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Potentiation of pentobarbital hypnosis by *Rosa damascena* in mice

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Rosa damascena has been found to act on central nervous system including brain. It inhibits the reactivity of the hypothalamous and pituitary systems in rat. In traditional medicine hypnotic effect of Rose is also suggested. In the present study hypnotic effect of ethanolic, aqueous and chloroformic extracts of *R. 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 ip in comparison with diazepam (3mg/kg) as positive control and saline as negative control. After 30 min of injection of extracts, pentobarbital (30mg/kg) was injected and increase in sleeping time by extracts was recorded. The results showed that the ethanolic and aqueous extracts in 500 and 1000 mg/kg doses significantly increased pentobarbital induced sleeping time which was comparable to diazepam. The chloroformic extract had no hypnotic effect.

Keywords: Hypnosis, Mice, Pentobarbital, *Rosa damascena*

Rosa damascena is an erect 1-2 meter high shrub. Flowers of the plant are large, showy and colorful. *R. damascena* is cultivated for its scent purposes[1]. The plant contains carboxylic acid, terpene, myrcene and vitamin C [1,2]. Flowers, petals and hip (seed-pot) of *R. damascena* are used for medical purposes[1]. Rose has been found to act on central nervous system including brain. It is well known that *Rosa* inhibits the reactivity of the hypothalamus and pituitary systems in rat and can suppress the activity of central nervous system[1]. Treatment for a long period 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]. Also anti-HIV[3] and anti-bacterial[2] effects of *R. damascena* have been reported. The present syudy has been undertaken to evaluate hypnotic effect of ethanolic, aqueous and chloroformic extracts of *Rosa damascena* in male mice.

Materials and Methods

Rosa damascena shrubs were collected from Kashan (middle part of Iran) in spring and were identified by the botanists of Ferdowsi University, Mashhad. Chopped, dried flowers (50 g) were extracted with 300 ml distilled water, ethanol (70% v/v) and chloroform to prepare aqueous, ethanolic and chloroformic extracts respectively. The solvent of all extracts was then removed under reduced pressure until the extract volume reached 50 ml and then was dried completely in room temperature within Petri dish.

Male Bulb C mice 70, obtained from the Pasteur Institute of Iran, weighing 20-28 g were used. The mice were fed *ad libitum* with rodent's chow and had free access to drinking water. The animals were kept in a room with controlled 12 hr light/dark cycle and temperature (22 ^{o}±3^{o} C).

Mice were divided in to 10 groups of 7 each and the extract was injected as per following protocol:

1 Saline as negative control
2 Diazepam (3mg/kg) as positive control
3 Ethanolic extract (100, 500, 1000 mg/kg dose, ip)
4 Aqueous extract (100, 500, 1000 mg/kg dose, ip)
5 Chloroformic extract (500, 1000 mg/kg dose, ip)

Hypnotic effect method[4] based on potentiation of pentobarbital induced sleeping time by extract was used to study the effect of extracts. After 30 min of administration of extracts pentobarbital (30mg/kg, ip) was given to induce sleep. The interval between loss and recovery of righting reflex was used as index of hypnotic effect[4]. The time interval between injection of pentobarbital and start of sleep was recorded as latency time. In the negative and positive control

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groups instead of extract, normal saline (10 ml/kg, ip) and diazepam (3mg/kg, ip) were injected respectively.

Statistical analysis—The data are expressed as mean±SE. Comparison of sleeping time in all groups was made using ANOVA. Significant was accepted at $P< 0.05$.

Results

Hypnotic effect of ethanolic extract—Sleeping time in animals receiving 100 mg/kg of ethanolic extract was increased to 31.66±4.08 min that was nonsignificantly more than that of negative control (20.05±3.53 min) and in those receiving 500 and 1000 mg / kg of ethanolic extract was increased to 46±3.64 min and 38±4.41 min respectively, that was significantly different compared to negative control value F(9,60)=8.3) ($P<0.001$; Fig. 1). However, there was no significant difference between diazepam (37.857± 3.83 min) and all three doses of ethanolic extract. The difference between 500 and 1000 mg/kg doses of ethanolic extract was not significant.

The time interval between injection of pentobarbital and onset of sleep in all groups (latency time) is presented in Fig.2. Ethanolic extract in the doses 100 and 500 mg / kg shortened the latency time of sleep to 5.66 ± 0.8 min and 6±1.43 min respectively which is lower than that of control (8.17±1.09 min) and is comparable to diazepam (5.28 ± 0.83 min) but the difference was not significant. The extract (1000 mg/ kg) decreased the time to 3.42 ± 0.42 min which was significantly different than control F(9,60)=2.587 ($P<0.05$).

Hypnotic effect of aqueous extract—Sleeping time between groups receiving 500 and 1000 mg/ kg of aqueous extract was significantly more than that of negative control and the group receiving 100 mg/kg of this extract (32.14±3.32 min) was also more than that of control but the difference was not significant. The effect of all doses of aqueous extract was comparable to that of diazepam. There was no significant difference between 500 and 1000 mg/kg of this extract.

All doses of the extract accelerated the initiation of hypnotic effect of pentobarbital comparable to effect of diazepam (Fig.2).

Hypnotic effect of chloroformic extract—Neither the doses of chloroformic extract (500 and 1000mg/kg) could potentiate the pentobarbital induced sleeping time (Fig.1).

Discussion

The present study demonstrated hypnotic effect for 500 and 1000 mg/kg doses of ethanolic and aqueous extracts from *R. damascena*. The hypnotic effect of both doses of extracts was comparable to diazepam. The effect of aqueous extract was dose dependent but in ethanolic extract 500mg/kg had the maximal effect.

Although the hypnotic effect of ethanolic and aqueous extracts from *R. damascena* was similar to that of diazepam the mechanism(s) of hypnotic effect of this plant cannot be concluded from the results of the present study. Family Rosaceae is known as a source of folk medicine used for nervous breakdown[5]. Noguerira and Vassilieff[5] have shown that the other genera of Rosaceae family have hypnotic effect

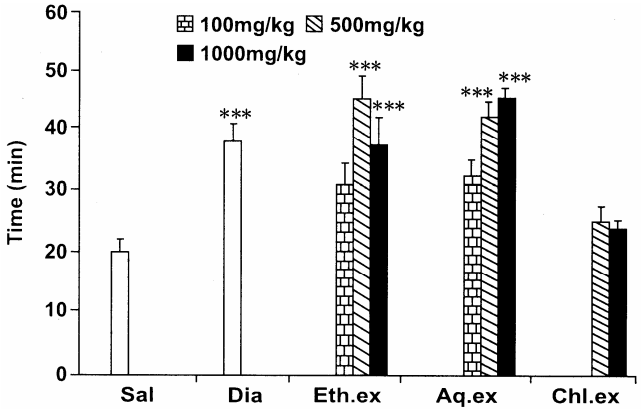

Fig. 1—Effect of ethanolic and aqueous extracts in 100, 500 and 1000 mg/kg doses and chloroformic extract in 500 and 1000 mg/kg on the penthobarbital–induced sleeping time in mice. [Data are presented as mean ±SE of 7 mice. Sal-Saline, Dia-Diazepam, Eth.ex-Ethanolic extract, Aq.ex-Aqueous extract, Chl.ex: Chloroformic extract. ***$P<0.001$ compared to negative control].

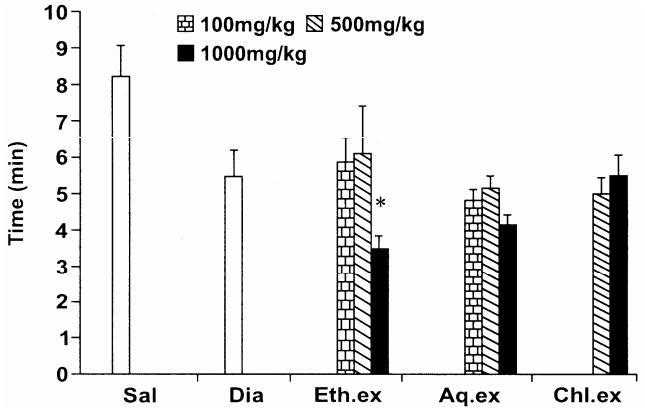

Fig. 2—Effect of ethanolic, aqueous and chloroformic extracts in 100, 500 and 1000 mg/ kg doses on the latency time of penthobarbital–induced sleeping time in mice. [Data are presented as mean ±SE of 7 mice. Sal-Saline, Dia-Diazepam, Eth.ex-Ethanolic extract, Aq.ex-Aqueous extract, Chl.ex-Chloroformic extract. *$P<0.05$ compared to negative control].

through $GABA_A$-system. Therefore, this system may be involved in the hypnotic effect of ethanolic and aqueous extracts of *R. damascena*.

Rosa damascena contains several components such as geraniol, citranellol, farnesol, nerol, linalol, eugenol, citral, terpene, myercene[6] vit C and bioflavonoids[1]. The responsible compound(s) for hypnotic effect of *R. damascena* is uncertain to us and can not be concluded from the result of the present study. Other plants contained compounds such as flavonoids, terpenes and saponins have been found to have hypnotic effects[7]. Therefore, it can be suggested that these compounds may be responsible for hypnotic effect of *R. damascena*. Flavonoids with anxiolytic and/or antidepressant activity have also been described in many plant species used in folk medicine to depress the CNS. This effect has been ascribed to their affinity for the central benzodiazepine receptor[8].

Geraniol possess methoxyphenol forms in 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 hypnotic effect of *R. damascena* through $GABA_A$-system. Also it has been reported that saponin regulates the effects of sedatives, hypnotic and convulsants[10], therefore saponins can contribute in the hypnotic effect of *R. damascena*. On the other hand, the other investigations have been found that Eugenol has anti-convulsant, analgesic and local anesthetic effects[11,12]. Thus this compound maybe involved in hypnotic effect of *R. damascena.*

In conclusion, the result of the present study indicated hypnotic effect of *R. damascena* which was comparable to that of diazepam but the exact mechanism(s) of this effect should be clarified in further studies.

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