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Analgesic, Anti-inflammatory and Other Pharmacological Activities of Methanol Extract of *Rhododendron campanulatum* from Nepal

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AP, SP, SA and MPA designed the study. Authors SA and TMS collected plant aerial parts and designed the protocol. Authors AP, SP and SS performed the experiments, statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Rhododendron campanulatum* D. Don is a plant of Ericaceae family that is found at an altitude of 3000-4200 meters. Traditionally, it has been used to cure rheumatism, hemicranias, sciatica and colds. This study aims to determine analgesic and anti-inflammatory activities and effect on gastrointestinal motility, brain function and skeletal muscle of *Rhododendern campanulatum* extracts in mice.
Methodology: Pharmacological activities were evaluated in mice. Analgesic activity was determined by hot plate and chemical writhing methods; anti-inflammatory activity by ability to cure carrageenan induced paw edema; effect on gastrointestinal motility by charcoal meal assay, effect on brain function by locomotor activity and skeletal muscle relaxant activity by traction test.
Results: Methanol extract from *R. campanulatum* increased the response time of mice from 6 secs

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to 20 secs in hot plate assay and reduced the stretching episodes from 49 to 14 in acetic acid induced writhing tests revealing its analgesic activity. The extract at a dose of 200 mg kg^{-1} inhibited carrageenan induced paw edema by 66.66% and reduced the gastrointestinal motility to 51.66%. The methanol extract inhibited locomotor activity and grasping power of mice suggesting its central nervous system depressant and skeletal muscle relaxant activities respectively.

Conclusion: The present study shows that *Rhododendron campanulatum* has analgesic, anti-inflammatory, anti-motility, central nervous system depressant and muscle relaxant properties. These results suggest that *R. campanulatum* could be a source for potential drug formulation.

Keywords: Rhododendron campanulatum; analgesic; anti-inflammatory; gastrointestinal motility; locomotor activity; skeletal muscle relaxant.

1. INTRODUCTION

Plants have formed the source for traditional medicinal systems for thousands of years. Knowledge about the medicinal properties of herbs have passed down usually within a family from generation to generation orally and from society to society [1]. Hence, very few written documents are available. The earliest written document was the Pen Ts'ao by Shen Nung of China in 2800 BC [2]. Previous records show that 80% people of the developing countries rely on the traditional medicine which is mainly derived from plant and many pharmacopoeias contain at least 25% drugs derived from plants [3].

The demand for medicinal plants has been increasing in both developing and developed countries because of their efficacy, availability, less side-effect, safety and affordability as compared to allopathic drugs. Medicinal plants are sometimes the only source of health care that poor people can afford [3,4]. Although plants are sources for drug seeds, only few have been studied and characterized. Hence, it is important to study the potential sources of drug seeds and characterize their properties. In this regard, this study was performed on *Rhododendron campanulatum* D. Don (herbarium number: PLP7431) [5].

Pain is unpleasant sensory symptom due to some stimuli [6] and inflammation is protective reaction of the immune system to harmful stimuli [7]. Both of the experiences are bodily way of removing or reducing/restricting damage to body [6,7]. Usually, pain and inflammation are associated at the same time. Non-steroidal anti-inflammatory drugs are commonly used for the treatment of pain and inflammation [8]. Gastrointestinal tract contract and relax in order to ingest and digest food, and/or eliminate waste from the body. During some pathological conditions such as diarrhea and constipation, the gastrointestinal motility is increased and decreased respectively. Anti-diarrheal agents or purgatives are prescribed for the respective conditions. Similarly, skeletal muscle relaxants and central nervous system depressants are given for muscular spasm or pain and as sedative or tranquilizers respectively. No matter what drug is used for an ailment, some side effects are seen. Therefore, many people have been relying more on herbal plant based treatment.

R. campanulatum D. Don belongs to family Ericaceae and commonly known as Nilo Chimal (Nepal). It is found at an altitude of 3000-4200 meters. We previously tested the chemical constituents of the crude extracts and showed that the crude extracts from *R. campanulatum* possessed antibacterial activities against few tested Gram-positive bacteria [9]. Other studies on *R. campanulatum* have reported its antimicrobial activity against Gram-negative bacteria as well [10,11]. Further, we sought to test the effect of the extracts in animal model to reveal its biological properties. Traditionally, the leaves of *R. campanulatum* have been used in headache, chronic rheumatism and sciatica. They are mixed with tobacco and used as sniff to cure hemicranias and colds. The dried twigs and wood are used in the treatment of phthisis and chronic fevers [11-14]. There is no report on pharmacological activities of *R. campanulatum*. Hence, this study was performed to determine effects of *R. campanulatum* extract in response to pain and inflammation, and test its effect on gastrointestinal motility, brain function and skeletal muscle in mice.

2. MATERIALS AND METHODS

2.1 Sample Preparation

Sample was prepared as previously described [9]. Briefly, aerial parts of *R. campanulatum* was collected from Langtang, Nepal, shade dried and powdered. Twenty grams of the powder was

extracted with 200 ml of petroleum ether, diethyl ether, chloroform, and methanol each in a stepwise manner. Extracts were concentrated and stocked until used for experiments. Methanol extract was dissolved in distilled water and used for all the assays to evaluate biological activities.

2.2 Analgesic Activity

Albino mice were obtained from Department of Plant Resources, Thapathali, Kathmandu, Nepal. Analgesic activity on intact mice was carried out by hot plate and chemical writhing methods as described [15]. Aspirin 100 mg kg^{-1} was taken as a positive control.

2.2.1 Hot plate method

Mice (25-30 g; n=4) were injected 50 mg kg^{-1}, 100 mg kg^{-1} and 200 mg kg^{-1} methanol extracts, 100 mg kg^{-1} aspirin, and vehicle intraperitoneally (IP). After 60 minutes, animals were individually placed on a hot plate maintained at a temperature of 55±5℃. The response of animals, such as paw licking or jumping was taken as the end point and the response time was noted for each mouse. A cut off time of 15 seconds was allowed to avoid any thermal injury to the paws.

2.2.2 Chemical writhing method

Mice (25-30 g; n=4) were injected 25 mg kg^{-1}, 50 mg kg^{-1} and 100 mg kg^{-1} methanol extract, 100 mg kg^{-1} aspirin, and vehicle IP. After 30 minutes, 0.6% acetic acid was injected IP and total number of stretching episodes was recorded for a period of 20 minutes.

2.3 Anti-inflammatory Activity

Anti-inflammatory activity was assayed by measuring paw edema induced by carrageenan as described [16]. Mice (20-25 g; n=6) were injected 50 mg kg^{-1}, 100 mg kg^{-1}, 150 mg kg^{-1}, 200 mg kg^{-1} methanol extract, 100 mg kg^{-1} aspirin and vehicle IP. After one hour, the mice were injected 0.05 ml of 1% of carrageenan suspension into sub-planter region of the right hind paw. The volume of the paw of each mouse was measured before and three hours after the carrageenan injection by the help of plethysmograph.

2.4 Gastrointestinal Motility Test

Gastrointestinal motility was determined by movement of charcoal in the intestine according to standard method [17]. Mice (20-25 g; n=3) were injected 50 mg kg^{-1}, 100 mg kg^{-1}, 200 mg kg^{-1} methanol extract, and vehicle IP. After 30 minutes, they were fed with 1 ml of charcoal meal (animal charcoal 12 g, tragacanth 2 g, and water 130 ml). These mice were killed after 15 minutes by cervical dislocation. Their abdomen was quickly opened and instestine was isolated, the small intestine from pylorus to ileocaecal junction was cut and length was measured. The distance, which the charcoal meal travelled was measured.

2.5 Spontaneous Locomotor Activity

Spontaneous locomotor activity was measured by their ability to crawl in open field according to published literature [18]. Mice (25-30 g; n=3) were injected 50 mg kg^{-1}, 100 mg kg^{-1}, 200 mg kg^{-1} of methanol extract, and vehicle IP. Each mouse was placed in an open square field 50x50 cm surrounded and subdivided into 25 squares of 10x10 after 30 minutes of administration of extract. The number of squares crossed by each mouse over a period of three minutes was recorded.

2.6 Skeletal Muscle Relaxation Test

Traction test was performed to test the effect of methanol extract upon skeletal muscle relaxation as described previously [19]. Mice (25-30 g; n= 10) were injected 50 mg kg^{-1}, 100 mg kg^{-1}, 200 mg kg^{-1}methanol extract, and vehicle IP. After 30 minutes, mice were suspended by means of their forepaws to a metallic wire, stretched horizontally 20 cm above the floor. Normal mice grasped the wire with forepaws and when allowed to hang free, put at least one hind foot on the wire within five seconds. Inability to put up at least one hind foot constituted failure to the traction.

2.7 Statistical Analysis

The data were analyzed by two-tailed unpaired t-test using prism software. A *P* value of < .05 was considered to be significant [95% confidence interval for mean]

3. RESULTS AND DISCUSSION

3.1 Analgesic and Anti-inflammatory Activities

Evaluation of analgesic activity was carried out by two methods: hot plate and chemical writhing. Mice, when put on hot plate, show licking of paw,

jumping etc. reactions due to feeling of pain. The time taken by mice to show these reactions is a measure of pain, the longer time required for the reaction the stronger is the analgesic activity. Similarly, when pain is induced chemically, the number of episodes of stretching correlates to the pain and lower the number of stretches stronger is the analgesic activity. On the evaluation of analgesic activity, administration of methanol extracts showed significant analgesic activity in the hot plate with the increase in response time dose dependently (Fig. 1a). The response time increased from 6 secs of that of vehicle treated control to 11, 14.5 and 20 seconds for 50, 100 and 200 mg kg^{-1} methanol extract respectively. The activities were statistically significant (*P* value < .0001). Similarly, number of stretching episodes decreased in acetic acid induced writhing test by methanol extract in dose dependent manner (Fig. 1b). The number of writhing episodes decreased from 49 of control to 34, 24, and 14 of 25, 50 and 100 mg kg^{-1} methanol extract respectively. The analgesic activities from both experiments were comparable to that of 100 mg kg^{-1} aspirin. The activities were statistically significant (*P* value < .0001).

Hot plate assay indicates the centrally mediated ability by response to pain, relief from such pain indicates centrally mediated analgesic activity. Similarly, episodes of the writhing response are thought to involve, in part, local peritoneal receptors [20] which may lead to the suggestion that the extract could interfere with such peritoneal receptors to bring the observed analgesic effect. Hence, these results indicate that the methanol extract possesses centrally and peripherally mediated analgesic properties. The analgesic activity of *R. campanulatum* justifies its use in hemicrania and sciatica.

To determine the effect of methanol extract in inflammation, mice were injected with methanol extract followed by carrageenan in their paw to induce acute paw oedema. The volume of paw edema reduced by treatment with methanol extract in dose dependent manner with 27%, 38%, 50% and 66% inhibition by 50, 100, 150 and 200 mg kg^{-1} of extract respectively (Figure 2). The activities were statistically significant (*P* value < .0001). The results indicate that methanol extract possessed anti-inflammatory activity. Alpha and beta- amyrins and beta-linalool were found to be present in leaves of *R. campanulatum* [21] which are thought to be responsible for anti-inflammatory activity [22,23].

Taken together, the crude extract of *R. campanulatum* possessed analgesic and anti-inflammatory activities which justify its traditional use in hemicranias, sciatica and rheumatism.

3.2 Effect on Gastro Intestinal Motility

The movement of charcoal meal in intestine gives the measure of the gastrointestinal motility and property of drug to exert antidiarrheal activity can be assessed. We injected methanol extract of the plant and fed charcoal meal to mice. We excised intestine of mice and measured the distance travelled by charcoal. Methanol extract inhibited charcoal movement in intestine of mice in dose dependent manner. The extract at a dose of 100 and 200 mg kg^{-1} reduced the gastrointestinal motility in mice significantly with

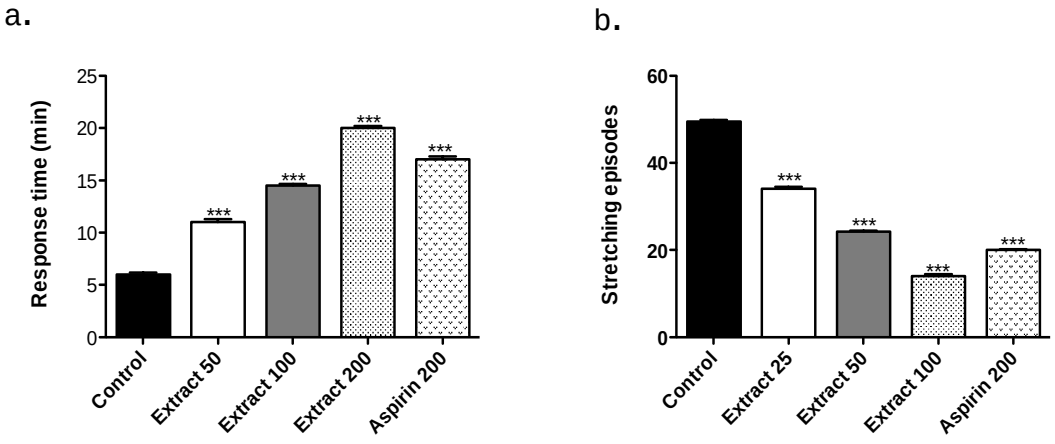

Fig. 1. Methanol extract of *R. campanulatum* exerted analgesic activity
a. hot plate method; b. chemical writhing method

P value of .01 and .001 respectively (Fig. 3). This activity indicates that it can be used to treat diarrhea as anti-motility agent. As methanol extract of *R. campanulatum* contains tannin [9], this may be responsible for anti-secretory activity to reduce gastrointestinal motility. However, a detailed study is required to get an insight of the responsible constituent (s).

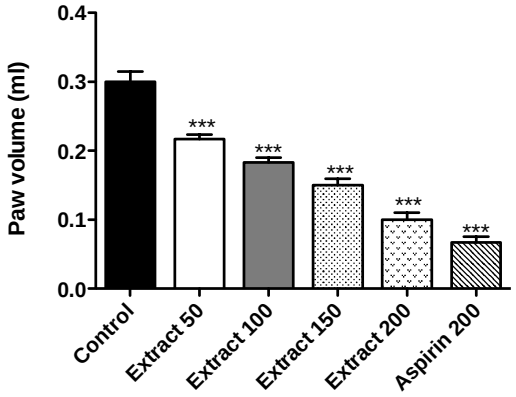

Fig. 2. *R. campanulatum* showed anti-inflammatory activity on carrageenan induced paw edema

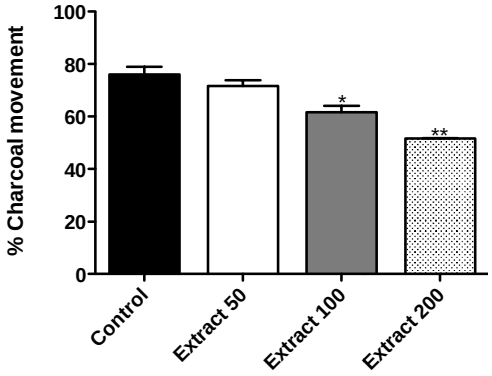

Fig. 3. Methanol extract of *R. campanulatum* decreased gastrointestinal motility
*(*P .01; **P .001)*

3.3 Effect on Spontaneous Locomotor Activity

Locomotion of mice in closed square fields give measure of the function of brain. When locomotion is retarded, it suggests that the brain function is depressed. Administration of methanol extract reduced the number of squares crossed by a mouse from 96 squares (control) to 60, 43, and 20 squares by 50, 100 and 200 mg kg^{-1} methanol extract respectively. The inhibition of locomotor activity was dose dependent with statistical significance (p value < .0001) (Fig. 4) indicating the central nervous system depressant property of extract. Further study to identify the responsible compound for this property is desired.

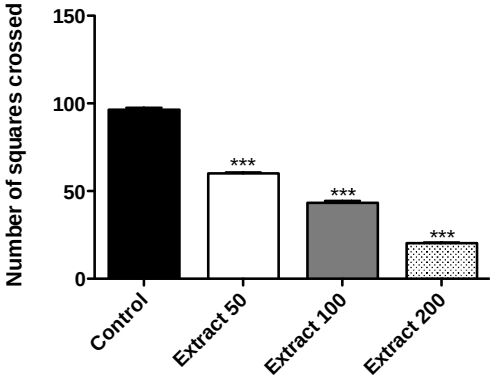

Fig. 4. *R. campanulatum* reduced spontaneous locomotor activity

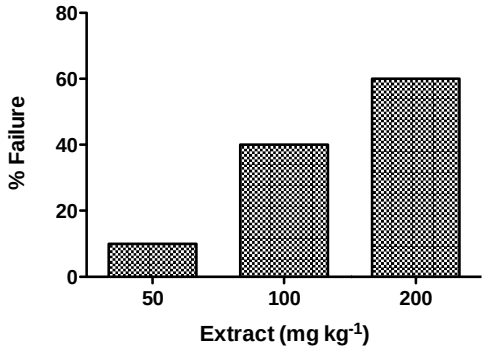

Fig. 5. *R. campanulatum* showed skeletal muscle relaxant effect on mice

3.4 Effect on Skeletal Muscle

Traction test evaluates the ability of muscle to grasp and it gives the idea of skeletal muscle relaxation effect. Administration of 50, 100 and 200 mg kg^{-1} ethanol extract led 10%, 40% and 60% mice to fail grasping. Thus, the grasping power was decreased in dose dependent manner indicating skeletal muscle relaxant property of the extract (Fig. 5). Further study is needed to determine the compound responsible for this activity.

4. CONCLUSION

This research work found out that the plant possessed significant analgesic, anti-

inflammatory, anti-motility, CNS depressant and skeletal muscle relaxant activities. The plant was found to possess analgesic and anti-inflammatory activities which justifies its traditional use in hemicranias, sciatica and rheumatism. It may, therefore, be concluded from this study, that the plant has medicinal values which may be helpful in generating the lead molecules for development of new drugs. This study, in addition, may assist in the establishment of scientific database of the plant that aids to improve knowledge on the activities and proper use of this plant. This is only a preliminary work and hence a detailed further investigation to identify and characterize the compounds responsible for the activities is desired.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All mouse protocols followed the regulations for animal care and use of the Tribhuvan University, Nepal. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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