

CNS Depressant Effect of the Crude Ethanolic Extract of the Flowering Tops of *Rosa Damascena*

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ABSTRACT

The ethanolic extract of the flowering tops of *Rosa damascena* (Rosaceae) was assessed for effect on the central nervous system (CNS) using a number of neuropharmacological experimental models in mice. The extract produced a dose-dependent reduction of the onset and duration of pentobarbitone-induced hypnosis, reduction of locomotor and exploratory activities in the open field, hole cross tests. At the same dose levels, the extract dose-dependently inhibited acetic acid-induced writhing in mice. These results suggest that the extract possess CNS depressant activity.

Keywords: *Rosa damascena*, Depressant activity, Central nervous system

Rosa damascena Mill. (Rosaceae) is a well known shrub. *R. damascena* is cultivated in rose gardens in several places in Bangle, Kashmir and Punjab. Enormous quantity of wild hill roses grows throughout the North West Himalayas and Kashmir [1]. Essential oil, quercetin, kampferol and cyanidin have been isolated from whole plant [1]. Cyanidine 3,5-diglucoside has been isolated from petals. Flowers also contain a bitter principle, tanning matter, fatty oil and organic acids [1]. The tetrahydroxyflavanone (kaempferol) isolated from the methanol extract was effective in reducing the maturation of infectious progeny virus apparently due to selective inhibition of the viral protease [2]. On the other hand the pentahydroxyflavone (quercetin) and two 3-substituted derivatives of kaempferol appeared to inhibit HIV-infection by preventing binding of gp120 to CD4 and 2-Phenylethanol-O-(6-O-galloyl)-beta-D-glucopyranoside interacted irreversibly with gp120 and neutralized virus infectivity [2]. The essential oil of *R. damascena* petals was evaluated for its antibacterial effects against three strains of *Xanthomonas axonopodis* spp. vesicatoria [3].

As a part of our on-going investigation on Bangladeshi plants for phytochemical, biological and pharmacological properties [4-6], we now report on the neuropharmacological potential of the ethanolic extracts of the flowering tops of *R. damascena* in mice.

MATERIALS AND METHODS

Plant Material

The flowering tops of *R. damascena* were collected from the Gazipur, Dhaka, Bangladesh in December 2005 and identified by the experts of Bangladesh National Herbarium, Dhaka, where a voucher specimen has been retained.

Preparation of Ethanol Extracts

Dried ground flowering tops (400 g) were extracted with 95% of ethanol in a Soxhlet apparatus at an elevated temperature. The extract was concentrated by evaporation under reduced pressure at 40°C using Buchi rotary evaporator to yield a gummy reddish black coloured extract (yield appx. 5.6%).

Animals

Swiss mice of either sex (20-25 g) were obtained from the Animal house of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were housed under standard laboratory conditions (relative humidity 55-65%, r.t. 23.0±2.0°C and 12 h light: dark cycle). The animals were fed with standard diet and water ad libitum.

Pentobarbitone Induced Sleeping Time Test

The animals were randomly divided into four groups consisting of five mice each. The test groups received of *R. damascena* flowering tops extracts at the doses of 250 and 500 mg/kg while positive control was treated

Table 1. Effect of *R. damascena* flowering tops ethanolic extract on pentobarbitone induced sleeping time in mice.

Treatment	Dose (mg/kg)	Route of administration	Onset of sleep (min)	Duration of sleep (min)
Control (1% aq. tween 80)	10 mL/kg	p.o.	7.4 ± 0.50	45.6 ± 1.36
<i>R. damascena</i> extract	250	p.o.	6.2 ± 0.37 ^b	60.5 ± 1.03 ^a
	500	p.o.	5.8 ± 0.37 ^a	73.4 ± 1.07 ^a

^a $p < 0.001$ ^b $p < 0.01$ ^c $p < 0.05$ vs. control, Student's *t*-test; values are mean ± S.E (N=6).

with diazepam (1 mg/kg i.p.) and control with vehicle (1% Tween 80 in water). Thirty minutes later, pentobarbitone (40 mg/kg, i.p., Sigma Chemicals, USA) was administered to each mouse to induce sleep. The animals were observed for the latent period (time between pentobarbitone administration to loss of righting reflex) and duration of sleep (time between the loss and recovery of righting reflex) [7].

Open Field Test

This experiment was carried out as described by Gupta et al. [8]. The animals were divided into control and test groups. The test groups received *R. damascena* ethanolic extracts of flowering tops at the doses of 250 and 500 mg/kg body weight orally whereas control group received vehicle (1% Tween 80 in water). The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had 40 cm height a wall. The number of squares visited by the animals was counted for 3 min, on 0, 30, 60, 120 and 240 min during the study period.

Hole Cross Test

The method described by Takagi et al [9] was adopted for this study. A steel partition was fixed in the middle of a cage having a size of 30×20×14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. The number of passages of a mouse through the hole from one chamber to other was counted for a period of 3 min on 0, 30, 60, 120 and 240 min after the oral treatment with *R. damascena* flowering tops ethanolic extracts at the doses of 250 and 500 mg/kg.

Antinociceptive Activity Study

Effect on nociception was studied using acetic acid induced writhing model in mice [6]. After an overnight fast, the animals were divided into control, positive control and test groups containing five mice in each group. The animals were fed with test substance at the doses of 250 and 500mg/kg body weight, reference drug (diclofenac-Na) and control vehicle 45 min before intraperitoneal administration of 0.7% acetic acid. After a five minutes interval for proper absorption of acetic acid, the mice were observed for specific contraction of body referred as 'writhing', which is an indication of pain

sensation in test animals. A comparison of writhing was made between positive control, control and test sample.

RESULTS AND DISCUSSION

In the pentobarbitone induced hypnosis test, the flowering tops extract at the doses of 250 and 500 mg/kg significantly induced the sleep at an earlier stage and also prolonged the duration of sleeping time in test animals as compared to control (Table 1).

In the open field test, the extracts showed a noticeable decrease in locomotion in the test animals from the third observation period at both dose levels (250 and 500 mg/kg body weight). The depressant actions were slowly reduced with the time. The results were dose dependent and statistically significant (Table 2).

In the hole cross test, the extracts also showed a decrease in locomotion in the test animals from the second observation period at both dose levels (250 and 500 mg/kg body weight). The results were dose dependent and statistically significant (Table 3).

In acetic acid induced writhing test, the extract significantly and dose dependently suppressed the frequency of acetic acid induced writhing in mice. At the dose 250 mg/ kg body weight the extract of *R. damascena* showed 37.07% writhing inhibition where as at 500 mg/ kg body weight produced 49.07 % writhing inhibition, which is comparable to a standard drug and all the result are statistically significant ($p < 0.001$) (Table 4). Diclofenac-Na, used as the positive control exhibited a writhing inhibition of 49.07 % as compared to control and the result was statistically significant ($p < 0.001$).

In vivo methods using intact animals are considered to be the best method for investigating the action of drugs on the CNS. The most important step in evaluating drug action on the CNS is to observe the behaviour of the test animals. To obtain meaningful results regarding the effect of *R. damascena* flowering tops ethanolic extracts on the CNS in mice, a number of methods namely pentobarbitone-induced hypnosis, open field, hole cross, hole-board and acetic acid induced writhing tests were adopted. In the pentobarbitone-induced hypnosis test, both extracts, at the doses of 250 and 500 mg/kg body weight, dose dependently induced sleep at a rapid stage as compared to control, and increased the duration of sleep (Tale 1). Pentobarbitone, a barbiturate type of hypnotic agent, when given at appropriate dose, induces sedation or hypnosis in animals by potentiating

Table 2. Effect of Rosa damascene flowering tops ethanolic extracts on Open field test in mice.

Treatment	Dose (mg/kg, p.o)	Number of Movements				
		0 min	30 min	60 min	120 min	240 min
Control (1% aq. tween 80)	10 mL/kg	86.4 ± 2.0	93.5 ± 1.8	96.2 ± 1.9	103.3 ± 1.5	105.2 ± 1.5
<i>R. damascena</i> extract	250	85.3 ± 1.6 ^a	72.4 ± 1.3 ^a	71.2 ± 1.4 ^a	65.1 ± 1.8 ^a	44.5 ± 4.2 ^a
	500	85.1 ± 2.0 ^a	70.0 ± 1.6 ^a	57.0 ± 3.2 ^a	46.4 ± 3.5 ^a	44.5 ± 3.9 ^a

^a $p < 0.001$ ^b $p < 0.01$ ^c $p < 0.05$ vs. control, Student's *t*-test; values are mean ± S.E (N=6).

Table 3. Effect of *Rosa damascena* flowering tops ethanolic extracts on Hole cross test in mice.

Treatment	Dose (mg/kg, p.o)	Number of Movements				
		0 min	30 min	60 min	120 min	240 min
Control (1% aq. tween 80)	10ml/kg	8.4±0.64	9.5±0.44	10.1±0.49	9.6±0.69	8.6±0.66
<i>R. damascena</i> extract	250	7.3±0.69 ^a	5.1±0.41 ^a	5.2±0.44 ^a	3.5±0.41 ^a	2.1±0.31 ^a
	500	8.5±0.66 ^a	5.0±0.49 ^a	4.1±0.31 ^a	2.3±0.43 ^a	1.5±0.21 ^a

^a $p < 0.001$ ^b $p < 0.01$ ^c $p < 0.05$ vs. control, Student's *t*-test; values are mean ±S.E (N=6).

the GABA mediated postsynaptic inhibition through an allosteric modification of GABA receptors [10]. Substances that have CNS depressant activity either decrease the time for onset of sleep or prolong the duration of sleep or both. The results obtained in this test, indicate that these extracts might have depressant action on the CNS.

An important step in evaluating drug action on CNS is to observe its effect on locomotor activity of the animal. The extract significantly decreased the locomotor activity as shown by the results of the open field and hole cross tests. The locomotor activity lowering effect was evident at the 3rd observation (60 min) and continued up to 5th observation period (240 min) Table 2. Thus decreased spontaneous motor activity and potentiation of pentobarbitone-induced sleep could be attributed to the CNS depressant activity of the extracts. Moreover, the validation of anxiety was carried out by measuring external signs, through hole-board and evasion tests. In the hole cross experiment, the depressing action of the extracts was evident from the second observation period in the test animals at the doses of 250 and 500 mg/kg body weight. Maximum depressant effect was observed from 4th (120 min) to 5th (240 min) observation period. The results were also dose dependent and statistically significant (Table 3).

The ethanolic extract of *R. damascena*, at the doses of 250 and 500 mg/kg body weight, showed significant and dose-dependent decrease in the acetic acid induced writhing in mice and the results followed a dose dependent response (Table 4). Intraperitoneal administration of acetic acid causes algnesia by liberating noxious endogenous substances including serotonin, histamine, prostaglandin, bradykinin and substance P that sensitize pain nerve endings [11-12]. Of the rostanoids, mainly prostacycline has been held responsible for the causation of pain following acetic acid administration [13]. It has been suggested that acetic acid stimulates the vanilloid receptor and bradykinin B2 receptor in the pathway comprising sensory afferent C-fibers [14]. The reason behind the observed activity of the ethanolic extract of *R. damascena*, may be due to the effect of the extract in decreasing the synthesis and/or release of those endogenous substances or depressant effect of the extract on the nerve fibers involved in the pain transmission pathway.

Finally overall results obtained from this study showed CNS depressant activity of the flowering tops on experimental animal models. Among the extracts the ethanolic extract of *R. damascena* flowering top 500 mg/kg dose showed more prominent depressant activity than the 250 mg/kg dose. The mechanism of this depression is not clearly understood at this point, but it can be assumed that the drug may exert CNS depressant effect by interfering with the function of cortex.

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Table 4. Effect of *Rosa damascena* flowering tops ethanolic extract on acetic acid induced writhing in mice.

Treatment	Dose* (mg/kg)	Route of administration	Writhings**	% of writhing inhibition
Control (1% aq. tween 80)	10 mL/kg	p.o.	21.6±1.28	100
<i>R. damascena</i> extract	25	p.o.	11.0±1.93 ^a	49.07
	250	p.o.	13.0±1.72 ^a	37.07
	500	p.o.	11.0±2.00 ^a	49.07

** Administered 45 min before 0.7% acetic acid administration (10 mL/kg, i.p.).

* Counted for 15 min, starting 5 min after acetic acid administration;

^a $p < 0.001$ ^b $p < 0.01$ ^c $p < 0.05$ vs. control, Student's *t*-test; values are mean ±S.E (N=6).

pp. 12–24.

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