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Original Article

Hypnotic Effect of *Rosa damascena* in Mice

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Abstract

Rosa damascena (Rosaceae) has been found to act on central nervous system including the brain. Several studies confirm that Rosa damascena inhibits the reactivity of the hypothalamus and pituitary systems in rat and can suppress the reactivity of central nervous system. In traditional medicine the hypnotic effect of rose is also suggested. In the present study hypnotic effect of ethanolic, aqueous and chloroformic extracts of Rosa damascena was investigated in mice. Hypnotic method was based on potentiation of pentobarbital induced sleeping time by extracts. Three doses of extracts (100, 500 and 1000 mg/kg) were injected intraperitoneally in comparison with diazepam (3 mg/kg) as the positive control and saline as the negative control. Thirty min after injection of extracts, pentobarbital (30 mg/kg) was injected and any increase in the sleeping time due to the extracts was recorded. Results showed that the ethanolic and aqueous extracts in doses of 500 and 1000 mg/kg significantly increased the pentobarbital induced sleeping time ($P<0.001$, compared to the negative control), which was comparable to diazepam. The chloroformic extract showed no hypnotic effect.

Keywords: Mice; *Rosa damascena*; Hypnotic effect; Pentobarbital.

Introduction

Rosa damascena is an erect shurb 1-2 meter in height. Flowers of this plant are large, showy and colorful. Today, *Rosa damascena* is highly cultivated for its scent (1). This plant contains carboxylic acids, terpenes, myrcene and vitamin C (1, 2). Flowers, petals and hips (seed-pots) of *Rosa damascena* have been used for medical purposes. Therapeutic effects of *Rosa damascena* which have been described in the Iranian ancient medical books include tonic, cardiac strengthening and anti-inflammatory effects. *Rosa* is also used in various conditions including menstrual bleeding, digestive disorders and headache. The essential oil obtained from *Rosa* is reported to have analgesic and antispasmodic effects (1, 2). *Rosa* is also used as a gentle laxative and to ease coughs (1). Furthermore, rose has been found to act on central nervous system including the brain. Several studies confirm that rose inhibits the reactivity of hypothalamus and pituitary system in rat and can suppress the reactivity of central nervous system (1). Long-term treatment with high doses of rose oil may lead to stress adjustment and the ability of brain to compensate by turning into a steady state of exhaustion (1). Anti-HIV (3) and anti-bacterial (2) effects for *Rosa damascna* have also been reported. In traditional medicine, rose is known for its hypnotic effect (1). Therefore in this study the hypnotic effect of the ethanolic, aqueous and chloroformic extracts of this plant was evaluated.

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Experimental

Plant and extract

Rosa damascena was collected from the Kalat region in north eastern part of Iran in spring 2003 and identified by Mr. Ahei. A voucher specimen was preserved in the Herbarium of the School of Pharmacy, Mashhad University of Medical Sciences (Herbarium No: 254-1804-01). The ethanolic extract was prepared as follows: 50 g of the chopped, dried plant was extracted with 300 ml ethanol (Darupakhsh) using a soxhlet apparatus. For the aqueous extract, 50 grams of the chopped and dried plant was extracted with 300 ml distilled water and for chloroformic extract, the same amount of plant material was extracted with 300 ml chloroform (Merck) by soxhlet apparatus. The extracts reduced to dryness with a rotary vacuum evaporator.

Animals

Male BALB/c mice weighing 20-28 g (The Pasteur Institute of Iran) were used throughout the study. All animals were maintained in groups of 8 per cage at a controled temperature of 21-25°C and a humidity of 55±5%. A standard pellet diet and tap water were provided *ad libitum*.

Methods

The hypnotic effect method was based on potentiation of pentobarbital (Sigma) induced sleeping time by the extracts. Animals were divided into groups of ten and the following solutions were injected (i.p.) to each group (n=8 for each group):

1- Saline as the negative control for ethanolic & aqueous extracts and saline plus a few drops of tween 80 (Merck) as the negative control for chloroformic extracts.

2- Diazepam (3mg/kg) (Darupakhsh) as positive control

3- Three groups (100, 500, 1000 mg/kg) of the ethanolic extract.

4- Three groups (100, 500, 1000 mg/kg) of the aqueous extract.

5- Two groups (500, 1000 mg/kg) of the chloroformic extract.

In the experimental group, the ethanolic and aqueous extracts were administered in doses of 100, 500 and 1000 mg/kg and the chloroformic in doses of 500 and 1000 mg/kg body weight (intraperitoneally). Thirty minutes later pentobarbital (30 mg/kg, ip) was given to induce sleep. The time interval between loss and recovery of righting reflex was used as an index of hypnotic effect (4). The time interval between injection of pentobarbital and onset of sleep was recorded as the latency time. In the negative and positive control groups normal saline (10 ml/kg, i.p.) and diazepam (3 mg/kg, ip) were injected respectively instead of the extract.

Statistical analyses

All data were expressed as mean±SEM. Comparison of sleeping time in all groups were made using ANOVA and with Tuky Cramer post test. Significant was accepted at $P< 0.05$.

Results and Discussion

Hypnotic effect of ethanolic extract

As shown in figure 1, sleeping time in animals receiving 100 mg/kg of the ethanolic extract was increased to 31.66±4.08 min. Thist was not significantly more in comparison with the negative control (20.05±3.53). On the other hand, those animals receiving 500 and 1000 mg/kg of ethanolic extract showed an increased up to 46±3.64 and 38±4.41 min respectively, this was significantly different compared to the negative control value ($p<0.001$). However, there was no significant difference between diazepam (37.86±3.83 min) and any of the other three doses of ethanolic extract. The difference between doses of 500 and 1000 mg/kg of the ethanolic extract was not significant.

Figure 2 shows the time interval elapsed between injection of pentobarbital and onset of sleep in various groups (latency times). As has been shown, the use of 100 and 500 mg/kg of ethanolic extract shortened the latency time of sleep to 5.66±0.8 and 6±1.43 min respectively, which is lower than that of the control (8.17±1.09 min) and is comparable to diazepam (5.28±0.83 min). However, the difference was not significant. A dose of 1000 mg/kg of extract decreased the latency time to 3.42±0.42 which was significantly different from the control group ($P<0.05$).

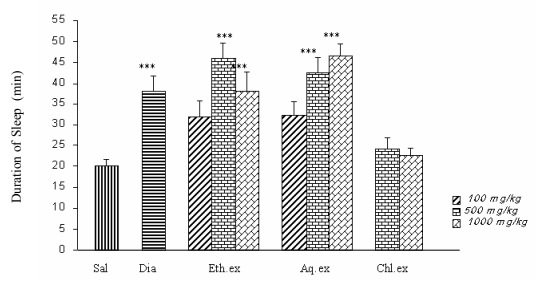

Figure 1. Effect of the ethanolic and aqueous extracts in dose of 100, 500 and 1000 mg/kg and the chloroformic extract in doses of 500 and 1000 mg/kg on the pentobarbital-induced sleeping time in mice. Data are presented as mean±SEM of seven mice. Sal: Saline, Dia: Diazepam, Eth.ex: Ethanolic extract, Aq.ex: Aqueous extract, Chl.ex: Chloroformic extract.
***P<0.001 compared to the negative control

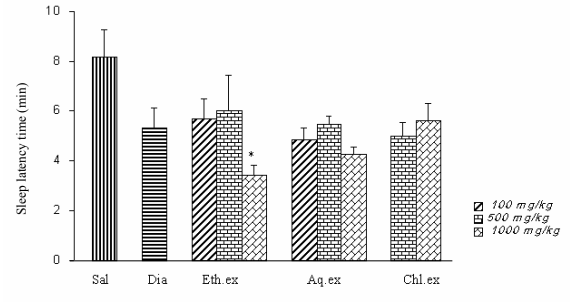

Figure 2. Effect of the ethanolic, aqueous and chloroformic extracts in doses of 100, 500 and 1000 mg/kg on the latency time of pentobarbital - induced sleeping time in mice. Data are presented as mean ±SEM of seven mice. Sal: Saline, Dia: Diazepam, Eth.ex: Ethanolic extract, Aq.ex: Aqueous extract, Chl.ex: Chloroformic extract.
*p<0.05 compared to the negative control

Hypnotic effect of the aqueous extract

Figure 1 shows that the sleeping time of groups receiving 500 and 1000 mg/kg of the aqueous extract (42.57±3.58 and 46.57±2.88 min) was significantly greater than that of the negative control (20.05±3.53 min). Furthermore, sleeping time of the group receiving 100 mg/kg of this extract (32.14±3.32 min) was also found to be more than that of the control group but the difference was not significant. The effect of various doses of the aqueous extract was comparable to that of diazepam. However, there was no significant difference between the doses of 500 and 1000 mg/kg of this extract.

As shown in table 1 and figure 2, all the doses of this extract accelerated the onset of hypnotic effect of pentobarbital compared with the effect of diazepam.

Hypnotic effect of chloroformic extract

Figure 1 shows that doses of 500 and 1000 mg/kg of the chloroformic extract could not potentiate the pentobarbital induced sleeping time.

Comparison of the hypnotic effect between three extracts

The pentobarbital induced sleeping time in animals receiving 500 mg/kg of the ethanolic extract was significantly more than those receiving a dose of 500 mg/kg of the chloroformic extract (P<0.001). The difference between groups of animals receiving 1000 mg of the ethanolic extract was significant compared to those receiving 1000 mg of the chloroformic extract (P<0.05).

The use of an aqueous extract in a dose of 500 mg/kg had a significantly greater effective on pentobarbital induced sleeping time compared to a dose of 500 mg/kg of the chloroformic extract (P<0.05). The sleeping time in animals receiving a dose of 1000 mg/kg of the aqueous extract was significantly different compared to those receiving 1000 mg/kg of the chloroformic extract (P<0.001) (Fig. 1).

There was no significant difference between the effect of various doses of ethanolic and aqueous extracts.

The present results indicated a relatively potent hypnotic effect for doses of 500 and 1000 mg/kg of the ethanolic and aqueous extracts obtained from Rosa damascena. The hypnotic effect of both doses of these extracts was comparable to diazepam. The effect of aqueous extracts was dose-dependent (r=0.9 in regression) while the ethanolic extract a dose of 500 mg/kg showed the maximal effect.

In the present study the hypnotic effect of Rosa damascena extract was evaluated using a standard method as previously described (4).

Although the hypnotic effect of the ethanolic and aqueous extracts from Rosa damascena were similar to that of diazepam, the mechanism(s) of hypnotic effect of this plant can not be concluded from the results of the present study. The family Rosaceae is known as a source of folk medicine used for treating nervous breakdown (5). Noguerira and Vassilieff have shown that the other genuses of Rosaceae

Table 1: Effect of ethanolic and aqueous extracts in 100, 500 and 1000 mg/ kg doses and chloroformic extract in 500 and 1000 mg/kg doses on the penthobarbital – induced sleeping time and on the latency time in mice. Dat a are presented as mean± SEM of seven mice .

	Dose	Latency time (minute)	Sleeping time (minute)
Saline	10 (ml/kg)	8.17±1.09	20.05±1.66
Diazepam	3 (mg/kg)	5.28±0.83	37.85±3.82 ***
Ethanolice xtract	100 (mg/kg)	5.66±0.8	31.66±4.08
	500 (mg/kg)	6.0±1.43	46±3.64 *** ###
	1000 (mg/kg)	3.42±0.42 *	38±4.71 *** +
Aqueous extract	100 (mg/kg)	4.85±0.45	32.14±3.32
	500 (mg/kg)	5.42±0.36	42.27±3.58 *** #
	1000 (mg/kg)	4.28±0.28	46.57±2.88 *** +++
Choloroformic extract	500 (mg/kg)	5.0±0.51	24.16±2.57
	1000 (mg/kg)	5.57±0.71	22.71±1.67

***P<0.001 compared to negative control.
# P<0.05, ###P<0.001 compared to 500 mg/kg of chloroformic extract.
+P<0.05 , +++ P<0.001 compared to 1000 mg/kg of chloroformic extract

family exert their hypnotic effect through GABAA-system (5). Therefore, this system could be involved in the hypnotic effect of ethanolic and aqueous extracts of Rosa damascena.

Rosa damascena contains several components such as geraniol, citranellol, farnesol, nerol, linalol, eugenol, citral, terpene, myercene (6), vitamin C and bioflavonoids (1). The responsible compound(s) for hypnotic effect of Rosa damascena is not clearly known and could not be concluded based on the result of the present study. Other plants containing compounds such as flavonoids, terpenes and saponins have been found to have hypnotic effects (7). Therefore, it is suggested that these compounds might be responsible for the hypnotic effect of Rosa damascna. Flavonoids with anxiolytic and/or antidepressant activities have also been described in numerous plant species used in folk medicine to depress the CNS. This effect has been ascribed to their affinity for the central benzodiazepine receptors (8). It could be suggested that flavonoids of the Rosa damascena contribute to the hypnotic effect of this plant through benzodiazepine receptors.

Geraniol possesses methoxyphenol forms in its structure. Behavioral studies have shown that a number of methoxyphenols and alkylphenols have hypnotic and anticonvulsant properties (9). It is conceivable that geraniol may be at least partially responsible for the hypnotic effect of Rosa damascena through GABAA-system.

Also, it has been reported that saponins regulate the effects of sedatives, hypnotics and convulsants (10). Therefore, saponins could contribute to the hypnotic effect of Rosa damascena.

Other investigations have found that eugenol has anti-convulsant, analgesic and local anesthetic effects (11,12). Thus, this compound could be involved in the hypnotic effect of Rosa damascena.

In conclusion, results of the present study indicate that the hypnotic effect of Rosa damascena which is comparable to that of diazepam, but the exact mechanism(s) of this effect should be clarified in further studies.

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