2
Flavanoid components of Turkish *Castenia sativa* propolis determined by GC/MS analysis (total 31.8%)

Flavanoids	Content (%)
Galangin	9.13
Quercetin	6.21
Kaempferol	5.84
Apigenin	4.57
Pinobanksin	1.96
Pinocembrin	0.82
Pinostobin	0.58
Others	2.69

Table 3
Enzyme activities in serum of rats given alcohol and alcohol + propolis for 15 days

Parameters	Groups		
	Control	Alcohol	Alcohol + Propolis
AST (U/l)	138 ± 7.40	230 ± 25.7[a]	167 ± 16.8
ALT (U/l)	64 ± 3.30	69.3 ± 2.85	76.6 ± 5.39
ALP (U/l)	371 ± 22.5	370 ± 29.9[b]	232 ± 13.5[a]
LDH (U/l)	1044 ± 92.4	718 ± 97.6[a,b]	1045 ± 107

[a] Significantly different from control group ($P \leqslant 0.05$).

the alcohol + propolis group and LDL level was the same as the control group. There were decreases in cholesterol and triglyceride levels in the alcohol + propolis group compared with the control and alcohol groups.

The biochemical analyses of rats in control and alcohol and alcohol + propolis groups are shown in Table 5. No significant changes in glucose, creatinine, uric acid or total bilirubin levels of rats given alcohol and alcohol + propolis were determined. While a marked reduction was observed in the amount of urea of rats given alcohol, there was no significant change in the amount of urea of rats given alcohol + propolis. The amounts of albumin were increased in rats given alcohol, while it decreased significantly in rats given alcohol + propolis. The amounts of total protein of rats given alcohol + propolis were decreased compared with those of the alcohol group.

Table 4
Lipid levels in serum of rats given alcohol and alcohol + propolis for 15 days

Parameters	Groups		
	Control	Alcohol	Alcohol + Propolis
HDL (mg/dl)	37.0 ± 0.96	7.85 ± 3.7[a]	29.8 ± 1.1[a]
LDL (mg/dl)	5.8 ± 0.73	19.0 ± 4.17[a,b]	4.28 ± 1.44
VLDL (mg/dl)	21.5 ± 1.43	29.3 ± 4.03	22.7 ± 2.94
Cholesterol (mg/dl)	62.6 ± 1.83	53.8 ± 1.19[a,b]	49.0 ± 1.54[a]
Triglyceride (mg/dl)	148 ± 7.66	146 ± 20.2[b]	114 ± 14.8[a]

[a] Significantly different from control group ($P \leqslant 0.05$).
[b] Significantly different from propolis group ($P \leqslant 0.05$).

Table 5
Results of biochemical analysis in serum of rats given alcohol and alcohol + propolis for 15 days

Parameters	Groups		
	Control	Alcohol	Alcohol + Propolis
Albumin (g/l)	37.7 ± 0.41	39.8 ± 0.22[a,b]	35.3 ± 0.26[a]
Creatinine (mg/dl)	0.60 ± 0.04	0.64 ± 0.02	0.60 ± 0.01
Glucose (mg/dl)	228 ± 13.8	228 ± 11.7	208 ± 12.6
Total protein (g/l)	69.2 ± 0.60	68.6 ± 0.86[b]	63.0 ± 0.70[a]
Total bilirubin (μmol/l)	7.88 ± 0.75	6.27 ± 1.03	9.36 ± 1.21
Urea (mg/dl)	56.8 ± 2.67	46.1 ± 3.02[a]	49.8 ± 2.03
Uric acid (mmol/l)	0.12 ± 0.01	0.11 ± 0.01	0.10 ± 0.01

[a] Significantly different from control group ($P \leqslant 0.05$).
[b] Significantly different from propolis group ($P \leqslant 0.05$).

4. Discussion

The plant species available in a geographic area determine the amounts of important compounds present in propolis (Greenway et al., 1991). A recent study of New Zealand propolis revealed that the important dihydroflavonoids, pinobanksin and pinocembrin, made up approximately 70% of the flavanoids in the samples analysed. A similar study of Brazilian, Uruguayan and Chinese samples showed dihydroflavonoids to comprise less than 10% in all but one sample, (which had 50%). Turkish *C. sativa* propolis mainly contain important flavanoids (31.80%) such as galangin, quercetin, kaempferol, apigenin, pinobanksin, pinocembrin and pinostobin. These flavanoids are very important in herbal medicine (Gonzales et al., 1995). The flavanoids concentrated in propolis are powerful antioxidants, and they have been shown to be capable of scavenging free radicals and thereby protecting against lipid peroxidation in the cell membrane. It is probable that active free radicals, together with other factors, are responsible for cellular ageing and degradation in such conditions as cardiovascular diseases, arthritis, cancer, diabetes, Parkinson disease and Alzheimer disease (Decastro & Higashi 1995; Dobrowolski, Vohora, Sharma, Shah,

Naqvi, & Dandiya, 1991; Frenkel et al., 1993; Hollants, Vidal, Gra, & Stolonga, 1991; Marcucci, 1995).

In the present study, there was an increase in AST activities of rats given alcohol. Propolis caused a reduction in AST activities compared with the alcohol group. Serum AST levels are increased in hepatic diseases (Hayes, 1994). There were significant decreases in LDH activities of rats given alcohol compared with those of the control, but LDH activities of rats in the alcohol + propolis group were similar to those of control rats. There were no significant changes in activities of ALT and ALP of rats in all groups. Furthermore, propolis prevented alcohol-induced acute liver damage and lipid accumulation, which was also demonstrated in the serum lipid profile. HDL levels were high and LDL levels were low in the control and alcohol + propolis groups, while HDL levels were decreased and LDL was increased in rats treated with alcohol. Propolis positively affected the HDL and LDL levels of the rats. The effects of alcohol on blood lipid levels are almost certainly influenced by metabolism. Ethanol is converted to aldehydes by alcohol dehydrogenase and eventually converted to acetyl CoA. Acetyl CoA enters the fatty acid synthesis pathway and promotes the secretion of VLDL by liver. This will inevitably affect plasma LDL levels. To our knowledge, no report of this pharmacological property of propolis up to now has been published.

No significant differences were seen in glucose, creatinine, uric acid or total bilirubin levels of rats given alcohol and alcohol + propolis. A marked reduction was observed in the amount of urea of rats given alcohol, but not those given alcohol + propolis. There was an increase in the amount of albumin of rats given alcohol. Albumin is synthesized by the liver. Albumin, one component of plasma proteins, most often transports or binds proteins, lipids, drugs or chemicals. So, an increase in the amount of albumin may be attributed to alcohol treatment. But the amount of albumin decreased in rats given alcohol + propolis. In addition, the amounts of total protein decreased in rats given alcohol + propolis compared with those of the control and alcohol groups. The decrease in the amount of total protein may be attributed to the decrease in the amount of albumin. These results parallelled with the LDL and HDL levels.

Additional studies on the effects of propolis on lipid peroxidation in cell membrane and enzymes involved in lipid metabolism such as MDA, are necessary to offer an insight into the mechanism of action of propolis at the molecular level. Also, it is necessary to demonsrate the effects of propolis at cell and tissue levels. It is important to investigate more than 150 compounds now identified in propolis. They may be able to induce hepatoprotective effects or scavenging actions against free radicals (Challem, 1995; Pascual, Gonzales, & Torricella, 1993) and play a role in the prevention of liver injury and atherosclerosis.

In conclusion, Turkish propolis caused a protective effect against alcohol-induced liver damage and improved lipid profile. We suggest that propolis may be used to protect against toxic effects of chronic alcoholism in liver and on other degenerative diseases.

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