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EFFECTS OF *RETAMA RAETAM* (FORSSK.) WEBB & BERTHEL. (FABACEAE) ON THE CENTRAL NERVOUS SYSTEM IN EXPERIMENTAL ANIMALS

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Abstract –Retama raetam (Forssk.) Webb & Berthel. (Fabaceae), commonly known as 'raetam' or 'broom bush', is a desert shrub that grows abundantly in North-African countries, Palestine and Syria. Traditionally, this plant has been used as an abortifacient, a purgative and a vermifuge. In the present study, the effect of the methanol (MeOH) extract of the aerial parts of *R. raetam* on the central nervous system (CNS) has been evaluated using a mice model. In the photoelectrical cell test, the extract of *R. raetam* (ERR) at a dose of 125 mg/kg body weight did not exhibit any effect on the spontaneous motor activity in mice. At a dose of 250 mg/kg body weight, ERR increased ambulatory movement, but had no effect on the non-ambulatory movement, while a dose of 375 mg/kg body weight decreased both ambulatory and non-ambulatory movements. The effect of ERR on the anxiety levels and behaviors of mice was investigated using the elevated plus-maze test. At doses of 125, 250 and 375 mg/kg body weight, ERR decreased anxiety levels without showing an effect on the total activity; it did not affect anxiety levels but increased the total activity; it increased anxiety levels and decreased the total activity, respectively. In the diazepam-induced sleep test, ERR increased the onset of sleep without affecting the duration of sleep at the dose of 250 mg/kg body weight. The dose of 375 mg/kg body weight decreased the onset of sleep while increasing the duration of sleep. ERR did not exhibit any effect on the diazepam-induced sleep in the presence of flumazenil or picrotoxin.

Key words: Retama raetam, Fabaceae, central nervous system (CNS), diazepam, North Africa

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INTRODUCTION

Retama raetam (Forssk.) Webb & Berthel. (Fabaceae *alt.* Leguminosae), commonly known as 'raetam' or 'broom bush', is a desert shrub native to several countries of North-Africa (*e.g.,* Algeria, Egypt, Libya, Morocco and Tunisia), temperate Asia (*e.g.,* Israel, Jordan, Lebanon, Palestine and Syria) and south-eastern Europe (*e.g.,* Sicily in Italy) (GRIN Database, 2011). This plant has long been used as an abortifacient, a purgative and a vermifuge in the traditional medicine systems of the source countries (Dr. Duke's Phytochemical and Ethnobotanical Databases, 2011). Previous pharmacological studies on this plant have revealed its antibacterial, antifungal, antihypertensive, anti-oxidant, antiviral, diuretic and hypoglycemic properties, and hepatoprotective, nephroprotective and cytotoxic effects (Maghrani et al., 2005; Eddouks et al., 2007; Hayet et al., 2007, 2008; Koriem et al., 2009; Algandaby et al., 2010; Edrizi et al., 2010). In

continuation of our phytochemical and pharmacological studies on the Libyan medicinal plants (Auzi et al., 2007, 2008; Elouzi et al., 2008; Geroushi et al., 2010), we now report on the effect of the MeOH extract of the aerial parts of *Retama raetam* on the CNS using the mice model.

MATERIALS AND METHODS

Plant materials

The aerial parts of *Retama raetam* (Forssk.) Webb & Berthel were collected from El-Khoms, Libya, in April 2009. The plant material was identified at the Herbarium of the Botany Department, Faculty of Science, Al-Fateh University, Tripoli, Libya, and a voucher specimen (RR-2009) was deposited at the Herbarium.

Extraction of plant material

Shade-dried and ground aerial parts (500 g) of *R. raetam* were macerated in MeOH (3.0 L) for three consecutive days with occasional stirring. The extract was concentrated under reduced pressure and a temperature not exceeding 45˚C to obtain a semi-solid mass (31.7 g).

Animals

Swiss albino mice weighing between 20-35 g of both sexes were used throughout this study for both test and control groups. The animals were obtained one week before the experiment from the local animal house of the Pharmacology Department, Faculty of Pharmacy, Al-Fatah University of Medical Sciences, Tripoli, Libya. They were housed in cages of five animals each and maintained under controlled laboratory conditions (25^{o}C ± 5). Standard animal food and tap water were provided *ad libitum*. The mice fasted overnight before each experiment. The studies involving mice were approved by the Ethical Review Committee, Al-Fateh University, Libya, and the experiments were carried out in strict accordance with the guidelines provided by the World Health Organization.

Measurement of spontaneous motor activity: the photoelectrical cell test

The photoelectrical cell (Columbus, OH) is a square arena (44 cm x 44 cm x 30 cm) in which the movements of the animals could be tracked by an automatic monitoring system (Gorzo et al., 2002). Animals were observed for exploratory activity by placing them in the centre of the open field arena for a period of 4 min (Lotufo et al., 2004; Maia et al., 2006). Horizontal (lines crossed) and vertical (rearing) activities were recorded (Ieraci and Herrera, 2006). Activity monitors were used to quantify horizontal activity and rearing. The test was performed in a dark environment between 8:00 a. m. and 4:30 p. m. A photoelectrical cell chamber was enclosed in a quiet room separated from the colony area. Horizontal distance was measured by the sequential breaking of infrared beams (5 cm on centre), in the horizontal plane of the x- and y-axes. Initiation of movement was incremented each time a break in ambulatory activity occurred for more than one second. Rearing movements were counted each time an animal passed above and then below the level of a sensor in the z-axis vertical plane (the mouse remained below the level of a sensor for at least one sec before it could score again (Kelly et al., 1998). The mice were divided into four groups of ten mice each and the test was performed one hour after oral administration of *R. raetam* extract (ERR) at the following doses: 125, 250 and 375 mg/kg body weight, to groups designated as 2, 3 and 4, respectively; 5% w/v gum acacia was administered to group 1 which was the control group.

The elevated plus-maze test

The apparatus was composed of two open arms (30 x 5 cm each) and two closed arms (30 x 15 cm each) that extended from a common central square (5 x 5 cm). The maze was constructed of wood with black-painted floor and walls (Vinader-Caerol et al., 2006), and elevated to a height of 45 cm above floor level (de Melo et al., 2006; Vinader-Caerol et al., 2006). The test was performed under

Table 1. The effect of the MeOH extract of the *Retama raetam* (ERR) suspended in 5% w/v gum acacia on spontaneous motor activity in mice.

Treatment		Number of ambulatory movements in every 4 min	Number of non-ambulatory movements in every 4 min
5% w/v Gum acacia (Control)		616.7 ± 71.4	250 ± 24.4
ERR	125 mg/kg body weight	795.8 ± 70.7	279 ± 25.8
	250 mg/kg body weight	884.2 ± 88.11*	285.7 ± 23
	375 mg/kg body weight	350.9 ± 87.1*	90.94 ± 28.7*

The values are the mean ± SE; (n=10 each group);
*Significantly differs from the control, $P < 0.05$.

Table 2. The effect of the MeOH extract of *Retama raetam* (ERR) suspended in 5% w/v gum acacia on anxiety measure in mice.

Treatment		Time spent in different arms (in sec)			Anxiety measure
		Open	Close	Zero	
5% w/v Gum acacia (Control)		75.18 ± 10.6	161.94 ± 10.1	2.80 ± 0.97	0.689 ± 0.04
ERR	125 mg/kg body weight	110.7 ± 18.5	117.56 ±19.6*	17.7 ± 9.59	0.47 ± 0.07*
	250 mg/kg body weight	91.26 ± 18	139.2 ± 18	9.54 ± 6.54	0.57 ± 0.07
	375 mg/kg body weight	22.38 ± 9.6*	216.3 ± 9.7*	1.44 ± 0.55	0.90 ± 0.04*

The values are the mean ± SE; (n=10 mice each group).
*Significantly differ from the control group ($P< 0.05$).

Table 3. The effect of the MeOH extract of *Retama raetam* (ERR) suspended in 5% w/v gum acacia on the total lines crossed in open and closed arms of the elevated plus-maze.

Treatment		Line crossed in different arm in 4 min		
		Open	Close	Total
5% w/v Gum acacia (Control)		15.4 ± 2.74	23.0 ± 2.24	38.4 ± 4.15
ERR	125 mg/kg body weight	26.0 ± 4.2*	2.29 ± 28.9	54.9 ± 4.5*
	250 mg/kg body weight	22.5 ± 4.57	29.7 ± 2.21*	52.2 ± 5.3
	375 mg/kg body weight	7.20 ± 2.7	15.9 ± 2.7*	23.1 ± 4.6*

The values are the mean ± SE; (n=10 mice each group).
*Significantly differ from the control group ($P< 0.05$).

Table 4. The effect of the MeOH extract of *Retama raetam* (ERR) suspended in 5% w/v gum acacia on the total number of entry in open and closed arms of the elevated plus-maze.

Treatment		Number of entry in different arms in 4 min		
		Open	Close	Total
5% w/v Gum acacia (Control)		3.40 ± 0.33	6.20 ± 0.64	9.60 ± 0.89
ERR	125 mg/kg body weight	5.80 ± 0.55*	6.50 ± 0.56	12.3 ± 1.10
	250 mg/kg body weight	6.40 ± 0.73*	7.10 ± 0.65	13.50 ± 1.30*
	375 mg/kg body weight	1.40 ± 0.47*	4.70 ± 0.83	6.10 ± 1.23*

The values are the mean ± SE; (n=10 mice each group).
*Significantly differ from the control group ($P< 0.05$).

Table 5. The effect of the MeOH extract of *Retama raetam* (ERR) suspended in 5% w/v gum acacia on diazepam-induced sleep in mice.

Treatment		Onset of sleep (in min)	Duration of sleep (in min)
5% w/v Gum acacia (Control)		7.25 ± 0.81	72.87 ± 5.40
ERR	125 mg/kg body weight	5.75 ± 0.25	67.20 ± 6.16
	250 mg/kg body weight	9.12 ± 0.61*	64.6 ± 4.64
	375 mg/kg body weight	1.50 ± 0.26*	93.2 ± 7.30*

The values are the mean ± SE; (n=10 mice each group);
*Significantly differ from the control group (P< 0.05).

subdued lighting. Each mouse was placed at the centre of the maze facing the closed arm and was allowed to explore the maze for 4 min (Kurt et al., 2003). In the plus-maze, several parameters were observed and recorded: time spent in open arms, closed arms, zero area, number of entries to open arm, closed arms and total number of entry, line crossed in open arms, closed arms and total lines crossed. Mice were considered to have entered an open or closed arm when full entry took place (all four paws) (Simone et al., 2005). In addition, the anxiety measure (level) was calculated as follows.

Anxiety measure = Time spent in closed arms / Time of the test (240 s.)

The animals were divided into four groups of ten mice each; the first group received gum acacia (5% w/v p.o.) and served as the control; the other three groups (groups 1, 2 and 3) received ERR at the doses of 125, 250 and 375 mg/kg body weight, respectively, one hour before the test.

Hypnotic effect (diazepam-induced sleep in mice)

The sleep-potentiating effects of ERR were studied in mice which received diazepam at a dose of 20 mg/kg body weight intraperitoneally one hour after the oral administration of ERR as described elsewhere (Ngo-Bum et al., 2004). The onset and the duration of the sleeping time (loss of righting reflex) were recorded. Four groups of mice (n=10 each group) were used in this experiment. The first group received 5% w/v p.o. gum acacia and served as the control group. The other three groups (groups 1, 2 and 3) were treated with ERR at the p.o. doses of 125, 250 and 375 mg/kg body weight, respectively.

Statistical analysis

Data generated from the above studies were statistically analyzed by the SPSS, a computerized statistical program (version 13.0). Results were expressed as mean ± S.E. The results were also analyzed for normality of distribution (i.e., if the results obtained are parametric or non-parametric), using the Kolmogorov-Smirnov test (Fras et al., 2000). Paired and unpaired t-Tests were used when comparing two means. The one-way analysis of variance (ANOVA) was used for comparing more than two means of a parametric data followed by the LSD`s (Least Significant Difference) *post hoc* multiple comparisons to determine which population means were different. The differences between data are considered to be significant if the P<0.05 and a highly significant if P<0.01.

RESULTS AND DISCUSSION

The effect of the MeOH extract of the aerial parts of *R. raetam* (ERR) on the central nervous system (CNS) has been evaluated using the mice model. In the photoelectrical cell test, ERR did not show any effect on spontaneous motor activity in mice at the dose of 125 mg/kg body weight (Table 1). At the dose of 250 mg/kg body weight, the ERR increased ambulatory movement but had no effect on the non-ambulatory movement, while a dose of 375 mg/kg body weight decreased both ambulatory and non-ambulatory movements. *R.*

raetam is known to produce various types of alkaloids (ElShazly et al., 1996; Abdel-Halim et al., 1997), and some of these alkaloids, *e.g.,* cytisine and anagyrine, are known to stimulate the brain at small doses (Abdel-Halim et al., 1997). At high doses cytisine causes the inhibition of locomotor activity and *N*-methylcytisine produces a sedation effect. The observed effect of ERR in the present study might have been attributed by the presence of these alkaloids.

The effect of ERR on the anxiety levels and behaviors of the mice was investigated using the elevated plus-maze test. The elevated plus-maze test is prone to false positive and false negative results, particularly when the drug in question alters locomotor activity. In the present study, to avoid misinterpretation of results, we used the photoelectrical cell test as another measure of locomotor activity. Different doses of ERR exhibited different effects in the elevated plus-maze test. At the low dose of ERR (125 mg/kg body weight), there was a significant decrease in the time spent in the closed arms, indicating a decrease in the anxiety measure (Tables 2-4). This dose induced an anxiolytic effect, but it did not show any effect on the total number of entries to open and closed arms. In addition, there was no change in the total activity, but ERR significantly increased the total lines crossed. ERR at the dose of 250 mg/kg body weight did not exhibit any effect on the anxiety, on the time spent in open and closed arms or the anxiety measurement, but it significantly increased the total number of entries to open and closed arms and the total lines crossed, suggesting an increase in the total activity of the mice (Will et al., 2000). The dose of 375 mg/kg body weight significantly decreased the time spent in open arms and increased the time spent in closed arms, leading to an increase in the anxiety measure. This data indicated clear induction of an anxiogenic effect. There was a noticeable decrease in the total number of entries to open and closed arms, and a profound decrease in the total lines crossed, showing a decrease in the total activity. This suggested a decrease in the total activity of the mice treated with this dose. Alkaloids, anagyrine and cytisine, reported from this plant, are known to have an activity similar to nicotine. They bind to the nicotinic cholinergic receptors in the CNS and autonomic ganglia (El-Bahri .et al., 1999). Neuronal nicotinic acetylcholine receptors (nAChRs) are also implicated in processes such as anxiety. Nicotine affects anxiety in different ways. In rodents, nicotine can be anxiolytic, anxiogenic, or have no effect on anxiety, depending on the dose used and the route of administration, even when the same behavioral test is performed (Salas et al., 2003). The results of the present study showed an activity pattern very similar to those alkaloids that act on these receptors.

In the diazepam-induced sleep test, ERR increased the onset of sleep without affecting the duration of sleep at the dose of 250 mg/kg body weight. The highest dose of 375 mg/kg body weight decreased the onset of sleep while increasing the duration of sleep (Table 5). The lowest dose of ERR (125 mg/kg) did not show any effect on the onset or the duration of diazepam-induced sleep. It could be assumed that the ERR doses of 250 and 375 mg/kg body weight could have exhibited the observed effects on sleep by acting on the nicotinic acetylcholine receptors. The effect of ERR on sleep reinforced the previous results of the locomotor activities of the animals when tested using the photoelectrical cell test, where the dose of 250 mg/kg increased the ambulatory movements and 375 mg/kg significantly decreased the ambulatory and non-ambulatory movements. It also supported the findings in the elevated plus-maze test, where ERR (375 mg/kg body weight) displayed a significant decrease in the total number of entries and total number of lines crossed, indicating a decrease in locomotor activity. ERR did not exhibit any effect on the diazepam-induced sleep in the presence of flumazenil or picrotoxin.

The presented results reveal the effects of the MeOH extract of the aerial parts of *Retama raetam* on the CNS of the experimental animals. However, the observed effects were different at different doses. The described effects were probably caused by the pharmacologically active alkaloids present in this plant.

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