Studying the Anti-Anxiety and Anti-Convulsant Impacts of Royal Jelly in Mice

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ABSTRACT

One of the secretory products of bees is called royal jelly, which has already been stated to have useful impacts on the nervous system. The purpose of this study was to study the effect of royal jelly in decreasing anxiety and seizures in mice. To survey the anticonvulsant impact of royal jelly, 25 male mice were randomly divided into 5 groups. After half an hour, strychnine was injected into the animals to induce convulsions. Mortality percentage, seizure duration, and seizure onset time were recorded. To investigate the effect on anxiety, 35 male mice were divided into 5 groups. Data analysis was done by one-way analysis of variance and $P<0.05$ was considered statistically significant. Based on the results, the doses of 200 and 400 mg/kg of royal jelly delayed the onset and decreased the duration of seizures compared to the group of control. The percentage of mortality in different doses of royal jelly showed a significant reduction compared to the control group ($P<0.05$). In the anxiety model, doses of 50 and 100 mg/kg of royal jelly, stopping time, and times of entering the arms compared to the control group demonstrated a significant increase ($P<0.05$). According to the findings obtained from the present study, it can be concluded that the performance of royal jelly can effectively decrease strychnine-induced convulsions and modulate anxiety behaviors in mice.

Key Words: *Royal jelly, Anxiety, Seizures, Mice*

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INTRODUCTION

Seizures are a type of brain dysfunction caused by abnormal electrical discharge of brain neurons. Several factors are involved in the exacerbation of neuronal stimulation and convulsive attacks, including hypoxia, metabolic alkalosis, infection, trauma, brain tumor, and hypoglycemia. Also, in a few patients, inherited genetic defects are considered the main cause of the disease [1-3].

Seizures may temporarily cause physical, behavioral, and consciousness changes in the afflicted person, which vary according to the severity and extent of the brain involvement. Seizures can also affect a wide range of psychological and individual functioning. This group of patients suffers from disorders such as depression, anxiety, and mental stress. Based on the findings of epidemiological studies, the anxiety prevalence in patients with convulsive attacks is 11-25%, and this anxiety may be

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the result of the unpredictability of convulsive attacks, reduction of daily activities, and social isolation in these people [4-6].

Anxiety and worry can be associated with important physiological changes like increased blood pressure, heart rate, sweating, and increased breathing rate. Anxiety disorders are one of the major human problems and cause a decrease in the quality of life and disruption of individual life [7, 8].

Several classes of drugs are available to control seizures and treat anxiety disorders. Carbamazepine, sodium valproate, topiramate, and ethosuximide are considered strong anticonvulsant drugs that can suppress seizures in many patients, but the important thing in this regard is the need for long-term drug therapy and often the simultaneous use of several drugs together, which can induce or Enzyme inhibition is the underlying cause of drug interactions and the occurrence of many unwanted side effects, including gastrointestinal disorders, neurotoxicity, and blood dyscrasias in patients [1, 9].

Also, for the treatment of anxiety disorders, new drug categories like serotonin/norepinephrine reuptake inhibitors and specific serotonin reuptake inhibitors by changing the levels of catecholamines in the brain are considered the first choice. Despite this, these drugs have little effect on the state of acute anxiety, and the side effects caused by them have reduced their acceptance and limited their use [10, 11].

Also, benzodiazepines are sedative/hypnotic drugs that can be used in the treatment of convulsive attacks, anxiety disorders, and panic attacks, but unfortunately, physical dependence, psychological addiction, drug withdrawal syndrome (if the drug is discontinued), and drowsiness are dangerous side effects. These drugs are considered [7, 12].

In this regard, nowadays, the use of complementary medicine has been promoted as a cost-effective method with limited side effects for the treatment and prevention of various diseases, including neuropsychiatric disorders [13].

One of the effective and safe methods of complementary medicine is the use of drugs and products of biological origin, among which royal jelly can be mentioned [14, 15]. Royal jelly is a milky white gelatinous substance that is secreted using the hypopharyngeal and maxillary glands of worker bees and is utilized to feed the queen bee. This substance has a lot of nutritional value and contains various vitamins, minerals such as calcium, magnesium, sodium, potassium, fatty acids (especially 10-hydroxy decanoic acid), flavonoids, and also a small amount of sterols are also found in royal jelly [16, 17].

From the results of the research conducted in the field of royal jelly, we can mention some things such as increasing the learning capacity, improving memory, reducing the symptoms of menopause, and reducing the symptoms of premenstrual syndrome [16, 17]. The effectiveness of royal jelly in improving depression and anxiety in the experimental model of Alzheimer's induced by the neurotoxin trimethyl tin chloride in rats has been shown that due to its antioxidant properties, this gel inhibits free radicals, including superoxide ions and hydroxyl radicals in the brain and can improve disorders. Cognitive and mood caused by Alzheimer's disease to be effective [18, 19].

Also, the effects of 10-hydroxy decanoic acid, the active ingredient in royal jelly, on the nervous system were evaluated in vivo and in vitro, and the positive effects of this substance in increasing the growth of neurons and reducing neuronal damage in the culture medium, as well as improving anxiety-like behaviors in mice. It has been reported in the desert [20]. Therefore, considering the many benefits of the above product, the current study was done to investigate the possible protective impacts of royal jelly in experimental models of seizures and anxiety in mice.

MATERIALS AND METHODS

Laboratory animals

In this study, 60 young male NMRI small white mice with a weight range of 25 to 30 grams were purchased, of which 25 were used to investigate the anticonvulsant effect and 35 were used to investigate the anti-anxiety effect of the royal jelly. The animals were acclimatized to the laboratory environment conditions for 1 week before the start of the study and were subsequently kept in special cages under a light cycle of 12 hours of darkness and 12 hours of light at a 22-25 C° temperature. Mice had free access to rodent chow and water except during the experiment.

Investigating the anticonvulsant impact of royal jelly in the strychnine-induced seizure model

In this study, royal jelly, strychnine, and phenobarbital were used. The animals were divided into 5 groups of 5 using a random number table using a computer. Negative control group: animals that received sterile physiological serum 30 minutes before administration of strychnine. Experimental groups: animals that received royal jelly at doses of 100, 200, and 400 mg per kilogram of body weight 30 minutes before administration of strychnine. Positive control group: animals that were treated with the anticonvulsant drug phenobarbital at a dose of 40 mg per kilogram of body weight 30 minutes before strychnine administration. All injections were done intraperitoneally and strychnine was used at a dose of 3 mg per kg of body weight to induce convulsions. Strychnine is a glycine receptor antagonist and causes tonic-clonic contractions in rats [21].

Immediately after injection of strychnine, convulsion indicators include the time of onset of convulsions (time interval from the moment of injection of strychnine to the beginning of tonic-clonic contractions in seconds), duration of convulsions (time interval from the moment of onset of convulsions to the end of convulsions or the death of the animal in seconds) and the percentage of death (ratio The number of mice that died due to strychnine injection (total mice in that group) during 30 minutes was measured and recorded.

Investigating the anti-anxiety effect of royal jelly using an elevated plus maze

In this study, royal jelly and diazepam were used. The animals were divided into 5 groups of 7 with the help of a random number table using a computer. Negative control group: animals that received sterile physiological serum 30 minutes before the anxiety assessment test. Experimental groups: animals that received royal jelly at doses of 50, 100, and 200 mg per kilogram of body weight 30 minutes before the anxiety assessment test. Positive control group: animals that were treated with diazepam at a dose of 2 mg per kilogram of body weight 30 minutes before the anxiety assessment test. The injections were done intraperitoneally and to assess the level of anxiety, a plus-shaped maze device was used.

The elevated plus maze device is a standard model for measuring anxiety in rodents, which includes 2 open arms (5 x 50 cm) and 2 closed arms (40 x 5 x 50 cm), which are located at a height of 50 cm from the ground. In this research, each of the mice was placed separately in the center of the elevated plus-maze device facing one of the open arms, and for 5 minutes, the number of entering the open arms and the duration of staying in these arms were recorded using Video Tracking. The device was cleaned with a wet wipe between each test. Increasing the frequency of entering the open arms and increasing the time spent in these arms is considered an indicator of reducing anxiety in rats.

Statistical analysis

Statistical analysis of data was studied using the software of SPSS 23. Tukey's test was used to compare groups 2 by 2 and a One-way analysis of variance was utilized to check the difference between groups. P<0.05 is considered a statistically significant difference between the tested groups.

RESULTS AND DISCUSSION

Anticonvulsant effect of different doses of royal jelly

According to the obtained results, the administration of royal jelly caused a delay in the onset of strychnine-induced seizures in a dose-dependent manner, and this difference was significant in the doses of 200 and 400 mg per kilogram of body weight compared to the negative control group (P = 0.001).

Intraperitoneal injection of phenobarbital at a dose of 40 mg per kilogram of body weight significantly delayed the onset of seizures compared to the negative control group (P = 0.000). Doses of 200 and 400 mg per kilogram of body weight of royal jelly caused a significant decrease in seizure duration compared to the negative control group (P = 0.001).

In the group treated with phenobarbital, the seizure duration decreased significantly compared to the negative control group (P = 0.000). The dose of 100 mg per kilogram of body weight of royal jelly caused a delay in the onset of seizures and a decrease in the duration of seizures compared to the negative control group, but this difference was not statistically significant. The percentage of death following the administration of doses of 100, 200, and 400 mg per kilogram of body weight of royal jelly was significantly reduced compared to the negative control group (P = 0.001). Also, the decrease in mortality in the group receiving phenobarbital was significant compared to the negative control group (P = 0.000).

Anti-anxiety effect of royal jelly different doses

In **Table 1**, the average duration of mice staying in the arms and the number of times they entered these arms in different experimental and control groups are presented.

Table 1. The impact of royal jelly on anxiety indicators using EPM.

Treatment	Mean ± SD	
	Duration of stay in the open arm (seconds)	Number of times entering the open-arm
Control (normal saline)	8.3 ± 6.67	4.2 ± 1.1
50 mg/kg of royal jelly	4.8 ± 4.78*	5.1 ± 4.4*
100 mg/kg of royal jelly	78 ± 1.10*	6.4 ± 6.1*
200 mg/kg of royal jelly	68 ± 1.4	8.2 ± 8.0
2 mg/kg diazepam	4.94 ± 3.9**	6 ± 2.1**

*Significant difference with the normal saline control group at P<0.05 level

**Significant difference with normal saline control group at the level of P<0.001

The staying duration in the open arms was significantly longer in the groups treated with doses of 50 and 100 mg per kilogram of body weight of royal jelly than in the negative control group (P<0.05) **(Figure 1)**.

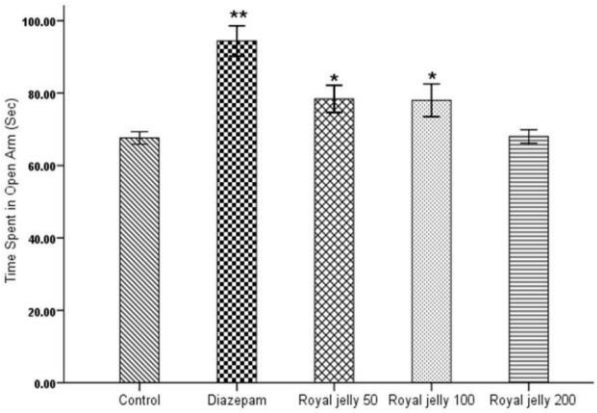

Figure 1. The effect of royal jelly on the duration of mice's presence in the open arms of the elevated plus-shaped maze (Data are expressed as Mean ± SD; * and ** indicate a significant difference, respectively, at the level of P < 0.05 and P < 0.001) with the control group).

The duration of stopping in the central plane did not differ among the different groups under the test (P = 0.24). Also, the number of times entering the open arms in the groups treated with doses of 50 and 100 mg per kilogram of royal jelly body weight increased significantly compared to the negative control group (P < 0.05) **(Figure 2)**.

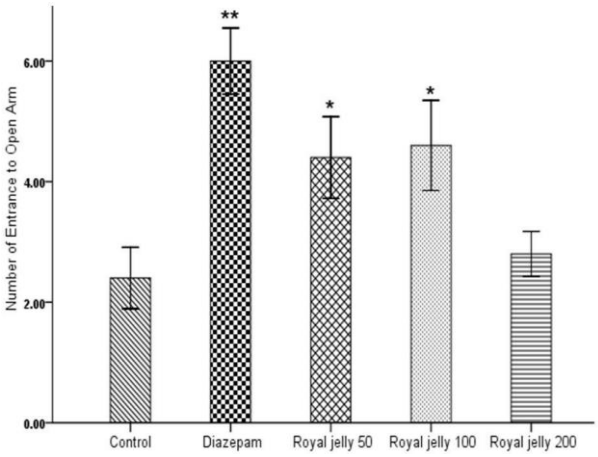

Figure 2. The effect of royal jelly on the number of times the mouse visited the open arms of the elevated plus-maze (Data are expressed as Mean ± SD; * and ** indicate a significant difference, respectively, at the level of P < 0.05 and P < 0.001) with the control group).

The dose of 200 mg per kilogram of body weight of royal jelly was not significantly different from the negative control group in the above indicators. In the group treated with diazepam in all the measured indicators, a significant difference was reported compared to the negative control group (P < 0.001).

During this research, using a strychnine neurotoxin-induced seizure test and anxiety behavioral test with the help of an elevated plus maze device, the protective effects of royal jelly were evaluated in small laboratory mice. In the convulsion test, strychnine is used to induce a simple local convulsion. A simple localized seizure is a type of seizure in which the electrical activity of the brain is disturbed only in a few areas of the brain and does not involve the entire 2 hemispheres, so consciousness is not lost. Some researchers have introduced strychnine-induced seizures as a model of treatment-resistant seizures [22].

In this study, the injection of royal jelly caused an increase in the delay time until the onset of seizures in a dose-dependent manner. Also, the duration of convulsions and the death of animals under the influence of royal jelly were significantly reduced. Consistent with the findings of previous studies, phenobarbital completely prevented strychnine-induced convulsions. In another test, which evaluated the anti-anxiety impact of royal jelly, the decrease in the time spent by the animals in the open arms of the elevated plus-shaped maze and the decrease in the number of times they enter these arms is considered as an indicator of the existence of anxiety behaviors [23].

In the present study, in the group receiving royal jelly, the duration of stopping and the number of times entering the open arms of the elevated plus-shaped maze increased compared to the rats receiving physiological serum, which indicates the positive effect of this substance on reducing anxiety. Based on the results of the current study, Sefirin *et al*. [24] reported that oral administration of royal jelly to ovariectomized female rats reduced anxiety in the open field test and the elevated plus-maze model. Also, the amount of flushing in these animals decreased, which suggests the estrogenic effects of royal jelly in improving behavioral symptoms in these animals.

Also, in another study, Ito *et al*. [25] reported using various behavioral tests that royal jelly, due to its 10-hydroxy decanoic acid fatty acid, improved mood in the stress-induced anxiety and depression model in small white mice. In the research that Pan *et al*. [26] showed, the oral consumption of royal jelly for 3 months reduced the possibility of cognitive disorders and Alzheimer's disease in rabbits under the test, and these effects of royal jelly are due to the reduction of oxidative stress, malondialdehyde, and acetyl enzyme, cholinesterase and beta-amyloid levels in the brain of these animals.

Several studies have suggested that oxidative stress and substances resulting from lipid peroxidation such as malondialdehyde can play a key role in the occurrence and exacerbation of convulsive attacks and anxiety behaviors. Oxygen free radicals cause an increase in the excitatory neurotransmitter glutamate and a decrease in GABA in the brain through the inactivation of the glutamine synthetase enzyme and also the inhibition of the glutamate decarboxylase enzyme [27, 28].

Royal jelly has high antioxidant activity and can inhibit hydroxyl radicals and superoxide ions. This antioxidant activity is often due to the presence of many bioactive compounds found in this substance, the most important of which are flavonoids, polyphenols, 10-hydroxy decanoic acid fatty acid, and vitamins. The most important flavonoids in royal jelly include flavonols, flavones, and flavanones [29, 30].

Recent studies show that these compounds, in addition to having strong antioxidant effects, are ligands for GABA-A receptors in the central nervous system and can act as benzodiazepine-like molecules [31]. On the other hand, behavioral disorders such as depression and anxiety are directly associated with the death of neurons in the hippocampus and inflammatory factors [32].

In a reported study, the administration of 10-hydroxy decanoic acid, the active fatty acid present in royal jelly, significantly inhibited neuronal death and reduced anxiety behaviors in aged rats [20]. It seems that in addition to the antioxidant effects, the anti-inflammatory properties and increased neurogenesis of this royal jelly can play a role in improving the behavioral findings of the current study.

CONCLUSION

Based on the results of the current study, the consumption of royal jelly reduced seizures and moderated mood in small laboratory mice. It seems that reducing inflammation and inhibiting oxidative stress by the active compounds in this gel, such as 10-hydroxy decanoic acid and flavonoids, play a role in the aforementioned impacts. One of the limitations of this research is the lack of investigation of the molecular mechanisms involved in the impacts of this substance on the central nervous system, and it is suggested that more research be done for this purpose in the future. Also, clinical trials should be conducted to prove the effectiveness and determine the exact dose of this gel in patients with neuropsychiatric disorders.

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