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Original Article

Comparative Hypnotic Effect of *Rosa damascena* Fractions and Diazepam in Mice

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Abstract

Rosa damascena has been found to act on central nervous system including the brain. Several studies confirm that *R. damascena* inhibits the activity of the hypothalamus and pituitary systems in rat and can suppress the central nervous system. In traditional medicine hypnotic effect of Rose is also suggested. In this study, the hypnotic effect of the ethanol crude extract of *R. damascena* and its fractions was investigated in mice. Hypnotic method was based on prolongation pentobarbital induced sleeping time by the extract and fractions (with water, ethyl acetate and n-butanol). Two doses of extract and fractions (250 and 500 mg/kg) was injected intraperitoneal in comparison with diazepam (3 mg/kg) as the positive control and saline (10 ml/kg) as the negative control. Thirty minutes after injection of extract and fractions, pentobarbital (30 mg/kg) was injected and the increase in the sleeping time due to the extract and fractions was recorded. The results showed that the ethanol extract and fractions of *R. damascena* at 250 and 500 mg/kg doses prolonged the pentobarbital induced sleeping time in mice ($P<0.05$ to $P<0.001$). Among all fractions, aqueous fraction has the least, and the ethyl acetate fraction at 500 mg/kg dosage has the best hypnotic effect. In conclusion, the results of this study showed hypnotic effect of *R. damascena* which was more potent in ethyl acetate fraction.

Keywords: *Rosa damascena*; Fraction; Hypnotic effect; Diazepam; Mice.

Introduction

R. damascena is an erect shurb 1-2 meter in high. Flowers of this plant are large, showy and colorful. *R. damascena* today is highly cultivated for their scent (1). This plant contains carboxylic acid, terpene, myrcene and vitamin C (1, 2). Flowers, petals and hip (seed-pot) of *R. damascena* used for medical purposes. Therapeutic effects of *R. damascena* that were described in Iranian ancient medical books include cardiotonic and anti-inflammatory effects. Rosa also used in various conditions include menstrual bleeding, digestive disorders and headache. Essential oil from Rosa is reported to have analgesic and antispasmodic effects (1, 2). Rosa also is used as a gentle laxative and to ease coughs (1). Rose has been found to act on central nervous system including the brain. Several studies confirm that Rose inhibits the activity of the hypothalamus and pituitary systems in rat and can suppress the central nervous system (1). Treatment for a long time with high doses of Rose oil can lead to stress adjustment and the ability of the brain to compensate by going into a steady state of exhaustion (1). Anti - HIV (3) and anti - bacterial (2) effects for *R. damascena*

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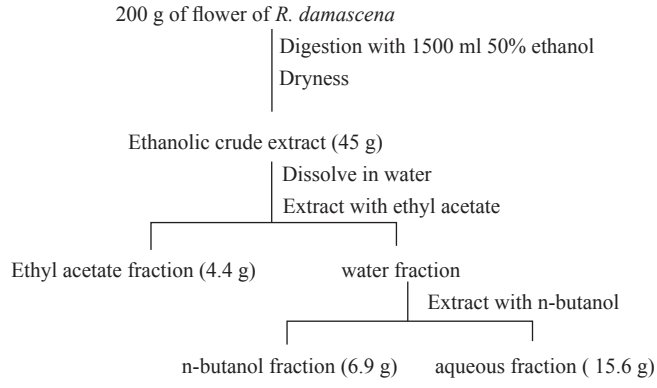

Figure 1. Separation of flowers of *R. damascena.*

were also reported. In traditional medicine hypnotic effect of Rose was suggested (1). In the modern pharmacological studies, ethanol extract of *R. damascena* was found to have hypnotic effect (4). Therefore, the hypnotic effect of ethyl acetate, aqueous and n-butanol fractions of this plant was evaluated in the present study.

Experimental

Plant and extract

R. damascena was collected from Kashan (middle part of Iran) in spring 2005 and identified by botanists in the School of Pharmacy, Mashhad University of Medical Sciences. A voucher specimen was preserved in the Herbarium of the School of Pharmacy, Mashad University of Medical Sciences (Herbarium No: 254-1804-01). Two hundred grams of the chopped, dried flowers of *R. damascena* was extracted for 3 days with 1500 ml 50% ethanol by digestion. The extract reduced to dryness with a vacuum rotary evaporator. A yield of 50 g (25%) was obtained. 45 g of extract was dissolved in 50 ml water and the solution was extracted with ethyl acetate and n-butanol. The ethyl acetate and n-butanol fractions were discarded to obtain aqueous fraction. The ethyl acetate fraction yield was 4.4 g (9.7%), n-butanol fraction yield was 6.9 g (15.3%) and the aqueous fraction yield was 15.6 g (34.6%). The separation steps are shown in Figure 1.

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 controlled temperature of 21-25 °C and a humidity of 55±5%. A standard pellet diet and tap water 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 ten groups and the following solutions were injected Intraperitoneally (I.P.) to each group (n=6 for each group):

1- Saline as negative control for aqueous fractions and ethanolic extract, and saline plus a few drops of tween 80 (art. no. 822181/1000, Merck chemical Co., batch number 5804) as negative control for ethyl acetate and n-butanol fractions (10 ml/kg).

2- Diazepam (3 mg/kg) (from Darupakhsh Pharmacutical Co., batch number 5804) as positive control.

3- Ethanol extract, two groups of 250 and 500 mg/kg.

4- N-butanol fraction two groups of 250 and 500 mg/kg .

5- Ethyl acetate fraction, two groups of 250 and 500 mg/kg.

6- Aqueous fraction two groups of 250 and 500 mg/kg.

Thirty minutes later Pentobarbital (30 mg/kg I.P., art. no. P 3761, Sigma) was given to induce sleep. The time interval between loss and recovery of righting reflex was used as index of hypnotic effect (5).

Statistical analyses

All data were expressed as mean ± SEM.

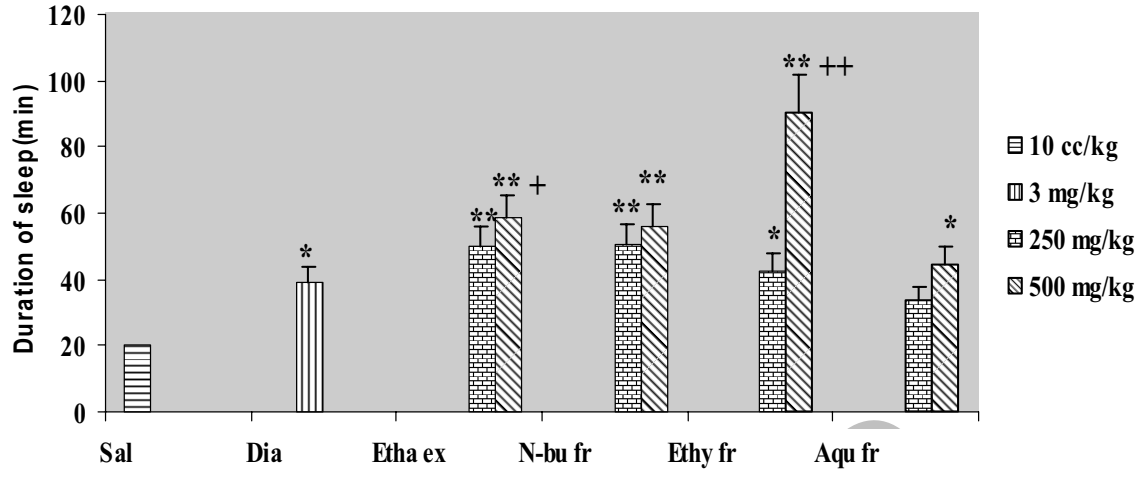

Figure 2. Effect of the ethanolic extract, n-butanol fraction, ethyl acetate fraction and aqueous fraction in doses of 250 and 500 mg/kg on the penthobarbital – induced sleeping time in mice (n=6 for each group).
Data are presented as mean±SEM. Sal: Saline, Dia: Diazepam, Etha.ex: ethanolic extract, N-bu fr: n-butanol fraction, Ethy fr: ethyl acetate fraction, Aqu.fr: aqueous fraction.
* p<0.05 compared to negative control.
** p<0.001 compared to negative control
+ p<0.05 compared to the positive control
++ p<0.001 compared to the positive control

Comparison of sleeping time in all groups were made using ANOVA with Tukey Cramer post test. Significance level was p<0.05.

Results

Hypnotic effect of ethanol crude extract

Sleeping time in animals receiving 250 mg/kg of the ethanol extract was increased to 50±4.76 minutes, which was significantly higher than the negative control (20.16±1.30) (p<0.01). However, the sleeping time was not significantly different from the positive control (38.83±3.8). Animals receiving 500 mg/kg of the ethanol extract showed an increased up to 58.66±4.98 minutes respectively, which was significantly greater than the sleeping times of both negative (p<0.001) and positive controls (p<0.05).

However, there was no significant difference between the effects of two doses of the ethanol extract.

Hypnotic effect of n-butanol fraction

The sleeping time of groups receiving 250 and 500 mg/kg of the n-butanol fraction (50.66±3.20 and 56.16±4.75 respectively) was significantly greater than that of negative control (p<0.001).The effect of both doses of the n-butanol fraction was comparable to that of diazepam. However there was no significant difference between the effects of two different doses of this fraction.

Hypnotic effect of ethyl acetate fraction

The sleeping time in animals receiving 250 mg/kg of the ethyl acetate fraction was increased to 42.66±4.60 minutes, which was significantly more than the negative control (P<0.05). But this was not significantly different from the positive control. On the other hand, those animals receiving 500 mg/kg of this fraction showed an increase up to 90.66 ± 5.42 minutes. This was significantly greater than the negative control, positive control and ethyl acetate fraction at 250 mg/kg dosage (P<0.001).

Hypnotic effect of aqueous fraction

The sleeping time of group receiving 250 mg/kg of the aqueous fraction was increased to 34±2.52 minutes, which was not significantly more than the negative control, and was lower than the positive control.

The sleeping time in animals receiving 500 mg/kg of this fractions was increased to 44.5±3.17 minutes, which was significantly more than the negative control (p<0.05). In addition,

the hypnotic effect in the group receiving 500 mg/kg of aqueous fraction was significantly greater than the group receiving 250 mg/kg aqueous fraction.

Comparison of the hypnotic effect between groups

The groups receiving 500 mg/kg showed more increase in pentobarbital induced sleeping time in comparison with the group at 250 mg/kg dosage. However, except ethyl acetate, the differences between the two doses were not statistically significant.

Among all fractions, aqueous fraction has the least and the ethyl acetate fraction of 500 mg/kg dosage has the higest hypnotic effect. However, the hypnotic effect in the group receiving only 500 mg/kg ethyl acetate fraction was significantly greater than groups receiving other fractions ($P<0.01$)

Discussion

In the present study the hypnotic effect of *R. damascena* extract and fractions was evaluated using standard method as previously described (5). Although the hypnotic effect of the ethanolic extract and three fractions of *R. damascena* were similar to that of diazepam, the mechanism(s) of hypnotic effect of this plant cannot 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 (6). Noguerira and Vassilieff have shown that the other geniuses of Rosaceae family exert their hypnotic effect through $GABA_A$- system (6). Therefore, this system could be involved in the hypnotic effect of ethanolic extract and three fractions of *R. damascena*.

R. damascena contains several components such as geraniol, citranellol, farnesol, nerol, linalol, eugenol, citral, terpene, myercene (7), vitamin C and bioflavonoids (1). The responsible compound(s) for hypnotic effect of *R. 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 effect (8) Therefore, it is suggested that these compounds might be responsible for the hypnotic effect of *R. damascena*. Flavonoids with anxiolytic and/or antidepressant activity have been also 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 (9). It could be suggested that flavonoids of the *R. damascena* contribute to the hypnotic effect of this plant through benzodiazpine receptors.

Geraniol possesses methoxyphenol forms in structure. Behavioral studies have shown that a number of methoxyphenols and alkylphenols have hypnotic and anticonvulsant properties (10). It is conceivable that geraniol may be at least partially responsible for the hypnotic effect of R. damascena through $GABA_A$-system.

It has been also reported that saponin could have effects on sedatives and hypnotic (11). Therefore saponins could contribute to the hypnotic effect of *R. damascena*.

Other investigations have found that eugenol has anti- convulsant, analgesic and local anesthetic effects (12, 13). Thus, this compound could be involved in hypnotic effect of *R. damascena*.

In conclusion the results of the present study indicate the hypnotic effect of *R. damascena* is comparable to that of diazepam, is more prominent in ethyl acetate fraction. However the mechanism(s) of this effect should be clarified in further studies.

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