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## Antinociceptive Effect of *Rosa damascena* in Mice

<sup>1,2</sup>Hassan Rakhshandeh, <sup>3</sup>Nasser Vahdati-Mashhadian, <sup>1</sup>Karim Dolati and <sup>4</sup>Mahmoud Hosseini  
<sup>1</sup>Department of Pharmacology,

<sup>2</sup>Pharmacological Research Center of Medicinal Plants, Ghaem Medical Center,  
Mashad University of Medical Sciences, Mashad, Iran

<sup>3</sup>Department of Pharmacodynamics and Toxicology, School of Pharmacy,  
Mashad University of Medical Sciences, Mashad, Iran

<sup>4</sup>Department of Physiology, Ghaem Medical Center, Mashad University of Medical Sciences, Mashad, Iran

**Abstract:** In this study, analgesic effects of the aqueous, ethanolic and chloroformic extracts of this plant were investigated. Mice were treated with IP injection of 100, 500 and 1000 mg kg<sup>-1</sup> aqueous, ethanolic and chloroformic extracts of the plant and analgesic effects were assessed using hot plate and tail flick methods. The results showed that ethanolic extract had significant analgesic effect comparable to morphine. As, pretreatment of animals with naloxone significantly reduced analgesic effect of the extract, the analgesic effect of ethanolic extract seems to be at least, in part through opioid system. No analgesic effects have been observed with aqueous or chloroformic extracts of the plant.

**Key words:** *Rosa damascena*, analgesic plant extract, hot plate, tail flick

### INTRODUCTION

*Rosa damascena* is a plant belonging to Rosaceae family that is cultivated for decorative and perfumery purposes (Zargari, 1992; Boskabady *et al.*, 2006). In Iran, it is also cultivated for the production of its essential oil and rose water (Boskabady *et al.*, 2006). In traditional medicine, it was introduced as having various therapeutic effects including treatment of fever and sore throat, constipation, gastrointestinal complaints, ophthalmic diseases, memory loss and breast tenderness in different parts of the world (Libster, 2002; Boskabady *et al.*, 2006).

The plant contains geraniol, citranellol, farnesol (Zargari, 1992; Caissard *et al.*, 2006), kaempferol and quercetin glycosides (Schiber *et al.*, 2005). It exhibited modest anti HIV (Mahmood *et al.*, 1996) and antibacterial activities against *Staphylococcus aureus* (Aridogan *et al.*, 2002). In the previous study the hypnotic effect of this plant was also investigated (Rakhshandeh and Hosseini, 2006). In ancient medical books, beneficial effects of this plant for treatment of abdominal and chest pain (Wood and Bache, 1839) and anti-inflammation (AveSina, 1990) are reported. In ancient Iranian literatures, it has been reported that Rosaceae family has analgesic effects (Miraldi *et al.*, 2001). In a pilot study, we showed that this plant had anti-inflammatory effect in carrageenan induced paw edema model of rat (Hosseini and Rakhshandeh,

2004). In this study, we investigated the analgesic effects of aqueous, ethanolic and chloroformic extracts of the plant. We used tail flick and hot plate methods to evaluate analgesic effects of *Rosa damascena*. These two methods have been repeatedly used to show analgesic effects of drugs (Morteza-Semnani *et al.*, 2006; Grass *et al.*, 1996). To our knowledge, this is the first study, of the analgesic of *Rosa damascena*.

### MATERIALS AND METHODS

**Animals:** Male albino mice weighing 25-30 g were obtained from a random bred colony in the animal house of Mashad University of Medical Sciences. Animals were housed in colony room 12/12 h light/dark cycle at 21±2°C and had free access to water and food. Experimental plan was approved by the Mashhad University Committee on Animal Research.

**Chemicals:** Ethanol and chloroformic were purchased from Ettehad and Merck Companies respectively. Morphine obtained as gift sample from Darou-Pakhsh Company (Tehran). Morphine, aqueous and ethanolic extracts were diluted in normal saline. Chloroformic extract was diluted to required concentrations in normal saline using a few drops of tween.

**Plant material:** *Rosa damascena* shrubs were collected from Kashan (middle part of Iran) in spring and were identified by the botanists of Ferdowsi University, Mashhad (Rakhshandah and Hosseini, 2006).

**Extraction:** 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 (Rakhshandah and Hosseini, 2006).

**Analgesic study:** This study carried out in Dept. of Pharmacology and Pharmacological Research Center of Medicinal Plants, of Mashhad University of Medical Sciences. Nine test groups (8 animals each) of mice were used in this part of study as test groups for each extract. Groups 1 through 3 of test animals received 100, 500 and 1000 mg kg<sup>-1</sup> of the extracts intraperitoneally (i.p.) and were used for hot plate test. Groups 4 through 6 received the same doses and were used for tail flick test. In groups 7 through 9, the same doses of the extracts were preceded, for 5 min, by morphine antagonist, naloxone (4 mg kg<sup>-1</sup>) and were used in tail flick test. Normal saline and morphine (9 mg kg<sup>-1</sup>) were used in negative control and positive control groups, respectively.

**Hot plate test:** The temperature of a metal surface was maintained at 55±0.2°C. Latency to a discomfort reaction (licking paws or jumping) was determined before and after drug administration. The cut-off time was 20 sec. The latency was recorded before and 30, 60 and 90 min following ip administration of the agents.

**Tail flick test:** In this test, light intensity was 99% and cut-off time was 20 sec. Light source was 2 cm distant from animals tail. Tolerance times were recorded for 0, 30, 60 and 90 sec after drug or extracts administrations.

**Statistical analysis:** Data were expressed as mean values ±SEM and the comparison between control and test groups was performed using ANOVA and Tukey tests. p<0.05 considered significant difference between groups.

## RESULTS

**Analgesic activity of aqueous extract:** Aqueous extract, in all concentrations used, did not produce a significant increase in animal reaction time in hot plate test (Fig. 1). Similar results were also seen in tail flick test.

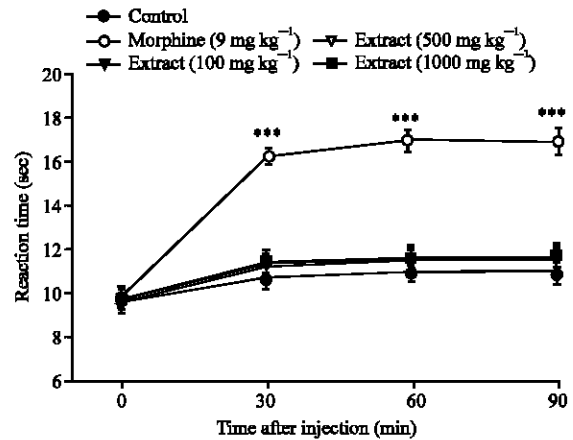


Fig. 1: Comparison of reaction time of the animals of control, morphine and three doses (100, 500 and 1000 mg kg<sup>-1</sup>) of aqueous extract of *Rosa damascena* in hot plate test. Data presented as mean±SEM (n = 8 in each group) \*\*\*p<0.001 compared to control group

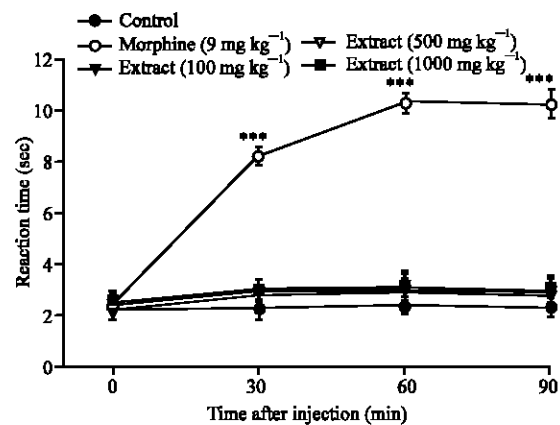


Fig. 2: Comparison of reaction time of the animals of control, morphine and three doses (100, 500 and 1000 mg kg<sup>-1</sup>) of chloroformic extract of *Rosa damascena* in hot plate test. Data presented as mean±SEM (n = 8 in each group) \*\*\*p<0.001 compared to control group

**Analgesic activity of chloroformic extract:** Figure 2 shows that chloroformic extract produced similar results to aqueous extract in both analgesic tests. Tail Flick test also produced similar results.

**Analgesic activity of ethanolic extract:** Results of hot plate study showed that ethanolic extract, produced significant increase in animal reaction time. Figure 3 shows that i.p., injection of 100 mg kg<sup>-1</sup> of the extract 30, 60 and 90 min prior to test increases animal reaction time

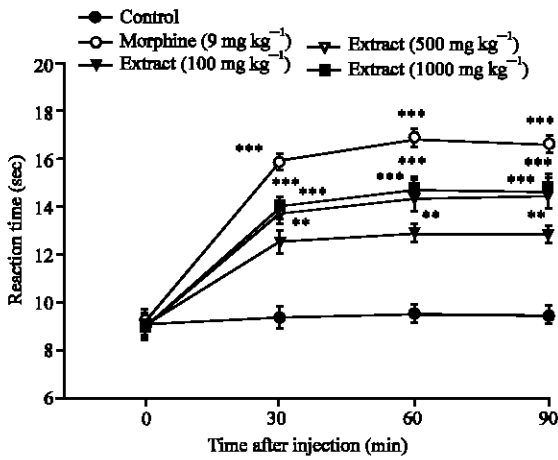


Fig. 3: Comparison of reaction time of the animals of control, morphine and three doses (100, 500 and 1000 mg kg<sup>-1</sup>) of ethanolic extract of *Rosa damascena* in hot plate test. Data presented as mean±SEM (n = 8 in each group) \*\*p<0.001, \*\*\*p<0.001 compared to control group

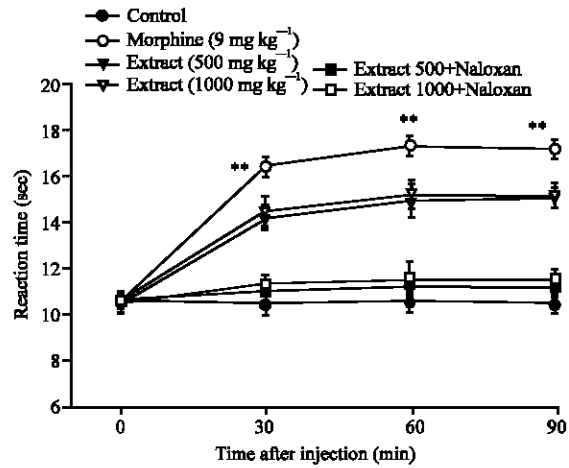


Fig. 5: Comparison of reaction time of the animals of control, morphine and two doses (500 and 1000 mg kg<sup>-1</sup>) of ethanolic extract of *Rosa damascena* ant after pretreatment with naloxone in hot plate test. Data presented as mean±SEM (n = 8 in each group) \*\*p<0.01 compared to control group

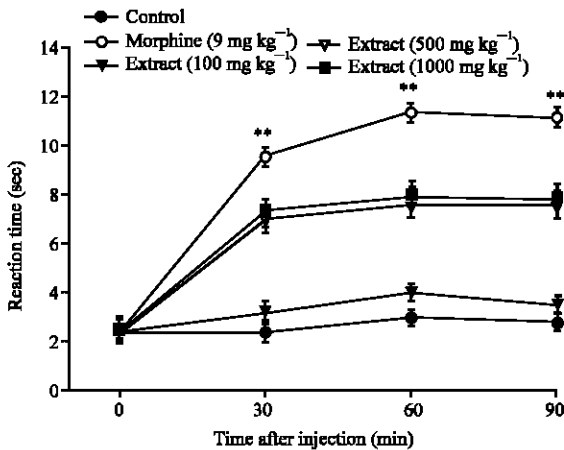


Fig. 4: Comparison of reaction time of the animals of control, morphine and three doses (100, 500 and 1000 mg kg<sup>-1</sup>) of ethanolic extract of *Rosa damascena* in tail flick test. Data presented as mean±SEM (n = 8 in each group) \*\*p<0.01 compared to control group

compared with negative control group (p<0.01). More significant effect was produced with 500 and 1000 mg kg<sup>-1</sup> (p<0.001). Peak effect was observed in 60 min after extract administration. Morphine produced comparable but stronger analgesic effect.

Figure 4 shows the results of tail flick experiments. The results show that ethanolic extract, in doses of 500 and 1000 mg kg<sup>-1</sup>, but not in 100 mg kg<sup>-1</sup>, significantly

increases the reaction time compared with negative control group in tail flick test (p<0.01). Morphine produced comparable but stronger analgesic effect.

Pretreatment with naloxone (4 mg kg<sup>-1</sup>) significantly diminished the analgesic activity of the extracts (p<0.01) in Hot Plate test (Fig. 5).

## DISCUSSION

Aqueous and chloroformic extracts of *Rosa damascena* did not show significant analgesic activity (Fig. 1, 2), but ethanolic extract showed significant activity comparable to 9 mg kg<sup>-1</sup> of morphine in hot plate and light tail flick tests (Fig. 3, 4). Naloxone produced potent inhibition of the extract analgesic effect (Fig. 5). These results imply that ethanolic extract of the plant has a major central analgesic effect and opioid receptors or pathways are contributed, at least, in part in its analgesic activity. This finding confirms the old opinion that *Rosa damascena* has analgesic effect (AveSina, 1990; Wood and Bache, 1839). There was not any previous study showing analgesic effect of this plant but in the previous study we showed that *Rosa damascena* has hypnotic effects (Rakhshandah and Hosseini, 2006). The present result another study showed that pretreatment with ethanolic extract of *Rosa damascena* could reduce inflammation in carrageenan induced paw edema model rats (Hosseini and Rakhshandeh, 2004).

The plant contains several flavonoids including kaempferol and quercetin glycosides. The kaempferol glycosides, along with the kaempferol aglycone, accounts for 80% of the total compounds in the distillate of its petals (Schiber *et al.*, 2005). Microsatellite genotyping demonstrated that *R. damascena* accessions from Bulgaria, Iran and India and old European Damask rose varieties possess identical microsatellite profiles, suggesting a common origin (Rusanov *et al.*, 2005). The high similarity between the varieties of the plant cultivated in different regions implies that it may preserve high identity in ingredients. Rylski *et al.* (1979) showed that quercetin decreases the pain threshold level (Rylski *et al.*, 1979). In *Xenopus* oocytes injected with 5-HT<sub>3A</sub> receptor cRNA, quercetin inhibited the 5-HT-induced inward peak current. Inhibition was competitive and voltage-independent. This receptor is involved in pain transmission, analgesia, vomiting and mood disorders (Lee *et al.*, 2005). Quercetin and its conjugates reduced COX-2 mRNA expression in both unstimulated and interleukin-1-beta stimulated colon cancer (CaCO<sub>2</sub>) cells and some of them inhibited COX-2 activity as well (O'Leary *et al.*, 2004). Analgesic activity of the ethanolic extract might be due to quercetin or its conjugates. Both central and peripheral effects may contribute to this activity.

Quercetin and kaempferol inhibit the release of inflammatory mediators IL-6, IL-8 and TNF- $\alpha$  (Kempuraj *et al.*, 2005; Kowalski *et al.*, 2005). These two flavonols also inhibited induction of NOS-2 protein in LPS-treated J774.2 cells. In addition, Kaempferol inhibited NOS-2 induction at the level of gene transcription (Olszanecki *et al.*, 2002). Anticociceptive effects in present results might be due to quercetin and kaempferol or some other flavonoids in the extract.

Results of this study shows significant analgesic activity of ethanolic extract of *Rosa damascena*. Analgesic effect has not been shown with aqueous or chloroformic extracts of the plant. This means that the analgesic ingredients are freely soluble in ethanolic but not in water or chloroformic. Quercetin and kaempferol are insoluble in water (O'Neil *et al.*, 2001) and can not enter aqueous extract to a significant amount for analgesia. We do not have any idea on the solubility of these two compounds in chloroformic. These data strongly suggest that quercetin and kaempferol are the main substances responsible for the analgesic and anti-inflammatory effects of *Rosa damascena*.

To present knowledge, this is the first report of systemic analgesic effect of *Rosa damascena*. We hope that more qualitative and quantitative analysis of the plant extracts together with more sophisticated pharmacological and toxicological experiments clarify some other useful aspects of this plant.

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