Original Research Article

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Anxiolytic activity of aerial part of *Mercurialis annua* aqueous extract in mice using light/dark and hole board tests

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Abstract

The present study was designed to study anxiolytic property of aqueous extracts of *Mercurialis annua*, an important and commonly used medicinal plant for its medicinal properties. The anxiolytic activity was evaluated in adult mice by hole board test and light/dark box test and motor coordination by rotarod test. The efficacy of the plant extract (100 - 600 mg/kg) was compared with the standard anxiolytic drug diazepam (1 mg/kg). The extract increased the time spent in the brightly lit chamber of the light/dark box, as well as in the number of times the animal crossed from one compartment to the other. Performance on the rotarod was unaffected. In the hole board test, the extract significantly increased head dip counts. *Mercurialis annua*, in contrast to diazepam, had no effect on locomotion. These results provide support for anxiolytic activity of *Mercurialis annua*, in line with its traditional medical use, and may also suggest a better side effect profile of *Mercurialis annua* relative to diazepam.

Keywords: Anxiety, *Mercurialis annua* aqueous extract, Rotarod test, Hole board test, Light/dark test, Morocco

1. Introduction

Anxiety disorders are the most common psychiatric illnesses [1], and considered a common emotional phenomenon in the human population, occurring in response to physiological and /or environmental factors. The pathophysiologic mechanisms associated with anxiety disorders are very complex and became a very important area of research interest in psychopharmacology during this decade [2]. Anxiety is characterized by a diffuse, unpleasant, vague sense of apprehension; negative emotional, cognitive, behavioral and physical components can all be present in anxiety.

At present, benzodiazepines (BZDs) and selective serotonin reuptake inhibitors (SSRIs) [3] are the major classes of compounds used to treat anxiety and they remained the most commonly prescribed treatment for anxiety [4]. As synthetic pharmacotherapies have significant potential side effect [5], there is a need for the development of new anxiolytic drugs that shows less adverse effects. Medicinal plants have been used from ancient times for their medicinal values as well as to impart flavor to food [6]. In folk medicine, some species belonging to the family Euphorbiaceae, such as *Mercurialis annua*, is reported to possess anxiolytic action [7].

Mercurialis annua L. (Euphorbiaceae) is a wind-pollinated annual plant that occupies ruderal and roadside habitats throughout central and western Europe and around the Mediterranean Basin [8]. The species is a winter annual in the Mediterranean region, and has long been known to have tranquillizing effects among the Moroccan people [7, 9]. Reaching 10–50 cm in height, *M. annua* contains large amounts of flavonoids and of the pyridinone-type alkaloid, hermidin [10]. Ethnobotanical reports attribute purgative, diuretic or antisyphilitic effects to the dried plant. Our previous studies involved evaluation of methanolic extract of *M. annua* for anxiolytic activity using elevated plus maze test and open field test. In the present study, the potential anxiolytic effect of several doses of the aqueous crude extract of *M. annua* was evaluated and compared with the effect produced by diazepam (1 mg/kg) in the light-dark box test, hole board test and rotarod test.

2. Materials and methods

2.1. Animals

Adult Swiss mice (20–30 g) of either sex were used for the study. The animals were acquired from the animal experimental centre of Mohammed V souissi University, Medicine and Pharmacy Faculty, Rabat, Morocco. The animals were maintained in a room with controlled temperature (25 ± 1 °C) and lighting (light/dark 12:12 h in polypropylene cages, with food and water ad libitum. Animals were acclimatized to laboratory conditions at least 1 h prior to initiation of experiments. The animals were divided into four groups, each consisting of six mice, implemented in all sets of experiments.

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2.2. Plant material

The aerial part of *Mercurialis annua* was collected from the north of Morocco near the town of Wazzan (Jaaouna el Basra), with assistance of a traditional medical practitioner. The plant was authenticated by botanists of scientific institute Pr. M. Ibn Tatou and Pr. Halim Khammar. A voucher specimen (N° RAB78984) was deposited in the Herbarium of Botany, Department of the Scientific Institute of Rabat, Morocco.

2.3. Preparation of the aqueous extract

The aerial parts of *Mercurialis annua* (100 g) were extracted with 1 L distilled water (70 °C, 30 min), concentrated and then freeze-dried producing aqueous extract (AE) with a 32.70 %w/w yield.

2.4. Drugs

The aqueous extract of *Mercurialis annua* was suspended in distilled water. Diazepam (ampoule 10 mg/2 ml) was diluted with saline to the required concentration before use. It is well known that benzodiazepines act as anxiolytics at low doses and that they induce sedation and muscle relaxant effects at higher doses [11]. Therefore, we used diazepam (1 mg/kg) as a positive control for anxiolytic-like effects.

2.5. Treatment schedule

Experimental groups of mice were treated orally (p. o.) with aqueous extract of *Mercurialis annua* at doses of (100 – 600 mg/kg), whereas control groups received normal saline by the same routes. Diazepam (1 mg/kg) was administered intraperitoneally (i.p.). All drugs were freshly prepared before each experiment. The doses of extracts were calculated to administer 0.25 ml of the suspension of extracts to the mice of 20 g. The trial was carried out 1 hr after the treatments. The anxiolytic activity was examined by using the light/dark box test and the hole board test, and motor coordination test was assessed with the rotarod equipment.

2.6. Acute toxicity study

The procedure was followed as per OECD 423 guidelines [12] (OECD/OCDE, 2002). The extract was administered orally at a dose of 2000 mg/kg body weight. Mice were kept under observation for 14 days to register possible mortality; their weights were registered and their behavioral neurological toxicity was studied.

2.7. Light/dark test

The apparatus consisted of two 20 cm × 10 cm × 14 cm plastic boxes with one compartment painted white and brightly illuminated and the other was painted black and dimly illuminated with red light. The mice were allowed to move from one box to the other through an open door between the two boxes. The illumination in the black compartment was 50 lux, whereas in the white area it was increased to 1000 lux, generated by an extra light source. A mouse was introduced into the light box facing the hole. The transition between the light and the dark box and time spent in the light box were recorded for 5 min [13].

2.8. Hole board test

The hole board apparatus [13] was used in this test. It is made of gray Perspex. The LETICA board (signo 720; Printer LE 3333) of dimensions 40 cm × 40 cm, containing 16 evenly spaced holes (3 cm diameter and 2.2 cm depth), with in-built infra-red sensors was used for the study. The matt finishing of the upper panel avoids reflections which may alter the animal behavior. An animal was placed in the center of the hole board and allowed to freely explore the apparatus for 5 min. The number of times an animal dipped its head into the holes was automatically counted and recorded by the instrument [14].

2.9. Rotarod test

The effect on motor coordination was assessed using a rotarod apparatus (LE 8500). Rotarod consisted of a base plant form and an iron rod of 3 cm diameter and 30 cm length, with a non-slippery surface. The rod was divided into four equal sections by three disks. The animals were pre-selected in a training session 24 hr before the test, based on their ability to remain on the bar (at 12 rpm) for 2 min. In actual test, four mice were allowed to walk on the rod at the speed of 12 rpm at the same time observed over a period of 30, 60, and 90 min. Intervals between the mounting of the animal on the rotating bar and falling off of it were registered automatically as the performance time. Time spent in the apparatus was observed for 5 min duration (300 s). Apparatus was cleaned thoroughly between trials with water. All behavioral recordings were carried out with the observer blind to the treatment the mice had received.

2.10. Statistical analysis

All results were expressed as mean ± standard error of the mean. The data were analyzed statistically using one way analysis of variance ANOVA, followed by the Tukey Kramer post hoc test for multiple comparisons. Statistical significance was set at $p < 0.05$.

2.11. Ethical approval

The study was conducted in accordance with the accepted principles outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the National Institutes of Health and all efforts were made to minimize animal suffering and the number of animals used. Ethics approval was obtained from the Central Laboratory of Animal, Facutly of Medicine and Pharmacy, Mohammed V University of Rabat.

3. Results

3.1. Acute toxicity study

Following oral administration, aqueous extract of *M. annua* at a dose of 2000 mg/kg, animals were observed for signs of toxicity such as convulsions, hypothermia, hyperactivity, and grooming continuously for 2 hr and for mortality up to 24 hr after administration of the doses. No toxicity and no significant changes in the body weight were observed between the treated and control groups.

3.2. Light/dark test

M. annua at the dose of 400 mg/kg and diazepam (1 mg/kg) induced a significant increment of the time spent by mice on the illuminated side of the apparatus compared to the respective control group ($p < 0.05$, $p < 0.01$), without significantly affecting other parameters (Fig. 1). Number of transition in the light-dark test showed a significant ($p < 0.05$) increment at dose of 400 mg/kg of *M. annua* (Fig. 2).

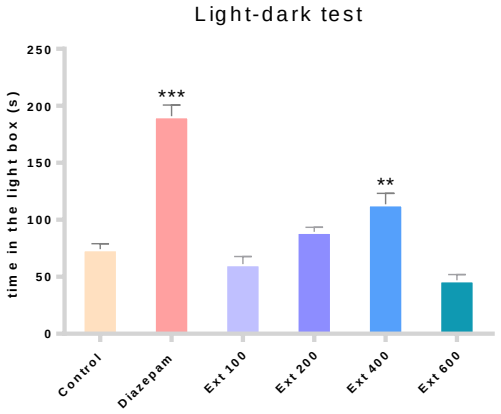

Figure 1. Effect of *M. annua* extract in light/dark test. Data are mean ± S.E.M. (n=6); One-way ANOVA, followed by Tukey Kramer post hoc test; ** p < 0.05, *** p < 0.01.

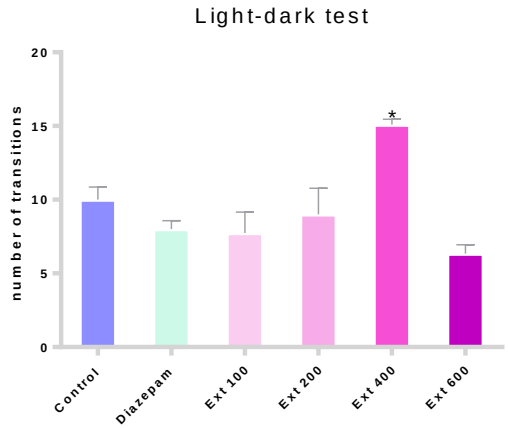

Figure 2. Effect of *M. annua* extract in light/dark test. Data are mean ± S.E.M. (n=6); One-way ANOVA, followed by Tukey Kramer post hoc test; *$p < 0.05$.

3.3. Hole board test

The dose 400 mg/kg of the plant extract significantly increased the number of head dipping as compared to control animals (Fig. 3).

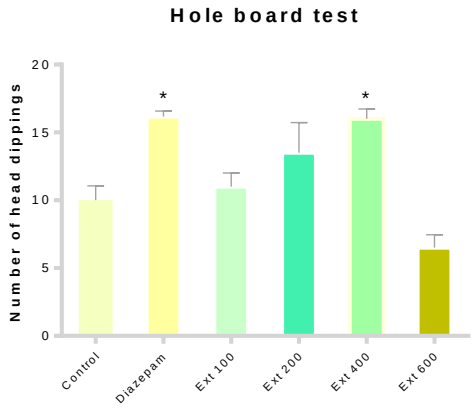

Figure 3. Effect of *M. annua* extract in hole board test. Data are mean ± S.E.M. (n=6); One-way ANOVA, followed by Tukey Kramer post hoc test; *$p < 0.05$.

3.4. Rotarod test

The results showed that the mice treated with 100, 200 and 400 mg/kg p.o. of the methanolic extract of *M. annua* were able to maintain equilibrium on the rotating rod and stayed on longer without falling (Fig. 4), whereas diazepam (1 mg/kg) and 600 mg/kg of our extract showed a significant decrease in the locomotor score when compared to other groups.

4. Discussion

Self-administration of herbal medicines is among the most popular of alternative therapies. There is a considerable interest in the development of new anxiolytic compounds that have fast onset of action with less side effects and a wider safety margin. In the current work, we examined the neurobehavioral effects of oral treatment with the aqueous extract from aerial part of *M. annua*. Various doses of the plant extract were tested using well-validated animal models of CNS activity, namely the light/dark test, hole board test and rotarod test. Furthermore, the effects of *M. annua* and diazepam on these animal models were compared to determine whether the behavioral profile of *M. annua* differed from an established anxiolytic drug.

In the light/dark box test, anxiety is generated by the conflict between the desire to explore and to retreat from an unknown and well-illuminated space [15] and can be evaluated according to the number of transition into and the time spent in the light chamber [16, 17]; where an increase in these parameters is considered to reflect anxiolytic-like properties.

The behavioral responses of animals evaluated in the light-dark test were found to be modified. Our results showed that *M. annua* treatment only at 400 mg/kg increased these two parameters and at doses lower than 400 mg/kg, there was no significant changes in the behavior parameter. Dose higher than 400 mg/kg produced sedative effects and were not considered suitable for further evaluation. (Fig. 1 and 2).

The hole board test is useful for modeling anxiety in animals; in this test the expression of an anxiolytic-like state may be reflected by an increase in head-dipping behaviors [18, 19]. Our results showed that *M. annua* increased the number of head dips (Fig. 3) indicating an anxiolytic-like effect.

A deficit in motor coordination would very likely affect performance in the behavioral tests. Therefore, we investigated the motor effects of *M. annua* in the rotarod test, a classical animal model used to evaluate peripheral neuromuscular blockade and the motor coordination [20]. Our finding showed that *M. annua* (100 – 400 mg/kg), unlike diazepam (1 mg/kg), had no significant effect on motor coordination (Fig. 4).

As expected, diazepam produced significant increase in time spent in light chamber, number of head dips. Diazepam was reported to potentiate the inhibitory effect of GABA by enhancing the frequency of chloride channel opening and thus enhance the chloride flux through the $GABA_A$ receptor/ chloride channel [21].

The aqueous extract of *M. annua* produced anxiolytic activity in these tests. However, the exact mechanism of action is unknown. In the future work, we should determine the mechanism underlying the anxiolytic-like effects of aqueous extract of *M. annua*.

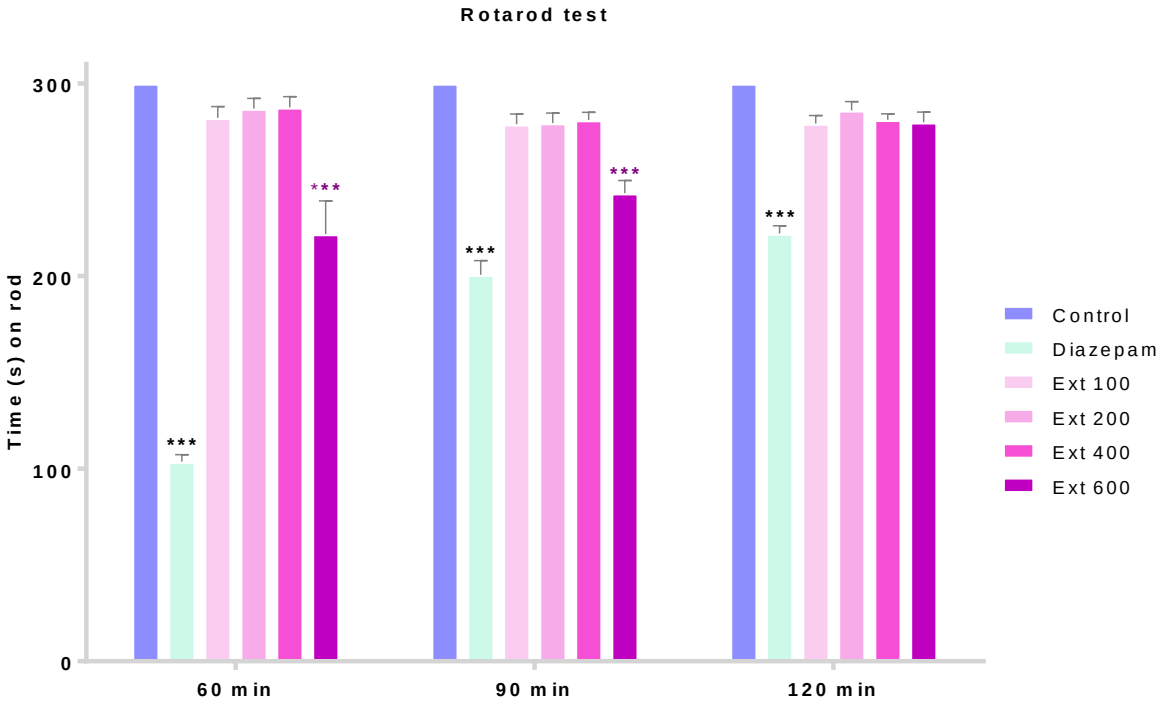

Figure 4. Effect of *M. annua* extract in rotarod test. Data are mean ± S.E.M. (n=6); One-way ANOVA, followed by Tukey Kramer post hoc test; ***$p < 0.01$.

5. Conclusion

In summary, the present results demonstrated an anxiolytic-like effect of aqueous extract of *M. annua* with a mild sedative action at dose (600 mg/kg). Future studies will be focused on the neurobiological mechanisms of action and possible interactions of *M. annua* with classical neurotransmitters. Furthermore, the phytoconstituent(s) responsible for the observed central effects need to be isolated and identified.

Competing interests

The authors declare that they have no competing interest.

Authors' contributions

DZ carried out all the studies and drafted the manuscript with the help of other authors. TK participated in this work and drafted the manuscript. MEJ helped in the chemistry part and NM carried out the behavioral tests. CY guided the experimental work. KA guided the experimental work and corrected the manuscript. All authors read and approved the final manuscript.

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