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Research Article

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Regulative Influence of Propolis on Oxidative Stress and Hormonal Changes in Chronic Unpredictable Mild Stress-induced Depression Model of Rats

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Abstract In Depression is an epidemic disease of today's life and, is one of the most important social problems that increases each year. Chronic stress-depression causes increased lipid peroxidation, release of free radicals, and hormonal imbalance. The protective properties of propolis, known as an antioxidant, on chronic unpredictable mild stress (CUMS) and its toxic effect in vivo formed the basis of our research. Four experimental groups were formed from Wistar Albino male rats: control, propolis, stress and stress + propolis. Various stressors were applied for 35 days to create a CUMS model. Propolis was given orally once a day for 35 days (100 mg / kg). Advanced oxidation products of protein, ferric reducing antioxidant power, thiobarbituric acid reactive substances, glutathione, catalase, superoxide dismutase, glutathione peroxidase, adrenocorticotropic hormone (ACTH), and cortisol (CORT) levels were examined in serum, kidney, and liver of experimental groups. In addition, ACTH and CORT stress hormone levels decreased with propolis in serum. It was observed that the induction of oxidative stress and the increase in stress hormones due to CUMS, decreased with propolis treatment. Our results show that propolis exhibits an antioxidant and hormone stabilizing potential, both protective against oxidative damage and regulating hormonal activity in depressed rats.

Keywords Chronic unpredictable mild stress, propolis, oxidative stress, adrenocorticotropic hormone, cortisol

Introduction

The main source of depression, which is defined as the disease of the modern age, is stress. Being under the influence of factors (stressors) threatening homeostasis, stress arises. The nerve pathways stimulated by stressors are the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system [1]. Stress responses encompass the relation between endocrine, nervous, and immune systems. Stress might lead to depression, cancer, increased susceptibility to infections, and immune dysfunctions, especially by increasing steroid hormone levels [2]. As a result of the effects of stressors such as daily sound stress, electric shock, and forced swimming, the "Chronic Unpredictable Mild Stress (CUMS) Model" emerges [1,3,4]. This stress model is frequently used in clinical research [5]. Exposure to unpredictable environmental stress is an important determinant of risk and severity in neuropsychiatric disorders (major depressive disorder, anxiety and post-traumatic stress) [6]. Stress-related depression is a psychological disorder associated with low mood and loss of interest in normal activities. Antidepressants are often used to struggle stress. However, some of them show undesirable side effects, and less than 50% of depression patients are treated with antidepressants [7]. Therefore, the use of safe and effective alternative therapies in the treatment of depression is increasing.

With the use of natural products and the development of folk medicine, interest in bee products such as honey, royal jelly, pollen, and propolis is growing [8,9,10]. Propolis, collected by honey bees (*Apis mellifera* L.) from plant extracts, leaves, and buds is a viscous mixture with complex resins [8,11]. Propolis, also called as bee glue, is enriched with bees' saliva and secretions containing various enzymes and is used in the construction, adaptation, and protection of hives [12,13,14], and for humans, these products are used to strengthen the immune system [11]. Until the beginning of the 21st century, 150 different compounds such as flavonoids, phenolics, various aromatic compounds, vitamins B, important minerals, and some trace elements have been identified in the content of propolis [15]. Propolis in nature consists of 30% wax, 50% resin and herbal balsam, 10% essential and aromatic oils, 5% pollen and other substances. In every region of the world, the chemical composition and biological properties of propolis have been extensively studied [16].

Stress increases the amount of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anion and hydroxyl ion, and reactive nitrogen species (RNS) such as nitric oxide (NO) produced by cellular metabolism. Locally produced ROS and RNS may spread rapidly and be neutralized by cellular antioxidants [12]. ROS and RNS are associated with cellular aging and death in conditions such as cardiovascular disease, arthritis, cancer, diabetes, Parkinson's and Alzheimer's diseases. Exposure of the organism to reactive species creates oxidative modifications with destructive impacts. Diverse compounds derived from propolis have been identified as potent inhibitors of oxidative stress [12]. The therapeutic properties of propolis are based on rich flavonoid, phenolic acid and terpenoid contents. Propolis is effective in cell membrane like vitamin C under oxidative stress conditions due to its flavonoid content. Caffeic acid phenethyl ester (CAPE) contents were stated to restrict ROS production [17]. Free radical scavenging and protective effects against membrane lipid peroxidation have also been demonstrated [18]. Propolis can reduce the cellular levels of H_2O_2 and NO. It has also been shown that the antioxidant activity of phenolic components of Turkish propolis can reduce DNA damage caused by H_2O_2 [19].

Furthermore, it has been suggested that abnormalities in the HPA axis play an important role in the development and maintenance of depressive symptoms and relapse of depression. It has been reported that traumatic or chronic stress associated with HPA abnormalities may cause long-term excessive CORT secretion and hippocampal atrophy due to neuronal death [20]. As a result of high CORT release, it can act as a mediator between major depression and associated coronary heart disease, type II diabetes, and osteoporosis [21].

Since ancient times, propolis, which has antiseptic and anesthetic properties, has been used by many civilizations to treat colds, wounds, and ulcers. Egyptians used propolis to embalm the dead. It has also been used frequently during wars to heal wounds and tissue damage [16]. Today, it is widely used in cosmetics, pharmaceutical industries and food industry for healthy foods, beverages, and nutritional supplements [12,16]. In addition, it is also widely used in alternative medicine due to its antiinflammatory, antitumor, antibacterial, antifungal, immunomodulatory, and antioxidant activities [1,2]. With this in mind, it is still not fully known whether propolis has therapeutic effects on depression [17]. Therefore, in our study, we aimed to investigate the status of oxidative changes in lipids and proteins, antioxidant systems and hormonal changes in serum, kidney, and liver of rats, which were modeled for CUMS.

Materials and Methods

Propolis Samples

The solution of propolis in water was prepared by Aksu Vital Natural Products Inc. at a concentration of 10 mg / mL. The concentration and content of the extract was pre-analyzed by Gas Chromatography-Mass Spectrometry (GC-MS, Agilent 7890A, 5975 MS; column: HP-5 (30mX0.25 mm; 0.25 µm thickness); Carrier gas: Helium). Percentage distribution of propolis content used according to GC-MS analysis results; Pinocembrin (19.30±5), Chrysin (10.97±5), Tectochyrsin (9.51±5), Alpha bisabolol (5.77±5), Pinostrobin chalcone (3.75±5), Galangin (1.12±5) ve other substances (49.58±5) [22].

Chemicals and Kits

Rat SOD and GSH-Px enzyme test kits, CORT and ACTH hormone test kits were obtained from Bioassay Technology Laboratory, China.

Animals and Experimental Design

Wistar albino male rats (250-350 g body weight (BW)) obtained from Kocaeli University Experimental Animal Medicine Research and Application Unit were used as experimental animals. They were supplied with food and water ad libitum, and kept in cages under a daily cycle of 12 h light and 12 h darkness, a temperature of 22 ± 2 °C, a humidity of 55-75%. For animals in the stress groups, the administration of CUMS was arranged in accordance with the experimental protocol [22] and propolis was treated orally every day (100 mg / kg).

Animals were divided into four identical experimental groups consisting of randomly selected 10 animals each.

I Group - Control group - were fed only normal pellet diet;

II Group - propolis treated group (P) - received a daily injection of propolis (100 mg / kg / day) for 35 days;

III Group - CUMS / stress treated group (S) - exposed to the stressors in the CUMS model for 35 days;

IV Group –CUMS and propolis treated group (S+P) - exposed to the stressors in the CUMS model for 35 days and propolis was treated orally at a dose of 100 mg / kg / day.

At the end of the 35-day period, the animals were fasted and anesthetized overnight. Blood was obtained by cardiac puncture and serum was obtained by centrifugation. Kidney and liver tissues were quickly removed, washed in 0.9% NaCl and kept on ice. Kidney and liver tissues were homogenized in cold 0.15M KCl (10%; w / v) and homogenates were centrifuged at 600 xg for 10 minutes at 4 °C. These supernatant fractions were used for biochemical analysis in tissues. Serum and tissue homogenates were stored at -80 °C until needed for analysis.

Biochemical Analysis

Thiobarbituric acid reactive species (TBARS): Serum, kidney and liver TBARS levels were determined with thiobarbituric acid [23]. Results were calculated using the molar extinction coefficient (1.56×10^{-5} M^{-1} cm^{-1}) of the resulting product.

Ferric reducing antioxidant power (FRAP): Antioxidant power was measured quantitatively in serum, diluted kidney and liver homogenates by FRAP method [24].

Advanced oxidation protein product (AOPP): AOPP levels in serum, diluted tissue homogenates were determined by the formation of triiodide ion upon oxidation of potassium iodide with chloramine-T [25]. Results are expressed in nmol / chloramine-T equivalent.

Glutathione (GSH): Measurement of GSH in diluted liver and kidney homogenates were performed using dithionitrobenzoic (DTNB) acid [26] and optical density was recorded at 412 nm. The GSH level was calculated using an extinction coefficient of 13.600 $M^{-1}cm^{-1}$ and results expressed as nmol / mg protein.

Catalase (CAT): The method applied by Worthington (1993) in diluted liver and kidney homogenates were applied. H_2O_2 was used as the substrate [27]. A CAT unit is defined as the amount of enzyme that metabolizes 1 μmol H_2O_2 at 240 nm and 25 ºC in one minute. Results were calculated using the extinction coefficient (40 M^{-1} cm^{-1}) and given as nmol / min / mg protein.

Superoxide dismutase (SOD) and Glutathione peroxidase (GSH-Px): Rat Superoxide Dismutase 1 Soluble ELISA kit and Rat Glutatione Peroxidase ELISA kit (Biossay Tecnology Laboratory, China) were used to measure SOD and GSH-Px activity in diluted liver and kidney homogenates. Tissue samples were measured at 450 nm using SpectrostarNano-BMG LABTECT GmbH Microplate Reader, according to the manufacturer's procedure. SOD results were defined as ng / L. GSH-Px results were defined as IU / mL.

Adrenocorticotropic hormone (ACTH) and Cortisol (CORT): Rat Adrenocorticotropic Hormone ELISA Kit and Rat Cortisol ELISA Kit (Biossay Tecnology Laboratory, China) were used for serum and diluted kidney homogenates ACTH and CORT levels. Samples were measured at 450 nm using SpectrostarNano-BMG LABTECT GmbH Microplate Reader, according to the manufacturer's procedure. ACTH results were defined as ng / L. CORT results were defined as ng / mL.

Protein levels: Protein levels were specified using bicinchoninic acid method [28].

Statistical analysis: The obtained results are presented as mean values ± standard errors (SE). One-way analysis of variance (ANOVA) followed by Tukey's fairly significant difference post-hoc test were used for equal variances. Statistical significance was defined as $p<0.05$.

Results

Serum: In all analyzes, propolis groups showed similar values with control groups ($p <0.05$). While AOPP and FRAP levels were not affected in the stress group, an increase in TBARS, ACTH and CORT levels were observed. Propolis decreased the levels of AOPP, which is a determinant of protein oxidation, in rats with CUMS. FRAP, which is an indicator of antioxidant capacity, was not affected by propolis treatment. Propolis reduced the increasing TBARS levels of stressed rats (Table 1). ACTH and CORT levels, which increased with stress, decreased post propolis treatment ($p <0.05$) (Figure 1,2).

Table 1: Regulative influence of propolis on serum function tests in rats with CUMS (ten rats in each group)

Groups	AOPP (µmol/L)	FRAP (µmol/L)	TBARS (µmol/L)
Control	235.2±20.4[a]	170.8±5.7[a]	0.46±0.03[a]
P	223.9±26.1[a]	162.0±8.9[a]	0.49±0.03[a]
S	327.3±34.5[a]	210.1±12.5[a]	0.59±0.04[b]
S+ P	219.4±19.0[b]	166.1±9.0[a]	0.48±0.02[c]

Data are presented as Means ± SE within each column followed by the different letter (a-c) indicate significant differences, $n = 10$, $p<0.05$.

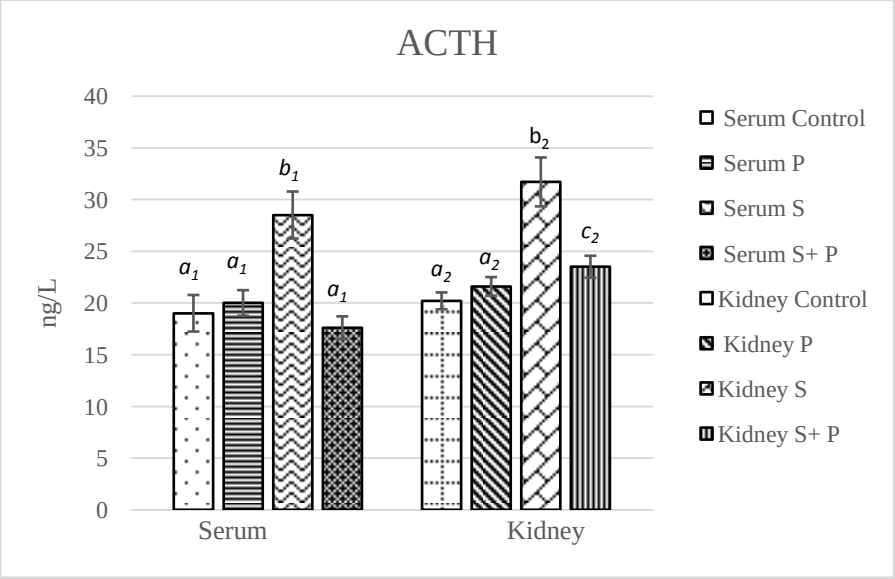

Figure 1: Effect of propolis on adrenocorticotropic hormone (ACTH) levels in serum and kidney tissues in stressed rats ($p <0.05$) (Mean ± SE, $n = 10$).

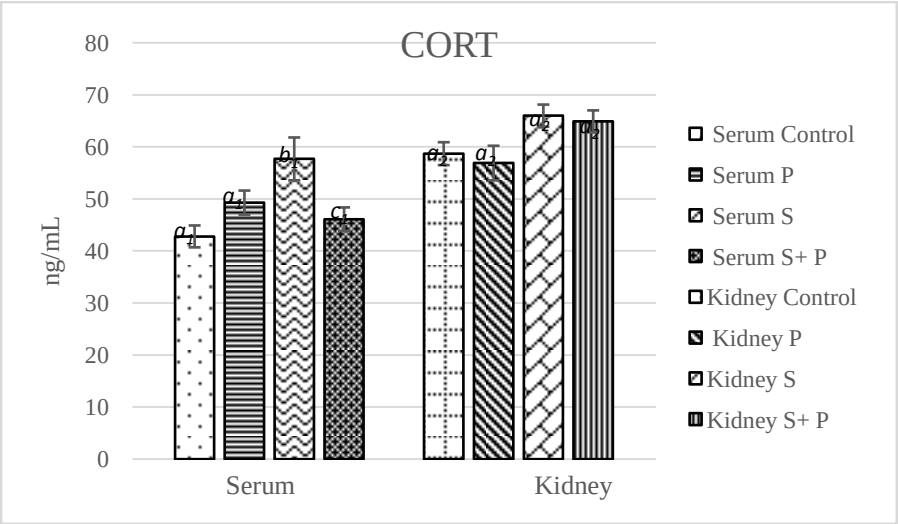

Figure 2: Effect of propolis on cortisol (CORT) levels in serum and kidney tissues in stressed rats (p <0.05) (Mean ± SE, n = 10).

Kidney: All analyzes in the propolis group did not exhibit a significant difference compared to the control group (p <0.05). In stressed rats, stress was effective in all parameters except CAT and CORT (Figure 2). Increased levels of AOPP, FRAP, and GSH in the kidney tissue of rats with CUMS, decreased with propolis treatment. Propolis did not notably reduce the lipid peroxidation that occurred in the kidney. While SOD and GSH-Px enzymes decreased in stressed and propolis-treated rats, no difference was observed among groups in CAT enzyme level (Table 2). In addition, while propolis cause a decrease in ACTH levels (Figure 1), there was no difference in CORT levels (Figure 2).

Table 2: Regulative influence of propolis on renal function tests in rats with CUMS (ten rats in each group)

Groups	AOPP (nmol/mg protein)	FRAP (nmol/mg protein)	TBARS (µmol/mg protein)	GSH (nmol/mg protein)	CAT (nmol/dk/mg protein)	SOD (ng/L)	GSH-Px (IU/mL)
Control	53.0±7.7^{a}	114.8±7.8^{a}	1.98±0.16^{a}	23.9±2.1^{a}	86.9±7.6^{a}	0.84±0.03^{a}	391.3±26.4^{a}
P	70.0±7.8^{a}	111.1±6.56^{a}	2.37±0.21^{a}	29.3±1.9^{a}	71.2±5.4^{a}	0.79±0.04^{a}	406.9±14.9^{a}
S	104.4±4.4^{b}	146.2±8.2^{b}	3.14±0.27^{b}	44.6±3.6^{b}	88.3±8.6^{a}	1.01±0.04^{b}	474.1±14.9^{b}
S+ P	65.2±4.4^{c}	117.1±7.1^{c}	2.67±0.23^{a}	28.3±3.8^{c}	57.9±3.9^{a}	0.86±0.07^{c}	410.4±14.3^{a}

Data are presented as Means ± SE within each column followed by the different letter (a-c) indicate significant differences, n = 10, p<0.05.

Liver: Propolis groups showed similar values in all analyzes compared to control groups (p <0.05). While FRAP, GSH, and CAT did not show the effect of stressors in the stress group, the levels of AOPP, TBARS, SOD and GSH-Px were increased (Table 3). Increased levels of AOPP in the liver tissue of rats with CUMS, decreased with propolis. Increased FRAP, TBARS and GSH levels did not remarkably decrease with propolis treatment. A decrease in antioxidant enzyme levels such as SOD and GSH-Px was also observed in stressed rats with propolis treatment. There was no difference in CAT enzyme levels among the groups (Table 3).

Table 3: Regulative influence of propolis on hepatic function tests in rats with CUMS (ten rats in each group)

Group	AOPP (nmol/mg protein)	FRAP (nmol/mg protein)	TBARS (µmol/mg protein)	GSH (nmol/mg protein)	CAT (nmol/dk/mg protein)	SOD (ng/L)	GSH-Px (IU/mL)
Control	77.7±6.4^{a}	102.5±5.7^{a}	1.00±0.16^{a}	50.2±7.8^{a}	198.3±15.3^{a}	0.89±0.02^{a}	458.5±16.3^{a}
P	76.6±8.9^{a}	108.0±8.5^{a}	1.15±0.17^{a}	52.1±6.5^{a}	253.0±17.1^{a}	0.79±0.03^{a}	467.1±22.5^{a}
S	118.2±7.6^{b}	123.4±8.3^{a}	1.82±0.21^{b}	69.1±4.2^{a}	255.8±19.2^{a}	1.01±0.08^{b}	627.4±23.7^{b}
S+ P	80.3±6.5^{c}	109.2±8.0^{a}	1.28±0.23^{a}	65.2±4.7^{a}	234.9±25.6^{a}	0.81±0.02^{c}	443.9±12.6^{c}

Data are presented as Means ± SE within each column followed by the different letter (a-c) indicate significant differences, n = 10, p<0.05.

Discussion

In recent years, the biological and therapeutic properties of propolis on many diseases have attracted great attention in the scientific world [29]. In particular, we tried to draw caution to the effect of propolis on stress, which is the disease of the modern age and the main cause of many diseases. Stress causes immunosuppression and is associated with the development of many diseases [2]. Animal studies have shown that, with stress, the organism becomes more susceptible to disease; it has been suggested that may cause immune system function [1], oxidative stress induction [15], hepatic damage [30] and hippocampal atrophy [31]. Many biological activities such as antimicrobial, antiparasitic, antiviral, antiinflammatory, antitumoral, antioxidant, free radical scavenger, and immunomodulator have been reported for propolis, which is accepted as an alternative medicine product [14,32]. In addition, there are several chronic studies involving different stressors, administration routes, and durations. The levels of NO, prostaglandin E2, inducible nitric oxide synthase, cyclooxygenase-2, and nuclear factor kappa B increased in the hippocampus and prefrontal cortex in rats treated with CUMS for 28 days [7]. In another chronic study group, it was reported that it caused an increase in plasma CORT and ACTH levels, an increase in brain tissue malondialdehyde (MDA) levels, and a decrease in GSH and SOD levels in mice exposed to 18-hour restraint stress [33]. To the best of our knowledge, there appears to be a lack of information in the literature on how chronic stress administration (CUMS) affects serum, kidney and liver oxidative stress and hormone activity, and the protective effect of propolis. In addition, no studies were found on AOPP, which reflects protein glyoxidation.

The effect of propolis on increased oxidative stress markers (AOPP, FRAP, TBARS, and GSH), antioxidant enzymes (CAT, SOD, and GSH-Px), and changes in hormone levels (ACTH and CORT) were investigated in rat serum, kidney, and liver. In our study, it was observed that while stress increased oxidative stress, renal and hepatic damage, propolis reduced these effects. Increases observed in TBARS, ACTH and CORT levels in serum indicated the presence of prooxidant state and activation of stress hormones due to CUMS. Propolis caused significant decreases in AOPP, TBARS and CORT levels, which increased due to CUMS, but did not cause a decrease in the high ACTH levels sufficiently. Kolankaya et al., (2002) has shown that Turkish propolis prevents alcohol-induced acute liver damage and lipid accumulation in the serum lipid profile [15]. The protective effect of five types of Argentine propolis collected from different regions against copper-mediated oxidative modification of serum lipids has been described [34]. In another study, it has been shown that daily intake of propolis in powder form for 15 days reduces plasma MDA concentration in men [35]. In contrast, treatment of propolis to stressed mice has been reported to result in a higher serum corticosterone production [2]. Treatment with propolis essential oil (PEO) has been reported to notably reduce CORT and ACTH levels in restriction stress mice, reduce MDA level in treatment with all PEO doses, and significantly improve SOD activity at high PEO doses [33]. Our data obtained in serum showed similar results with other studies, and it was seen that propolis had therapeutic effects on depression by weakening oxidation mechanisms in serum lipids and proteins, and reducing stress hormone secretion.

Propolis decreased AOPP, FRAP and ACTH levels in the kidneys of rats with CUMS, but did not adequately affect high TBARS and CORT levels. Increasing AOPP levels in liver tissue decreased with propolis treatment, while no effect of propolis was observed on TBARS levels. In addition, while propolis did not affect the CAT and GSH levels in both tissues, the antioxidant enzymes SOD and GSH-Px showed the opposite effect. In the study conducted by Valente et al. (2011), they showed that Portuguese propolis extracts in primary cell cultures isolated from human kidney tissues could prevent or reduce lipid peroxidation and hemolysis caused by peroxyl radicals [18]. It has been reported that GSH depletion caused by acetaminophen is restored by propolis extract treatment in the modulation of GSH metabolism [36]. Badr (2016), showed that propolis water extract is associated with therapeutic and hepatoprotective effects on methotrexate-induced liver toxicity in mice [37]. In several other studies, hepatoprotective and therapeutic effects of ethanol extracts of propolis on liver damage have been reported [36,38]. They found that with low levels of MDA, propolis exhibited a dose-dependent inhibitory effect on lipid peroxidation, and considerably increased SOD and CAT activity [38]. On the other hand, daily intake of low or high doses of propolis in diabetic rats has been shown to reduce hepatic and pancreatic MDA and protein carbonyl concentrations [39]. The chemical compound of propolis is variable and directly depends on the geographical origin and local flora. Season, climate, bee species or genus and extraction method impres the composition of propolis

extract [11,40]. CAPE, another active ingredient of propolis, has been reported in many studies as a powerful agent in preventing oxidative stress in the liver for diverse reasons [30,41]. It was observed that high MDA levels decreased remarkably, and CAT and SOD enzyme activities increased with CAPE treatment. These results showed that CAPE has a hepatoprotective effect against hepatotoxicity induced by carbon tetrachloride, alcohol and rich greasy feed in rats [41]. It has been reported that antioxidant enzyme activities and GSH levels increased considerably compared to the chronic stress group as a result of chronic stress and CAPE treatment [30]. When compared with previous studies, our data showed similarity in TBARS levels, which are the indicator of renal and hepatic oxidative damage. In addition, we determined that increased AOPP and FRAP (just in kidney tissue) levels, which were not evaluated in many studies, reached normal levels with propolis treatment. We have demonstrated that propolis removes or inhibits the formation of free radicals, reducing or inhibiting protein glycation and lipid peroxidation in rats treated with CUMS. However, contrary to these studies, an increase owing to propolis was not observed at a sufficient level in the amount of GSH stores and antioxidant enzyme activities. The reason for this can be considered as the differences in the amount of active ingredients in the regional propolis content used or in the daily dose intake. In addition, many researchers concluded that the hepatoprotective effect of propolis may be owing to its inhibitory, free radical synthesis inhibitory or free radical scavenging effect on lipid peroxidation mechanisms [41].

Conclusions

Since the presence of free radicals in the pathogenesis of stress-induced toxicity is well known [15], the free radical scavenging effect of propolis is well supported in this study. We observed that propolis has an inhibitory effect on the HPA axis, and this can reduce or reverse the damage caused by depression. Although there are a few studies confirming the antioxidant activity of propolis, there are no strict datas regarding the safe dose that can be recommended. Therefore, it is suggested that clinical studies using propolis and its active ingredients are needed [12]. Overall, our findings determined that propolis has a protective effect against stress, improves protein and lipid profile, and reduces stress hormone levels. At the same time, we think that propolis can be used as a promising therapeutic agent in chronic stress and other degenerative diseases related to stress.

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