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RESEARCH ARTICLE

The Ameliorative Effects of Propolis against Cyclosporine A Induced Hepatotoxicity and Nephrotoxicity in Rats [1] [2]

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Summary

This study was planned to determine the effects of propolis in rats applied Cyclosporine A (CsA). In this study, 24 male Sprague-Dawley rats were used. Rats were randomly divided into 4 groups including control and 3 treatment groups. Group 1 (Control) were no supplement; CsA (group 2) were given as s.c. 15 mg/kg body weight (BW) every day; Propolis (group 3) were given by gavage 100 mg/kg BW every day; CsA+Propolis (group 4) were given as s.c. 15 mg/kg BW of CsA and by gavage 100 mg/kg BW of propolis every day. The feed intake were significantly higher (P<0.01) in Control and Propolis groups than CsA and CsA+Propolis groups within time period of 21 days. Further, body weight was significantly lower (P<0.01) in groups administrated with CsA (Group 2 and 4) than the other groups. Cortisol, AST, ALT and urea levels in serum of Control, Propolis and CsA+Propolis groups were found significantly lower (P<0.01) than those of CsA group. Malondialdehyde levels in kidney and liver tissues were significantly higher (P<0.01) than in the CsA groups compared to other groups. The catalase and reduced glutathione activities in kidney tissue of CsA+Propolis group were significantly higher (P<0.01) than those of CsA group. The present study demonstrated that propolis provided amelioration in terms of hepatotoxicity and nephrotoxicity consisting rats applied to CsA.

Keywords: *Cyclosporine A (CsA), Propolis, Hepatotoxicity, Nephrotoxicity, Rat*

Ratlarda Hepatotoksisite ve Nefrotoksisite Oluşturan Siklosporin A'ya karşı Propolisin İyileştirici Etkileri

Özet

Bu çalışma, Siklosporin A (CsA) uygulanan ratlarda propolisin etkilerini belirlemek amacıyla planlanmıştır. Çalışmada 24 adet Sprague-Dawley erkek rat kullanılmıştır. Ratlar tesadüfi olarak kontrol ve 3 muamele grubuna ayrılmıştır. Grup 1 (Kontrol)'e katkı yapılmadı; CsA her gün canlı ağırlığa (BW) 15 mg/kg s.c. olarak verildi (grup 2); Propolis her gün 100 mg/kg BW gastrik gavajla verildi (grup 3); CsA+Propolis her gün 15 mg/kg BW CsA s.c. olarak ve 100 mg/kg BW propolis gastrik gavajla verildi (grup 4). 21 günlük peryottaki yem tüketimi, Kontrol ve Propolis gruplarında CsA ve CsA+Propolis gruplarından önemli derecede daha yüksek oldu (P<0.01). Ayrıca, canlı ağırlık CsA uygulanan gruplarda (Grup 2 ve 4) diğer gruplardan önemli derecede daha düşüktü (P<0.01). Kontrol, Propolis ve CsA+Propolis gruplarında serum kortizol, AST, ALT ve üre düzeyleri, CsA grubundan önemli derecede düşük bulundu (P<0.01). Böbrek ve karaciğer dokularının malondialdehid düzeyleri CsA gruplarında, diğer gruplarla karşılaştırıldığında önemli derecede yüksekti (P<0.01). CsA+Propolis grubunun böbrek dokusu katalaz ve redükte glutatyon aktiviteleri CsA grubununkinden önemli derecede daha yüksek oldu (P<0.01). Bu çalışma, propolisin CsA uygulanan ratlarda oluşan hepatotoksisite ve nefrotoksisite açısından iyileşme sağladığını gösterdi.

Anahtar sözcükler: *Siklosporin A (CsA), Propolis, Hepatotoksisite, Nefrotoksisite, Rat*

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INTRODUCTION

Cyclosporine A (CsA) is an immunosuppressive drug that has considerably improved the survival of transplant patients in recent years [1-3]. However, several side effects have been associated with CsA treatment, such as hypertension, nephrotoxicity and neurotoxicity [4]. All alterations in mitochondrial functions, covalent binding of CsA metabolites to proteins, elevated thromboxane synthesis, and lipid peroxidation have been implicated in the CsA-mediated cell damage. Whereas its precise toxic mechanisms remain to be investigated, lipid peroxidation ascribable to oxygen radicals produced in the kidney has been proposed to be one of the major mechanisms for CsA nephrotoxicity and cell injury, which are partly reversed by some antioxidants [5].

The antioxidant serves as a defensive factor against free radicals in the body. Enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and non enzymatic antioxidant such as reduced glutathion (GSH) are the main system that opposes oxidation. If production free radicals overwhelm the capacity of enzymatic system, the second line of defense (vitamins) may come to action [6,7]. Antioxidants such as vitamin C and E extinguish free radicals and become oxidized and non-active [8,9]. Propolis contains about 300 constituents. In these days, propolis has gained popularity in connection with oxidative stress [10] and used widely as a food additive to improve health and prevent diseases such as inflammation, heart disease, diabetes and even cancer [11,12]. Flavonoids of propolis are one of the most important compounds. Compounds of propolis are being used for many biological and pharmacological activities including anticancer, anti-inflammatory, antimicrobial and antioxidant [10,13]. Flavonoids and various phenolics in propolis have been appeared to be capable of scavenging free radicals and thereby defending lipids and other compounds such as vitamin C from being oxidized or break down during oxidative stress [7]. Propolis widely began to attract the attention of scientists. The results of many animal researches showed that propolis may relieve the negative effects of oxidative stress on the body's defense system [10,14,15].

This study was planned to determine the effects of propolis on feed intake (FI), body weight (BW), body weight change (BWC), some blood parameters and antioxidant status in rats applied CsA which induced neprotoxicity and hepatotoxicity.

MATERIAL and METHODS

Drugs

CsA (Sandimmun® enj. sol., 50 mg/ml, Novartis) and propolis (Ari Dunyasi Firm, Istanbul-Turkey) were both dissolved in ethanol. CsA was injected as sub-cutaneous (s.c.) 15 mg/kg and propolis was given by gavage daily 100 mg/kg during the experimental period (for 21 days). CsA and propolis doses have been chosen, respectively, according to Rezzani et al.[3] and Seo et al.[16].

Animals, Diet and Treatment

Twenty-four healthy adult male Sprague-Dawley rats (8-10 weeks old, 280-300 g BW) were used in this study. The animals were obtained from Firat University, Experimental Research Centre (Elazig, Turkey) and were housed in stainless steel cages under standard laboratory conditions (24±3°C, 40-60% humidity, 12 h dark/light cycle). A standard commercial pellet diet (Elazig Food Company, Elazig/Turkey) containing 23% crude protein and 2.650 kcal/kg metabolic energy, and fresh drinking water were given *ad libitum*. The protocol for the use of animals was approved by the National Institutes of Health and Local Committee on Animal Research. This study was approved by the Animal Ethical Committee of Firat University (18.04.2012/57).

Rats were randomly divided into the Control and 3 treatment groups. Rats were housed in individual cages. During a 21 days period, while Control group: No supplement, group 2: CsA were given s.c. 15 mg/kg BW of CsA every day; group 3: Propolis were given by gavage 100 mg/kg BW of propolis every day; group 4: CsA+Propolis were given by s.c. 15 mg/kg BW of CsA and 100 mg/kg BW of propolis were given by gavage every day. Rats were individually weighed initially and then weekly to monitor the BW. In addition, FI and BWC at 7, 14 and 21 days of the experiment were determined. No rat died during experimental period.

Sample Collection

After 24 h of last application, rats were anaesthetized by light inhalation of diethyl ether and were decapitated, then 1.5 ml blood sample from each rat was collected for biochemical analysis. The kidney and liver tissues were removed for biochemical analysis. These samples were stored at -20°C until further analysis.

Biochemical Analysis

Serum cortisol, glucose, albumin, globulin, total protein, urea, triglycerides, HDL, VLDL, LDL, total cholesterol, creatinine, AST and ALT values were determined using autoanalyzer.

Extraction Procedure of Propolis

0.1 g sample was extracted, in 3 parallels, with 25 mL 60% ethanol and incubated for 6 days, vortexing every day. At the end of the 6th day of incubation, the extracts were sonicated for 10 min and then centrifuged for 10 min at 4.000 rpm and 4°C [17]. The extracts were then used for the spectrophotometric analysis of total phenolic content, total flavonoid content, and total antioxidant capacity. HPLC analysis was also performed to determine the phenolic profile of the propolis sample.

Spectrophotometric Assays

Analysis of Total Phenolics: The amount of total phenolics in extracts was determined with the Folin-Ciocalteau reagent using the method of Velioglu et al.[18]. To 0.1 mL of each sample (three replicates), 0.75 mL 0.1 N Folin-Ciocalteau reagent and 0.75 mL Na_2CO_3 (6%, w/v) were added. After 1.5 h, the absorbance was measured at 725 nm using spectrophotometer. Results were expressed as mg gallic acid equivalent (GAE)/g fresh weight sample *(Table 1)*.

Analysis of Total Flavonoids: The total flavonoid content was determined using the colorimetric method reported by Kim et al.[19]. 1 mL extract was mixed with 0.3 mL 5% $NaNO_2$ at t=0 min. Then 0.3 mL 10% $AlCl_3$ was added at t=5 min. After 6 min, 2 mL 1 N NaOH was added and the solution was mixed. The absorbance was measured against prepared water blank at 510 nm. Total flavonoid content was expressed as mg quercetin equivalents (QE)/g fresh weight sample *(Table 1)*.

Analysis of Total Antioxidant Capacity - DPPH Method: The antioxidant activity of the propolis extracts were assessed on the basis of the radical scavenging effect of the stable DPPH free radical [20]. 0.1 mL extract was added to 2 mL 0.1 mM DPPH in methanol solution in a test tube. After incubation for 30 min at room temperature, the absorbance of each solution was determined at 517 nm against blank (methanol). The results were expressed as mg trolox equivalent antioxidant capacity (TEAC)/g fresh weight sample *(Table 1)*.

Analysis of Total Antioxidant Capacity - CUPRAC Method: The CUPRAC (Cupric Reducing Antioxidant Capacity) method was utilized using the method described by Apak et al.[21]. First, 1 mL of 0.01M copper (II) chloride ($CuCl_2$), 1 mL of 0.0075 M neocuproine (Nc), 1 mL of ammonium acetate (NH_4Ac) buffer (pH 7.0) was mixed in a test tube. Subsequently, 0.1 mL of sample extract or Trolox was added to this mixture. Lastly, 1 mL of MQ water was included to make the final volume 4.1 mL. After 1h reaction time, absorbance was measured at 450 nm. The results were expressed as mg TEAC/g fresh weight sample *(Table 1)*.

Table 1. *The total phenolic content, total flavonoid content and total antioxidant capacity values of propolis*

Tablo 1. *Propolisin toplam fenolik madde, toplam flavonoid madde ve toplam antioksidan kapasite değerleri*

Content of Propolis	Amount in 1 g Propolis *
Total Phenolics	139.1±1.8 mg GAE
Total Flavonoids	397.6±1.2 mg QE
Total Antioxidant Capacity - DPPH	269.5±0.4 mg TEAC
Total Antioxidant Capacity - CUPRAC	494.5±1.3 mg TEAC

** Values are given as mean ± standard deviation of the values found for three replicates;* ***GAE:*** *Gallic Acid Equivalent;* ***QE:*** *Quercetin Equivalents;* ***TEAC:*** *Trolox Equivalent Antioxidant Capacity*

Determination of Phenolic Profile by HPLC Analysis

Filtered extracts were analysed using a W600 Waters HPLC system coupled to a Waters 996 photodiode array (PDA) detector as described previously [22,23]. Compounds were separated using a C18 column (150x4.6 mm, 3 µ) and applying a gradient from 95% to 25% MQ and a 5-75% acetonitrile, both in 0.1% trifluoroacetic acid (TFA) (1 mL/min flow rate) across a period of 50 min. Phenolics of propolis were detected at 280, 312, and 360 nm. For quantification, dose-response curves of available pure standards (0-500 µg/mL) were used as reference *(Table 2)*.

Lipid Peroxidation

The levels of MDA were measured as described by Candan and Tuzmen [24]. One gram sample was homogenized in 4 ml of 20 mM phosphate buffer (pH 7.4). Then the homogenate centrifuged at 15.000 x g for 15 min. The supernatant was used for analysis. Tissue lipoperoxides were hydrolyzed in dilute sulfuric acid (H_2SO_4, 1%) and then by boiling in phosphoric acid (H_3PO_4). MDA is reacted with thiobarbituric acid (TBA) to form MDA-TBA. Tissue proteins are precipitated with methanol and removed from the reaction mixture by centrifugation. HPLC analysis was performed using Scimadzu LC-20AT HPLC system. A mobile phase consisted of 40:60 (v/v) methanol-KH_2PO_4. The C_{18} column (150x4.6 mm, 5 µm, Fortis) was used with a flow rate of 0.6 ml/min (30°C), sample run was 10 min, injection volume was 20 µl and fluorescence detector wavelengths were set at 532 nm (excitation) and 553 nm (emission). Results were expressed µg/ml homogenate.

Reduced Glutathione

The GSH levels were measured spectrophotometrically at 412 nm using the method of Ellman [25]. The protein content in the kidney and liver was measured using by method of Lowry et al.[26] with bovine serum albumin as the standard.

Table 2. *Phenolic substances and quantities defined in propolis*

Tablo 2. *Propoliste tanımlanan fenolik maddeler ve miktarları*

Phenolics	Amount (mg/g) *
Pinobanksin	8.9±0.5
Pinostrobin	5.5±0.3
Pinocembrin	3.4±0.2
Chrysin	2.7±0.1
Galangin	2.2±0.2
Apigenin	1.7±0.1
Kaempferol	0.7±0.0
Luteolin	0.6±0.0

** Values are given as mean ± standard deviation of the values found for three replicates*

The GSH level was expressed as nmol/mg protein.

Catalase

The kidney and liver tissue CAT activity was determined according to the method of Aebi [27]. The principle of the method is based on the determination of the rate constant (*k*) for the H_2O_2 decomposition rate at 240 nm. Results were expressed as k/g protein.

Statistical Analysis

All values were presented as mean±SD. Differences were considered to be significant at $P<0.05$. Statistical analysis was performed using one-way ANOVA and *post hoc* Duncan's significance difference test by SPSS 21 [28] program.

RESULTS

The FI were significantly higher in Control and Propolis groups than CsA and CsA+Propolis groups in period of 21 days ($P<0.01$) *(Table 3)*. FI of Control, CsA, Propolis and CsA+Propolis groups were found as 4.11, 3.79, 4.22 and 3.83 g/day/animal in period of 21 days, respectively ($P<0.01$). The BW were significantly lower in groups administrated with CsA than other groups ($P<0.01$). Body weight of Control, CsA, Propolis and CsA+Propolis groups were found as 330.20, 257.50, 338.11 and 280.33 g/animal in period of 21 days, respectively ($P<0.01$) *(Table 3)*. The decrease of BW were significantly highest in CsA group compared with the other groups ($P<0.01$). Further, BWC of Control, CsA, Propolis and CsA+Propolis groups were found as 1.95, -1.58, 1.79, -0.83 g within a period of 21 days, respectively ($P<0.01$). The results indicate that CsA had negative effects on the FI, BW and BWC. Based on the BWC values, the negative impact of CsA on BWC was decreased by oral administration of propolis *(Table 3)*. Cortisol, HDL, LDL, VLDL, total cholesterol, AST, ALT and urea levels of Control group were significantly lower than those of CsA group ($P<0.01$) *(Table 4)*. Cortisol, AST, ALT and urea levels of Control, Propolis and CsA+Propolis groups were found significantly lower than those of CsA group ($P<0.01$) *(Table 4)*. Furthermore, MDA levels in kidney and liver were significantly the highest in the CsA groups compared to Control, Propolis and CsA+Propolis groups ($P<0.01$) *(Table 5)*. The CAT and GSH activities of CsA+Propolis groups in kidney were significantly found higher than those of CsA group ($P<0.01$). GSH activity of CsA+Propolis groups in liver was determined significantly higher than that of CsA group ($P<0.05$) *(Table 5)*.

DISCUSSION

Cyclosporine A (CsA) is a drug most frequently used in transplant surgery because of its potent immunosuppressive action. However, its clinical use is accompanied by adverse side effects such as hypertension, nephrotoxicity and hepatotoxicity [29]. Previous studies established that reactive oxygen species production and oxidative stress situation are involved in CsA hepatotoxicity [30,31]. The present work investigated the effect of propolis supplementation on the severity of CsA-induced oxidative stress, nephrotoxicity and hepatotoxicity.

The chemical composition and biological activities of propolis depend mainly upon the local flora, the geographic region, and the climate. Thus, development of analytical methods to evaluate the antioxidant capacity and to discriminate the floral origin of propolis is necessary. There are numerous methods for determining the antioxidant capacity of soluble natural extracts as well as for insoluble food components [32].

Table 3. *Effects of propolis on feed intake (FI), body weight (BW) and body weight change (BWC) of experimental groups (g)*
Tablo 3. *Deneme gruplarının, yem tüketimi (FI), canlı ağırlığı (BW) ve canlı ağırlık değişimi (BWC) üzerine propolisin etkileri (g)*

Performance	Days	Control	CsA	Propolis	CsA+Propolis	P
FI	1-7	3.96±0.09^a	3.54±0.08^b	4.02±0.14^a	3.57±0.09^b	**
	8-14	4.06±0.06^a	3.77±0.11^b	4.04±0.04^a	3.81±0.05^b	**
	15-21	4.31±0.06^b	4.01±0.10^c	4.58±0.04^a	4.08±0.10^{bc}	**
	1-21	4.11±0.05^a	3.79±0.08^b	4.22±0.06^a	3.83±0.05^b	**
BW	IW	291.20±6.35	289.16±8.76	302.17±6.31	297.00±6.61	NS
	7	302.80±5.22	279.25±9.99	311.33±8.15	289.78±7.61	NS
	14	316.90±4.99^a	268.67±5.71^b	324.67±7.39^a	281.98±5.29^b	**
	21	330.20±4.82^a	257.50±4.26^b	338.11±7.59^a	280.33±7.10^b	**
BWC	1-7	1.93±0.56^a	-1.65±0.49^b	1.52±0.32^a	-1.20±0.88^b	**
	8-14	2.01±0.43^a	-1.51±0.67^b	1.90±0.41^a	-1.11±0.86^b	**
	15-21	1.90±0.40^a	-1.59±0.51^c	1.92±0.37^a	-0.43±0.37^b	**
	1-21	1.95±0.34^a	-1.58±0.28^c	1.79±0.13^a	-0.83±0.19^b	**

IW: *Initial weight;* ^{a,b,c} *Mean values with different superscripts within a row differ significantly;* ***NS:*** *Non significant; ** P<0.01*

Table 4. *Effects of propolis on some blood parameters of experimental groups*
Tablo 4. *Deneme gruplarının, bazı kan parametreleri üzerine propolisin etkileri*

Parameter	Control	CsA	Propolis	CsA+Propolis	P
Glucose (mg/dL)	105.67±3.34	108.33±5.23	105.50±6.25	105.33±3.82	NS
Cortisol (ug/dL)	0.47±0.06^{b}	0.93±0.04^{a}	0.53±0.09^{b}	0.60±0.07^{b}	**
HDL (mg/dL)	13.97±0.55^{c}	17.43±0.49^{a}	14.78±0.40^{bc}	16.75±1.16^{ab}	**
LDL (mg/dL)	5.00±0.37^{b}	12.00±1.75^{a}	7.33±0.61^{b}	11.67±0.42^{a}	**
VLDL (mg/dL)	10.00±1.63^{b}	14.83±2.02^{a}	9.50±0.99^{b}	11.50±1.61^{ab}	**
Total Cholesterol (mg/dL)	29.03±1.82^{b}	44.30±2.57^{a}	31.62±1.72^{b}	39.88±1.44^{a}	**
Triglyceride (mg/dL)	50.33±8.41^{ab}	74.33±10.19^{a}	47.50±5.09^{b}	57.33±7.92^{ab}	*
AST (U/L)	201.17±18.81^{c}	398.50±38.51^{a}	189.83±16.64^{c}	290.16±17.69^{b}	**
ALT (U/L)	72.00±6.32^{b}	96.83±2.95^{a}	69.84±3.78^{b}	80.16±3.70^{b}	**
Total Protein (g/dL)	6.13±0.06^{a}	5.55±0.09^{c}	5.97±0.07^{ab}	5.73±0.09^{bc}	**
Albumin (g/dL)	3.75±0.06^{a}	3.23±0.08^{b}	3.68±0.06^{a}	3.53±0.07^{a}	**
Urea (mg/dL)	58.00±4.21^{b}	84.16±6.37^{a}	55.33±1.76^{b}	60.50±4.99^{b}	**
Creatinine (mg/dL)	0.25±0.02	0.24±0.01	0.27±0.02	0.27±0.01	NS
Globulin (g/dL)	2.38±0.06	2.32±0.03	2.28±0.04	2.40±0.05	NS

[a,b,c] Mean values with different superscripts within a row differ significantly; ***NS:*** *Non significant; * P<0.05; ** P<0.01*

Table 5. *Effects of propolis on MDA (µg/ml homojenat), CAT (k/g protein) and GSH (nmol/mg protein) activities of experimental groups*
Tablo 5. *Deneme gruplarının, MDA (µg/ml homojenat), CAT (k/g protein) ve GSH (nmol/mg protein) aktiviteleri üzerine propolisin etkileri*

Tissues		Control	CsA	Propolis	CsA+Propolis	P
Kidney	MDA	1.15±0.00^{4}c	3.03±0.02^{a}	1.18±0.002^{c}	2.26±0.06^{b}	**
	CAT	5.22±1.^{3}4a	3.24±0.12^{c}	4.60±0.39^{ab}	4.18±0.49^{b}	**
	GSH	64.38±1.58^{a}	31.38±2.11^{b}	59.12±7.29^{a}	49.93±9.21^{a}	**
Liver	MDA	1.14±0.002^{c}	2.43±0.13^{a}	1.16±0.009^{c}	1.90±0.016^{b}	**
	CAT	4.43±0.41^{a}	2.72±0.12^{c}	4.13±0.42^{b}	3.64±0.28^{bc}	**
	GSH	62.30±2.08^{a}	47.17±1.61^{b}	61.38±2.93^{a}	55.94±2.64^{a}	*

*[a,b,c] Mean values with different superscripts within a row differ significantly; * P<0.05; ** P<0.01*

In this study, two methods (CUPRAC and DPPH) were used to determined total antioxidant capacity for propolis. Total antioxidant capacity were investigated to the two different methods which were contained polyphenols (quercetin, catachin, naringenin, ferulic acid, caffeic acid), vitamins (ascorbik acid, α-tocopherol), thiols (glutathione cysteine), plasma antioxidants (uric acid and bilirubin), and synthetics (butylated hydroxy, anisole, tert- butyl, hydroquinone) CUPRAC assay which is based on reduction of Cu^{+2} to Cu^{+} by antioxidants. This method is simultaneously cost-effective, rapid, stable, selective and suitable for a variety of antioxidants regardless of chemical type or hydrophilicity [33].

The DPPH method, though simpler and of lower cost, has been reported to be much influenced by light, air oxygen, pH and type of solvent. Since DPPH is essentially soluble in organic solvent media. Flavonoids and other complex phenols generally exhibit moderate-to-slow reaction with DPPH. In this study, it was found higher total antioxidant capacity of propolis by the CUPRAC method than DPHH method *(Table 2)*. This may be due to DPPH assay is brings an important limitation to the determination of hydrophilic antioxidants [34].

In this study, FI and BW of rats applied CsA decreased significantly in comparison with that of Control in periods of 21 days (*P<0.01*) *(Table 3)*. This might be caused due to anorexia as aside effect of CsA [35]. Supplementation of rats with antioxidant compounds would attenuate partially the side effects of CsA-induced-oxidative stress [3]. The present study demonstrated that, FI and BW with propolis supplementation in rats applied CsA increased numerically, besides BWC in CsA+Propolis group ameliorated significantly in comparison with that of CsA group (*P<0.01*). The other a study [36] demonstrated that the CsA-treated animals lost the BW compared to those treated with control. The decrease in BW was certainly because of a parallel reduction in food intake following CsA administration. Similar finding has been reported in previous publications [37,38]. These results are in agreement with the present study. However, an increased metabolic rate caused by the catabolic effect of CsA could not be ruled out because other studies have also reported a decreased BW in CsA-treated rats although the amount of food intake remained unaltered [36,39,40]. Propolis has delicious substances like resin, wax, honey and vanillin [41]. In the present study, the attenuate in BWC of CsA+Propolis group could be connected to the tasty characteristic and flavonoid content of propolis *(Table 1, Table 2)*. It could be linked to flavanoids show antioxidant characteristics by chelating with trace elements or radicals [42,43].

Nephrotoxicity and hepatotoxisity can be determined via changes in serum biochemical parameters. Hirano et al.[44] reported that in nephrotic syndrome patients, serum total and LDL cholesterol concentrations were significantly higher than those of 15 healthy subjects ($P<0.005$). We observed that serum cholesterol parameters (HDL, LDL, VLDL, total cholesterol) ($P<0.01$) and triglyceride ($P<0.05$) of CsA administration in rats increased significantly more than those of Control group [44]. Glucose and globulin values were similar between all of groups. In this study, CsA induced hepatotoxicity characterized by increased biochemical parameters such as AST and ALT that are indicators of liver toxicity which is in accordance with our study *(Table 4)*. The transaminase enzymes such as AST and ALT are the most sensitive markers that play a majör role in the diagnosis of the liver injury. The changes in the levels of transaminases are the indicators of impaired liver function state [45,46]. Kim et al.[47] have suggested that the significant increase in the activities of hepatic marker enzymes such as AST, ALT and ALP manifested by CsA induced hepatocellular damage. Administration of propolis in rats significantly decreased the activities of AST and ALT ($P<0.01$) *(Table 4)*, suggesting that they offer protection by preserving the structural integrity of the hepatocellular membrane against CsA [48]. Similarly our study, other researchers showed that the protective effects of caffeic acid phenethyl ester is an active component of propolis obtained from honeybee hives on hepatotoxicity induced by lead acetate [49] and nephrotoxicity induced by CsA [36]. Similarly with present study, other authors [29,46] suggested that a significant decrease in serum total proteins associated with a significant elevation in hepatic thiobarbituric acid reactive substances and a decline in GSH, GSH-Px and CAT concentrations. Urea is a waste product made when protein is broken down and it is made in the liver. It is well known that blood urea nitrogen (BUN) measures the amount of urea in blood and increased BUN levels show kidney dysfunction in clinical practices [50]. We showed that CsA administration caused renal damage, which was reflected by a significant increase in serum urea levels in the CsA group in comparison with Control, Propolis and CsA+Propolis groups ($P<0.01$). In this study, propolis supplementation restored the normal values of some blood parameters (Cortisol, AST, ALT, albumine, urea) which were deteriorated after inoculation of CsA *(Table 4)*, similarly to caffeic acid supplementation [36].

We observed that the MDA levels in the kidney and liver tissues were significantly higher in the CsA group compared to the Control group ($P<0.01$). Whereas, CsA signeficantly increased kidney and liver ($P<0.01$) MDA, and decreased kidney ($P<0.01$) and liver GSH ($P<0.05$) as well as their CAT ($P<0.01$) contents [36,45,51]. Furthermore, GSH activities in kidney and liver along with CAT activity in kidney of CsA+Propolis groups were found significantly higher than CsA group ($P<0.01$). Propolis treatment partially ameliorated the CsA-induced lesions in hepatic and renal tissues. Flavonoids and various phenolics in propolis have been appeared to be capable of scavenging free radicals and thereby defending lipids and other compounds such as vitamin C from being oxidized or destroyed during oxidative damage *(Table 3)* [7,10]. Additionally, flavonoids inhibit lipid peroxidation, platelet aggregation, capillary permeability and fragility, and the activity of enzyme systems including cyclooxygenase and lipoxygenase [52]. These results clearly demonstrate the important role of oxidative stress and its relation to renal dysfunction and hepatic toxisity and also point out the protective potential of propolis against CsA nephro and hepatic toxicities. At least in part, the protection afforded by propolis is mediated through inhibiting renal and liver lipid peroxidation and increasing or maintaining the GSH and CAT contents in that tissues.

Together, it can be concluded that CsA administration in rats decreases BW and increases oxidative stress in blood and tissues. Propolis appeared to improve reduction in BW and ameliorate the toxicity of CsA by scavenging the free radicals and increasing the antioxidant activities. Therefore, propolis as an antioxidant compound administration might be appropriate to prevent CsA-induced renal and hepatic toxicity in proper dose.

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