Neuropharmacological effects of methanolic extracts of *Rubus fruticosus* L.

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Aim: To investigate methanol extracts of various parts of *Rubus fruticosus* L. (Rosaceae) for various neuropharmacological activities, such as anxiolytic, muscle relaxant, antidepressant, and sedative activities.

Materials and methods: The extracts were administered to albino mice orally at doses of 100, 300, and 500 mg/kg. The antidepressant activity was determined by using the forced swimming test, while line crossing in a special box was used for assessment of locomotor activity.

Results: All extracts were found to be anxiolytic in nature, while no muscle relaxing activity or sedative effect was observed. The order of central nervous system (CNS) depressant effect for various parts of *R. fruticosus* was fruit > root > leaves > stem.

Conclusion: Our results indicate that methanolic extract of various parts of *R. fruticosus* possess anxiolytic and CNS depressant effects but do not possess significant sedative or muscle relaxing potential.

Key words: Neuropharmacological, *Rubus fruticosus* L., methanolic extracts

1. Introduction

Rubus fruticosus L. (Rosaceae) grows wild in northern areas of Pakistan like Malakand, Kotli, Chitral, Mansehra, and Dir (1–6). It is well known by local people due to its nutritional and medicinal importance. The fruit is commonly known as berry or blackberry. The fruit is edible and used for preparing jams and jellies (7). A tea is prepared from the dried leaves (8) of the plant while salad is prepared from its young shoots (9). All parts of the plant are associated with diverse pharmacological activities. The leaves are believed to possess diuretic, carminative, and antidiabetic properties and are used to cure diarrhea, cough, fever, hemorrhoids, and cystitis (10). A decoction of the leaves is useful as a gargle in treating thrush, mouth ulcers, and gum inflammations (8,11–12). The leaves are also used to treat skin and gastrointestinal tract infections and for healing of wounds (13–15). Leaves are used to manage digestive disorders of calves and piglets (16). A decoction of root bark is used in diarrhea and dysentery. The root bark and the leaves are strongly astringent, depurative, tonic, and vulnerary. The plant has reported antiinflammatory action and antiviral activity, and it forms part of a herbal deodorant composition against allyl methyl monosulfide (17–19). Various bioactive constituents, like triterpenes, sterols, glycosides, and anthocyanins (20–22), have been isolated from the plant. The plant also contains phytoestrogen, fatty acid, tocols, flavonoids, carotenoids, and rare earth elements (23–27).

Previously, psychoactive synthetic drugs were recognized as most effective in the management of central nervous system (CNS)-related disorders. However, their continuous and indiscriminate use has led to various side effects affecting the endocrine, autonomic, allergic, hematopoietic, and neurological systems of the human body. Thus, scientists are searching for new therapeutic agents with minimum side effects and maximum potency from medicinal plants, which are believed to be safe and cost-effective, and there is an increasing trend of screening botanicals for neuropharmacological effects. Neuropharmacological screening includes various activities like anxiolytic activity assessed by open field, head dip test, cage cross and rearing test, muscle relaxing activity via traction test, and antidepressant activity via forced swimming test. Anxiety affects one-eighth of the population worldwide and has become an important research area in the field of psychopharmacology (28). It is well known that the sedative effect of drugs on the CNS can be evaluated by the measurement of spontaneous motor activity in laboratory animal models.

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A literature survey about *R. fruticosus* confirmed that no CNS-related research work has been carried out. Therefore, in continuation of our research on Pakistani medicinal plants (29–31), we recently screened methanolic extracts of various parts of *R. fruticosus* for neuropharmacological activities.

2. Materials and methods

R. fruticosus L. fruit, leaves, root, and stem were collected from Lower Dir district, Khyber Pakhtunkhwa, Pakistan, and were identified by Prof Dr Mansoor Ahmad. A voucher specimen, number RIPS-0012, was deposited in the herbarium at the Research Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Karachi. The plant parts were chopped and dried in shade to prevent photochemical degradation and to avoid fungus growth. Dried plant parts were soaked in methanol for 15 days. The extracts obtained were filtered through filter paper under vacuum and concentrated under reduced pressure in a rotary evaporator (model Q-344B, Quimis) using a warm water bath (model Q-214M2, Quimis) to obtain a thick gummy mass, which was further dried in a desiccator and stored in a vial in a refrigerator at 4 °C until further use.

2.1. Animals used

Albino mice of either sex (25–30 g) were used in all experiments. Animals were purchased from HEJ Research Institute of Chemistry, University of Karachi. The animals were maintained in standard laboratory conditions (25 °C and light/dark cycles of 12/12 h) and fed with standard food and water ad libitum.

2.2. Assessment of neuropharmacological activity

Neuropharmacological activity was studied by open field test, traction test, head dip test, rearing test, and swimming induced depression test. All tests were performed in a calm and peaceful environment. For each test, animals were divided into 5 groups (i.e. Group A for control; Group B, Group C, and Group D for 100 mg/kg, 300 mg/kg, and 500 mg/kg oral doses of crude extract, respectively; and Group E for standard). Each group comprised 5 animals. Diazepam at 2 mg/kg was used as the standard. Standard drugs were also administered orally. The control animals were treated with the same volume of saline as the crude extract. The observations were made after 30 min of oral dose of the test substance.

2.3. Open field activity

The open field apparatus was designed in the laboratory and consisted of a 76 × 76 cm square area with opaque walls 42 cm high. The floor was divided by lines into 25 equal squares. The test was performed in a quiet room under white light as described earlier (32–34). Mice were taken out from their cages and placed in the center square of the open field (one at a time). The number of squares crossed with all 4 paws was counted for 30 min. Activities of control mice and drug-treated mice were monitored simultaneously. The apparatus was cleaned with 10% alcohol after mouse exposure.

2.4. Rearing test

A 1000-mL glass beaker lined with white paper on the bottom was used in this study. Upward movements of mice positioning the body in an erect position in the beaker were counted (27,28,31).

2.5. Head dip test

A specially designed square-shaped head dip box having 3 holes in each side was used in this study. The number of head dips by mice through these holes in a specified time (32–34) was counted. The control and drug-treated animals were placed individually in the head dip box and the observations were made for 30 min.

2.6. CNS depressant activity

CNS depressant activity was evaluated by the forced swimming test. All mice were first trained for swimming in a bath with dimensions (42 × 19 × 19 cm) as reported previously (34,35). Mice were placed individually for 6 min in a glass tub filled with water at room temperature (25 ± 2 °C) up to a marked level. When placed in water, mice suddenly start to move their front and hind paws. The activity time was determined with the help of stop watch out of a total observation time of 6 min. Mice were considered immobile when they ceased struggling and started making the minimum movements necessary to keep afloat. This is the most commonly used method to evaluate depression.

2.7. Traction test

This observation was made to determine the time taken by the animal to travel on an iron rod of 1 m in length. Mice were first trained to walk on the iron rod. Any increase or decrease in the time taken by the drug-treated animals from that of the control animals to travel the rod describes the sedative or stimulant activity of the drug, respectively (35–37).

2.8. Cage crossing movement

The test was performed with a specifically designed instrument having a rectangular shape. Both control and treated mice were placed in the cage and their cage crossing movements were noted over 30 min. This test was performed according to the method described previously (38,39).

2.9. Statistical analysis

The results are expressed as the mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used for comparison tests of significant differences among groups, followed by Dunnett's multiple comparison post-test.

3. Results

3.1. Anxiolytic effect

Anxiolytic effects of extracts were evaluated via open field, head dip test, cage cross, and rearing tests (Figure 1) and results were compared with the negative control group, i.e. mice without any treatment, and with the positive control group, i.e. mice given standard drugs of diazepam 2 mg/kg, imipramine 15 mg/kg, and caffeine 15 mg/kg.

Fruit extract increased the motor functions in the open field test and decreased them in the head dip test. For doses of 100, 300, and 500 mg/kg, the open field results in terms of readings were 474.2 ± 2.44, 404 ± 1.66, and 435 ± 1.94, while the head dip results were 34.8 ± 2.48, 26.6 ± 2.92, and 16.4 ± 2.28, respectively. The control showed 287 ± 2.62 for open field and 43.8 ± 3.90 for head dip. Leaf extract increased the motor functions in the open field test and decreased them in the head dip activity. For doses of 100, 300, and 500 mg/kg, open field results in terms of readings were 255.4 ± 2.74, 259.4 ± 1.29, and 306.2 ± 2.54, while head dip results were 11.8 ± 2.32, 17.6 ± 1.97, and 15.4 ± 2.19, respectively. Root extract increased the motor functions in the open field test and decreased them in head dip activity. For 100, 300, and 500 mg/kg of root extracts, open field results in terms of readings were 422.2 ± 0.58, 358.4 ± 2.21, and 306.6 ± 1.92, while head dip results were

14.6 ± 1.73, 9.6 ± 1.94, and 11 ± 1.79, respectively. Stem extract increased the motor functions in the open field test and decreased them in head dip activity. For doses of 100, 300, and 500 mg/kg, open field results in terms of readings were 293.8 ±1.47, 197.6 ± 1.51, and 186.8 ± 2.09, while head dip results were 33.2 ± 0.86, 19 ± 1.27, and 32.4 ± 1.21, respectively.

In the cage cross, the activity observed for the control group was 65.4 ± 4.13. For fruit-treated mice at doses of 100, 300, and 500 mg/kg, the means ± SEMs of crossing numbers were 65.8 ± 2.81, 70 ± 1.71, and 90.6 ± 1.64, respectively. Similarly for leaf extracts (44.6 ± 1.87, 62.6 ± 2.12, 71.4 ± 1.40), fruit extracts (56.4 ± 1.17, 52.2 ± 1.86, 46.2 ± 1.78), and stem extracts (65.8 ± 2.34, 50.2 ± 2.18, 82.2 ± 1.94), the means ± SEMs of crossings were found with the same dose pattern.

The exploratory rearing activity observed for the control group was 50.6 ± 1.53. For fruit-treated animals at doses of 100, 300, and 500 mg/kg, the means ± SEMs of crossing numbers were 34 ± 1.21, 46 ± 1.43, and 55.4 ± 1.61, respectively. Similarly for leaf (28 ± 1.51, 41 ± 1.11, 57.5 ± 0.81), fruit (23.5 ± 1.15, 33.8 ± 0.81, 18.6 ± 1.43), and stem (24 ± 1.42, 36.4 ± 1.13, 45.4 ± 0.83) extracts, the means ± SEMs of crossings were found with the same dose pattern as for fruit-treated animals.

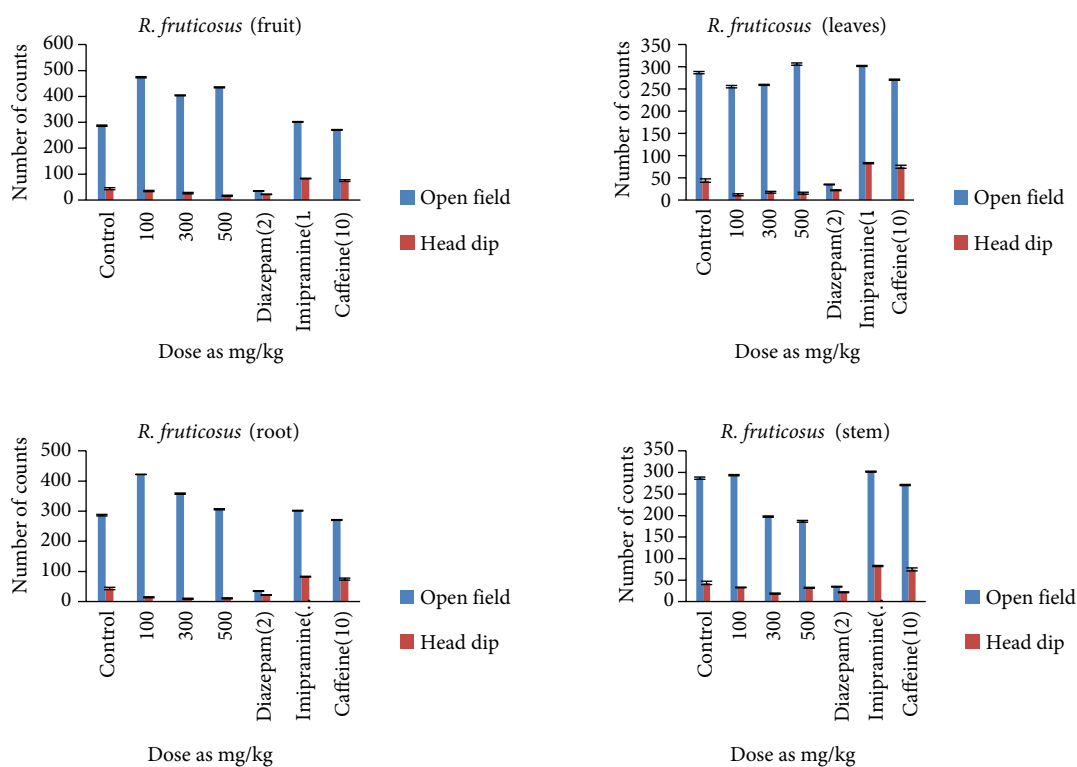**Figure 1.** Assessment of open field and head dip activity.

3.2. Muscle relaxant effects

The results of motor coordination activity in a traction test were noted for 3 h. The time for crossing the rod and the numbers of falls were observed and compared with the control and standard drugs. The data were represented ±SEM at P ≤ 0.05. No muscle relaxing effect was observed for any extract. Alertness was comparatively higher and so there was no decrease in the tone of muscle, as illustrated by the findings in the traction time test (Table 1).

3.3. CNS depressant activity

The immobility time was recorded in a forced swimming test. The mean observations ±SEM are presented in Table 2 at doses of 100, 300, and 500 mg/kg. The immobility time in minutes:seconds for the control group was 2:15 ± 0.059. Immobility time for fruit-treated groups of mice at doses of 100, 300, and 500 mg/kg were 4:46 ± 0.0323, 5:35 ± 0.062,

and 4:35 ± 0.0338. Similarly for leaf (3:33 ± 0.059, 3:38 ± 0.076, 3:56 ± 0.028), root (4:41 ± 0.0193, 3:53 ± 0.0341, 4:49 ± 0.2016), and stem (3:32 ± 0.0316, 3:57 ± 0.030, 2:57 ± 0.0353), the immobility times (mean ± SEM) were found for 100, 300, and 500 mg/kg doses, respectively. Increases in immobility times in this test indicate a decrease in swimming and struggling.

4. Discussion

Anxiety disorders are increasing day by day with modernization, and so people rely heavily on medications that help in reliving these anxieties. Mostly benzodiazepines are prescribed for anxiety disorders, but their use is limited due to clinically proven adverse effects, such as psychomotor impairment, potentiating other central depressant drugs, and dependence (40). Therefore, the search for new and

Table 1. Assessment of traction time.

Drug/dose	30 min	60 min	90 min	120 min	150 min	180 min
Control	9.8 ± 1.11	10.05 ± 1.12	9.8± 1.09	10.0 ± 2.08	10.02 ± 1.02	9.5 ± 0.89
Fruit						
100 mg/kg	9.2 ± 1.21	9.6 ± 0.61	9.7 ± 0.63	10.0 ± 0.55	9.8 ± 0.51	9.3 ± 0.33
300 mg/kg	8.5 ± 0.35	9.6 ± 1.20	9.4 ± 0.83	9.7 ± 0.51	9.7 ± 0.41	9.3 ± 0.21
500 mg/kg	8.2 ± 0.18	7.3 ± 1.03*	7.3 ± 0.53*	7.0 ± 0.57*	7.4 ± 0.21*	8 ± 0.27
Leaf						
100 mg/kg	9.4 ± 1.31	9.8 ± 0.71	9.9 ± 0.73	10.2 ± 0.65	10.0 ± 0.61	9.5 ± 0.43
300 mg/kg	8.7 ± 0.45	9.8 ± 1.30	9.6 ± 0.93	9.9 ± 0.61	9.9 ± 0.51	9.5 ± 0.31
500 mg/kg	8.7 ± 0.28	7.8 ± 1.13*	7.8 ± 0.63*	7.5 ± 0.67*	7.9 ± 0.31*	8.5 ± 0.37
Root						
100 mg/kg	9.3 ± 1.20	9.6 ± 0.63	9.8 ± 0.61	9.8 ± 0.54	10 ± 0.52	9.5 ± 0.30
300 mg/kg	9.00 ± 0.65	9.8 ± 1.70	9.6 ± 0.63	9.5 ± 0.31	9.8 ± 0.48	9.4 ± 0.71
500 mg/kg	8.8 ± 0.39	8.3 ± 1.23	9.0 ± 0.58	9.0 ± 0.77	9.4 ± 0.41	9.3 ± 0.29
Stem						
100 mg/kg	9.7 ± 1.22	10.0 ± 1.32	9.6 ± 1.29	9.8 ± 1.20	10.0 ± 1.42	9.2 ± 0.79
300 mg/kg	9.5 ± 1.33	9.9 ± 1.20	9.4 ± 1.25	9.6 ± 1.33	10.0 ± 1.66	9.5 ± 0.73
500 mg/kg	9.4 ± 1.27	9.7 ± 0.72	9.5 ± 0.29	9.3 ± 1.38	9.7 ± 0.40	9.2 ± 0.83
Diazepam						
300 mg/kg	12.2 ± 0.35*	14.4 ± 0.82*	17 ± 0.32**	17.7 ± 1.31**	18.2 ± 0.66**	15 ± 0.72**
Imipramine						
15 mg/kg	8.4 ± 1.23	9 ± 2.07	8.4 ± 1.22	9.2 ± 0.72	9 ± 0.53	8.5 ± 0.64

Mean ± SEM; n = 5; * = significant, ** = highly significant.

Table 2. Assessment of forced swimming test.

Extract/drug	Dose mg/kg	Immobility time
Control	0.5 mL saline	2.15 ± 0.059
Fruit	100 mg/kg	4.46 ± 0.0323**
	300 mg/kg	5.35 ± 0.062**
	500 mg/kg	4.35 ± 0.0338*
Leaf	100 mg/kg	3.33 ± 0.059
	300 mg/kg	3.38 ± 0.076
	500 mg/kg	3.56 ± 0.028*
Root	100 mg/kg	4.41 ± 0.0193**
	300 mg/kg	3.53 ± 0.0341*
	500 mg/kg	4.49 ± 0.2016**
Stem	100 mg/kg	3.32 ± 0.0316
	300 mg/kg	3.57 ± 0.030*
	500 mg/kg	2.57 ± 0.0353
Diazepam	2 mg/kg	4.05 ± 0.08**
Imipramine	15 mg/kg	2.15 ± 0.09

Mean ± SEM; n = 5; * = significant, ** = highly significant.

safe medications having anxiolytic properties that are free of the complications of benzodiazepines would be of great importance in the treatment of anxiety-related disorders. It is a common perception that plant extracts and natural products isolated from them are safe. *R. fruticosus* is an important component of herbal compositions that modulate cytokines to regulate inflammatory or immunomodulatory conditions, including Alzheimer's disease (41), but there are no research findings about its neuropharmacological effects. Therefore, methanolic extracts of various parts of *R. fruticosus* were screened for various neuropharmacological effects with the hope of finding safe and effective natural medicines.

After administration of extracts of *R. fruticosus*, an increase in exploratory functions was observed as compared to the negative control group. The anxiolytic effect was dose-dependent, i.e. it increased with increases of dose and decreased with decreases of dose. The exploratory activity increased as the dose of leaf extract increased in open field tests, as well as in head dip tests, in comparison with the control group. Root extract showed a decrease in the open field test as well as in the head dip test, indicating an anxiolytic effect. In the open field test, stem extract-
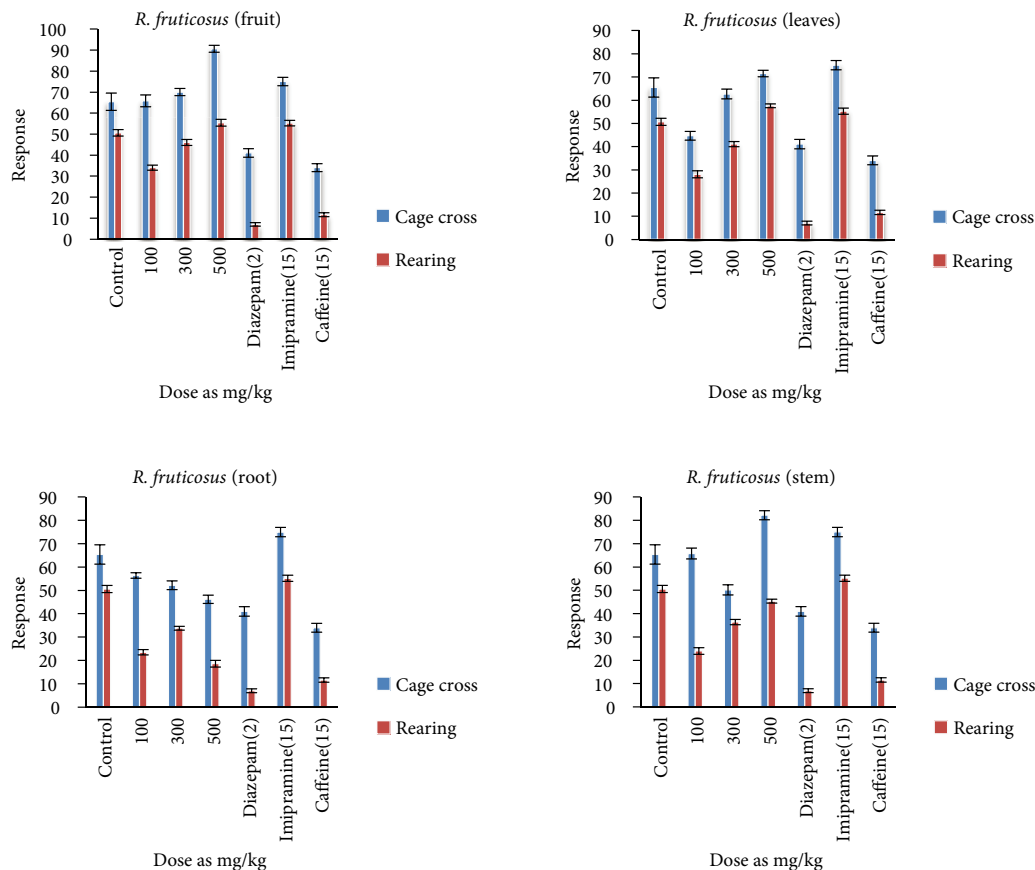

Figure 2. The effect of cage cross and rearing activity.

treated mice showed an almost normal response compared to the control at 100 mg/kg, but as the dose increased, the activity reduced (Figure 1). In cage cross activity there was a dose-dependent stimulatory effect, while in rearing the activity was lower at the minimum dose but comparable to normal at a maximum dose of 500 mg/kg, such that no sedative effect was observed. In the cage cross and rearing tests the activity was slightly reduced at 100 and 300 mg/kg but normal at 500 mg/kg. There was a slight calming effect in cage cross and rearing tests with an increase in dose (Figure 2). Locomotor activity is considered as an index of alertness and a decrease in it indicates a sedative effect.

The forced swimming test is frequently used for the assessment of antidepressant-like activity in animal models. The shortening of immobility duration indicates antidepressant activity in this model, while prolonged immobility duration reflects a CNS depression-like effect (42). No significant antidepressant effect was observed;

however, a dose-dependent CNS depression was observed in the forced swimming test. The order of CNS depressant effect for various parts was fruit > root > leaves > stem.

The animals remained healthy throughout all experiments; this might be due to the food nature of the extracts, which are a good source of various nutrients. All extracts at all doses were safe and mice showed symptoms of alertness followed by relaxation or decreased activity. The results indicate that the fruit extract may be further scrutinized for CNS depressant effect to explore its possible mechanisms of action. Preclinical studies should be performed on active principles for toxicological purposes and dose extrapolation for use in future clinical trials.

Acknowledgment

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