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Research Article

Neuropharmacological Evaluation of Methanolic Extract from *Mercurialis Annua* a Plant used in Moroccan Traditional Medicine

Zouhra Doukkali[1]*, Mohamed Amine Boumahdi[2], EL Houcine Bouidida[3], Khalid Taghzouti[4]

Abstract

Current therapeutic for the treatment of anxiety is associated with a wild variety of prominent side effects. The traditional use of plant extract to health care can indicate an important source of new pharmaceuticals. In Morocco traditional medicine, the use of *Mercurialis annua* is commonly recommended for relief of anxiety. Nevertheless, despite its popular use there are no studies related to its possible neuropharmacological effect. Here, we investigated the possible anxiolytic effect of the extract of *M.annua* after acute treatment in mice.

The methanolic extract from the aerial parts of *M.annua* (100, 200 or 400 mg/kg) was orally administered, and its anxiolytic effect was evaluated in hole board test, the light–dark box test, and motor coordination with the rota rod test. Diazepam was employed as standard drug 1mg/kg.

The methanolic extract of Ma 100 mg/kg 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 rota rod was unaffected. In the hole board test, the extract significantly increased head-dip counts.

These results provides support for anxiolytic activity of *Mercurialis annua*, in line with its medicinal traditional use, and may also suggest a better side-effect profile of *Mercurialis annua* relative to diazepam.

Keywords: Anxiety, *Mercurialis annua*, Rota rod test, Hole board test, Light–dark test, Morocco

Abbreviations: Ma: *Mercurialis annua*

Introduction

Anxiety is considered a common emotional phenomenon in the human population, occurring in response to physiological and/or environmental factors [1]. When anxiety becomes excessive, it may be considered as an anxiety disorder, and can critically decrease the quality of life inducing several psychosomatic diseases.

Benzodiazepines are the major class of compounds used in anxiety and they have remained the most commonly prescribed treatment for anxiety [2]. Benzodiazepines can lead to disturbing effects, such as amnesia, dependence liability, and sedation [3,4].

Therefore, the development of other anxiolytic drugs without such adverse effects is important for the treatment of anxiety disorders.

Several traditionally used plants exhibit pharmacological properties with great potential for therapeutic applications in the treatment of central nervous system disorders, such as anxiety disorders [5]. In folk medicine, some species belonging to the family Euphorbiaceae, such as *Mercurialis annua*, is known to possess anxiolytic action [6].

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 [7]. The species is a winter annual in the Mediterranean region, and has long been known to have tranquillizing effects among the Moroccan people [6,8].

Reaching 10–50 cm in height, *M. annua* contains large amounts of flavonoids [9] and of the pyridinone-type alkaloid, hermidin [9]. Ethnobotanical reports

Affiliation:
[1]*Pharmacodynamy Research Team, ERP, Laboratory of Pharmacology and Toxicology, Faculty of Medicine and Pharmacy, University Mohammed V of Rabat, Morocco*

[2]*Laboratory of Physiology and Pathophysiology, Faculty of Science, University Abdelmalek Essaâdi, Tetouan*

[3]*National Laboratory of Drug Control, Directorate of Pharmacy, Ministry of Health, Rabat, Morocco*

[4]*Laboratory of Animal Physiology, Department of Biology - Faculty of Science - University Mohammed V of Rabat, Morocco*

***Corresponding author:**
Zouhra Doukkali, Pharmacodynamy Research Team, ERP, Laboratory of Pharmacology and Toxicology, Faculty of Medicine and Pharmacy, University Mohammed V of Rabat, Morocco,
E-mail: d.zouhra@yahoo.fr

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attribute purgative, diuretic or antisyphilitic effects to the dried plant.

Despite the wide spread use of *M.annua* as an anxiolytic, there are no pharmacological data to support such effect, therefore, we explored the anxiolytic-like effects of Methanolic extract of Ma by using the hole board test, the light–dark box test, and the rota rod test, classic experimental models.

Materials and Methods

Animals

Balb/c mice of either sex (20-30g) were employed in the present study. Animals were procured from the animal experimental center of Mohammed V. University, Medecine and pharmacy Faculty, Rabat. Animals were provided normal diet and water ad libitum and were maintained in a room with controlled temperature of 20-25°C, and lighting (light/dark 12:12 hour) in polypropylene cages. The animals were acclimatized to the laboratory condition before experiments at least 1h. The animals were kept fasted 2h before drug administration, the Open field and the elevated plus maze were performed between 2p.m and 6p.m.

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.

Preparation of the methanolic extract

The aerial part was dried at room temperature and crushed. 700 g of plant material was extracted with six liter of methanol by maceration at room temperature (25°C) over period of 48 hours. Methanol containing the extract was then filtered through Whatman paper and the solvent was vacuum-distilled at 60 °C in a rotary evaporator. The remaining extract was finally dried by desiccator. Final extract was a dark green paste, with 21.22 % dry weight. The residue was dissolved in water for final suitable concentrations.

Drugs and chemicals

The methanolic extract of *Mercurialis annua* was suspended in distilled water. Diazepam 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 [**10**]. Therefore, we used diazepam (1mg/kg; ip) as a positive control for anxiolytic-like effects.

Treatment schedule

Animals were divided into five groups, each consisting of six mice. Experimental groups of mice were treated orally (p. o.) with methanolic extract of *Mercurialis annua* at doses of (100, 200 and 400 mg/kg), whereas control groups received normal saline by the same routes, the trial was carried out 1H after the treatments. Diazepam (1 mg/kg) was administered intraperitoneally (i.p.) the trial was carried out 30 min after the treatments. 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 anxiolytic activity was examined by using the hole board test, the light–dark box test, and the rota rod test.

Behavioral paradigms

Light/Dark test: The apparatus consisted of two 20 cm X 10 cm X 14 cm plastic boxes: one light compartment painted white and brightly illuminated and the other was dark 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, in the white area it was increased to 1000 lux, generated by an extra light source. A mouse was put 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.

Hole Board test: The hole board test [**11**] was adopted in this test. It is made of gray Perspex. The LETICA board (signo 720; Printer LE 3333) of dimensions 40 cm X 40 cm, contained 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 [**12**].

Rota rod test: The effect on motor coordination was assessed using a rota-rod apparatus (LE 8500). Rota rod consisted of a base plant form and aniron 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 h before the test, based on their ability to remain on the bar (at 12 rpm) for 2 min, and then allowing four mice 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.

Statistical analysis

All the results were expressed as mean ± SEM. All statistical analysis was done using one way analysis of variance (ANOVA) followed by the Tukey's post hoc test. $P<0.05$ was considered as significant when compared to their respective control group.

Ethics 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 of Facutly of medecine and pharmacy from the Mohammed V University of Rabat.

Results

Light/Dark test

Mercurialis annua at the dose of 100 mg/kg and diazepam (1 mg/kg) induced a significant increment of number of transition and time spent by mice on the illuminated side of the apparatus compared to the respective control group (P <0.001) (Table A).

Hole board test

The dose 100 mg/kg of the plant extract significantly increased the number of head dippings as compared to control animals (P< 0.01) (Table B).

Rotarod test

The data shows that on average the mice treated with 100, 200 and 400 mg/kg p.o. of the methanolic extract of *Mercurialis annua* were able to maintain equilibrium on the rotating rod and stayed on longer without falling (Table C), whereas diazepam (at 1 mg/kg only) showed a significant decrease in the locomotor score when compared to other groups.

Discussion

The pathophysiologic mechanisms associated with anxiety disorders are very complex. Dysregulation of the GABAergique, serotoninergic, dopaminergic and adrenergic neurosystems have all been implicated in the pathophysiology of anxiety [13].

Benzodiazepines are the major class of compounds used in anxiety and they have remained the most commonly prescribed treatment for anxiety [2]. BZDs produce their pharmacological actions via specific high affinity binding sites on a supramolecular complex composed of GABA-A and a BZD receptor coupled with a chloride ion channel. Other anti-anxiety medications include antidepressants, buspirone and b-blockers which though effective in many cases, also possess side effects like sedation, myorelaxation, ataxia and amnesia, and can cause pharmacological dependence [2,14].

Treatment	Dose (mg/kg)	Time in the light box	No. of transition
Saline		72,98 ± 18,39	10,5 ± 1,910
Diazepam	1	193,4 ± 17,00 ***	8,5 ± 1,928
Plant extract	100	141,4 ± 5,247***	18,33 ± 1,145***
Plant extract Plant extract	200 400	102,1 ± 4,824* 94,05 ± 3,850≠≠	14,25± 1,250 13,20 ± 1,114

All values are mean ± SEM (n=6); *p<0.05, ***p<0.001 when compared to control. One- way ANOVA, Tukey's Multiple Comparison post hoc tests.

Table A: Light/dark test.

Treatment	Dose (mg/kg)	Number of head dipping
Saline		10,50 ± 1,3
Diazepam	1	14,83 ± 1,6
Plant extract	100	23,17 ± 2,358**
Plant extract Plant extract	200 400	17,33 ± 2,060 18,17± 3,005

All values are mean ± SEM (n=6); **p<0.01 when compared to control. One- way ANOVA, Tukey's Multiple Comparison post hoc tests. The dose 100 mg/kg of the plant extract significantly increased the number of head dippings as compared to control animals (P< 0.01).

Table B: **Hole board test.**

Treatment	Dose (mg/kg)	Time (sec) of animals remained without falling from rod		
		30 min	60 min	90 min
Saline	1ml	300	300	300
Diazepam	1	92,33± 22,45 ***	199,8± 35,34*	217,5 ± 32,58
Plant extract	100	237±33,87	255,7±18,39	275,2 ± 31,19
Plant extract Plant extract	200 400	260,5±22,48 250,2± 34.51	264,8±22,99 273,1± 17.25	275,2 ± 22,83 284,1 ± 19.90

All values are mean ± SEM (n=6); *p<0.05, ***p<0.001 when compared to control. One- way ANOVA, Tukey's Multiple Comparison post hoc tests.

Table C: Rotarod test.

In the current work we examined, for the first time, the anxiolytic effects of methanolic extract of *Mercurialis annua*, using the light/ dark test and the hole board, and to examine motor coordination we used Rota rod test. Furthermore, the effects of *Mercurialis annua* and diazepam on these animal models were compared to determine whether the behavioral profile of *M.annua* differed from an established anxiolytic drug.

The light/dark test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour of rodents in response to mild stressors, that is, novel environment and light [15].

Thus, in the light/dark test, drug-induced increase in behaviours in the white part of the box, in which white compartment is illuminated and black compartment is darkened, is suggested as an index of anxiolytic activity. An increase in transitions is considered to reflect anxiolytic activity. Our results showed that the extract (100 mg/kg) increased time spent in the light chamber, suggesting anxiolytic action of M.annua.

The hole board test is useful for modeling anxiety in animals, in this test an anxiolytic-like state may be reflected by an increase in head –dipping behaviors [16,17]. Our results showed that methanolic extract (100 mg/kg) of *Mercurialis annua* increased the head dipping corroborating the anxiolytic-like effect previously shown in the light- dark test.

Rota rod test a classical animal model used to evaluate peripheral neuromuscular blockade and the motor coordination [18], a deficit in motor coordination would very likely affect performance in the behavioral tests. Our findings showed that *Mercurialis annua* (100-200 mg/kg), unlike diazepam (1 mg/kg), had no significant effect on motor coordination. Furthermore, the extract didn't affect motor coordination, is additional evidence of centrally mediated actions and not blockade of neuromuscular system [19,20]. The *M.annua* extract showed promising anxiolytic effects without causing any neuromuscular side effects.

Conclusions

The data presented hereby reinforce the traditional use of *Mercurialis annua* by Moroccan people to treat anxiety [6]. Despite the wide spread traditional use of *Mercurialis annua* for treating various disorders there are no reports of scientific evaluation of its anxiolytic activity.

Our study shows that the *Mercurialis annua* extract had marked effects on the anxiety-related behavioural parameters on exposure to the light/dark test and the hole board in mice.

Mercurialis annua extract causes an "anxiolytic" behavior comparable with the effects of diazepam. Future studies will be focused on the neurobiological mechanisms of action and possible interactions of *Mercurialis annua* with classical neurotransmitters and the phytoconstituent(s) responsible for the observed central effects has to be isolated and identified.

Conflict of Interest Statement

All authors assert that none has any commercial or financial involvements that might present an appearance of a conflict of interest in connection with the submitted manuscript.

Authors' Contributions

DZ, I carried out all the studies and drafted the manuscript with the help of the above authors, as regards TK participated in this work and drafted with me the manuscript. EHB helped us in the chemistry part and NM carried out the behavioral tests with me, MAB helps me in the behavioral tests,and CY is the director of the laboratory he advises me and guides me always in my work, after my PhD supervisor KA she corrects the manuscript, guides me and advises me. All authors read and approved the final manuscript.

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