Original Article

# Investigating the effect of hydro-alcoholic extract of *Salix aegyptiaca* on anxiety in male rat

Alireza Komaki, Nasrin Hashemi-Firouzi, Sahar Kakaei[1], Siamak Shahidi, Abdolrahman Sarihi, Iraj Salehi

Neurophysiology Research Center, [1]Department of Physiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

## Abstract

**Background:** Anxiety disorders are frequently common neuropsychiatric disorders. Herbal medicines are widespread and used universal as a treatment compound for anxiety. The present study investigated the effects of hydro-alcoholic extract of *Salix aegyptiaca* blossom on rat behavior in the elevated plus-maze (EPM) and compared results with the effects of diazepam, as a positive control drug.

**Materials and Methods:** Seventy adult male Wistar rats were divided into seven groups ($N = 10$). Animals received *S. aegyptiaca* extract (25, 50, 100 mg/kg) or Diazepam (0.3, 0.6, or 1.2 mg/kg) intraperitoneally and the control group was given the vehicle (10 ml/kg) 30 min before submitting into plus-maze test. The number of entries into the open and closed arms, the percentage of entries into the open arms of the EPM, and the time spent in the open arms were recorded.

**Results:** The results revealed significant increases in percentage of entries into the open arms ($P < 0.01$) and in the time spent in the open arms ($P < 0.01$) after administration of diazepam (0.3, 0.6) and *S. aegyptiaca* (50, 100 mg/kg) in compare with control group. *S. aegyptiaca* extract has no effects on the total distance covered by animals and number of closed arms entries, whereas diazepam decreased these parameters. The locomotor activity was not significantly changed by *S. aegyptiaca*.

**Conclusion:** Single-session administration of optimum doses of total extract of *S. aegyptiaca* has anxiolytic effects in rat similar to the low dose of diazepam. More research is needed for better understanding of anxiolytic properties and neurobiological mechanisms of action and probable interactions of *S. aegyptiaca* extract with neurotransmitters.

**Key Words:** Anxiety, *Salix aegyptiaca*, diazepam, elevated plus maze, rat

**Address for correspondence:**
Dr. Alireza Komaki, Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran. E-mail: alirezakomaki@gmail.com
**Received:** 30.12.2013, **Accepted:** 25.10.2014

## INTRODUCTION

Anxiety is among the most common, most treatable mental disorders. Emotional, cognitive, behavioral and physical components all can be present in anxiety. Anxiety affects one-eighth of the total population word-wide and has become an important area of research interest in psychopharmacology during this decade.[1] Benzodiazepines are the major class of compounds used in anxiety and they have remained the most commonly prescribed treatment for anxiety.[2] Although the benzodiazepines have been known as an effective treatment for anxiety disorders but they cause several undesirable side effects. Therefore, many researches are necessary

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| | DOI: 10.4103/2277-9175.170683 |



**How to cite this article:** Komaki A, Hashemi-Firouzi N, Kakaei S, Shahidi S, Sarihi A, Salehi I. Investigating the effect of hydro-alcoholic extract of *Salix aegyptiaca* on anxiety in male rat. Adv Biomed Res 2015;4:258.

to find new anxiolytic drugs with less adverse effects.[2-5]

*Salix aegyptiaca*, a plant well-known as Musk Willow, is a member of Salicaceae family.[6] In Iranian traditional medicine, *S. aegyptiaca* (Bidmeshk in Persian), has been used for a long time for treating inflammation, gastrodynia, osteodynia palpitation, heart failure and also it is suggested for neurological disorders.[7-10]

There is a variety of animal tests for the investigation of anxiolytic or anxiogenic effects of substances.[11,12] Behavior in the elevated plus-maze (EPM) is a model of anxiety for rodents and it may serve as a new basis for developing anxiolytic agents and investigating psychological and neurochemical factors of anxiety.[13-15] Rats were allowed to freely explore two elevated open and two elevated closed arms of the EPM apparatus that has been confirmed applicable to rats and mice. According to Barrett (1991), an anxiolytic effect is suggested when the drug increases open arms entries without altering the total number of arm entries.[12-14,16] An increase of the time and the proportion of the entrances into the open arms without a changed locomotor activity are regarded as a powerful marker for an anxiolytic substance effect.[15] Locomotor activity of the animals was assessed by measuring the total distance travelled by the animal.[17-20]

Pharmaceutical studies of *S. aegyptiaca* have shown its antioxidant,[9,21] anti-inflammatory, analgesic and anti hyperchlosterolemic effects.[9,10,22] There is an evidence for anxiolytic activity from flowers extract of *S. aegyptiaca* in optimal doses in mice.[23]

In order to more precisely extrapolate these findings for treatment of anxiety, research using behavioral, in rat species is beneficial. On the basis of these considerations the purpose of this study was to characterize the anxiolytic-like activity of the hydro-alcoholic extract prepared from the blossom of *S. aegyptiaca* on rat behavior in the EPM and compared results with the effects of diazepam. The diazepam (as a benzodiazepine) is a standard anxiolytic and is also employed in behavioral pharmacology.[5,24,25] Benzodiazepines have been the most extensively used anxiolytics for many years.[2,5] Diazepam is known to be anxiolytic in humans and reduced anxiety-like behavior in several animal models of behavior.[4,16,26]

## MATERIALS AND METHODS

### Experimental animals

Male Wistar rat weighing 240–300 g were purchased from Razi institute, Tehran, Iran. These animals were transported to a room adjacent to the test laboratory 72 h before starting the experiments. They were housed in groups of four per cage under a 12:12 dark/light cycle (lights on at 07:00 AM) at 23 ± 2°C and given free access to food and water. Rats were randomly assigned to different treatment groups ($N$ = 10). Animals were tested under the same experimental conditions. All experiments were carried out in a quiet room under controlled light conditions between 11:00 AM and 3:00 PM. Behavioral observations took place in soundproof rooms at the same period of the day to reduce the confounding influence of diurnal variation in spontaneous behavior. Each animal was tested only once. All research and animal care procedures were approved by the Veterinary Ethics Committee of the Hamadan University of Medical Sciences and were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1985). The minimum number of animals and duration of observation required to obtain consistent data were employed.

### Drugs and preparation of the extract

The blossom of *S. aegyptiaca* collected in the spring, and identified at the Botanic Institute of Hamadan University of Medical Sciences (plant code number: 1537/12; faculty of Pharmacy, Central Herbarium of Hamadan University of Medical Sciences). The plant material was dried at 40°C with air circulation, ground, and extracted with 70% ethanol by percolation at room temperature. The extracts were dried at 40°C under vacuum and finally freeze-dried.[27,28] The pharmacological assays were carried out with aqueous suspensions of the dried extract. The doses are expressed as mg of dried extract/kg of rat body weight.

Diazepam (Sigma-Aldrich) was diluted with deionized water containing 0.5% propylene glycol (Sigma-Aldrich) and administrated in different doses (0.3, 0.6 and 1.2 mg/kg). Three different concentrations (25, 50, 100 mg/kg) of the *S. aegyptiaca* extract were prepared by dissolving the extracts in 10 ml deionized water with 0.5% propylene glycol to form a homogeneous suspension. All compounds were administrated intraperitonealy (i.p.) 30 min before placing in EPM session.

### Elevated plus maze

Anxiolytic activity of extract was measured using the EPM apparatus. This test has been widely validated to measure anxiety in rodents.[13-15] Briefly, for rats, the apparatus consisted of two open arms (50 × 10 × 1 $cm^3$ each), two enclosed arms (50 × 10 × 50 $cm^3$ each) and a central platform (10 × 10 $cm^2$), arranged in such a way that the two arms of each type were opposite to each

other. The maze was elevated 100 cm above the floor. Thirty minutes after the i.p. injection of the extract (25, 50, 100 mg/kg) or diazepam (0.3, 0.6 and 1.2 mg/kg) or the solvent (10 ml/kg), each animal was placed at the center of the maze facing one of the enclosed arms. During the 10 min test period the number of open and enclosed arms entries, the time spent in open and enclosed arms[15,16] and the percentage of entries into the open arms were recorded.[28] Entry into an arm was defined when the animal places all four paws onto the arm.[15,16]

Animal behavior was tracked by using a video camera located above the maze. Two 100 W lamps brightly illuminated the arena. The experimental sessions were recorded by video camera interfaced with a monitor and a VCR in an adjacent room. This apparatus allowed the measurement of activity (i.e. movement time spent) or time and distances covered in each part of the maze during test. In order to record displacements and other behaviors, the image of the elevated plus-maze was divided into 10 cm squares in a transparent mask placed on the TV screen. This allowed recording of the behavioral category end-arm exploration when the animal reaches the extremity of the open arms.[14,15] After the test, the maze was carefully cleaned with a wet tissue paper (10% ethanol solution).

### Statistical analysis

The data of following parameters were analyzed in our study: Total time spent in the open arms compare to total time in EPM, number of entries into the closed arms, percentage of entries into the open arms compare to total entries in EPM as a whole. The recorded videotapes were analyzed by a person not involved in the experimental procedure.[13] Calculation of the percentage time and number of entries on the open arms with 95% confidence limits and comparisons of the results were performed using computerized analysis. The statistical analysis of data were performed by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. In all cases differences were considered significant if $P < 0.05$.

## RESULTS

An IP injection of diazepam before EPM trial significantly increased the percentage of open arm entries and the time spent in the open arms. This substance decreased the total distance covered by the animals and the number of closed arm entries.[25]

The total distance covered by the *S. aegyptiaca* extract treated rats during the 10 min test was not significantly different from controls [Figure 1a]. The ANOVA revealed a significant effect of *S. aegyptiaca* extract treatment on open arms exploration. Tukey's *post hoc* test showed that, *S. aegyptiaca* has a significant increase in percentage of entries into the open arms in concentrations of 50 mg/kg ($P < 0.05$) and 100 mg/kg ($P < 0.01$), but not at 25 mg/kg in compare to the control group [Figure 1b]. In addition, the extract-treated group spent more time in the open arms in the doses of 50 ($P < 0.05$) and 100 mg/kg ($P < 0.01$), but not at 25 mg/kg [Figure 1c]. The number of entries into the closed arms was not significantly different between the *S. aegyptiaca*-treated and control groups [Figure 1d].

## DISCUSSION

The present study investigated the behavioral effects of the hydro-alcoholic extract of the *S. aegyptiaca* blossom. Our results demonstrated that the extract is able to produce anxiolytic effect in rats. In the other word, the extract of *S. aegyptiaca* (50 or 100 mg/kg, i.p.) increased both of the percentages of entries and time spent in the open arms of the maze, similar to the effects observed after the reference anxiolytic drug diazepam. The effect of *S. aegyptiaca* was not induced by changes in motor activity for such doses, because the total distance traveled by the rats was not altered.

Diazepam was applied as anxiolytic positive control drug.[25,29-31] Amount of closed arm entry indicates the animal locomotion activity.[32] In this method high dose of diazepam could not induce anxiolytic effect because it reduces animal locomotion activity in result of severe sedative effect.

*S. aegyptiaca* extract did not induce changes in motor activity in their anxiolytic doses. In the other word, the total distance covered by the rats was not altered. The high dose of 100 mg/kg induced the most marked effects and raised the anxiolytic behavior. In the other word, extract-treated rats showed a significant increase of both the percentage of entries and the percentage of time spent in the open arms of the maze, similar to the effects observed after the reference anxiolytic drug diazepam. The results of this experiment are consistent with an anxiolytic-like activity from the extract of *S. aegyptiaca* in mice.[23] In addition of animal model, the manner of extract administration in the two studies was different. In the previous study, the mice had treated by subcutaneous and oral administration extract of the flower parts of the plant at doses of 100 and 200 mg/kg. Also, the results of extract effects on animal locomotion in the two studies were different. It has been reported that *S. aegyptiaca* can increase the percentage of time spent in the open arms of the EPM.[23] An increase of the time and the proportion of the entrances into the open arms

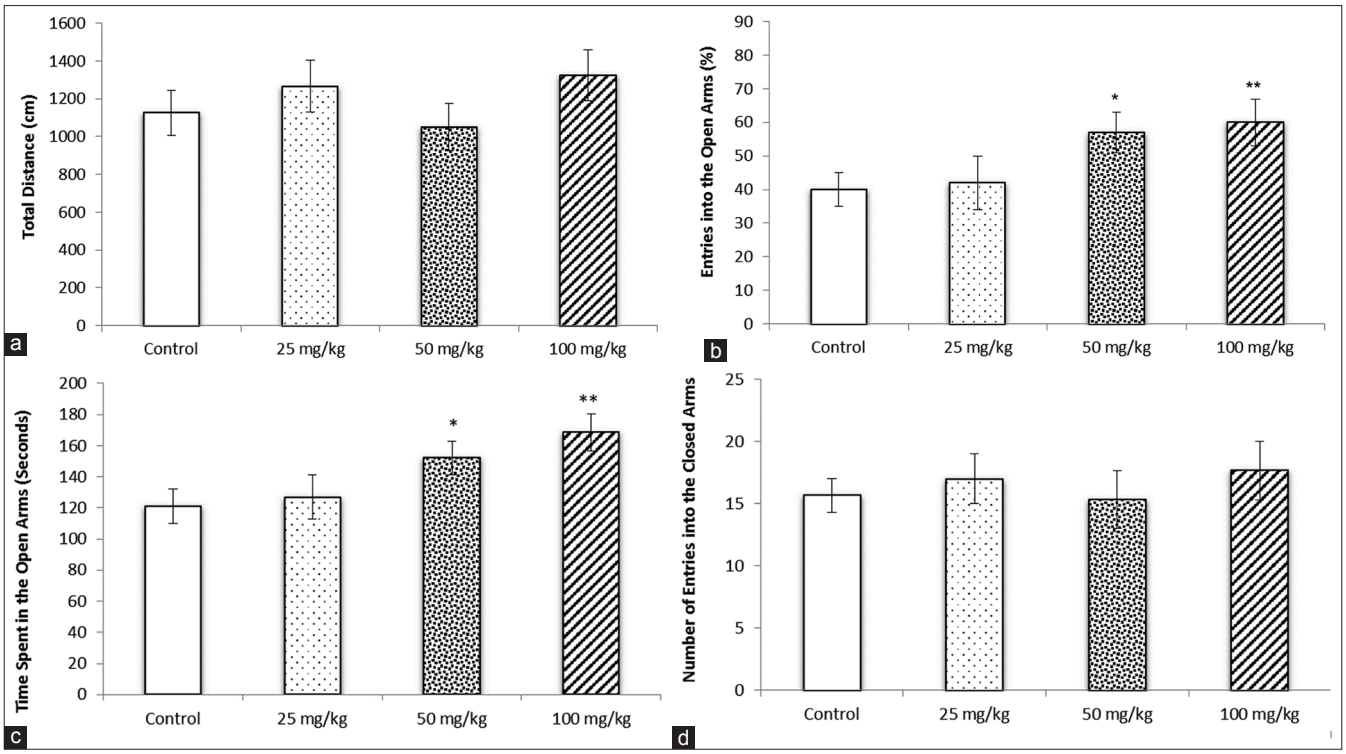


**Figure 1:** The effects of *salix aegyptiaca* extract (25, 50, 100 mg/kg, i.p.) on total distance covered by rats (a), the percentage of entries in the open arms (b), time spent in the open arms (c) and number of closed arms entry (d) during the 10 min test session in EPM. Data represent mean±SEM ($n$=10). Comparisons were made using one-way ANOVA followed by post hoc Tukey's test. *: $P$<0.05, **: $P$<0.01 in compare with control group

lacking a changed locomotors activity are confirmed as a potent sign for an anxiolytic substance effect.[15]

Recently, antioxidant activity of *S. aegyptiaca* has been reported.[21] Extensive research has been conducted to reveal multiple neural substrates and mechanisms that contribute to the etiology of depression and anxiety, among which the imbalance between oxidation and antioxidant defense system have gained attention.[33] Some studies have demonstrated the role of oxidative stress in anxiety of rodents.[34-36] Furthermore, it has been recently reported that two agents that induce oxidative stress, l-buthionine-(S, R)-sulfoximine (BSO) or xanthine plus xanthine oxidase (X + XO), cause increased anxiety-like behavior in rats.[37-39] Also, increasing evidence suggests that the impairment of antioxidant defense and the neuronal cell death are important in the process of emotional disorders, such as depression and anxiety.[33] According to this, Masood *et al*. (2008) reported that the induction of oxidative stress in mice hypothalamus occurs in parallel with anxiety.[36] The consumption of diets with high levels of sucrose was reported to increase the oxidation of proteins in frontal cortex and to cause anxiety in rats. Increased anxiety has been positively correlated with the increase of ROS levels. In another study, oxidative stress in hippocampus of adult rats was reported to be anxiogenic.[40,41] Interestingly, the induction of oxidative stress by a nonpharmacological method also leads to anxiety like behavior in rats.[37] Moreover, the increase in anxiety-like behavior is reversed by antioxidant tempol treatment, suggesting direct involvement of oxidative stress in mediating anxiety-like behavior of rats.[38]

The pharmacological mechanism of *S. aegyptiaca* is suggested to nerve tonic and laxative actions.[10] *S. aegyptiaca* contains phytochemicals compounds such as flavonoids, phenolics, volatile, endogenous salicin, myricetin, kaempferol, quercetin, rutin and luteolin.[9,10,21,23,42] The high amounts of phenols and flavonoids in *S. aegyptiaca* extracts may be responsible for its anxiolytic effects. However, the exact mechanism(s) and the active compound(s) involved in anxiolytic property have not been fully clarified and further biochemical and pharmacological studies are warranted.

## CONCLUSION

In conclusion, our results demonstrate that acute administration of hydro-alcoholic extract of *S. aegyptiaca* produced the anxiolytic properties due to their active components and did not change the locomotion activities in rat. Possibly, the anxiolytic activity observed in this work is dependent on the phenols, flavonoid or other substances with antioxidant activity in *S. aegyptiaca*

extract. Future work will be needed to find the active compound (s) and the neurobiological mechanisms as well as probable interactions of *S. aegyptiaca* extract with neurotransmitters systems.

## ACKNOWLEDGMENTS

This study was supported by Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran.

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**Source of Support:** This research was supported by a grant (grant number: 16/70/2/43873) from the Hamadan University of Medical Sciences, **Conflict of Interest:** None declared.