

Effect of *Matricaria recutita* Hydroalcoholic Extract on Anxiety Behavior in Mice by Hole-Board Test

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Article information	Abstract
<p>Article history: Received: 30 Aug 2012 Accepted: 20 Oct 2012 Available online: 12 Mar 2013 ZJRMS 2014; 16(3): 21-24</p> <p>Keywords: Matricaria recutita Anxiety Mouse</p> <p>*Corresponding author at: Department of Biology, Faculty of Sciences, Shahid Chamran University, Ahvaz, Iran E-mail: m.kesmati@scu.ac.ir</p>	<p>Background: An anxiolytic effect of chamomile has been shown in various studies. In the previous study was indicated that the Iranian species of chamomile, <i>Matricaria recutita</i> (<i>M. recutita</i>) hydro alcoholic extract acts sex dependent in the elevated plus maze. It showed anxiolytic effect in the presence and absence of male mice gonads but not in female mice. In this study we examined the anxiety model dependent of <i>M. recutita</i> in another unconditioned anxiety model, hole-board test, because there are various model for evaluating anxiety with specific properties.</p> <p>Materials and Methods: Adult male and female of N-MARI mice (N=120) were prepared and each sex divided into 5 groups (each group consist of 12 animals): control group, saline and 3 experimental groups that received different doses (10, 30, and 50 mg/kg, intraperitoneally) of <i>M. recutita</i> hydro alcoholic extract. Hole-board instrument was used to anxiety measurement, and delay time, the devour number and maintained time in the holes, as anxiety indices in this device, were evaluated.</p> <p>Results: There were not any significant differences between anxiety indices in control and saline groups in both sexes. <i>M. recutita</i> extract (10, 30 and 50 mg/kg via i.p.) reduced significantly an anxiety in both male and female mice and an anxiolytic effects of 30 mg/kg than the other doses were considerably higher.</p> <p>Conclusion: It seems an anxiolytic effect of <i>M. recutita</i> is independent to anxiety model and the similarity effect at male and female mice in this model emphasizes the validity of the model.</p> <p>Copyright © 2014 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

Anxiety is a spread unpleasant sense and usually is imprecise care that is with one or many unpleasant bodily sense [1]. This disorder is a numbered century widespread disorders that everybody experienced it and freedom of it sometime is not possible. Multiple neural and hormonal centers play role in anxiety outbreak [2]. In this field GABA (Gamma Amino Butyric Acid), serotonin, dopamine and central areas, like amygdala and hippocampus involve in anxiety regulation [3-6]. Understand of intermediated mechanism in anxiety and finding suitable methods for treatment has been research topic for many investigators. In this way multiple chemical and plant drugs for treatment of anxiety have introduced [7, 8].

Side effect of anxiolytic drugs like benzodiazepines and barbiturates lead to usage of plant drugs be attractive for investigators [9]. Plant drugs have lower cost and side effect also there is another component with effective component in them that in more cases potentiate therapeutic effects and reduces side effects of them [10]. chamomile has been attractive for investigators that is a very aromatic and growing wild in champs and beside of pathways [11]. The flowers of this plant are used in industry and medicine. This plant from the past has been

high usage in many clinical disorders as anti-inflammatory, anti-spasm, analgesic drug and etc. [12, 13].

Studies show that has been attention to this plant for anti-anxiety effects and difference methods confirm anti-anxiety effects of it [12-16]. Yamada et al. shown that inhalation of Commomile extract gas in ovariectomized mice, lead to reduce plasma ACTH and reduce anxiety in them [17]. Also Della-Loggia et al. shown oral usage of chamomile in mice has anxiolytic effects [18]. In spite of above investigations, in previous study at this laboratory has been shown that hydro alcoholic extract of *M. recutita* flower in elevated plus maze test at the male and female mice significantly change anxiety indexes. In male mice with and without gonads had anxiolytic effect while in female didn't show this effect. So suggested that anxiolytic effect of *M. recutita* has been sex dependent [19].

Also in HPLC technique revealed that Iranian *M. recutita* has phytoestrogenic components such as Apigenin and Chrysin. And probably by the effect of these components has been observed difference in anti-anxiety effect of it [20]. By attention to exist of many kind of anxiety measurement models that every measure

special aspect for answer to this ambiguity that the anti-anxiety effect of *M. recutita* is related to the model of evaluation of anxiety or not? In this study the effect of this plant drug investigate in another test. For this aim in this study hole-board apparatus as another unconditional model to make and measurement of anxiety was used [21-23]. This model in evaluation of anti-anxiety effect of *M. recutita* has been lowering noteworthy by investigators. So in this study with new insight, by hole-board apparatus, we investigate and compare the effect of hydro-alcoholic extract of *M. recutita* in adult male and female mice.

Materials and Methods

In this experimental study were used adult male and female N-MARI mice (3 months) weighting 32 ± 2 g for male and 28 ± 2 g for female that were purchased from Razi Institute of Hesarak of Kraj. All animals in this study kept in $22 \pm 2^\circ\text{C}$ with wet percentage 30-50 and light/dark system 12 hours dark and 12 hours light with air ventilation. Animals randomly divided in special cages (the number of animal in each group was 12) and were fed with tap water and animal food from Dam Pars, Tehran, Iran. During the test and before it tried to appropriate quiet and out of stress place for animals. All procedures were carried out in accordance with institutional guidelines for animal care and use that accepted by Medical Science University of Ahvaz.

M. recutita flowers from Gol-Daru company (Isfahan, Iran) prepared and confirm by expert. In this investigation soak method was used to preparation of hydro alcoholic extract of *M. recutita* flower. For this reason evaporate branch with flowers was powdered by electrical instrument. In another stage 20 g of powder was get and put in 200 ml ethanol 70% (hydro alcoholic solvent) and mouth of vessel was banned. Prepared mixture kept for 48 hours in laboratory (shaked every 12 hours for few minutes) and then vessel components cleared by Wathman paper and funnel and after kept liquid in oven with 40°C temperature, dried powder of plant extract prepared and was used to preparation doses of it.

In this study to evaluation of anxiety hole-board apparatus was used. This apparatus is an unconditional model for evaluation of anxiety and make of flat square disk (35×35 cm) that has 16 regulate holes (4×4). Diagonal of each hole was 3 cm and apparatus elevated 50 cm above the floor on the chair [23-25]. To evaluation the anxiety animal first sit in the center of apparatus and its behavior evaluated during 5 minutes. In this apparatus each animal was used just for one and there was no any prior instruction and learning. After each test apparatus cleaned by cotton for next test. In this study three anxiety indexes from this apparatus including latency time: time that animal for the first time plunged its head in one of the holes, head-dip in (count of the animal inserts the head into the hole in 5 min), head-dipping (duration of head-dip in seconds).

In this method if latency time being higher, this show anxiety reducing also if head entries in holes and time

spent were higher these show anxiety increased and inverse of these cases show anxiety reduced [23-25]. In this experiments two class containing 60 adult male and female mice were used that divided randomly into 5 groups containing 12 animals. Every of this class were containing: control, group receiving saline and 10, 30, 50 mg/kg of hydro alcoholic extract of *M. recutita*. Animals 30 minutes after i.p. injection of saline or hydro alcoholic extract of *M. recutita* placed in the center of hole-board apparatus and anxiety indexes were evaluated during 5 minutes for each of them [25-29].

Data analyzed by SPSS-16 and one way ANOVA with post hock LSD. In all experiment $p < 0.05$ used as significant level and bars show mean \pm SD.

Results

Statistical comparison between control and saline groups in anxiety indexes (latency time, head-dipping and head-dip in the hole-board test show that saline injection hasn't any effect and there is no any difference between male and female (Fig. 1, 2, 3).

Also figure 1, 2, 3 show that between saline group and groups that receiving different doses of hydro alcoholic extract of *M. recutita* in both male and female mice there is significant increasing in latency time while number of head-dipping and head-dip in significantly reduced. All of these results showing *M. recutita* extract reduced the anxiety in male and female mice.

Statistical analysis between both of male and female in equal doses didn't show significant differences so we did not presented them in this section but seems anxiolytic effect of *M. recutita* on female mice partially was more effective.

Many studies confirmed that *Matricaria chamomilla* has anxiolytic effect [11, 16]. Della-Loggia et al. shown that oral usage of chamomile in mice can make anxiolytic effects [18]. Also Yamada et al. shown that inhalation of chamomile gas in ovariectomized female mice, lead to reduce blood plasma ACTH and reduce stress and anxiety in them [17]. In a clinical study identified that Apigenin isolated from chamomile is effective in some of drugs withdrawal syndrome including anxiety [30, 31]. Palladini et al. shown that Apigenin isolated from chamomile has anxiolytic effects and can act as stimulator neurotransmitter in central nervous system [32]. In addition in another similar study identified that Apigenin and Chrysin flavonoids extracted from chamomile show anxiolytic and benzodiazepines like effects [16, 20, 33].

Studies show that phytoestrogens, like flavonoids isolated from chamomile, can be responsible for anxiolytic effects of it in both of male and female mice and oral administration of them in mice lead to make anxiolytic effects in lower doses [18, 26]. In this study identified that hydro alcoholic extract of *M. recutita* has anxiolytic affects in male and female mice. Thought difference between male and female in equal doses was not significant so didn't present in this study but anxiolytic effects of *M. recutita* in female according to the level of significant partially seems is more effective.

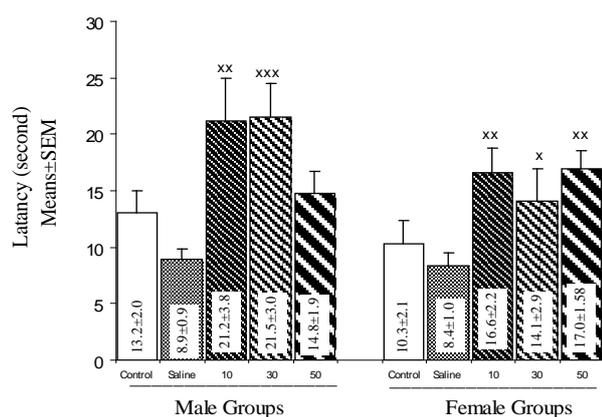


Figure 1. Comparison of latency time between saline group with control and groups receiving 10, 30, 50 mg/kg of hydro-alcoholic extract of *Matricaria recutita* in hole-board apparatus. Significant level for every male and female group has been shown in compared to saline group of same sex. The number of animal in each group was 12. ($x=p<0.05$, $xx=p<0.01$, $xxx=p<0.001$)

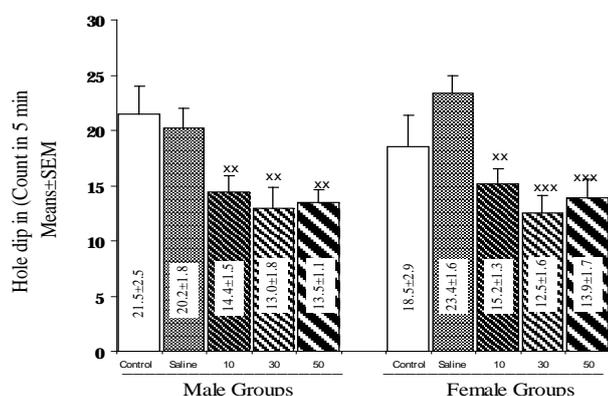


Figure 2. The comparison of head dip in (count of the animal inserts the head into the hole in 5 min) between saline group with control and groups receiving 10, 30, 50 mg/kg of hydro-alcoholic extract of *Matricaria recutita* has in hole-board apparatus. Significant level for every male and female group has been shown in compared to saline group of same sex. The number of animal in each group was 12. ($xx=p<0.01$, $xxx=p<0.001$)

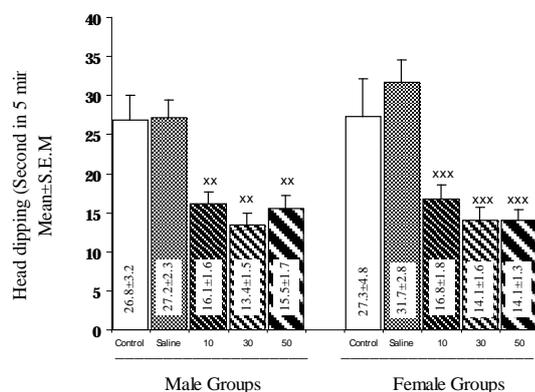


Figure 3. The comparison of head-dipping (duration of head-dip in seconds), between saline group with control and groups receiving 10, 30, 50 mg/kg of hydro-alcoholic juice of *Matricaria recutita* has in hole-board apparatus. Significant level for every male and female group has been shown in compared to saline group of same sex. The number of animal in each group was 12. ($xx=p<0.01$, $xxx=p<0.001$)

Today it has been demonstrated that flavonoids materials isolated from botanic drugs like: Apigenin that with high affinity bind to benzodiazepines site of GABA-A receptors [34]. Studies show that Apigenin by binding to these receptors in central nervous system, play its sedative and anxiolytic effect [35]. So it possible that powerful effect of *M. recutita* in reduce anxiety is due to this material. In addition identified that Apigenin as flavonoid material has its best anxiolytic effect at dose of 2 mg/kg in elevated plus maze test [26], and it has more similarity with our finding, actually maximum anxiolytic effect of *M. recutita* is at dose of 30 mg/kg in animals.

Main point in this study in contrary with our pervious finding, about the effect of *M. recutita* extract on mice in elevated plus maze test, is the effect of this extract on female mice anxiety in hole-board test [19]. This difference probably can be related with different aspects of anxiety measuring and strong and weak points of these tests. For example it has been identified that elevated plus maze test as unconditional model of anxiety in rodent because it need animal travels on the surface of open arm of apparatus make unusual anxiety in animals and so the validity of some results of it can have poor confidence in compared to others tests [36]. On the other hand Borrin et al. said that of two evaluated parameters anxiety and locomotor activity, in male genus, anxiety, and in female genus, locomotor activity, affected in anxiety test when usage of effective materials [37]. So the reason of didn't change in anxiety behaviors in female in elevated plus maze test can be due to different in the kind of utilized test.

So according to the findings from this study can conclude that the *M. recutita* probably with containing flavonoids has anxiolytic effect in male and female mice in hole-board test and affecting central nervous system through the change in GABA-A receptors activity or/and stress hormones and makes this plant drug good candidate to produce anti-anxiety drug. Also because of similarity in anxiolytic effect of this plant drug in both of male and female mice, this model to measurement of anxiety has higher validity in compared to elevated plus maze test to evaluation of anxiety and investigation of the effects of drugs.

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Authors' Contributions

The first author had role in design and manuscript writhing, the second one in experimental work and the third one in manuscript edition.

Conflict of Interest

The authors declare no conflict of interest.

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