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Research Article

EVALUATION OF CNS ACTIVITIES OF *MATRICARIA CHAMOMILLA L.* ESSENTIAL OIL IN EXPERIMENTAL ANIMALS FROM MOROCCO

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ABSTRACT

Matricaria chamomilla L. (*Asteraceae*) has been widely used in the traditional medicinal system for the treatment of a variety of diseases in Morocco, as a calming sleep aid, a remedy to ease an upset stomach, and for its wonderful anti-inflammatory and antispasmodic properties.

The aim of this study is to investigate central nervous system (CNS) activity of the Essential Oil of *Matricaria chamomilla L.* (EOMC) using different models based on mice and rats behavior.

In the present study, Steam distillation of *Matricaria chamomilla L.* was carried out using a Clavenger apparatus in order to obtain the volatile oils. The essential oil extract was analyzed by capillary gas chromatography with mass spectrometric detector (GC-MS).

Twenty-five compounds were identified in the essential oils and the main constituents of the essential oils were Chamazulene (25.21 %), Cis-beta-farnesene (12.51 %), Eucalyptol (9.19 %), Coumarin (7.72 %), Galaxolide (6.28 %), and Camphor (4.3 %).

The results of the psychopharmacological screening revealed that EOMC produced significant sedative effect at the doses of 300,400 and 500mg/kg (p.o.) It affected curiosity (Hole-Board Test), and caused a remarkable decrease in muscle relaxant activity (Rota-Rod , and Traction Tests), also potentiate the hypnotic effects of sodium thiopental in rats but did not present any hypnotic action or catalepsy effect.

It can be concluded that the essential oil of Matricaria chamomilla L. exhibit CNS depressant activity in tested animal models. This effect might be due to the presence of different chemical compounds in this oil.

Keywords: Acute toxicity, Psychotropic Activity, Hole-board test, CNS depressant activity.

INTRODUCTION

Herbs have been highly valued and used regularly for thousands of years as traditional medicine. The rapid increase in consumption of herbal remedies worldwide has been stimulated by several factors, including the notion that all herbal products are safe and effective.

Medicinal plants have been used to treat such psychotropic and behavioral conditions as anxiety, depression, seizures, poor memory, dementia, insomnia, and drug intoxication.

Matricaria chamomilla L. (*Asteraceae*), popularly known as chamomile is an ancient as well as a modern herb with many uses. *Matricaria chamomilla L.* has been reported to exhibit anti-inflammatory, antispasmodic, anti-oxidative, antibacterial, anti-fungal, anti-cancer, anti-allergic, and anti-pyretic [1].

Therefore, because of synthetic and chemical drug's unwanted side effects, nowadays Compounds of plant origin could represent a valid alternative to synthetic drugs.

The aim of this study is to investigate the central nervous system (CNS) activity of the Essential Oil of *Matricaria chamomilla L.* (EOMC) using different models based on mice and rats behavior.

MATERIALS AND METHODS

Extraction of the essential oil

Samples of *Matricaria chamomilla L.* (MC) were purchased at a farmer's market in Hay Nahda-Rabat, between the months of March and April 2011. Samples of the plant were identified with botanist of Department of Plant Biology, Ibn Tofail University, Morocco.

Freshly harvested aerial part biomass (300g per sample) of Chamomile was hydrodistilled in Clevenger apparatus for 4h to obtain the essential oil with 0.4% (v=w) yield. Extracted oil was stored in a refrigerator at 4°C [2]. The essential oil was diluted with peanut oil to obtain the desired doses and was immediately administered orally (p.o.) to mice and rats as a single dose expressed as ml/kg.

Identification of the essential oil composition

GC-MS analyses were performed on a PolarisQ quadrupole ion trap mass spectrometer coupled to a TRACE GC Ultra. A VB-5 column (coated with Methyl polysiloxane) (30m X 0.25mm X 0.25µm film thickness) was used as the stationary phase. Helium was the carrier gas at 1.4mL/min flow rate. Temperature was programmed from 40º to 300ºC at 5ºC/min ramp rate. The injector and the GC-MS interface temperatures were maintained at 220ºC and 300ºC, respectively. Mass spectra were recorded over 40–400 amu range at one span/s with 70eV ionization energy and EI mode of ionization. The ion source and the detector temperatures were maintained at 200ºC and 300ºC, respectively. The samples (1 µL) were injected with a 1:50 split ratio. The compounds were identified by comparing their retention times and mass spectra with those obtained from the authentic samples and/or the MS library.

Pharmacological evaluations

Drugs and essential oil administrations

The drugs used were:

- Bromazepam
- Thiopental sodium

Peanut oil was obtained from a local market. All drugs were freshly prepared by dissolving in distilled water.

Animals

Normal healthy male Wistar rats weighing 200-250g and Swiss Mice weighing 20-30g were used in pharmacological and females Swiss mice in the LD50 calculation tests.

They were housed in standard conditions (approximately 26°C, 60–70% relative humidity and 12h-dark/12h-light cycle). The animals received a standard rodent diet and water *ad libitum* except when fasting was required in the course of the study.

The animals were obtained from the animal experimental centre of Mohammed V Souissi University, Medicine and Pharmacy Faculty, Rabat.

Acute toxicity test

Acute oral toxicity study was performed according to the Organization for Economic Co-operation and Development OECD guidelines 423 [3].

Three animals are used for each step, they fasted for 3-4h prior to the experiment then they were given essential oil of *Matricaria chamomilla L.* up to 5g/kg p.o. Mice were closely observed for 2h post-treatment for detect any behavioural changes and signs of toxicity. Mortality in each group within 24h was recorded and surviving animals were observed for a further 14 days for any signs of delayed toxicity according to OCDE 2001. The purpose of this study is to allow selection of the appropriate starting dose for the main study.

Psychopharmacological screening

The MCEO administered p.o., was evaluated for its psychopharmacological activities in several experimental models using Swiss mice and Wistar rats.

Reference drugs

All drugs and essential oils were freshly prepared on the day of the experiments. A control group received peanut oil as vehicle.

Bromazepam (30mg/kg p. o., a conventional sedative) or with Thiopental sodium (40mg/kg i.p., a conventional hypnosis) were used as positive control.

Traction test

Placing the forepaws of the mice in a small twisted wire (1mm diameter, 15cm long) rigidly supported above the bench top did the screening of animal.

Normal mice grasped the wire with forepaws and when allowed to hang free, placed at least one hind foot on the wire within 5 seconds. Inability to put up at least one hind foot considered failure in the traction test; also, the behaviours of animals were recorded during this experiment [4, 5].

Chimney Test

Chimney test of Boissier 1961 was used where each mouse was introduced into the vertical glass tube 30cm in length and 28mm diameter, with the head forward.

When the mouse reached the other end of the tube, the tube was moved to a vertical. The mouse tried to climb backwards.

The time required for the mouse to climb backwards out of the cylinder was noted. Cut off time was 240 sec. A normal mouse typically attempts to escape in thirty seconds, and the mice considered as subject to the sedative effect when performing the rise of cylinder greater than 30sec [6, 7].

Hole-Board Test

The board is 40cm x 40cm and 2.2cm thick. It has 16 holes of 3cm diameter and it is made of grey Perpex. The matt finishing of the upper panel avoids reflections which may alter the animal behavior. The mice were injected with drugs or vehicle and, thirty minutes later, each animal was placed in the center of the hole-board, and allowed to freely explore the apparatus for 5 min. The numbers of head pokes and the time of dipping during a 5 min period were recorded [8].

Rota-Rod Test

In this test mice were selected 24h prior to the test by choosing only those that were able to remain successfully on the revolving bar (14 rpm) of the Rota-Rod apparatus (Ugo Basile, Model 7600) for two consecutive periods of 60s. Motor performance was evaluated at 30, 60, and 120 min following treatments, and the amount of time of permanence(s) on the revolving bar during a 60 second period was recorded [9].

Hypnotic test

The animal is considered as in state of hypnosis, when placed on his back, it loses the righting reflex. Following the oral administration of *M. chamomilla L. essential oil at the doses (*300, 600 and 900mg/kg) the time between loss and recovery time of righting reflex was recorded as sleeping time [10, 11].

Catalepsy test

Catalepsy test consists of placing an animal into an unusual posture and recording the time taken to correct this posture.

The animal is considered as in state of cataleptic when they agree to pass the forelegs with the hind legs ipsilateral. After administration of different doses (300, 600 and 900mg/kg) of the essential oil, 5 rats are labeled differently so as to monitor the evolution of catalepsy individually over time. Every 15 minutes we follow the evolution of catalepsy. For each rat the onset time of this catalepsy was recorded [11, 12].

Thiopental sodium Induced sleeping time

Hypnotic effect method based on potentiation of thiopental induced sleeping time by essential oil of *Matricaria chamomilla L.* was used to study the effect of plant materials [2]. A sub-hypnotic dose of thiopental (40mg/kg) was injected via intraperitoneal route, 30min after a similar injection of vehicle or the drug. The standard drug used was Bromazepam (30mg/kg P.O.).The effect was recorded for disappearance (latency) and reappearance (duration) of the righting reflex. Hypnotic sleeping time was considered to be the time interval between disappearance and reappearance of the righting reflex [13].

Statistical Analysis

All Results were expressed as Mean±SEM. The significance of difference between means was determined by student's t-test, followed by One-way ANOVA. A level of significance (P<0.05 or 0.01) was considered for each test.

Table 1: The chemical constituents of the essential oil of *Matricaria chamomilla L.* obtained by GC-MS.

Retention time (Rt) (min)	Components	Relative percentage
7.90	Cis-Ocimene	1.68
8.37	Camphene	2.48
9.33	2-α-pinene	3.41
11.22	Eucalyptol	9.19
15.18	Camphor	4.3
15.74	Quercetin	1.23
16.10	Pregnane	0.41
16.79	Linoleic acid	1.33
17.22	Triacontane	1.33
17.65	Lucenin-2	2.9
21.96	Thymol	3.44
22.11	Galaxolide	6.28
24.65	Benzoic acid	3.11
24.86	Salicylic Acid	2.62
25.82	Trans- caryophyllene	6.85
26.38	Terpinen-4-ol	0.8
27.32	Naphthalene	2.12
28.74	α-Cedrol	4.9
35.67	Anisaldehyde	0.86
36.56	Coumarin	7.92
37.15	α-linolenic acid	0.13
37.24	β-pinene	0.27
37.54	Benzopyran	2.31
40.56	Chamazulene	25.21
42.02	Cis-beta-farnesene	12.51

RESULTS

Composition of the essential oil

Analysis of the essential oil of *Matricaria chamomilla L.* by GC/MS revealed Twenty-five components (monoterpenes and

sesquiterpenes), accounting for 99.67% of the whole composition of the samples (Table 1).This oil is predominantly composed of sesquiterpenes (trans- caryophyllene, α-Cedrol, chamazulene, Cis-beta-farnesene) (49.47%) and monoterpenes (cis-Ocimene, Camphene, 2-α-pinene, Eucalyptol, Camphre, Thymol, terpinene-4-ol, b-pinene) (25.27%) (Fig 1 and Table 1).

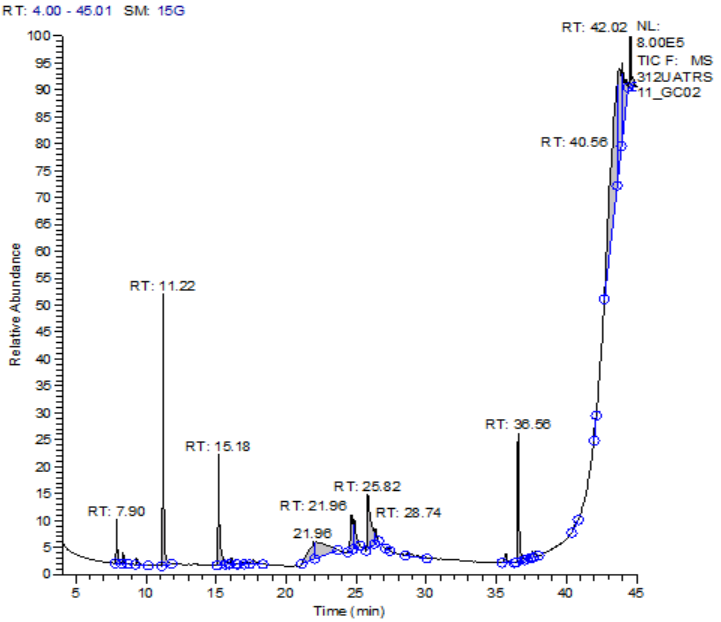

Fig. 1: Gas chromatography- mass spectrometry (GC-MS) of *Matricaria chamomilla L.* essential oil.

Acute toxicity test

All mice were free of any toxicity up to the dose of 5g/kg without any mortality. From this data, the LD50 was higher than 5000mg/kg. Thus, the essential oil is considered safe for long term administration.

Psychopharmacological screening

The results of psychotropic effects of MCEO were expressed by comparison with control and reference groups.

Traction test

The mice treated with the essential oil of *Matricaria chamomilla L.* showed a significant failure in traction at all doses tested when compared with the control group (Table 3).

Chimney Test

Animals treated with the essential oil of *Matricaria chamomilla L. at 300*, 400, and 500mg/kg, do show loss of initiative and curiosity, *i.e.* The animal did not attempt to amount of the tube for escape ($P < 0.001$) (Table 3).

Hole-Board Test

In the hole board test the essential oil of *Matricaria chamomilla L.* at doses (300,400 and 500mg/kg, p.o.) significantly reduced head-dip (both $P < 0.01$) when compared with vehicle alone (Table3).

Rota Rod Test

The effect of the essential oil on motor coordination is shown in Table 2. In this test the essential oil of *Martricaria chamomilla L.* (300,400 and 500mg/kg) showed a significant effect on the spontaneous motor activity as determined by the Rota-Rod performance. The standard drug (Bromazepam) also showed highly significant effect when compared to control ($P<0.01$).

Hypnotic test

The results demonstrated that MCEO at doses of 300, 600 and 900mg/kg have no hypnotic effect on the animals during this experiment.

Catalepsy test

The result indicated that MCEO did not induce catalepsy in rats.

Thiopental sodium Induced sleeping time

In the potentiation of Thiopental sleep test, *Matricaria chamomilla L.* essential oil significantly increased the sleeping time of the rats at the dose of 100mg/kg compared to control (Table 4).

Table 2: Sedative effect of the essential oil of *Matricaria chamomilla L.* in mice.

Test		Control	Reference (30mg/kg)	MCEO (300mg/kg)	MCEO (400mg/kg)	MCEO (500mg/kg)
Traction test	Re-establishment Time	0.05 sec ± 0 n = 5	12 sec ± 1.58 n = 5	7.8 sec ± 0.83 n = 5	4 sec ± 1.58 n = 5	2.2 sec ± 0.83 n = 5
Chimney Test	Time to go back the tube in seconds	6.0 sec ± 0.6 n = 5	> 2min n = 5	> 2min n = 5	> 2min n = 5	> 2min n = 5
Hole-board test	Explored holes during 5 minutes	5 ± 1 n = 5	1 ± 0 n = 5	3 ± 1 n = 5	2 ± 0 n = 5	1 ± 0 n = 5

Values are mean ± S.E.M (n = 5); p.o. means oral route; n means number of mice per group; sec means seconds; MCEO means *Matricharia chamomilla L.* essential oil; $P < 0.001$ versus the control group.

Table 3: The effect of the essential oil of *Matricaria chamomilla L.* on the Rota-Rod test in mice.

Treatment	Dose (mg/kg)	Interval following treatment (min)		
		30 min	60 min	120 min
		Time of permanence (s)/60s		
Control		60±0	60±0	60±0
MCEO (p.o.)	300	12.8±0.8	26.4±1.51	35.4±6.7
	400	8.7±2.7	21±3	33±5
	500	4.0±1.6	10.8±2.4	13.2±4.8
Reference (p.o.)	30	0±0	1.2±1.09	1.0±0.4

MCEO means *Matricharia chamomilla L.* essential oil .Values are Mean time in seconds spent on the Rota-Rod **±** S.E.M, (n = 5 mice per group).

Table 4: The effects of the essential oil of *Matricaria chamomilla L.* on Thiopental sodium Induced sleeping time.

	Control Thiopental sodium 40 mg/kg	Reference Thiopental sodium 40 mg/kg + Bromazepam 30mg/kg	Thiopental sodium 40 mg/kg + 100mg/kg MCEO
Sleep latency (min)	3 ± 1	1.75 ± 0.95	3 ± 0.70
sleeping time (min)	45 ± 2	3h51 ± 13.25	1h45 ± 10

MCEO means *Matricharia chamomilla L.* essential oil. Data are expressed as mean ± S.E.M (n = 5); P < 0.001 versus the control group.

DISCUSSION

In this study, chemical profiling by GC–MS analyses of the hydrodistilled essential oil of *Matricaria chamomilla L.* revealed a mixture of monoterpenes and sesquiterpenes (Chamazulene, coumarin, Trans- caryophyllene, etc) as the main components.

There was no mortality and no sign of toxicity observed after oral administration of the essential oil up to 5000mg/kg body weight in mice. However, mice manifested signs of sedation like quiescence and reduced locomotion when observed for 2h post-treatment.

The Effects of essential oil of *Matricaria chamomilla L.* on some psychopharmacological activities were studied in animal models by Traction Test, Chimney Test and motor coordination (Rota-Rod Test), muscle relaxant activity (Hole-Board Test) and Thiopental sodium induced sleeping time.

For the hole-board test, the effect of essential oil at doses of 300,400 and 500mg/kg was carried out by measuring external signs, in which we obtained a decrease in the exploratory conduct (curiosity) of the mice.

In the experiment concerned with muscle relaxant activity, MCEO (300,400 and 500mg/Kg p.o.) was found to produce significant decrease in motor coordination (Rota-Rod Test) and muscle tone (Traction Test and Chimney Test) of the animals. Decrease in spontaneous locomotor activity reveals depression effect on the central nervous system [14].

It also showed a marked sedative effect as evidenced by a significant potentiation of thiopental sodium induced sleeping time at dose of 100mg/kg but did not present any hypnotic or catalepsy effect at doses of (300,600 and 900mg/kg). The potentiation of thiopental sodium induced sleeping time is possibly through a CNS depressant action [15] or a tranquilizing action [16]. The CNS depressant activity may be due to the increase in the concentration of GABA (GABA is the most important inhibitory neurotransmitter in the human central nervous system also is involved in epilepsy, sedation and anxiolysis, and works via binding to GABAA receptors) in brains [17]. Our GC-MS analysis revealed Chamazulene (25.21%),Coumarin (7.92%) and Trans- caryophyllene (6.85%) as major compounds in EO and it can be able by inhibitory effects on CNS of animal models observed during pharmacological screening after treatment with EO [18]. In fact some authors have shown that chamomile oil might affect the GABAergic system in the rat brain, similarly as the benzodiazepine agonist [19] and mediate these central depressant actions.

The result of this study is consistent with the report of Bennaghmouch and Zellou [20] in which there was a significant reduction in the CNS activity in the animal models treated with Ajuga iva.

CONCLUSION

Thus, we concluded that, the *Matricaria chamomilla L.* essential oil induces sedative, muscle relaxant, anxiolytic effects and may be grown commercially to be formulated as sedative plant in Morocco. However, further studies will be necessary to evaluate the exact mechanism by which exerts its action and await the isolation and identification of the active principle responsible for such actions.

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