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Research Article

CNS depressant activity of Chamomile methanol extract in mice

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ABSTRACT

Matricaria chamomilla L. (Asteraceae) is a well-known plant in most ancient traditional medicine and highly used for the cure of numerous medical disorders, insomnia included. Using a sleep disturbed model, pentobarbital sodium and ether induce loss of righting reflex were applied for the evaluation of hypnotic and narcosis effects respectively. In the present study, the methanol extract of Matricaria chamomilla flower, at the intraperitoneal (IP) dose 300 mg/kg, showed considerable restful effect and significantly potentiate the hypnotic effect of pentobarbital sodium, both the latency to sleep and duration time, with a decrease value of 31.9% (P<0.001) and increase values of 53.3% (P<0.01) respectively. On the contrary, the Matricaria chamomilla flower methanol extract did not prolong the narcosis effect induced by ether. These findings indicate that Matricaria chamomilla methanol extract, at the dose used has a potential sedative-hypnotic effect as well as providing a scientific evidence for its traditional use as a sleep inducer.

Keywords: *Matricaria chamomilla*; CNS depressant activity; Sedative; Loss of righting reflex; Pentobarbital, mice.

INTRODUCTION:

Insomnia is considered as a symptom of difficulty in starting and maintaining sleep and usually associated with a day time efforts (Roth and Roehrs, 2003). Among sleep-related complaint insomnia is believed, after pain, the most common overall complaint (Mahowald *et al.,* 1997). Studies have indicated that if insomnia is not addressed appropriately it can cause significant morbidity (Attarian, 2003). Moreover, the use of sleep inducer, benzodiazepines, in the control of insomnia had been shown associated with certain troubles including dependence and addiction, withdrawal symptoms and drug resistance (Tyrer, 1989).

All over the recent years, the interest in the plant-based medicine has encouraged noticeably worldwide. Chamomile, *Matricaria chamomilla* L. (*Matricaria recutita,* syn.), belongs to the family Asteraceae. *M. chamomilla* is an annual plant of 6–24 inches tall found near populated areas all over Europe and temperate Asia. It has long and narrow leaves and the flowers, bloom in early to midsummer, have a strong aromatic smell. *M. chamomilla* is widely used and well documented medicinal plant in the world. It is commonly distributed equally in tropical and subtropical countries, and has long been applied in traditional medicine to treat many diseases. *M. chamomilla* is one of the most popular herbal teas used as sedative and tonic. It is also known to be effective in treatment of various gastro-intestinal disorders such as intestinal colic, flatulence, ulcer

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and diarrhea (McKay and Blumberg, 2006). Several studies were reported to possess antipyretic activity, rheumatic pain management and effective in hemorrhoid, relieve anxiety and have anti-inflammatory effects (McKay and Blumberg, 2006). It has been suggested that *M. chamomilla* inhibits the reactivity of hypothalamus and pituitary system in rodents and can suppress the reactivity of central nervous system. In traditional medicine, *M. chamomilla* is well known for its hypnotic effect (Murti *et al.,* 2012). Keeping this in view, the present study was undertaken to find out the validity of the folkloric uses of this plant in brain disorders, lack of sleep included. Therefore, we aimed to evaluate whether the *M. chamomilla* flower methanol extract could influence the sleeping time induced by pentobarbital sodium and the narcosis effect induced by ether in mice.

MATERIAL AND METHODS

Plant materials and drugs

The following drugs were used: Chamomile methanol extract (7.2%), Camomilla Setacciata (Prontofoods S.P.A Brescia, Italy, 25 sacs per box of 1.3 g each) was bought from private pharmacy shop located at center part of the capital, Tripoli, Libya. Chlorpromazine as a standard drug was administered by IP at a dose of 1 mg/kg body weight (Yemitan and Adeyemi, 2005). Pentobarbital sodium (Ceva Sante Animale B.V. France), as a hypnotic agent, diluted with saline to 6 mg/ml and used at the dose 40 mg/kg as described previously in albino mice (Shetty *et al.,* 2009).

Methanol extract

The dried powdered flowers (13 g) were extracted with 200 ml methanol (70% v/v in water) (decoction) for 30 min. The methanol extracts were filtered and the filtrates were combined, evaporated and then concentrated almost to dryness using a water bath at 60–70°C for 6 h. The greenish sticky residue yield of the extraction procedure was 7.2% with respect to dried plant material. Thereafter, the concentrated methanol extract was kept in a refrigerator for subsequent pharmacological evaluation at a dose 300 mg/kg (Shinomiya *et al.,* 2005).

Animals

In this study we used inbred male and female albino mice aged 2 months (weight 20-35g) procured from the Animal House, department of Pharmacology and Clinical Pharmacy, Tripoli University, Libya. Animals were selected and divided randomly and fed on standard animal diet pellet and water *ad libitum* throughout the experiments period. All animal cages were kept in well-ventilated and 12h time regulated light-dark cycle place. Room temperature was adjusted at 26$\pm$2ºC. The study was approved by the Animal Ethical Committee of Tripoli University. Animal care and use were in strict compliance with the standard guidelines (Canadian Council). Fifty two mice of both sexes male and female were used.

Pentobarbital sleeping time

The hypnotic effect was based on potentiation of pentobarbital sodium induced sleeping behavior by the extract. The method was performed as previously described (Ali *et al.,* 2015). Briefly, mice of both sexes were randomly divided into three groups each consist of 12 mice. Group1, control group treated with saline, as a negative

control (10 ml/kg). Group 2, treated with chlorpromazine. Group 3 treated with methanol extract of dried flowers *M. chamomilla*. Pentobarbital sodium was injected IP 30 minutes post to administration of saline, chlorpromazine or methanol extract of *M. chamomilla* to mice. The animals were observed and the latency to exhibit loss of the righting reflex (time between pentobarbital injection to loss of righting reflex) and the total sleep time (time between the loss and the recovery of the righting reflex) were measured. The mouse was considered as being awake if it could right itself (return to upright position). Once a mouse righted itself, it was placed on its back twice more and allowed to right itself second and third time for confirmation.

Ether anesthesia

This was examined according to the method previously described by Sethi *et al* (Sethi *et al.,* 1987). Briefly, by using pairs of mice, of which one animal was administered with normal saline and served as control, while the other mouse was treated with 300 mg/kg of methanol extract of *M. chamomilla* flower. After 30 min. mice were placed in a bell jar and exposed to a cotton swab soaked with 10 ml of anesthetic ether. The anesthetic effect in this experiment was characterized by a reversible loss of the righting reflex without the loss of a spinal reflex (the withdrawal of the foot upon pinching). Time to the loss of righting reflex (onset time) was recorded. Thereafter, the animals were removed from the bell jar and the time taken for regaining the righting reflex was recorded (duration time) and the percentage increase of the ether anesthesia was calculated. Eight mice were used per each group.

Statistical analyses

All data are expressed as mean ± SEM. In sleep-disturbed model the differences in mean of latency and duration time to loss of righting reflex among different treated groups were statistically analyzed by one-way ANOVA. The uniformity of groups was proved by Tukey's *post hoc* test at α level equal to 5%. In the study of narcosis effect induced by ether, comparisons between groups were made using unpaired *t*'test.

RESULTS AND DISCUSSION

The present study showed that the IP administration of *M. chamomilla* flower methanol extract at 300 mg/kg produced a significant suppression in sleep latency induced by pentobarbital sodium in mice (31.9% reduction; $p<0.001$, n=12 each, figure 1). The reference drug, chlorpromazine, at 1 mg/kg produced 72.4% significant decrease in onset time towards pentobarbital sodium induced sleep in this sleep-disturbed model ($p<0.001$, figure 1). Furthermore, it was also shown that administration of *M. chamomile* flower methanol extract produced a significant increase in the duration of sleep induced by pentobarbital sodium compared to control mice (53.3% increase, $p<0.01$, n=12 each, figure 1). In this model, the tranquilizer reference drug chlorpromazine significantly increased the duration of sleep time by 280.7% ($p<0.001$, figure 1). In second experiment, the methanol extract of *M. chamomile* flower at 300 mg/kg failed to prolong, significantly, the effect of ether to induce anesthesia assessed by no change in both the latency and duration time of narcosis compared to control mice (Table 1).

Table 1: Effects of the IP administration of *M. chamomilla* flower methanol extract on the onset and duration of loss of righting reflex (narcosis effect) induced by inhalation of ether.

Treatment	Dose (mg/kg, i.p)[a]	Loss of righting reflex (narcosis)	
		latency (min.)	duration (min.)
control + ether	10 ml/kg	5.4±0.25	36.9±1.2
M. chamomilla + ether	300 mg/kg	6.1±0.27 (12.9%)	38.3±1.7 (3.8%)
	p value	0.08	0.5

[a]Administered 30 min before exposure to ether (10 ml/cotton, inhalation). Values are mean (8 mice per group) ± SEM. Percentages of increase are in parentheses. Unpaired *t*' test.

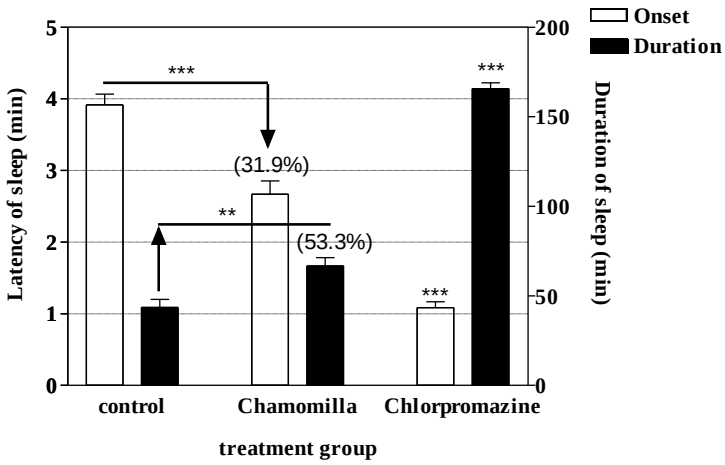

Figure 1: Effects of the IP administration of *M. chamomilla* flower methanol extract and chlorpromazine on the onset and duration of sleep induced by injection of pentobarbital sodium. Columns and vertical bars represent means ± SEM of sleeping time. Twelve mice were tested per group. Significant difference from control are shown as **p < 0.01 and ***p < 0.001.

In the present study *M. chamomilla* flower methanol extract showed significant CNS depressant effect. The methanol extract of *M. chamomilla* flower is less potent than chlorpromazine. This results indicate that *M. chamomilla* possess depressive central effect, in particular sedative-hypnotic effect. Our results are in line with the findings by others (Bozorgmehr *et al.,* 2012). Moreover, Shinomiya and colleagues (Shinomiya *et al.,* 2005) had demonstrated that chamomile extract has a benzodiazepine-like hypnotic effect in rodents. So, we assume that the methanol extract may act through $GABA_A$-chloride ion channel complex prolongs pentobarbital induced sleep duration, therefore, an involvement of GABAergic system may be suggested (Ma *et al.,* 2009: Shrestha *et al.,* 2012).

Earlier studies available specified that chamomile extract is an effective inhibitor, *in-vitro*, of the CYP3A4 microsomal enzyme (Budzinski *et al.,* 2000). Therefore, the observation that the *chamomile* flower methanol extract-induced prolongation of pentobarbital hypnotic effect and that the *chamomile* extract did not show any effect on the narcosis induced by a non-metabolized anesthetic, ether, suggest that the dual mechanisms is in some manner appears unlikely. So that although it cannot totally exclude that the dose used might play a role in the response produced and/or the constituents required to induce narcosis might not be extracted by methanol, *chamomile* flower methanol extract might exert a single effect in this experiment, a non-direct depressant effect *per se* possibly through drug interaction, at the microsomal level, to reduce the rate of metabolism of the pentobarbital sodium. These findings led us to conclude that the methanol extract of *M. chamomilla* flower has indirect central nervous depression effect. Furthermore, the results of this

study support the use of *M. chamomilla* flowers as a sedative, as claimed in traditional medicine.

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Conflict of interest and funding

Authors have declared that no conflict of interest was existed during running of this study.

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