ORIGINAL ARTICLE

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Evaluation of the Antidepressant Effect of Propolis in Chronic Unpredictable Mild Stress-Induced Depression Model in Rats

Sıçanlarda Kronik Öngörülemeyen Hafif Stres Kaynaklı Depresyon Modelinde Propolisin Antidepresan Etkisinin Değerlendirilmesi

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ABSTRACT

Aim: In this study, the antidepressant effect of propolis was investigated in a model of chronic unpredictable depression in rats.

Materials and Methods: Wistar-Albino male rats were used in the study and were divided into four groups as propolis, stress, stress + propolis, and control groups. Eight animals were assigned to each group. The experimental protocol was applied to the stress groups for 60 days, and the animals were exposed to different stressors. Propolis extract (100 mg/kg) was administered orally to propolis and stress + propolis groups throughout the experimental protocol. As a result of depression modeling, the Forced Swimming Test, Sucrose Preference Test, and Elevated Plus Maze Test were applied for behavioral evaluation. Twenty-four hour urine samples were collected for quantitative analysis of serotonin 5-hydroxytryptamine (5-HT) and its metabolite 5-hydroxy indole acetic acid (5-HIAA) in urine by liquid chromatography-tandem mass spectrometry method. The animals were sacrificed as a result of the experiment process.

Results: It was seen that there was a statistical difference for behavioral tests between the groups ($p<0.05$). The administration of propolis to rats under stress has been shown to alter sugar consumption in rats ($p<0.05$). For Forced Swimming Test, there was a statistical difference between the stress group and the other groups. For 5-HT and 5-HIAA levels, there was no significant difference between the groups ($p>0.05$).

Conclusion: The findings have shown that propolis extract may help to prevent depression, thanks to its antidepressant-like effects.

Keywords: Serotonin, depression, propolis, chronic unpredictable stress model

ÖZ

Amaç: Bu çalışmada, sıçanlarda kronik öngörülemeyen depresyon modelinde propolisin antidepresan etkisi araştırıldı.

Gereç ve Yöntem: Çalışmada Wistar-Albino erkek ratlar kullanıldı ve propolis, stres, stres + propolis, ve kontrol olmak üzere 4 gruba ayrıldı. Her gruba sekiz hayvan atandı. Deney protokolü stres gruplarına 60 gün süreyle uygulandı ve hayvanlar farklı stresörlere maruz bırakıldı. Propolis ekstresi (100 mg/kg) propolis ve stres+propolis gruplarına deney protokolü boyunca oral yoldan verildi. Depresyon modellemesi sonucunda davranışsal değerlendirme için Zorunlu Yüzme Testi, Sükroz Tercih Testi ve Yükseltilmiş Artı Labirent Testi uygulandı. Sıvı kromatografi-tandem kütle spektrometresi yöntemi ile idrarda serotonin 5-hidroksi triptamin (5-HT) ve metaboliti 5-hidroksi indol asetik asidin (5-HIAA) miktarsal analizi için 24 saatlik idrar örnekleri toplandı. Deney işlemi sonucunda hayvanlar sakrifiye edildi.

Bulgular: Gruplar arasında davranış testleri açısından istatistiksel olarak anlamlı fark olduğu görüldü ($p<0,05$). Sıçanlara stres altında propolis verilmesinin sıçanlarda şeker tüketimini değiştirdiği gösterildi ($p<0,05$). Zorunlu Yüzme Testi için, stres grubu ile diğer gruplar arasında istatistiksel olarak fark vardı. 5-HT ve 5-HIAA düzeyleri için gruplar arasında anlamlı fark yoktu ($p>0,05$).

Sonuç: Bulgular, propolis özütünün antidepresan benzeri etkileri sayesinde depresyonu önlemeye yardımcı olabileceğini göstermiştir.

Anahtar Kelimeler: Serotonin, depresyon, propolis, kronik öngörülemeyen stres modeli

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INTRODUCTION

Depression is one of the most common mood disorders in society. It is also a common health problem associated with a high threat of death from other attendant medical disorders. Depression is characterized by wakefulness or inordinate somnolence, fatigue or loss of energy, loss of sense of control, and private experience of great torture. It affects the thinking and performing processes of the existent, greatly reducing his social part and productivity[1].

When the basic physiology and pathophysiology of depression are examined, it has been seen that some neurotransmitters in the brain are directly active in this process. It is known that serotonin 5-hydroxytryptamine (5-HT), dopamine, and noradrenaline neurotransmitters fall to critical levels in depressive moods, and the working mechanisms of these chemicals are interrupted. Antidepressant drug treatments developed from this point of view are responsible for increasing the levels of these neurotransmitters in the brain to healthy mood levels and controlling various inhibition and activation processes[2].

In recent years, it has been seen that alternative medicine applications are used as treatment options as well as existing drug treatments. It is thought that the reasons for this situation are the reduction of chemical damage with alternative medicine applications, problems in accessing the drug required for treatment, and the treatment process with fewer drugs by using alternative medicine applications in diseases with high comorbidities such as depression[3].

Propolis, which is the most studied substance among bee products, is known as a product that benefits the body in many ways. Considering the usage areas of propolis as a treatment tool, it has been seen that it is used as a treatment option in cancer, neurological disorders, dentistry, cardiovascular, digestive, and dermatological diseases. Propolis components (caffeic acid phenethyl ester, flavonoids) are known to exhibit neuroprotective effects against oxidative damage in a model of induced ischemia and neurodegenerative disorders, including Alzheimer's disease[4]. Concerning its effect on the central nervous system (CNS), several studies have suggested that propolis has neuroprotective effects in both in vitro and in vivo models. However, the effect(s) of propolis on the CNS, such as depressant and anxiolytic effects, have been poorly reported[5]. Therefore, investigating the effect of propolis on changing behavior and mood and on neurotransmitter levels is considered substantial to define its relationship with depression. Since it is known that it is a crucial requirement for alternative medicine application areas such as apitherapy and phytotherapy to offer proven activities, it is scientifically invaluable that such studies both obtain new results and provide results that support existing research[6].

Considering this information, in this study, it is aimed to investigate the antidepressant effect of propolis using a chronic unpredictable depression model in rats. The behavior of animals exposed to the stressors presented in the experimental procedure, in proportion to their current mood, was examined with determined behavioral tests, and the levels of 5-HT and its metabolite, 5-hydroxy indole acetic acid (5-HIAA) were compared in urine samples collected from animals.

MATERIALS AND METHODS

Chemicals

The 12% sucrose solution used in the experimental process was obtained from Bilgi Kimyevi Laboratory Products Manufacturing Consultancy Analysis Services Industry and Trade Limited company (İstanbul, Turkey). To determine 5-HT and its metabolite 5-HIAA in urine, CE-IVD certified and validated Jasem HVA-VMA-5HIAA in Urine LC-MS/MS Analysis Kit was used (Sem Laboratuvar Cihazları Pazarlama San. ve Tic. Inc., İstanbul, Turkey). Glycerin (E422) and propolis extract (96 mg) were obtained from Aksu Vital Natural Products Joint Stock Company (İstanbul, Turkey) to prepare propolis extract in 50 mL glass bottles (192%; m/v) for the use in the experimental process. Other chemicals were obtained from Sigma-Aldrich, United States of America.

Animals

Wistar albino rats aged 12-16 weeks and weighing 300-400 g were used in this study. The rats were housed in a temperature and light-controlled room (12 h dark-light cycles, 22±2 °C, and humidity 60±5%). All animals were free to access water and pellet food, and experiments were performed according to national laws and guidelines. The Laboratory Animal Care and Use Guidelines were taken into account. The protocol used in this study was approved by the Üsküdar University on the Ethics of Animal Experiments of (ÜÜ-HADYEK), İstanbul, Turkey (decision no: 2020-17, date: 22.01.2021).

Experimental Design

In this study, 32 rats were divided into four groups, including eight in each group. For the control group, 0.4 mL of saline (0.9% NaCl) was administered to group animals by gavage for 60 days. In the propolis group, 0.4 ml/day (~ 100 mg/kg) propolis extract (192%; m/v) was administered by gavage to group animals daily for 60 days. The given propolis dose (100 mg/kg) was determined by taking into account a similar study[7]. In the stress group, the animals were exposed to various stressors for 60 days. At the same time, 0.4 mL of physiological saline (0.9% NaCl) was administered to the group animals by gavage. In the stress+propolis group, 0.4 mL/day

(~ 100 mg/kg) propolis extract (192%; m/v) was administered by gavage to the animals daily for 60 days. At the same time, group animals were exposed to various stressors for 60 days.

Depression Model and Behavioral Tests

Chronic Unpredictable Stress Model

As a depression model in the study, the chronic unpredictable stress procedure defined by López-López et al.[8] was applied as feed restriction (12 hours), water restriction (12 hours), permanent light (24 hours), crowded cage (24 hours), no stressor applied (24 hours), float in cold water (15 minutes), immobilization (1.5-2 hours), insulation (24-48 hours), wet sawdust (12 hours), lattice tilting 45° (5 hours), foreign object (5 hours), and changing animals between cages (12-24 hours).

The stress procedure mentioned above was applied to the stress and stress+propolis group experimental animals every day for 60 days. Each stress procedure was applied 8-10 times. To prevent the experimental animals from predicting the applied stress procedure, the same procedure was tried not to be applied consecutively. In addition, the stress procedure was applied at different times of the day. The body weights of the rats were determined before starting the stress model and after 60 days of exposure.

Forced Swimming Test

The 40 cm high and 20 cm in diameter cylindrical glass container was filled with water up to 30 cm. The temperature of the water was kept at 24-26 °C. The animals in all groups were allowed to swim for 15 minutes on the first day to adapt to the experimental environment and learn, and then they were dried and placed back in their cages. After 24 hours, the subjects were allowed to swim for five minutes[9]. Video recording was made to score the animals' immobility (swimming periods where only the head is above the water but motionless), swimming, and climbing movements during the total time. Recordings were calculated by scoring (swimming, climbing, and immobility) at 5-second intervals by an unbiased observer[10]. The water in the bowl was changed after each animal. The animals taken from the lantern were dried and brought into a warm cage. Prolonged inactivity of rats is correlated with helplessness behavior, which is one of the important markers of depression, but it is a depression-like behavior. Studies have shown that the duration of inactivity is shortened as a result of antidepressant treatments applied[11].

Sucrose Preference Test

The Sucrose Preference Test (SPT) measures aversion to pleasure (Anhedonia) in experimental animals. Anhedonia, one of the main symptoms of major depression, is measured with SPT, which is used to Anhedonia in experimental animals. Initially, two different water bottles were placed on the right and left sides of the cage. The experimental animals were allowed to drink water from both bottles for 24 hours, and the water bottles were changed every 12 hours. After two training days, 200 mL of water containing 2% sucrose was randomly placed in one of the bottles. The vials were weighed before and 24 hours after administration to the rats. Percent sucrose consumption was calculated according to the following formula in Equation 1[12,13].

% Sucrose Consumption=Sucrose consumption x100/Total consumption *Equation 1*

Total consumption: water and sucrose consumption was evaluated as the total consumption[12,13].

The Elevated Plus Maze Test

The apparatus used for the elevated plus maze test is "+" shaped, perpendicular to two opposing open arms (25x5x0.5 cm) and a center and two closed arms (25x5x16 cm) with platform (5x5x0.5 cm). Open arms have a very small (0.5 cm) wall to reduce the number of falls, while closed arms have a high (16 cm) wall to surround the arm. The whole apparatus is 50 cm above the ground. The device is made of plastic materials. The platform is black, and the walls are opaque. All test rats were transferred to the behavioral test chamber 30 minutes before starting the first experiment to acclimate to the condition of the behavioral test chamber. A test trial using an application animal has two purposes. The first step is to ensure everything in the registry to be okay. Another important thing is to keep the test condition as monotonous as possible[14,15]. A rat was placed in the middle area of the maze, with its head directed towards a closed arm. The elevated plus maze test was recorded using a video camera connected to a computer controlled by a remote device. The number of entries in each arm (one entry is defined as the mouse's center of gravity entering the arm) and time spent in the open arms were recorded, and these measurements serve as indicators of anxiety-like behavior. Rats were allowed to move freely in the maze for 5 minutes. After each trial, all arms and core areas were cleaned with 70% alcohol, an effective deodorizing agent with a relatively weak odor compared to other cleaning solutions, to avoid bias based on olfactory cues[14].

Sample Preparation Procedure for Quantitative Analysis of 5-HT and Its Metabolite 5-HIAA in Urine by LC-MS/MS Method

Twenty-four-hour urine samples were collected from rats in each group to analyze 5-HT and its metabolite 5-HIAA molecules in urine by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Urine samples were analyzed using

the colorimetric method to determine creatine levels. Since the urine samples of three rats in the experimental groups were insufficient, creatine and quantitative analyses could not be performed in these rats. Quantification of 5-HT and 5-HIAA in urine samples was carried out by Sem Laboratories using CE-IVD certified validated Jasem HVA-VMA-5HIAA in Urine LC-MS/MS Analysis Kit (Sem Laboratuvar Cihazları Pazarlama San. ve Tic. Inc., İstanbul, Turkey).

Sample Preparation Procedure for Creatine Analysis in Urine Samples by Colorimetric -Jaffe Method

Urine samples were normalized via the creatinine level to provide a reduction for inter-individual variations in urine samples[16]. In order to evaluate the amount of 5-HT and its metabolite 5-HIAA in the urine, the creatine levels of the urine were determined, and the creatine amounts were included in the calculation. Using the formula in Equation 2 and Equation 3, normalized 5-HT and 5-HIAA values were calculated in the urine.

Normalized 5HT (mg/g crea)=5-HT (ppm)/ (Creatine(mg/dL)/100)
Equation 2

Normalized 5-HIAA (mg/g crea)=5-HIAA (ppm)/ (Creatine(mg/dL)/100)
Equation 3

Urine samples were centrifuged at 3500 rpm for 5 minutes. Urine samples given to the Cobas Integra 400 Plus biochemistry autoanalyzer device are automatically diluted with 1/25 distilled water in the device. The results are then obtained by reading at a 512/583 nm wavelength. After reading, the result is reached by automatic multiplication.

Statistical Analysis

Data analysis was done using Statistical Package for the Social Sciences (SPSS) software. Considering the distribution of the data, they were found to be normally distributed ($p>0.05$). From this point of view, the t-test and one-way analysis of variance (ANOVA) test were used to examine the amount of variation between the groups. The Tukey and Tamhane tests, which are post-hoc tests, were used for the comparison between the groups in ANOVA tests that gave statistical significance, taking into account the homogeneity of population variances. All data were tested at a 95% confidence interval.

RESULTS

Body Weight

The bodies of the animals were planned to be ahead of the days when the training was scheduled. The groups were compared among themselves and the analysis was done using the t-test in Table 1. There was a significant difference between the initial and final weights in the control, propolis, and stress + propolis groups ($p<0.05$) (Figure 1).

Evaluation of Behavioral Experiments

With the Forced Swimming Test, the immobility times of the animals were compared, and when the values in the groups were examined, there was a statistical difference between the stress group and the other groups (Figure 2).

Supportive periodontal therapy was applied to measure the anhedonia behavior, which is one of the depressive mood characteristics of animals exposed to stress procedures for 60 days, and the results were compared with a one-way ANOVA. Sucrose consumption was lower in the stress group than in all groups ($p<0.05$) (Figure 3).

In the elevated plus maze test performed to examine the effects of the animals' anxiety on their behaviors, the times of staying in the closed arm were compared between the groups and a significant difference was found between the stress group and the propolis and propolis + stress groups (Figure 4).

The open arm durations in the elevated plus maze test were compared between the groups and a significant difference was found between the propolis and stress groups, and between the stress + propolis group and the stress group (Figure 5).

Quantitative Analysis of 5-HT and its Metabolite 5-HIAA in Urine

Chromatograms of 5-HT and its metabolite 5-HIAA analyzed in urine are given in Figure 6. The results of the quantitation of 5-HT and its metabolite 5-HIAA molecules in the urine, measured by the LC-MS/MS method and calculated according to the device analysis results and urinary creatinine levels, are given in Table 2. In each experimental group, three out of the rats, numbered from one to eight, could not have their creatinine and quantity determination analyses conducted due

Table 1. Weights of the animals on the first and last days

Experimental groups	Day zero measurements (g) (mean ± standard error)	60th day measurements (mean ± standard error)	p value (t-test)
Propolis	354±8.88	402±10.17	<0.05
Stress	384.37±13.85	390.37±13.87	>0.05
Stress+propolis	349.37±10.78	391.31±8.96	<0.05
Control	361.14±20.63	422.28±10.17	<0.05

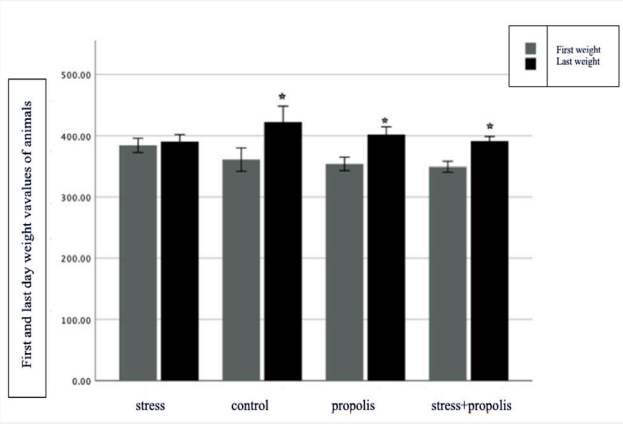

Figure 1. First and last day weight values of animals. *p<0.05 shows that there is a significant difference between the initial and final weights in the control, propolis and stress+propolis groups (t-test)

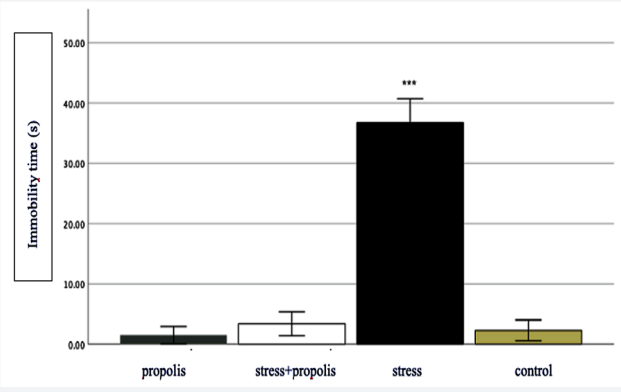

Figure 2. Immobility times in the forced swim test. ***p<0.05 shows a significant difference in the comparison of the stress group with the other groups (ANOVA). The differentiation status between the groups was analyzed by the Tamhane from Post-Hoc tests

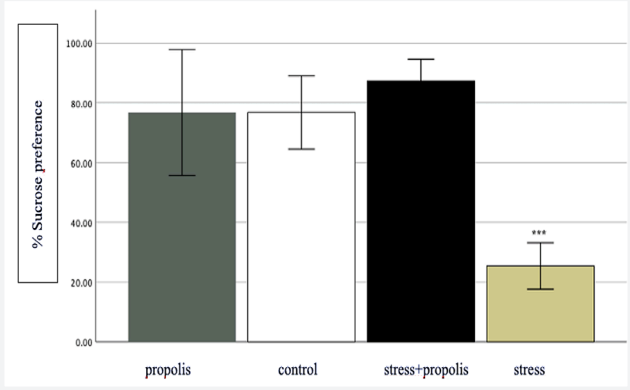

Figure 3. Sucrose consumption of two experimental groups. ***p<0.05 indicates that the stress group consumed significantly less sucrose than the other groups (ANOVA). The Tukey test was used as a Post-Hoc test

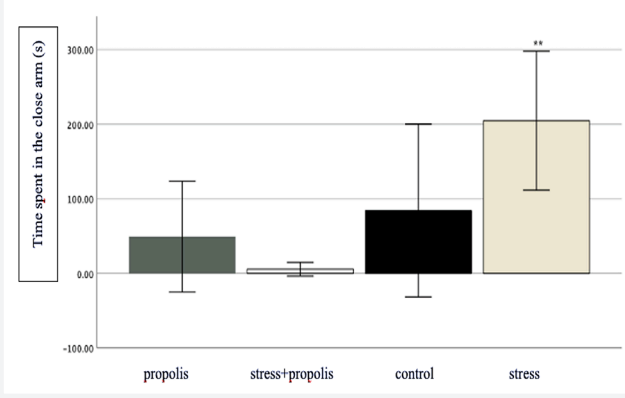

Figure 4. Time spent in the closed arm in the 4 elevated plus maze test. **p<0.05 shows that the duration of the stress group is significantly higher than the propolis and stress + propolis groups (ANOVA). The Tamhane test was used as a Post-Hoc test

to insufficient urine samples collected during the study. The analyses for the quantitative determination of rats labeled control 7, propolis 2, and propolis 5 were not performed and thus were not included in the calculations. The values for quantitative determination of the analyzed rats are presented in Table 2.

Figure 7 shows the calculated results of 5-HT and 5-HIAA levels. Table 2 and Figure 7 show that there is no significant difference between the groups (p>0.05).

DISCUSSION

Whether propolis has a positive effect on mood disorders, especially depression, is one of the issues that researchers have focused on in recent years. It is thought that the potential antidepressant effect of propolis, as an apigenin-containing product, stems from this[17-20]. Studies have shown that apigenin has an antidepressant-like effect on dopamine and norepinephrine, and according to a study with mice, it has a reversal effect on decreased sucrose consumption due to depressed mood and increased inactivity times in floatation tests. In this study, which aimed to achieve supportive results for being an alternative and easily applicable treatment option, the effect of propolis on the 5-HT level was investigated in a model of chronic unpredictable mild stress-induced depression in rats by biochemical analysis. In addition to biochemical parameters, the effectiveness of propolis on behavioral tests was also examined, and in general, it was seen that propolis created differentiation in behavioral tests. Still, there was

no statistically significant difference despite observable differences in biochemical parameters.

In previous studies, it has been shown that rats exposed to chronic, unpredictable mild stress procedures have a decrease in body weight. Similarly, in this study, there was a stress-induced reduction in the weight of the animals, as seen in Table 1. The findings obtained were statistically significant, as shown in Figure 1. However, propolis, our focus of study, had a remarkably positive and significant effect on both the decrease in body weights and the recovery of the behaviors observed in the increased plus labyrinth test with the forced swimming test due to anhedonia and returning to normal values. This is a promising indication of the potential of propolis in stress management. In another study on the use of propolis in animals exposed to a chronic unpredictable stress procedure, a significant decrease was reported in the weight of stress group animals. It was noted that there was an increase in weight in the groups after applying of propolis[8], further reinforcing the positive effects of propolis.

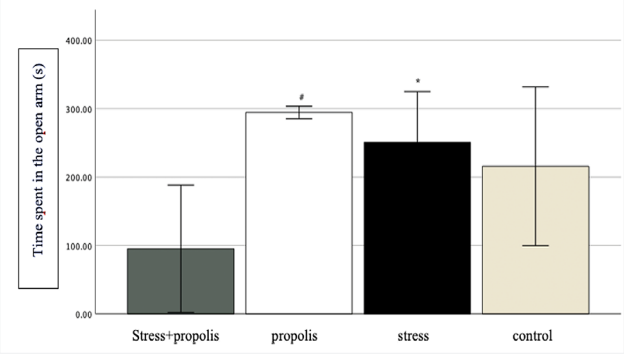

Figure 5. Open arm times in the elevated Plus Maze test, *p<0.05 propolis group spent longer time on the open arm than the stress group, #p<0.05 stress+propolis group spent longer time on the open arm compared to the stress group

The increase in the immobilization time in the forced swimming test is an indicator of depressive mood. Zangen et al.[21] studied the effect of antidepressant treatment on the change in 5-HT and 5-HIAA levels in genetically selected flinders susceptible strain (FSS) rats. In the selection of FSS rats, they exhibited the characteristic behavioral features of depression, such as decreased movement, increased anhedonia, increased amount of rapid eye movement (REM) sleep, decreased REM sleep onset, and cognitive difficulties in response to chronic mild stress[21]. In the study, animals were subjected to mandatory swimming tests before and after antidepressant treatment. It was observed that depressed animals remained inactive for a longer period compared to the control group, and this period was shortened after treatment. When the immobilization times in our study were evaluated, it was concluded that the stress group remained motionless in water significantly longer than the other groups. As shown in Figure 2, the results obtained are statistically significant (p<0.05). It was observed that animals in the stress group remained inactive longer than the control group did, which was shortened after propolis treatment (Figure 2). Our study results are similar to those in the literature. It has been demonstrated by the forced swimming test model that propolis has potential antidepressant activity. In addition,

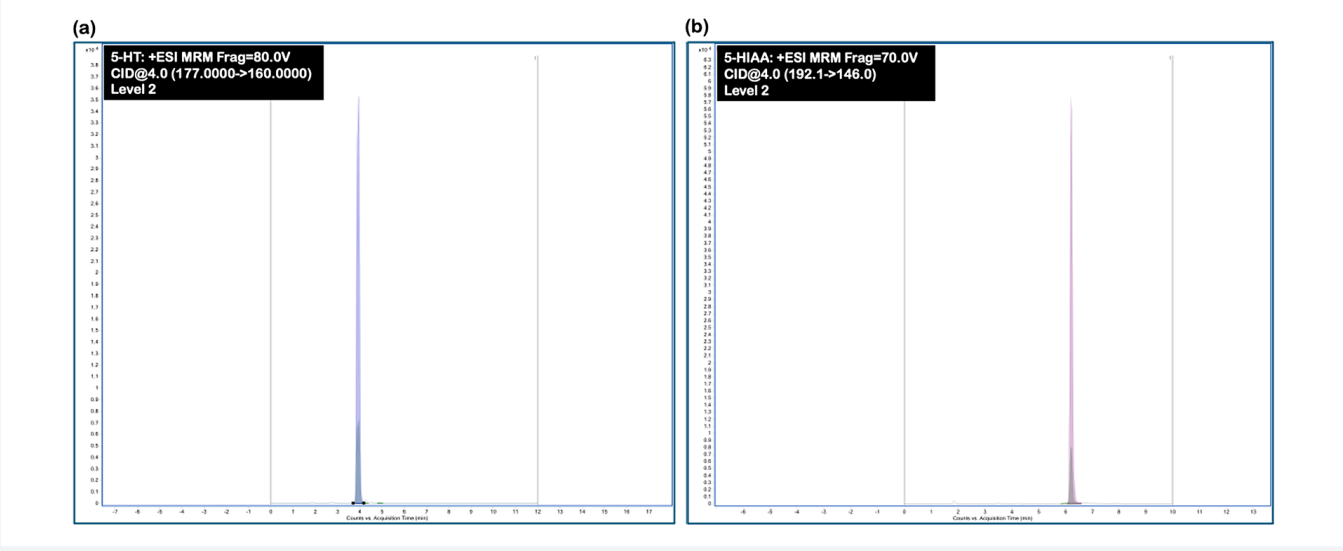

Figure 6. Chromatograms of serotonin 5-HT and its metabolite 5-HIAA in urine. (a) 5-HT (m/z: 177.0>160.0), (b) 5-HIAA (m/z: 192.1>146.0)
5-HT: 5-hydroxytryptamine, 5-HIAA: 5-hydroxy indole acetic acid

Table 2. Results of 5-HT, 5-HIAA, and creatine assays

Sample	5-HT (ppm)	5-HIAA (ppm)	5-HIAA/5-HT	Creatine (mg/dL)	Normalized 5-HT (mg/g crea)	Normalized 5-HIAA (mg/g crea)	Normalized (5-HIAA/5-HT)
Stress 1	0.386	4.656	0.01	93.25	0.414	4.993	12.06
Stress 2	2.065	13.708	0.00	250.47	0.825	5.473	6.63
Stress 3	0.373	9.065	0.02	195.88	0.191	4.628	24.23
Stress 4	0.223	1.754	0.00	71.7	0.311	2.447	7.87
Stress 5	ND	14.364	NC	ND	NC	NC	NC
Stress 6	0.676	2.531	0.00	60	1.127	4.219	3.74
Stress 7	0.879	8.992	0.01	156.25	0.563	5.755	10.22
Stress 8	0.932	15.079	0.01	197.55	0.472	7.633	16.17
Stress+propolis 1	0.465	7.890	0.01	129.14	0.360	6.110	16.97
Stress+propolis 2	ND	9.788	NC	ND	NC	NC	NC
Stress+propolis 3	0.287	10.264	0.03	128.41	0.224	7.993	35.68
Stress+propolis 4	0.531	9.241	0.01	138.74	0.383	6.661	17.39
Stress+propolis 5	0.586	7.617	0.01	134.98	0.434	5.643	13.00
Stress+propolis 6	0.535	6.772	0.01	108.52	0.493	6.240	12.66
Stress+propolis 7	0.662	9.577	0.01	ND	NC	NC	NC
Stress+propolis 8	0.722	7.004	0.00	123.08	0.587	5.691	9.69
Control 1	0.385	4.385	0.01	95.42	0.404	4.596	11.38
Control 2	0.979	11.718	0.01	153.14	0.639	7.652	11.97
Control 3	0.415	7.410	NC	111.79	0.372	6.628	17.82
Control 4	1.194	2.693	0.00	62.5	1.911	4.309	2.26
Control 5	0.150	3.402	0.02	74.37	0.203	4.574	22.53
Control 6	1.138	1.062	0.00	35.65	3.194	2.980	0.93
Control 8	0.382	5.157	0.01	107.02	0.357	4.818	13.50
Propolis 1	0.603	2.181	0.00	66.86	0.903	3.262	3.61
Propolis 3	0.440	3.333	0.00	195.88	0.225	1.702	7.56
Propolis 4	0.304	5.889	0.01	71.7	0.425	8.214	19.33
Propolis 7	0.294	3.254	0.01	156.25	0.188	2.083	11.08
Propolis 8	0.216	5.752	0.02	197.55	0.109	2.912	26.71

Study groups in rats: Animals in each group are numbered from one to eight individually. The analyses for the quantitative determination of rats labeled Control 7, propolis 2, propolis 5, and propolis 6 were not performed due to insufficient urine samples collected during the study and thus were not included in the calculations.
5-HT: 5-hydroxytryptamine, 5-HIAA: 5-hydroxy indole acetic acid, ND: Not detected, NC: Not calculated, no significant results were obtained between the groups ($p>0.05$) (ANOVA)

as seen in Figure 3, low consumption of sucrose, one of the anhedonic behavioral characteristics, is consistent with the literature, especially in the stress group[22,23]. Sugar consumption in the propolis groups is similar to that in the control group. The administration of propolis to rats under stress has been shown to alter sugar consumption in rats (Figure 3).

The elevated plus maze test is one of the tests used to measure anxiety in experimental animals. According to the evidence presented in the literature, the time spent in the closed arm increases compared to the time spent in the open arm in rats with anxiety behavior[15]. When the rats stayed in the closed and open arms were compared in the study, results consistent with those in the previous study were obtained. According to the findings obtained in Figure 4 and Figure 5, there was a significant difference between the groups regarding duration ($p<0.05$). When the data of propolis groups were evaluated, it was found that propolis decreased anxiety-like behaviors in rats.

Studies examining the relationship between depression and serotonin metabolism have shown that elevated 5-HIAA levels may be associated with depression and that the 5-HT cycle in patients increases during depression, especially according to 5-HT conversion measurement results in patients with major depressive disorder[24,25]. 5-HT is found in various tissues and platelets of the digestive system and CNS and is widely distributed in our body. As a hydrophilic

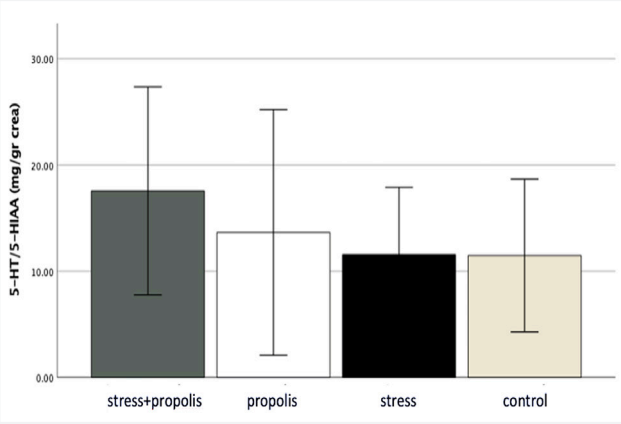

Figure 7. Average values of 5-HT/5-HIAA device result in urine. No significant results were obtained between the groups (p>0.05) (ANOVA)

5-HT: 5-hydroxytryptamine, 5-HIAA: 5-hydroxy indole acetic acid

substance, 5-HT cannot cross the blood-brain barrier[26,27]. Monoamine oxidase is primarily responsible for the metabolism of 5-HT, which is converted to 5-HIAA, the main metabolite, by the aldehyde dehydrogenase enzyme. It provides a stable means to measure the amount of serotonin in the body[28]. It has been shown in many studies that the change in serotonin levels is important, particularly for patients with depression, and that this condition is characterized by a decrease in serotonin levels. This decrease is thought to be due to the location of the raphe nuclei in the memory and cognition regions[29]. The measurement of serotonin levels and their metabolites in different biological fluids is given in the literature. Studies to identify possible biomarkers of depression have provided substantial evidence. To this end, Zhao et al.[30] compared the concentrations of monoamine neurotransmitters and amino acid neurotransmitters in plasma samples taken before and after fluoxetine administration in depression model rats. Considering the results, it was determined that the 5-HT, 5-HIAA concentrations of the depressive group were lower than those of the healthy controls, and it was emphasized that fluoxetine might have a crucial role in increasing the plasma concentrations of 5-HT, 5-HIAA[29]. However, in our study, no significant difference was found between the groups for 5-HT and 5-HIAA levels. The fact that the 5-HT and 5-HIAA levels shown in Table 2, which we obtained in our study, were not statistically significant (p>0.05), as seen in Figure 7, maybe because the sample we used was urine. Although there are consistent and reliable studies on using urine samples for metabolite determination, as a depression-related biochemical, 5-HT levels can be measured in blood and cerebrospinal fluid, and the tissues responsible for 5-HT release or inhibition can be directly examined. It may be possible to obtain a significant difference between the groups regarding 5-HT and 5-HIAA levels if they are examined in such different fluids and tissues. Some in the literature have obtained significant results from the studies conducted with these samples and tissues[27,31]. On the other hand, as is known, the monoamine hypothesis, which is the most accepted hypothesis regarding major depressive disorder, posits that the concentration of dopamine, 5-HT, and noradrenaline neurotransmitters in synaptic gaps decreases in the depressive state. This hypothesis is significant in the context of other hypotheses about major depressive disorder, as it provides a framework for understanding the role of neurotransmitters in depression[32]. Therefore, considering that multiple neurotransmitters are involved in the process, it is evident in our study that it is important to examine the levels of other neurotransmitters rather than just a single neurotransmitter when investigating the antidepressant effect of any substance. Although the findings obtained from behavioral tests in our study indicate that propolis extract may help prevent depression due to its antidepressant-like effects, the reason for these effects has not been explained solely based on serotonin levels.

The chronic unpredictable stress model is frequently used for depression modeling[23,31]. The use of natural stressors and making it possible to observe anhedonic behaviors in animals increase its validity. On the other hand, this model also has the disadvantage that it does not decrease the stress and anxiety levels of animals to the expected degree in case the animals adapt to stressors depending on their application for a certain period and routinely[33]. Therefore, different stressors were applied to the rats at different times during the experiment.

It is seen that the monoamine hypothesis is losing its validity with the studies conducted in recent years. The fact that one-third of people diagnosed with major depression do not respond to pharmacological treatments based on the current monoamine hypothesis shows that this hypothesis alone is not sufficient to explain depression, and other explanations are needed. These results may explain the significant behavioral results, although statistically significant results were not obtained in the chemical tests examined in our study. Considering that behaviors occur due to complex processes, especially in mammals, the meaningful results in behavioral tests show the effectiveness of propolis[23].

Study Limitations

Due to the difficulties experienced at collecting urine samples from animals, a sufficient number of samples could not be taken from some animals, and these samples could not be included in the analysis. It is thought that the lost data caused by such reasons affect the results of the study.

CONCLUSION

As a result, it was seen that the study created differentiation in terms of behavioral tests between animals exposed to chronic unpredictable stress due to propolis use and healthy subjects, and this literature supported this situation. As a result of behavioral tests, the findings showed that propolis extract might help prevent depression, thanks to its antidepressant-like effects. However, when the biochemical parameters in the urine samples were examined, no difference was found between the groups in the levels of serotonin and metabolites. The reasons for the lack of statistical significance in biochemical parameters were focused on, and in this direction, 5-HT, and 5-HIAA levels could be examined in different body fluids and tissues for future research. Considering that the results of the studies may have a lower efficiency due to the unpredictable conditions of the animals in the laboratory environment and controllable size, it is recommended to advance the experiments.

Ethics

Ethics Committee Approval: The protocol used in this study was approved by the Üsküdar University on the Ethics of Animal Experiments of (ÜÜ-HADYEK), İstanbul, Turkey (decision no: 2020-17, date: 22.01.2021).

Informed Consent: Animal experiment.

Authorship Contributions

Surgical and Medical Practices: A.T., F.C., S.N.Y., B.Ç., Concept: A.T., Design: A.T., Data Collection or Processing: A.T., S.N.Y., B.Ç., Analysis or Interpretation: A.T., F.C., Literature Search: A.T., Writing: A.T.

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