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Investigation of dose related effects of propolis on anxiety and some biochemical parameters with sympathetic skin response and increased T-maze

Propolisin anksiyeteye ve bazı biyokimyasal parametrelere etkilerinin sempatik deri cevabı ve yükseltilmiş T labirent ile araştırılması

Mustafa Nisari [1], Memet Hanifi Emre [2], Nazan Dolu [3], Hale Acer [4], Ferhat Pektaş [5]

[1] Department of Nutrition and Dietetics, Nuh Naci Yazgan University, Faculty of Health Science, Kayseri, Turkey
[2] Department of Physiology, Bahçeşehir University Faculty of Medicine, İstanbul, Turkey
[3] Department of Physiology, Başkent University School of Medicine, Ankara, Turkey
[4] Talas Toplum Sağlığı Merkezi, Kayseri
[5] Department of Physiology, BahçeAhi Evran şehir University Faculty of Medicine, Kırşehir, Turkey

ORCID ID of the author(s)

MN: 0000-0001-7469-8921
MHE: 0000-0001-8695-5913
ND: 0000-0002-3104-7587
HA: 0000-0002-7823-2034
FP: 0000-0002-1862-9515

Corresponding author/Sorumlu yazar:
Mustafa Nisari
Address/Adres: Nuh Naci Yazgan Üniversitesi Sağlık Bilimleri Fakültesi Beslenme ve Diyetetik Bölümü, Kayseri, 38090, Türkiye
e-Mail: mnisari@nny.edu.tr

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Abstract

Aim: Propolis has been shown to have anti-microbial, antioxidant, anti-tumor, anxiolytic and anti-inflammatory effects. However, to the best of our knowledge, there are no studies on its anxiogenic effects. In this study, we aimed to investigate the effects of different doses of propolis on anxiety in rats with cold stress via sympathetic skin response (electrodermal activity) and elevated T maze.

Methods: Forty Wistar albino male rats were used in the study, divided into four groups: The control group, low dose (10 mg/kg PRO), medium dose (30 mg/kg PRO) and high dose propolis groups (50 mg/kg PRO). Propolis was administered via gavage to all rats except the control group. Twenty minutes after injection, the anxiety scores of the rats were evaluated with an elevated T-maze, and their electrodermal activities (EDA) were measured. At the end of the experiment, some enzymatic and lipid values were measured with malondialdehyde (MDA) in blood samples.

Results: The percentage of time spent on open arms and the number of open arm entries were lower in the 10mg/kg PRO group, while an increase was observed in the 30 mg/kg PRO group. EDA values were lower in the 30 mg / kg PRO (P=0.012; P=0.02, respectively) and 50 mg / kg PRO (P=0.013, P=0.02, respectively) groups as compared to the control group. MDA was significantly lower in the 30 mg/kg PRO and 50 mg/kg PRO groups. While AST value increased in the 30 mg / kg PRO group, ALT value decreased. Total cholesterol and triglyceride values were significantly lower in the 50 mg / kg PRO group. HDL value increased significantly after administration of propolis and LDL value decreased significantly only in the 10 mg/kg PRO group.

Conclusions: According to the results obtained by EDA and T labyrinth methods, while low and high dose propolis, which was administered to rats after cold stress, showed an anxiogenic effect, medium dose propolis exerted an anxiolytic effect. It also decreased MDA values in the medium and high dose groups and influenced enzymatic and lipid values in favor of the rat. It was concluded that the anxiety-related effects of propolis were dose-dependent.

Keywords: Propolis, EDA, Anxiety, T-maze

Öz

Amaç: Propolisin anti-mikrobiyal, antioksidan, anti-tümör, anksiyolitik ve anti-inflamatuar etkilere sahip olduğu gösterilmiştir. Ancak literatürde propolisin anksiyojenik etkisi konusundaki çalışmalara rastlanılmamıştır. Bu çalışmamızda, soğuk stresi oluşturulmuş sıçanlarda propolisin farklı dozlarının anksiyeteye etkilerinin sempatik deri cevabı (elektrodermal aktivite) ve yükseltilmiş T labirent ile araştırılması amaçlandı.

Yöntemler: Çalışmada 40 adet Wistar albino erkek sıçan kullanıldı. Kontrol grubu, düşük doz (10 mg/kg PRO), orta doz (30 mg/kg PRO) ve yüksek doz propolis (50 mg/kg PRO) grupları oluşturularak, kontrol grubu hariç diğer gruplara propolis gavaj yoluyla uygulandı. Enjeksiyondan 20 dakika sonra, sıçanların anksiyete skorları yükseltilmiş T-labirent ile değerlendirildi ve daha sonra da elektrodermal aktiviteleri (EDA) ölçüldü. Deney sonunda kan örneklerinde, spektrofotometrik olarak malondialdehit (MDA) ile bazı enzimatik ve lipid değerleri ölçüldü.

Bulgular: 10 mg/kg PRO grubunda açık kolda harcanan zamanın yüzdesi ve açık kola giriş sayısı, diğer gruplara göre daha düşük bulunurken 30 mg/kg PRO grubunda artış gözlendi. EDA, 30 mg/kg PRO (sırasıyla, P=0,012, P=0,02) ve 50 mg/kg PRO (sırasıyla, P=0,013, P=0,02 gruplarında kontrole kıyasla daha düşüktü. MDA'nın 30 mg/kg PRO ve 50 mg/kg PRO gruplarında önemli oranda düşük olduğu görüldü. 30 mg/kg PRO grubunda AST değeri artış gösterirken ALT değerinde azalmanın olduğu görüldü. Toplam kolesterol ve trigliserit değerleri 50 mg/kg PRO grubunda anlamlı olarak düşüktü. HDL değerinin düşük doz propolisten uygulamasından itibaren anlamlı yükseldiği, LDL değerinin ise sadece 10 mg/kg PRO grubunda anlamlı azaldığı olduğu görüldü.

Sonuçlar: EDA ve T-labirent yöntemleriyle elde edilen sonuçlara göre düşük ve orta doz propolis anksiyetik etki yaparken, yüksek doz propolis anksiyolitik etki göstermiştir. Ayrıca sıçanlara soğuk stres sonucunda uygulanan propolisin MDA değerlerini orta ve yüksek doz gruplarında düşürdüğü, enzimatik ve lipid değerlerinde ise organizma lehine olumlu değişimlere sebep olduğu görüldü. Dolayısıyla propolisin anksiyeteye ilişkin yanıtlarının, doza bağlı etkilerine göre değiştiği sonucuna varılmıştır.

Anahtar kelimeler: Propolis, EDA, Anksiyete, T-labirent

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Introduction

Anxiety disorders, one of the most common psychiatric diseases worldwide, constitute an important health problem [1]. There are many types of anxiety, including post-traumatic stress disorders, phobias, panic disorders, and obsessive–compulsive disorders, all of which usually share important mental and physical symptoms such as nervousness, tremor, racing thoughts, agitation, emotional discomfort, and insomnia [2]. Although new drug types have not been adopted since the introduction of selective serotonin uptake inhibitors (SSRIs) and other antidepressants for the treatment of anxiety, advancement in anxiolytic drugs has been a major focus of the pharmaceutical industry and academic neuropsychiatric investigations [3]. Anxiety research relies on similarities between human emotional behavior and behaviors in animals, such as the rat and the mouse [4]. There are many rodent behavioral paradigms that aim to model anxious behavior, such as anxiety-related defense behavior (ARDEB), the elevated plus maze (EPM), the light-dark box (LD) and the open field (OF) [3].

In psychological research, one of the most utilized indices of the autonomic nervous system is electrodermal activity (EDA), which usually measures the level of skin conductivity (SCL) [5]. SCL reflects tonic arousal [6]. EDA, for which the change in skin potential or resistance of sweat gland reaction that is controlled by the sympathetic nervous system (SNS), is measured, has been used in studies examining emotion [7], attention [8], and psychopathology. The relationship between anxiety and sympathetic skin response (SSR), in other words, SCL, has been widely studied. The results of studies on human subjects and animals indicate that anxious subjects have greater sweat gland activity [9,10]. However, data are limited in animals. These studies were conducted to define the anxiolytic activities of some drugs, and to measure anxiety, fear response, stress, and arousal [11,12].

The elevated T maze test is a widely used anxiety measurement method based on the natural avoidance of rodents from high and open fields. Untreated animals usually spend more time in closed arms. The percentage of time spent in open arms is regarded as an index of anxiety. It is generally known that anxiolytic drugs increase the number of entries in open arms (OAEs) and the time spent in there (TSOA), while the anxiogenic drugs decrease these parameters. Certain pharmacological compounds may affect anxiety-related behaviors depending on the dose [9].

Natural products have been used in medicine for various purposes for centuries. Propolis, which is a natural product, is gaining increasing importance due to its antioxidant effect against pathogenic microorganisms [13]. Propolis has antibacterial, antifungal, anti-inflammatory, antioxidative, adaptogenic, and anxiolytic effects [14-19].

Studies investigating the effect of propolis on anxiety have generally been studied in one or two doses. These studies were conducted directly on the rats without anxiety, and anxiety was measured in the open area, forced swimming test and plus maze. In the literature, there is no study investigating the effect of different doses of propolis on anxiety by creating an anxiety model. The aim of this study was to determine the effect of propolis on the behavioral scores of rats, assessed by elevated plus-maze (ETM) and EDA, and which mechanism plays a more effective role on anxiety.

Materials and methods

Experimental animals

All protocols were approved by the Animal Care and Use Committee (3/11/2015, 15/65 Ethics Committee) of Erciyes University. About 3-4 months-old male Wistar albino rats (average body weight of 250-300 g) were supplied by the Laboratory Animal Unit of Experimental and Clinical Research Center, Erciyes University and kept under controlled conditions (25±1°C temperature, 55% relative humidity and 12 h dark/light cycles). Food and water were allowed ad libitum during the experimental period. All animal experiments were conducted in the Laboratory of Brain Dynamics, Erciyes University Faculty of Medicine, Department of Physiology.

Preparation of propolis

1.2 g of propolis extract was weighed on a precision scale. It was placed in the measuring cup and dissolved in a magnetic stirrer. Propolis was prepared for each subject with ethanol and distilled water at a dose of 0.012 g/ml/kg. First, 50 ml of ethanol was added slowly. Then, a total of 500 ml of water was added and the propolis was allowed to dissolve in the magnetic stirrer for 40 minutes. The undissolved particles were passed through filter paper and mixed after re-addition to the medium. Following these procedures, the mouth of the container was left open for 1 week to remove alcohol. For low dose propolis, medium dose propolis and high dose groups, 50 µl, 75 µl and 250 µl were withdrawn from the propolis solution and completed to 5 ml with distilled water.

Cold stress

Animals were kept in a cold room (+4ºC) between 08:00 and 10:00 for 2 hours each day during the 5-day experiment. The body weights of the rats were measured to determine the effect of the cold stress procedure. Also, the rectal temperatures of the animals exposed to cold were measured immediately after this application [20]. The rat groups and each of the rats were studied individually. In cold-exposed rats, propolis was administered after cold exposure, when rectal temperatures normalized. Propolis was administered 30 minutes prior to the ETM. The sympathetic activity via skin conductivity was then recorded with A/AgCl electrodes attached to the plantar surfaces of the posterior extremities for EDA measurement.

Forming the experimental groups

In the study, 40 male rats, 10 rats in each group, were used.

Control group: Physiological saline was administered by gavage to each rat.

Low dose propolis group (10 mg/kg PRO): 10 mg/kg of propolis was administered by gavage to each rat.

Medium dose propolis group (30 mg/kg PRO): 30 mg/kg of propolis was administered by gavage to each rat.

High dose propolis group (50 mg/kg PRO): 50 mg/kg of propolis was administered by gavage to each rat.

Elevated T-maze test (ETM)

The rats were subjected to an ETM, to determine if propolis affected anxiety-related behavior [12]. Briefly, the ETM

consisted of a central platform (5 cm×5 cm), with two open arms (50 cm×10 cm×50 cm), and one closed arm (50 cm×10 cm×40 cm). The arms were arranged in such a way that each type was opposite each other. The maze was 50 cm above floor level and tests were conducted under a dim red light. The animals were placed individually on the central platform of the T-maze facing an open arm. Two observers recorded the number of times spent in the open and closed arms and the number of entries into each arm during a 5-minute period. The percentage of time spent in the open arms and the number of entries into these arms were used to measure anxiety [9].

Electrodermal activity (EDA)

The physiological recordings took place in a dimly lit, electrically and acoustically shielded experimental room. EDA was measured using the MP30 system (MP30, Biopac Systems Inc, Santa Barbara, CA). EDA was recorded between the paw pads of both hindlimbs using 2 Ag/AgCl electrodes after ETM. NaCl electrode (0.05 M) jelly was placed between the skin and the electrodes. The two electrodes were connected to the MP30 system. The signals received from the skin were converted to digital signals by MP30 data acquisition unit and processed for off-line analysis on an IBM-AT computer located in a separate room. Digital signals were stored in the computer for data analysis. The mean of skin conductance (SC) was expressed as SCL [ln (μmho)/cm^2 per electrode area]. Two recordings were obtained in 2 sections for all animals. Tonic section was recorded over 2 minutes without any stimuli. The phasic section was recorded by giving 15 auditory stimuli, which were of 1-second duration, 1000 Hz tones with 50-ms rise and fall times. The sound chip of a computer produced the tones. They were amplified with an audio amplifier (Harvard). The intensity of the tones was 90 dB as measured by a sound level meter positioned at the approximate location of the rat's ear. The tones were presented against a 50-dB pink noise background. They occurred at pseudorandom intervals ranging from 30 s to 65 s and averaging 45 s. The mean SCL values were also calculated off-line for phasic EDA. Values of phasic SCL averaged at 10 rats per group during the test period, which used 15 auditory stimuli [9].

Procedure

The animals were conscious during the recordings. Propolis was administered to the rats in three different doses at once. The ETM measurements started 30 min after the gavage. The ETM apparatus was wiped clean with a sponge and dried with a cloth between tests. After ETM, SCL was recorded, without losing any time.

Collection and analysis of blood samples

Blood was collected from anesthetized rats to measure total cholesterol, HDL-LDL cholesterol, phospholipid, triglyceride, total protein, albumin, glucose, AST and ALT enzyme levels and transferred to heparinized polyethylene tubes for determination of lipid peroxidation level +4°C at 3000 rpm. They were centrifuged for 10 minutes and their plasma was obtained, which were stored at -20°C until analysis. -80°C was used for the storage of plasma separated for MDA measurements. The determination of the specified biochemical values was studied by spectrophotometric method using an auto analyzer. Blood plasma MDA values were also determined spectrophotometrically (UV-2100 Shimadzu, Japan) with the help of Oxis Research (Bioxytech) test kits.

Statistical analysis

The distribution of the data was evaluated by histogram, q-q graphs, and Shapiro-Wilk test. Values were expressed as means (Standard deviation). Data from the T-maze test and EDA were analyzed with Mann-Whitney U test and independent t-test was used for comparison of 2 groups. One-way analysis of variance was used for comparison of biochemical parameters between the groups, and Tukey and Tamhane tests were used for multiple comparisons. SPSS program was used to analyze the data. A *P*-value of less than 0.05 was considered statistically significant.

Results

Effects of propolis rats in the T-maze

The number of entries into open (NEOA) and closed arms (NECA), total time and the percentage of time spent (% TSOA) in the open arms were measured in the T-maze test (Table 1).

Table 1: Distribution of parameters evaluated in T-maze test according to experiment groups. Mean (SD)

	NEOA	NECA	TSOA	% TSOA
Control	1.5(0.52)	2.4(0.69)	38(11.83)	14.5(3.43)
10mg/kg PRO	0.5(0.52)*#	1.4(0.56)*#	6.5(3.01)*#	2.2(1.09)*#
30mg/kg PRO	2.6(0.84)	2.7(0.42)	24(6.41)	9.6(2.66)
50mg/kg PRO	0.6(0.96)*#	1.3(0.63)*#	0.6(0.83)*#	0.6(0.34)*#
P-value	<0.011	<0.001	<0.001	<0.001

* Different from the control group, PRO: propolis, # Different from the medium dose group (*P*<0.05, Kruskal-Wallis, post-hoc Mann-Whitney U), NEOA: Number of entries into open arms, NECA: Number of entries into closed arms, TSOA: Time spent in open arms, % TSOA: Percentage of time spent in open arms

The NEOA, NECA, TSOA, and % TSOA values of the control group were significantly different from the propolis groups except for the 30 mg/kg PRO.

The number of entries into the open arms and percentage of time spent there were lower in 10 mg/kg PRO group than in the other groups. The open and closed arm entries (*P*<0.001, *P*<0.001, respectively), time spent in the open arms (*P*<0.001) and the percentage of time spent in the open arms (*P*<0.001) decreased significantly in the 50 mg/kg PRO group as compared to the control group. This finding indicated that administration of 50 mg/kg PRO increased anxiety and sympathetic activity. Administration of 30 mg/kg propolis increased the number of open arm entries (*P*=0.011), the number of enclosed arm entries (*P*=0.013), time spent in the open arms (*P*=0.009) and percentage of time spent in the open arms (*P*=0.009), when compared with administration of 50 mg/kg propolis. The anxiolytic potential of 30 mg/kg PRO was stronger than that of other groups in the elevated T-maze model. There were no significant differences between the 30 mg/kg PRO and the control groups (Figure 1, 2).

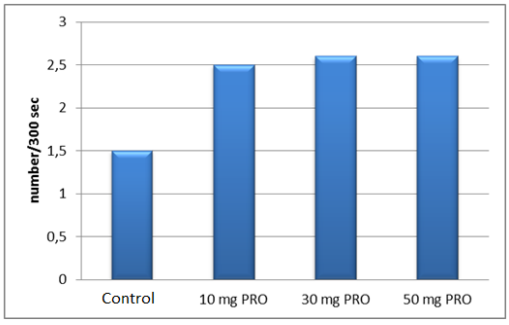

Figure 1: Distribution of NEOA (The number of entries into open arms)

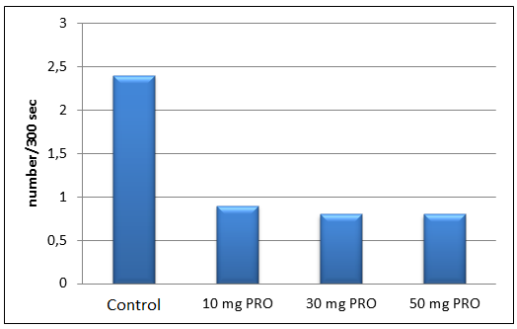

Figure 2: Distribution of NECA (The number of entries into closed arms)

Effects of propolis on skin conductance level (SCL)

The increase in SCL in Tonic EDA, which is independent of the stimuli, and the phasic EDA measured by stimulating, indicate increased anxiety.

Tonic and Phasic SCLs were statistically higher in the 50 mg/kg PRO group than the control (P=0.02, P=0.04, respectively) and 30 mg/kg PRO groups (P=0.013, P=0.02, respectively). Tonic and Phasic SCLs were statistically lower in the 30 mg/kg PRO group than the control (P=0.012, P=0.02, respectively) and 50 mg/kg PRO group (P=0.013, P=0.02, respectively). These findings showed that 50 mg/kg PRO exhibited an anxiogenic effect, while 30 mg/kg PRO showed an anxiolytic effect (Table 2).

The results of biochemical parameters

Evaluation of lipid peroxidation product MDA to measure antioxidant revealed that it was significantly lower in the 30 mg/kg PRO and 50 mg/kg PRO groups. This decrease was more pronounced in 30 mg/kg PRO (P<0.001) group. AST value increased in the 30 mg/kg PRO group, while a decrease in ALT was observed. No differences were observed between the groups in terms of LDH values (Table 3).

There was a decrease in triglyceride and total cholesterol values, especially in 50 mg/kg PRO group (P<0.001). It was observed that HDL value increased significantly starting from the administration of high dose propolis and LDL value decreased significantly in the 10 mg/kg PRO group only (P<0.001). A significant increase was observed in the phospholipid values in the 30 mg/kg PRO and 50 mg/kg PRO groups (Table 3).

Table 2: Tonic and phasic SCL values of the groups.

SCL (µmho)	10 mg/kg PRO	30 mg/kg PRO	50 mg/kg PRO
Tonic SCL	9.53(1.46)	6.32(0.23)*	12.12(1.12)
Phasic SCL	8.74(2.15)	5.75(1.22)*	11.17(1.19)
P-value	<0.001	<0.001	<0.001

Mean (Standard deviation), * Compared with 30 mg/kg PRO, # Compared with 50 mg/kg PRO

Table 3: Average blood values and statistical results of rats

Parameter/Group	Control group	10 mg/kg PRO	30 mg/kg PRO	50 mg/kg PRO	*P*-value
MDA (µM)	2.46(0.37)^{a}	2.25(0.26)^{a}	1.83(0,27)^{b}	0.76(0.16)^{c}	<0.001
AST (IU/L)	116.59(21.30)	114.48(15.21)	112.07(21.34)	114.14(19.74)	0.874
ALT (IU/L)	57.68(10.24)^{a}	58.34(7.44)^{a}	52.14(10.72)^{ab}	48.32(8.57)^{b}	0.018
LDH (IU/L)	1502(294.14)	1633(314.72)	1672(201.24)	1508(276.12)	0.248
Triglycerides	169.04(43.01)^{a}	145.27(32.76)^{ab}	141.25(25.42)^{bc}	114.13(11.78)^{c}	0.001
Total cholesterol	97.25(6.28)^{ab}	101.14(9.8)^{a}	92.23(4.8)^{b}	76.94(10.48)^{c}	<0.001
HDL	24.28(4.89)^{a}	31.76(8.75)^{b}	39.89(5.85)^{c}	41.23(4.28)^{c}	<0.001
LDL	36.27(3.89)^{a}	30.27(6.57)^{b}	21.54(7.98)^{c}	16.49(6.21)^{c}	<0.001
Phospholipids	178.43(17.25)^{a}	154.76(22.58)^{b}	216.23(24.89)^{c}	230.49(22.76)^{c}	<0.001

Data are expressed as mean (standard deviation), The same letters in the same line indicate the similarity between the groups and the different letters indicate the difference between the groups, MDA: malondialdehyde, AST: Aspartate Aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, HDL: high density lipoprotein, LDL: low density lipoprotein

Discussion

Emotional, cognitive, and physical behaviors include changes in peripheral autonomic activity. Electrodermal activity (EDA), the so-called galvanic skin response or sympathetic skin response, reflects sympathetic tone, and is therefore frequently used as an indirect measure of attention, cognitive effort, or emotional arousal [21]. EDA is a multisynaptic sympathetic reflex that may be evoked by a variety of internally generated or externally applied arousal stimuli. It is considered an index of psychological processing properties of stimuli, such as significance, novelty or emotional relevance, and effortful processing [22]. Skin conductance level (SCL) is a parameter of EDA. A high SCL may result from increased eccrine sweat gland activity and sympathetic activity [5].

Anxiety can be explained as fear, tension, distress during daily life and is part of a widely spread group of psychiatric illnesses which are a cause of major concern [1]. The defense behavior assays of rodents have usually been used as preclinical models of anxiety. The EPM and EDA are established methods for testing animal anxiety.

Studies examining the effects of propolis on sympathetic nerve activation and blood pressure are very limited. In the literature, there is no study on whether propolis affects anxiety. Thus, in our study, it was investigated whether different doses of propolis affect anxiety level and electrodermal activity, which is considered an indicator of sympathetic nervous system activity.

Considering the role the autonomic nervous system plays in its formation, EDA can be evaluated as the window of the central nervous system (CNS) opening to the periphery. From this point of view, it is possible to extract information about CNS through observations made in the periphery. It is also stated that EDA can be used to study the effectiveness of various anesthetic conditions and drugs in the CNS. Dolu et al. [23] reportedly used EDA and plus maze methods in their studies to investigate the acute effects of L-tryptophan on anxiety in different doses. Anxiety mainly stems from the CNS. The attention and perception centers of CNS, which are also responsible for anxiety, play a significant role in EDA formation. Therefore, according to the level of anxiety, the electrical activity of the skin changes as a result of variable emotional sweating in the sweat glands innervated by the sympathetic nervous system, which is controlled by the autonomic nervous system in CNS. The reflection of the changing electrical activity of the skin in the DIS is measured by EDA.

According to the results of EDA measurements in our study, the anxiety levels of 30 mg/kg PRO and 50 mg/kg PRO groups were found to have significantly changed. In the 30 mg/kg PRO group, tonic and phasic SCL were quite low. This showed us that propolis suppresses sympathetic activation and reduces anxiety levels. The results obtained by EDA measurements were supportive of the behavior scoring in the T-maze test.

MDA measurements for determination of antioxidant levels revealed that it was significantly lower in the 30 mg/kg PRO and 50 mg/kg PRO groups. This decrease was thought to stem from propolis flavonoids. Mohammadzadeh et al. [24] reported that the samples with low flavonoid concentrations were more effective in MDA inhibition in their studies comparing the flavonoid contents (mg/g propolis) and MDA formation inhibition values (%) of the propolis samples they collected from different regions. They emphasized that the structural type of

flavonoid may be more important than the total flavonoid amount in the antioxidant effect expected from flavonoids. Hosnuter et al. [25] similarly reported a significant decrease in MDA values compared to the control group with the administration of CAPE, an important compound of propolis, in rats.

AST enzyme did not change significantly in intergroup averages, while ALT activity decreased in the 50 mg/kg PRO group. A similar situation was observed when examining the regulatory effect of long and short-term propolis supplementation by applying sodium fluoride, and it is noteworthy that the ALT enzyme is higher and the AST enzyme is lower in the groups treated with propolis compared to the sodium fluoride group [26]. In the studies of Kolankaya et al. [27] on alcohol-induced hepatotoxicity, the AST activity of the alcohol-administered group increased significantly more than ALT, whereas it was observed that this increase reached a normal course in the alcohol+propolis group. No such relationship could be detected in the mentioned groups in terms of ALT levels. Similarly, Mani et al. [28] reported that did not detect any changes in AST levels in rats which were administered 1, 3 and 6 mg/kg/day propolis.

The level of MDA and ALT values decreased in 30 mg/kg PRO group. No significant difference was observed in intra-group and inter-group comparisons of LDH. While Mani et al. [28] did not find any differences in LDH enzyme values in rats which were administered propolis for a short and long time, Newairy et al. [29] reported that there was a significant decrease in LDH activity in the rat group with AlCl3 toxicity which were given propolis. Kolankaya et al [27] found that LDH activity was low in rats that were administered alcohol compared to the propolis group.

There was a decrease in triglyceride and total cholesterol values and a statistically significant decrease in the 30 mg/kg PRO group only. It was observed that HDL value increased significantly after administration of low dose propolis and LDL decreased significantly only in the PRO-30 mg/kg group. A significant increase was observed in the phospholipid values in the 30 mg/kg PRO and 50 mg/kg PRO groups. The results are compatible with many other studies on this subject [27,29,30].

Limitations

Blood samples could have been evaluated at the beginning and end of the experimental study, along with other biochemical parameters such as TAS, and TOS. However, due to lack of time and funding, it could not be done. We believe that this study will contribute to future work as a basis.

Conclusions

This is the first study to investigate the effect of propolis on anxiety and electrodermal activity. According to the results obtained by EDA and T labyrinth methods, low and medium dose propolis showed anxiogenic effects while high dose propolis exerted an anxiolytic effect. In addition, positive changes in enzymatic and lipid values in favor of the organism were observed in which propolis administered to rats decreased MDA values in the middle and high dose groups. Therefore, it was concluded that the anxiety responses of propolis depend on dosage.

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