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Effect of a Hydroalcoholic Extract of *Rosa Canina* Flowers on Anxiety in Rats

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We investigated effects of the hydroalcoholic extract of *Rosa canina* (dog rose) petals on behavior of rats in the elevated plus-maze (EPM) test; adult male Wistar rats weighing 200-240 g were used. Oral everyday administration of the *Rosa* extract in three doses (150, 300, and 450 mg/kg) was done for one week. Animal behavior in the EPM was videotaped for 10 min, and conventional indices considered to be related to the anxiety level were scored. Introduction of the *Rosa canina* extract significantly increased the number of open arm entries in a dose-dependent manner and also increased the time of stay in the open arms at a high dose (450 mg/kg). At the same time, the number of closed arm entries interpreted as a correlate of the locomotion intensity did not differ from the control at all doses. Thus, the *Rosa canina* extract, when orally administered, demonstrates an anxiolytic profile in rats. Future investigations are essential for better understanding of the anxiolytic properties of the extract and neurobiological mechanisms of its action (probable interactions of the *Rose* extract active agents with neurotransmitter systems.

Keywords: *Rosa canina* (dog rose), anxiety, elevated plus-maze, rats.

INTRODUCTION

Anxiety is a natural psychophysiological reaction of animals and humans directed against known, unknown, and even imagined dangerous situations [1]. High levels of anxiety are characterized by a diffuse, unpleasant, and vague sense of apprehension. This state in humans is often accompanied by autonomic symptoms (headache, perspiration, palpitations, tightness in the chest, and mild stomach discomfort) [2]. High-anxiety states are among most important factors responsible for the development of pathological stresses.

Benzodiazepines constitute the major class of pharmacological agents used for suppression of anxiety, and they have remained the most commonly prescribed treatment for anxiety in the respective cases [3]. Benzodiazepines, however, present a narrow safety margin between the anxiolytic effect and those causing unwanted side effects, and this situation has prompted many researchers to evaluate new compounds in the hope that other anxiolytic drugs will have less undesirable effects [4, 5].

Drugs derived from traditional plants may have possible therapeutic relevance in the treatment of anxiety. Research has been conducted to investigate natural anxiolytic agents in the search for alternative means [5, 6]. The effectiveness of a remedy obtained from *Rosa damascene* in the treatment of anxiety in animals has been described [7], and there is some evidence concerning the sedative effect of remedies from *Rosa canina* in humans [8, 9].

Rosa canina (*Rosaceae*), also known as dog rose, rose hip, or briar rose, is an extensively known prickly shrub (1-3 m high) with fragrant pink or white flowers. The respective genus is widely distributed in Europe, Asia, the Middle East, and North America [9-11]. This species has been evaluated for thousands of years for its food-related biological properties, and its multiple functional uses have been suggested. Teas and syrups made from pseudofruits of this plant are widely known as accessible valuable sources of vitamin C [12]; teas made from these fruits demonstrated laxative and diuretic effects [9]. Extracts of this plant were shown to possess a large amount of phytochemicals that include flavonoids and polyphenols having the potential to serve as antioxidants [6]. These extracts have also been extensively used as anti-inflammatory agents, in particular in alternative treatment for osteoarthritis [13, 14].

On the basis of these considerations, we examined

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the anxiolytic effect of a hydroalcoholic extract prepared from *Rosa canina* flowers in experiments on rats using the elevated plus-maze (EPM) test.

METHODS

Plant Material. The samples of flower petals of *Rosa canina* were collected in summer in the Ganjnameh Mountain, Hamadan, Iran. The flowers were picked in the morning and stored at a low temperature for further use.

Preparation of the Extract. The flowers were air-dried and milled. The milled flower material (180 g) was extracted with 80% ethanol. After the 3rd day, the extract was separated by filtering and subjected to evaporation and concentration using a rotary evaporator at 40°C. Then the concentrate obtained was dried at the laboratory temperature and, when used, dissolved in saline.

Animals. Male Wistar rats weighing 200-240 g were purchased from the Razi Institute, Tehran, Iran. During three days before the tests, animals were housed in groups of four per cage under a 12:12 dark/light cycle (lights on at 07:00 AM at 22 ± 2°C) with free access to food and water. Rats were randomly assigned to one control and three treated groups (n = 10 in each). All experiments were carried out under the same experimental environment in a soundproof room with controlled light conditions, between 11:00 AM and 3:00 PM. Each animal was tested only once.

Elevated Plus-Maze Test. The EPM design was standard and similar to that originally described [15]. In brief, the apparatus was composed of two open (50×10×0.25 cm) and two closed (50×10×30 cm) arms radiating from a central platform (10×10 cm). A slightly raised edge on the open arms (0.25 cm) provided an additional grip for the animals. The plus-maze was elevated to a 50 cm height above the floor level by a single central support.

Daily oral administration of the extract in three doses (150, 300, and 450 mg/kg) or saline was done for 7 days. Animal behavior in the EPM was videotaped for 10 min and saved in the computer. The number of entries into and the times spent in each of the two types of arms were counted within a 10-min-long test period. The number of open-arm entries and open-arm time were used as indices correlating with the anxiety level, while the number of entries into the closed arms was believed to be a correlate of the intensity of spontaneous locomotion. A rat was considered to have entered the arm when all four paws were on the floor of the latter.

Statistical Analysis. Numerical results are expressed as means ± s.e.m. The intergroup differences between the means were estimated by one-way analysis of variance (ANOVA) followed by the Tukey *post-hoc* test. In all cases, differences were considered significant if $P < 0.05$.

RESULTS

The effects of different doses of the *Rosa canina* extract on the time spent in the open arms are shown in Fig. 1. One-way ANOVA indicated that, compared to the control group, the animals treated with the extract spent more time in the open arms. The Tukey *post-hoc* test showed that extract-treated groups spent more time in these arms at all doses (150 mg/kg, $P < 0.05$; 300 and 450 mg/kg, $P < 0.01$). Normalized increments of the means related to the above doses of the extract were 53, 95, and 93%, respectively; thus, the effect can be considered to be dose-dependent.

The effects of different doses of the extract on the number of entries into the open arms are shown in Fig. 2. One-way ANOVA indicated that, compared to the control group, the extract, in general, increased this index. Results of the Tukey *post-hoc* test showed that the extract induced a significant increase in percentage of entries into the open arms only at the highest daily dose of 450 mg/kg ($P < 0.05$, increment about 24%). At the same time, introductions of the extract in 150 and 300 mg/kg daily doses induced practically no changes in the above-mentioned index.

The number of entries into the closed arms was not significantly different between the extract-treated

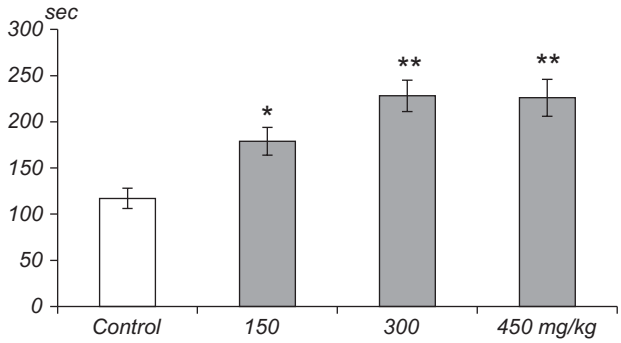
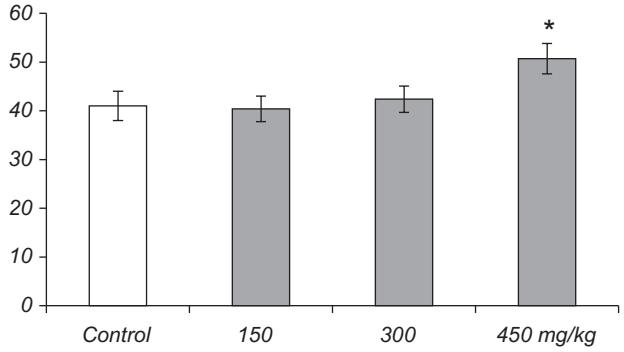
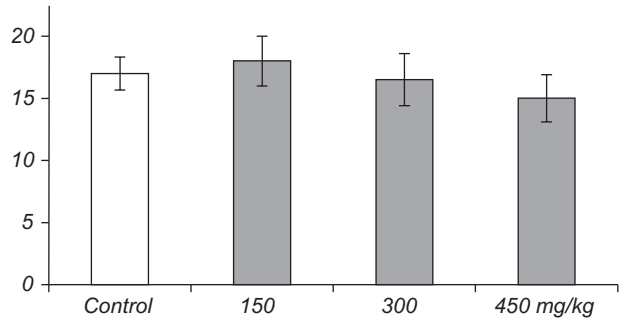


Fig. 1. Effects of the *Rosa canina* extract (150, 300, 450 mg/kg) on the time, sec, spent in the open arms of the elevated plus-maze within 10 min (n = 10 in each group). *$P < 0.05$, **$P < 0.01$ compared to the control (saline) group.



F i g. 2. Effects of the *Rosa canina* extract on the number of entries into the open arms of the elevated plus-maze. Other designations are similar to those in Fig. 1.



F i g. 3. Effects of the *Rosa canina* extract on the number of entries into the closed arms of the elevated plus-maze. Other designations are similar to those in Figs. 1 and 2.

and control groups. At the highest dose of the extract (450 mg/kg), the mean of the above index was slightly (by 13%, on average) smaller than in the control, but the difference was insignificant (Fig. 3).

DISCUSSION

We investigated the behavioral effects of the hydroalcoholic extract from flower petals of *Rosa canina*. Our results demonstrated that peroral repeated introductions of the extract increased both normalized values of the number of entries and time spent in the open arms of the maze. In other words, the extract was able to produce a clear anxiolytic effect in rats after one-week-long oral administration. The effect of *Rosa canina* was not related to changes in general motor activity of the animals because the number of entries into the closed arms practically did not changed. An increase in the time and proportion of the entries into the open arms in the absence of changes in locomotor activity should be interpreted as a potent sign for a specific anxiolytic action of the extract [16, 17].

A number of animal tests are used for investigation of anxiolytic/anxiogenic effects of a variety of agents [18]. The EPM is a well-established animal test rather extensively used for estimation of changes in the anxiety level in small terrestrial rodents (e.g., rats and mice) [19]. These animals, in general, avoid spending time on open elevated surfaces. Changes in the number of entries into the open arms and time spent in these arms are interpreted as reliable correlates of changes in the level of anxiety in experimental animals. Thus, the EPM is considered a proven and reliable test for detecting both anxiolytic- and anxiogenic-like effects of different drugs [16, 20, 21].

The benzodiazepine family is known to be an effective group of pharmacological agents used for the treatment of anxiety disorders. At the same time, it is well known that benzodiazepines are capable of inducing certain rather significant side effects. Therefore, research directed toward finding new anxiolytic drugs with lesser adverse effects is considered highly expedient [3, 5, 22-24]. Various types of herbal medicines have been used as anxiolytics in different parts of the world [5].

Various plants have been used for medicinal purposes long before the beginning of recorded history [25], and their utilization in medicine is still extensively practiced around the world [26, 27]. *Rosa canina* L. has been used for the prevention and treatment of common cold, influenza-like infections, infectious diseases, fever, general exhaustion, gastric spasms, prevention of gastritis and gastric ulcers, diarrhea, gallstones and gallbladder discomforts, urinary tract diseases and discomforts, inflammatory disorder, arthritis, nephritis, rheumatism, gout, sciatica, diabetes, inadequate peripheral circulation, and lung ailments [12]. Using of *Rosa canina* fruits as a source of vitamin C has been mentioned above.

Volatile oils are responsible for the unique and pleasant flavor of *Rosa canina* flowers [11]. Previous phytochemical studies of volatile oils of different *Rosa* species have led to identification of more than 400 compounds, classified into several chemical groups. The latter include hydrocarbon, esters, alcohols, aromatic ethers, aldehydes, and norisoprenoids [28]. Phenols (eugenol and linalool) are among the major active components of *Rosa canina* [29, 30]. The dose-dependent sedative effects of linalool (introduced i.p. or inhaled), including hypnotic and hypothermic effects, are manifested as an increase in sleeping time [31, 32].

In our study, the *Rosa canina* extract noticeably decreased the level of anxiety in rats. It should be mentioned that linalool inhalation was also shown to reduce anxiety [33]. As was reported, linalool prevents glutamate (i.e., the main excitatory neurotransmitter) from binding to its receptors in the rat neocortex [34]. Therefore, it seems that active phenol compounds of the *Rosa* extract work by inhibiting the excitatory neurotransmitter system rather than by potentiating the action of GABA, the main inhibitory neurotransmitter.

The flavonoid rutin (quercetin-3-rutinoside) is a flavonol glycoside comprised of the flavonol quercetin and the disaccharide rutinose [35]. Rutin and quercetin are readily identified among flavonoids of *Rosa canina* [36]. Rutin is known to have anti-anxiety properties [37, 38] related to its action on the serotonin and GABA systems [37]. GABA is widely known to be involved in the etiology of anxiety considering the short-term effectiveness of diazepam, a GABA agonist, in relieving anxiety [39]. Quercetin also decreases the expression of corticotropin-releasing factor in the brain. The above factor is commonly implicated in high-anxiety states [40]. It is released from the hypothalamus, and consequent secretion of adrenocorticotropic hormone (ACTH) form the anterior pituitary and of glucocorticoids from the adrenal cortex is the major endocrine response to stress [38].

Thus, our results demonstrated that repeated oral administration of the *Rosa* flower extract exerts a clear anxiolytic effect in rats. The presence of flavonoids and linalool in the extract is probably the main factor responsible for manifestations of anxiolytic activity. Further studies would be necessary to evaluate the contribution of other substances present in the extract. The source of the extract is quite cheap and accessible. At the same time, it should be taken into account that significant anxiolytic effects of the extract were manifested only at rather high doses of the latter.

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All experiments were conducted in accordance with international standards of animal welfare recommended by the Society for Neuroscience (Handbook for the Use of Animals in Neuroscience Research, 1997). The minimum number of animals and duration of observation required to obtain consistent data were employed.

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