The effects of propolis on sperm quality, reproductive organs and testicular antioxidant status of male rats treated with cyclosporine-A

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Abstract

This study was designed to determine the effects of propolis on the quality of sperm and reproductive organs in male rats treated with cyclosporine-A. In this study, 24 male Sprague-Dawley rats (280-300 g BW) at 8-10 weeks of age were used. The rats were randomly divided into control and 3 treatment groups. Each rat was placed into a cage. During 21 days (experimental period), Group 1 served as control group; Group 2 (CsA) was given 15 mg/kg BW/day of CsA by subcutaneously; Group 3 (P) was given 100 mg/kg BW/day of propolis by gavage; Group 4 (CsA+P) was given 15 mg/kg BW/day of CsA subcutaneously and 100 mg/kg BW/day of propolis by gavage. Administration of CsA to rats decreased sperm motility ($P < 0.01$) and sperm concentration ($P < 0.01$), and it increased total abnormal sperm rates ($P < 0.05$) as compared with the control group. Significant improvements were observed in the sperm motility ($P < 0.01$) and total abnormal sperm rates ($P < 0.05$) in CsA+P group. No significant differences were observed in the weights of right and left testes among all groups. The weight values of right and left epididymis were found similar to each other in CsA administrated groups. Seminal vesicle ($P < 0.01$) and prostat glands weights ($P < 0.01$) were significantly higher in CsA+P group than CsA group. As a result, it was determined that oral supplementation of 100 mg/kg of propolis had amendatory effect on the quality of sperm and reproductive organs treated with CsA administered rats. CsA treatment caused a significant increase in MDA level ($P < 0.01$) and significant decreases ($P < 0.01$) in GSH level and CAT activity when compared with the control group. Ingestion of propolis by CsA-treated rats significantly decreased the MDA level and significantly increased the GSH level when compared with the CsA alone group ($P < 0.01$).

Keywords: cyclosporine-A, propolis, rat, reproductive organs, sperm characteristics.

Introduction

Many environmental, physiological, and genetic factors have been implicated in the poor sperm functions and infertility (Vine, 1996; Auger et al., 2001; Kenkel et al., 2001; Sharlip et al., 2002).

Thus, it is very important to identify the factors/conditions which affect normal sperm functions.

Among various causes, oxidative stress has been attributed to affect the fertility status and physiology of spermatozoa (Agarwal et al., 2008). The term oxidative stress is generally applied when oxidants outnumber antioxidants (Du Plessis et al., 2008). The imbalance between the production of reactive oxygen species (ROS) and a biological systems ability to readily detoxify the reactive intermediates or easily repair the resulting damage is known as oxidative stress (Agarwal et al., 2003). The main destructive aspects of oxidative stress are the production of ROS, which include free radicals and peroxides (Bansal and Bilaspuri, 2011).

Cyclosporine-A (CsA) is an immunosuppressive drug that has considerably improved the survival of transplant patients in recent years (Xue et al., 1987; Sander and Victor, 1995; Rezzani et al., 2005). However, several side effects have been associated with CsA treatment, such as hypertension, nephrotoxicity and neurotoxicity (Tavares et al., 2003). The previous studies reported that CsA caused toxicities such as testicular (Sirinivas et al. 1998; Masuda et al., 2003) and spermatozoal toxicity (Seethalakshmi et al., 1990a, b; Iwasaki et al.,1996; Misro et al., 1999; Xu et al., 2003). 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 (Seethalakshmi et al., 1987). 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 (Kumano et al., 1989).

Propolis contains approximately 300 constituents. Latterly, propolis has gained popularity in connection with oxidative stress (Tatli Seven et al., 2012) and used widely as a food additive to improve health and prevent diseases such as inflammation, heart disease, diabetes and even cancer (Burdock, 1998; Banskota et al., 2000). Propolis flavonoids are one of the most important compounds. Propolis compounds have been used for many biological and pharmacological activities including anticancer, anti-inflammatory, antimicrobial and antioxidant (Sathiavelu et al., 2009; Tatli Seven et al., 2012). 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 (El-Khawaga et al., 2003;

Mannaa *et al*., 2011; Tatli Seven *et al*., 2012). Propolis protects the reproductive system from toxicity, especially flavonoids and phenolic compounds are responsible for antioxidant activity and show protective effects against aluminium chloride which caused testicular dysfunction, deterioration in semen quality and testosterone levels (Yousef and Salama, 2009; Ogretmen *et al*., 2014). Previous studies have reported that propolis provides increments in testosterone level, body and reproductive organ weights, the percentage of motile sperm and normal-shaped sperm and seminal plasma enzymes, and it causes reductions in the levels of free radicals, lactate dehydrogenase in rabbits (Yousef *et al*., 2010). In addition, propolis decreases dead and abnormal sperm numbers and increases testosterone levels in rats (Meurer *et al*., 2009).

This study was planned to determine the effects of propolis on the quality of sperm and reproductive organs, and antioxidant status of testis tissue in rats treated with applied CsA.

Material and Methods

Drugs

CsA was obtained from Novartis, Istanbul, Turkey (Sandimmun® enj. sol. 50 mg/ml) and propolis (dissolved in ethanol) was purchased from Ari Dunyasi Co., Istanbul, Turkey. The CsA was subcutaneously injected at the dose of 15 mg/kg and propolis was given by gavage daily at the dose of 100 mg/kg for 21 days. The CsA and propolis doses have been chosen, respectively, according to Kyung *et al*. (2003) and Rezzani *et al*. (2005).

Animals, diets and treatments

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 standart commercial pellet diet (Elazig Food Company, Elazig, Turkey) containing 23% crude protein and 2650 kcal/kg metabolic

energy, and fresh drinking water were provided *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 (Elazig, Turkey). Rats were housed in individual cages.

The rats were randomly divided in four experimental groups of six rats each: control, CsA, propolis, and CsA+propolis groups. The rats in the control group were not given anything but commercial pellet diet and fresh water. The CsA group received CsA subcutaneous injection at 15 mg/kg/BW per day for 21 days. The propolis group received propolis by gavage at 100 mg/kg BW per day for 21 days. The CsA plus propolis group received CsA subcutaneous injection of CsA + propolis for 21 days. No rat died during the experimental period. After 24 h from last administration, rats were euthanized by cervical dislocation under the anesthesia with diethyl ether and reproductive tissues were removed for analysis. These samples were stored at -20°C until further analysis.

Extraction procedure of propolis

Chromatographic analysis was carried out by Prevail C_{18} reversed-phase column (Grace Davison) (15 × 4.6 mm, 5 mm, USA); the mobile phase was methanol/water/ acetonitrile (46/46/8, v/v/v) containing 1% acetic acid (Zu *et al*., 2006). Quantification was done by the integration of the peak using external standard method. All chromatographic procedures were carried out at the temperature of 25°C.

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*. (1998). 0.75 ml 0.1 N Folin-Ciocalteau reagent and 0.75 ml Na_2CO_3 (6%, w/v) were added to 0.1 ml of each sample. This procedure was replicated for three times. 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).

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

	Amounts in 1 g Propolis*
Total phenolics	139.1 ± 1.8 mg GAE
Total flavonoids	397.6 ± 1.2 mg QE
Total antioxidant capacity - CUPRAC	494.5 ± 1.3 mg TEAC

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

Analysis of total flavonoids

The total flavonoid content was determined using the colorimetric method reported by Kim *et al*. (2003). 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 – CUPRAC method

The CUPRAC (cupric reducing antioxidant capacity) procedure was utilized using the method described by Apak *et al*. (2004). Firstly, 1 ml of 0.01 M copper (II) chloride ($CuCl_2$), 1 ml of 0.0075 M neocuproine (Nc), 1 ml of ammonium acetate (NH_4Ac)

buffer (pH 7.0) were mixed in a test tube. Subsequently, 0.1 ml of sample extract or Trolox was added to this mixture. Finally, 1 ml of MQ water was supplemented to make the final volume 4.1 ml. After 1 h reaction time, the absorbance was measured at 450 nm. The results were expressed as mg Trolox Equivalent Antioxidant Capacity (TEAC)/g fresh weight sample (Table 1).

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 (Bino *et al*., 2005; Ahn *et al*., 2007). The compounds were separated using a C18 column (150 x 4.6 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/minute flow rate) across a 50 min period. 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 standard (Table 2).

Table 2. Phenolic substances and quantities defined in propolis.

Phenolics	(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 parallel.

Determination of epididymal sperm concentration, motility and abnormal sperm rate

The sperm concentration was determined counting the spermatozoa in the right epididymis (Yokoi *et al*., 2003). The right epididymis was finely minced with anatomical scissors in 1 ml of isotonic saline in a Petri dish. It was completely squashed by a tweezers for 2 min., and then allowed to incubate at room temperature for 2 min. to provide the migration of all spermatozoa from epididymal tissue to fluid. After incubation, the epididymal tissue-fluid mixture was filtered via strainer to separate the supernatant from tissue particles. The supernatant fluid containing all epididymal spermatozoa was drawn into the capillary tube up to 0.5 lines of the pipette designed for counting red blood cells. The solution containing 0.595 M sodium bicarbonate, 1% formalin and 0.025% eosin was pulled into the bulb up to 101 lines of the pipette. The contents of the pipette were mixed by holding the ends of the pipette between the thumb and the index finger and shaking it vigorously in 100 back-and-forth 30 cm movements. The bulb of the pipette contains a small glass bead that makes thorough mixing possible. Sufficient solution was then blown from the pipette to ensure that the diluents containing no sperm were flushed from the capillary. This gave a dilution rate of 1:200 in this solution. Approximately 10 ml of the diluted sperm suspension was transferred to both counting chambers of an Improved Neubauer (Deep 1/10 mm, LABART, Darmstadt, Germany) and allowed to stand for 5 min. The sperm cells in both chambers were counted with the help of a light microscope at 200X magnification (Table 2).

The percentage of forward progressive sperm motility was evaluated using a light microscope with heated stage as described by Sonmez *et al*. (2007). For this process, a slide was placed on a light microscope with a heated stage warmed up to 37°C, and then several droplets of Tris buffer solution (0.3 M Tris (hydroxymethyl) aminomethane, 0.027 M glucose, 0.1 M citric acid) were dropped on the slide and a very small droplet of fluid obtained from left cauda epididymis with a pipette was added to the Tris buffer solution and mixed by a cover-slip. The percentage of forward progressive sperm motility was evaluated visually at 400X magnification. Motility was performed in three different fields in each sample. The mean of the three successive estimations was used as the final motility score.

To determine the percentage of morphologically abnormal spermatozoa, the slides stained with eosinenigrosin (1.67% eosin, 10% nigrosin and 0.1 M sodium citrate) were prepared. The slides were then viewed under a light microscope at 400X magnification. A total of 300 sperm cells was examined on each slide (2100 cells in each group), and the head, tail and total abnormality rates of spermatozoa were expressed as a percentage.

Lipid peroxidation and antioxidant status

Malondialdehyde (MDA) determination

The MDA level was measured as described by Candan and Tuzmen (2008). 1 g testis tissue sample was homogenized in 4 ml of 20 mM phosphate buffered saline (pH = 7.4). Then the homogenate centrifuged at 15000 x g for 15 min. The supernatant was used for the analysis. Tissue lipoperoxides were hydrolyzed in diluted sulfuric acid (H_2SO_4, 1%) and then these were boiled in phosphoric acid (H_3PO_4). MDA is reacted with thiobarbituric acid (TBA) to form MDA-TBA-2 adduct. 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 (150 x 4.6 mm, 5 μm, Fortis) was used with a flow rate of 0.6 ml/minute (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 (GSH) level

The GSH level was measured spectrophotometrically at 412 nm using the method of Ellman (1959). The protein content in the testis was measured using the method of Lowry *et al.* (1951) with bovine serum albumin as the standard. The GSH level was expressed as nmol/mg protein.

Catalase (CAT) activity

The testis tissue CAT activity was determined according to the method of Aebi (1984). 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 are presented as mean ± standard deviation (SD). Differences were considered to be significant at P < 0.05. Statistical analysis was performed using the one-way ANOVA and post hoc Duncan's significance difference test by SPSS 21, IBM, 2012 program.

Results

Sperm characteristics

Epididymal sperm motility, sperm concentration, abnormal sperm rates and reproductive organ weights are presented in Table 3. Although a significant decrease (P < 0.01) was found for sperm motility and concentration in the CsA group when compared with the control group, no difference was observed in sperm motility of the CsA+P group as compared with the control group. Administration of propolis to CsA-treated rats was able to prevent the CsA-induced reduction in sperm motility but not in sperm concentration in comparison with the CsA alone group. A significant increase (P < 0.05) was observed in abnormal sperm rate in CsA group alone when compared with the control group but propolis administration to CsA-treated rats significantly decreased this rate. No statistically significant differences were found in the values of right and left testis weights between the groups. CsA treatment significantly reduced the weights of epididimydes (P < 0.05) and all accessory glands (P < 0.01) when compared to the control group but propolis administration to CsA-treated rats increased these weights. However, the increments that were found only in the weights of right cauda epididymis and accessory glands after propolis administration had a statistical significance (P < 0.01).

CsA treatment caused a significant increase (P < 0.01) in MDA level and significant decreases (P < 0.01) in GSH level and CAT activity when compared with the control group. Ingestion of propolis by CsA-treated rats significantly decreased the MDA level and significantly increased the GSH level when compared with the CsA alone group (P < 0.01; Table 4).

Table 3. The effects of propolis on the quality of sperm and reproductive organs of experimental groups (g).

	Control	CsA	Propolis	CsA+Propolis	P
Motility (%)	70.00 ± 4.47[a]	45.83 ± 4.55[b]	68.33 ± 4.77[a]	61.67 ± 5.43[a]	**
Concentration (milion/cauda)	370.00 ± 18.44[a]	238.33 ± 26.88[c]	323.33 ± 28.13[ab]	260.00 ± 20.33[bc]	**
Abnormal spermatozoid rate (%)	2.33 ± 0.33[a]	4.50 ± 0.44[b]	2.66 ± 0.42[a]	3.16 ± 0.30[a]	*
Right Testis Weight (g)	1.38 ± 0.06	1.13 ± 0.15	1.42 ± 0.08	1.22 ± 0.07	NS
Left Testis Weight (g)	1.40 ± 0.06	1.24 ± 0.12	1.38 ± 0.06	1.20 ± 0.16	NS
Right Epididymis (g)	0.54 ± 0.02[a]	0.39 ± 0.07[b]	0.56 ± 0.03[a]	0.47 ± 0.03[ab]	*
Left Epididymis(g)	0.60 ± 0.03[a]	0.43 ± 0.05[c]	0.56 ± 0.03[ab]	0.45 ± 0.05[bc]	*
Seminal vesicles (g)	1.16 ± 0.03[a]	0.51 ± 0.09[c]	1.16 ± 0.08[a]	0.85 ± 0.09[b]	**
Prostate (g)	0.61 ± 0.07[a]	0.33 ± 0.05[b]	0.56 ± 0.02[a]	0.49 ± 0.05[a]	**
Right Caudo (g)	0.22 ± 0.01[a]	0.13 ± 0.03[b]	0.21 ± 0.01[a]	0.16 ± 0.01[b]	**

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

Table 4. Effects of propolis on MDA (μg/ml homogenate), CAT (k/g protein) and GSH (nmol/mg protein) activities in testis tissue of experimental groups.

		Control	CsA	Propolis	CsA+Propolis	P
	MDA	1.14 ± 0.004[c]	5.74 ± 0.55[a]	1.14 ± 0.001[c]	3.04 ± 0.03[b]	**
Testis	CAT	0.63 ± 1.39[a]	0.34 ± 0.10[c]	0.60 ± 0.07[a]	0.47 ± 0.08[b]	**
	GSH	64.30 ± 14.21[a]	47.50 ± 5.6[b]	46.63 ± 7.29[b]	52.57 ± 9.21[b]	**

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

Discussion

CsA treatment is probably responsible for the pathogenesis of testicular and spermatozoal toxicity associated with the oxidative stress. Although CsA-induced nephrotoxicity and hepatotoxicity are related with oxidative stress, there is no evidence concerning the relationship between cellular/biochemical

mechanisms of gonadal damage and oxidative stress (Turk *et al*., 2007). ROS are normally synthesized in several essential metabolic processes for living cells including the spermatozoa. Spermatozoa are especially sensitive to peroxidative damage because of high concentration of polyunsaturated fatty acids, which are involved in arranging of sperm maturation, spermatogenesis, capacitation, acrosome reaction and eventually in membrane fusion, and low antioxidant capacity. The lipid matrix of sperm membrane is destroyed by the peroxidation of lipids, and it is associated with rapid loss of intracellular ATP leading to axonemal damage, reduced sperm viability and increased mid-piece morphological defects, and besides it fully prevents spermatogenesis in extreme status (Sanocka and Kurpisz, 2004). In this study, it was indicated that CsA treatment significantly decreased sperm concentration and motility, and increased abnormal sperm rate in comparison with the control group. These results are in agreement with previous studies at which was indicated that CsA caused spermatozoal damage (Seethalakshmi *et al*., 1987; Seethalakshmi *et al*., 1990a, b; Iwasaki *et al*., 1996; Misro *et al*., 1999; Xu *et al*., 2003; Turk *et al*., 2007, 2010). Especially, sperm motility is an important functional measurement to predict sperm fertilizing capacity. Any negative impact on motility would seriously affect fertilizing ability (ElMazoudy *et al*., 2011). Moreover, treatment with propolis extract prevented CsA-induced reduction in sperm motility and, it increased sperm motility of rats in the propolis group (P < 0.01; Table 3). Consistent with the present results, Capucho *et al*. (2012) and Rizk *et al*. (2014) reported that propolis extract can increase the sperm count of rats. According to these findings, propolis may be have an adjuvant therapy. Testicular tissue that induced by CSA was protected from oxidation by propolis administration. Rizk *et al*. (2014) reported that administration of propolis extract to normal rats increased sperm count by 31.20% as compared with the control group. Sperm motility may be affected by altered enzymatic activities of oxidative phosphorolytic process. Oxidative phosphorolytic process is required for ATP production, a source of energy for the forward movement of spermatozoa (ElMazoudy *et al*., 2011).

It has been reported that the CsA administration causes a decrease in the reproductive organ weights of non-transplanted male rats (Turk *et al*., 2007, 2010). CsA caused significant negative effects on reproductive organ weights except for testis weights in the present study. The secretory activity of accessory glands is dependent on testosterone produced by the Leyding cell. It was reported that CsA administration leads to decrease in the level of testosterone (Sikka *et al*., 1988a, b). The decline in reproductive organ weights except for testis weights observed in the study may be explained by the fact that the secretion of this organ likely decreased because testosterone levels were reduced by CsA. Consistent with the findings of a study done by ElMazoudy *et al*. (2011) propolis administration increased (P < 0.01) seminal vesicles and prostate weights and alleviated the negative effects of CsA in the present study.

CsA treatment caused a significant increase in testicular tissue MDA level in comparison with the control, and co-administrations of propolis with CsA caused significant decrease in MDA levels, and significant increase in CAT, when compared with the CsA group. This finding is in agreement with the findings of the studies done by Turk *et al*. (2007, 2010). The increment in MDA levels are probably because of the extreme generation of ROS by blocking the permeability transition pore, providing an increase in Ca^{2+} concentration, and distruption of mitochondrial electron transport chain (Fournier *et al*., 1987; Nicolli *et al*., 1996). When ROS began to be kept under control, testes exhibit a defensive mechanism using different antioxidant enzymes. The primary detoxifying systems for peroxides are CAT and GSH. CAT is an antioxidant enzyme, which overthrows H_2O_2 that can form a highly reactive OH in the presence of iron as a catalyst (Sikka, 1996). After entering into redox cycle of gluthation, H_2O_2 and lipid peroxides were coverted to nontoxic products by GSH and GSH-Px (Nicolli *et al*., 1996; Sharma and Agarwal,1996; Sikka, 1996; Sanocka and Kurpisz, 2004). Decreasing activity of antioxidant systems is due to the toxic effect of CsA that causes an increase in lipid peroxidation and oxidative stress and subsequently testicular and spermatozoal toxicity. Phenolic compounds derived from pomegranate, vitamins C, E, melatonin, lycopene have been used as antioxidant agents to prevent different organs from various lipid-peroxidation-induced damages (Turk *et al*., 2008). Propolis treatment partially ameliorated the CsA-induced imbalance in oxidant-antioxidant system of testes (Table 4). Especially, propolis supplementation to rats treated with CsA significantly decreased the MDA level (P < 0.01), increased CAT activity (P < 0.01) but had no effect on GSH activity in comparison with the CsA group.

The first mechanism of this effect of propolis may involve the scavenging of free radicals that causes lipid peroxidation. The second mechanism can be its ability to prevent the xanthine oxidase activity, which is known to cause generation of free radicals. The reason for increase in CAT activity and decrease in MDA level of CsA+P group in the present study may be explained by potent free radical scavenging activity of flavonoid content of propolis (Attia *et al*., 2012). Flavonoids and other phenolic compounds found in propolis seem to be capable of scavenging free radicals avoiding lipids and other substances such as vitamin C to be oxidized or destroyed during oxidative damage (Seven *et al*., 2014). Otherwise, flavonoids inhibit lipid peroxidation, platelet aggregation, capillary permeability and fragility, and the activity of enzyme systems including cyclooxygenase and lipoxygenase (Seven *et al*., 2014). These results clearly demonstrate the important role of oxidative stress and its relation to testicular toxicity and also point out the protective potential of propolis against CsA. At least in part, the protection afforded by propolis is mediated through inhibiting testicular lipid peroxidation and increasing CAT contents in that tissue (Seven *et al*., 2014).

In conclusion, propolis appeared to ameliorate the toxicity on sperm quality and reproductive organs of CsA by scavenging the free radicals and increasing the antioxidant activities. Therefore, propolis administration may be a good option to diminish the negative effects of CsA on male reproduction in transplanted patients.

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