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Preliminary Investigation of Pharmacological Activities of *Curcuma caesia* Methanol Extracts

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Abstract

Curcuma caesia (Kalo haledo) is the plant reported for its variety of ethnic medicinal value. To screen antimotility, CNS depressant, muscle relaxant and antimuscarinic activities of methanolic root and rhizome extracts of this plant. Antimotility, muscle relaxant along with CNS depressant and antimuscarinic activities were evaluated by charcoal meal test, spontaneous locomotory test and isolated tissue test respectively in animal model (mice and rat). Preliminary phytochemical studies revealed the presence of alkaloids, terpenoids, flavonoids, deoxysugars as well as C-glycosides. Methanolic extract at 200 mg/kg (*C.caesia*) i.p doses significantly ($P < 0.05$ versus control) reduced the motility of GIT which was found comparable to that of atropine at dose of 5 mg/kg i.p. Methanol extract inhibited locomotor activity of mice in a dose dependent manner indicating its CNS depressant property. Furthermore the extract significantly ($P < 0.05$ versus control) inhibited the acetylcholine induced contraction of ileum of rat in dose dependent manner indicating a possibility of muscarinic receptor blocking action of the extract.

Key-Words: *Curcuma caesia*, methanolic extract, antimotility, CNS depressant, muscle relaxant

Introduction

Plant resources are the main part of medicinal world. In Nepal, about 80% of the population still relies on herbal traditional medicines. Despite being major component of health care system, many traditional medicines have not been properly investigated or the findings have not been correlated with phytochemical and pharmacological studies. As a result, knowledge of their potential adverse effects is very much limited.[1]

Curcuma caesia is used as a carminative, and for the treatment of headaches, rheumatic pains as well as a pain reliever, an antifungal and an anthelmintic.[2] It is a traditional medicine used for the treatment of jaundice and liver ailments. Other traditional uses it affords are treatment of bloating, colds and chills, diarrhea, digestion, inflammation, menstruation problems, appetite and much more.[3] As long as 4,000 years ago, records from traditional healers of India and China mention its oral use as a remedy for many conditions, including eye infections, intestinal worms, leprosy and different skin diseases.[4] Fresh tubers of *Curcuma caesia* are pale yellow and aromatic and are used as cosmetics. They are used externally for sprains and bruises. They are also used as spices and coloring agents in food.[5]

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However, the safety, efficacy and adverse effects of *Curcuma caesia* have not been scientifically investigated and proved. There are only few research, investigations and studies carried out for *Curcuma caesia* as compared to other species of the same family e.g. *Curcuma longa* and thus, the formal and rationale use of *Curcuma caesia* has been missing.

This preliminary study aimed to investigate methanol extract of *Curcuma caesia* for its selected pharmacological activities (antimotility, CNS depressant, and muscle relaxant as well as anti-muscarinic) in animal model (mice and rat).

Material and Methods

Collection and Extraction of Plant Materials

The fresh plants were collected from Lalitpur District and were duly identified as *Curcuma caesia* in Department of Plant Resources (Banaspati Bibhag), Thapathali, Kathmandu. The plant materials were cut into pieces and were shade dried at room temperature. Dried sample was crushed into powder by electric blender and subjected to extraction by using Soxhlet apparatus. 40 grams of dried and powdered material was extracted separately and successively with 200 ml of methanol.

Pharmacological Studies

A. Anti-motility Activity

Charcoal meal test[6]

Charcoal meal test evaluates the efficacy of a test compound to either inhibit small intestinal propulsive motility or prevent inhibition of gastrointestinal propulsive motility. This test is based on the intestinal transport of charcoal meal along the small intestine.

Requirement: Mice (25-30g), charcoal meal, methanol extract, normal saline, syringes (1ml), feeding needle, scissor, and ruler.

Procedure: Mice were divided into four groups of three mice each. First group was given 100mg/kg of methanol extract made in normal saline solution by intra-peritoneal route. Second group was given 200 mg/kg of methanol extract; and third group was given 5mg/kg of atropine and only normal saline solution to the control group. After 30 minutes they were fed with 0.3ml of charcoal meal (animal charcoal 12g, tragacanth 2g, and water 130ml) by a feeding needle.

The mice were killed after 60 minutes by cervical dislocation. The abdomen was opened, the small intestine was quickly isolated and cut from pylorus to ileocaecal junction by a scissor and its length was measured by a ruler. The distance, which the charcoal meal traveled, was also measured and expressed as percentage of the total length of the small intestine by using the following formula:

$$\% \text{ Intestinal motility} = \frac{\text{Distance traveled by the charcoal meal}}{\text{Total length of the small intestine}} \times 100$$

B. CNS Depressant and Muscle Relaxant Activity

Spontaneous locomotor activity[7]

Most CNS acting drugs influence the locomotor activities in man and animals. The CNS depressant drugs reduce the motor activity while the stimulants increase the activity. The locomotor activity can be an index of wakefulness of mental activity. The test consists of counting, in a square open-field arena, the number of passages of the animal through the open-field.

Requirement: Mice (25-30g), open square field 50X50 sq. cm. (having 25 squares of 10X10 sq.cm. each), methanol extract, normal saline, syringes (1ml)

Procedure: The mice were divided into four groups, each group containing three mice. The first group was given 50mg/kg of methanol extract made in normal saline solution by intra-peritoneal route. Second group was given 100mg/kg and third group was given 200 mg/kg of methanol extract. The fourth group was the control group receiving only normal saline. After 60 minutes of administration of extract, each mouse was placed in an open square field 50X50 sq. cm surrounding and subdivided for swing into 25 squares of 10X10 sq. cm. The number of squares crossed by each mouse over a period of 3 minutes was recorded. The percentage inhibition was calculated as:

$$\% \text{ Inhibition} = 100 - \frac{\text{No. of squares covered in test}}{\text{No. of squares covered in control}} \times 100$$

C. Anti-muscarinic Activity

Test on isolated tissue[8]

Requirement: Rat (250-300g) intestine, methanol extract, kymograph equipment, tyrode solution, organ bath, syringes (1ml)

Procedure: 3 to 5cm long terminal portion of ileum of rat was taken from a freshly killed rat weighing 250 to 300g and suspended in aerated, tyrode solution maintained at 30-37°C in an isolated organ bath of 10 ml capacity. The preparation was set up under a tension of 1g and was allowed to equilibrate in the above solution for about 60 minutes before the experiment was started. Effects of standard dose of acetylcholine (0.2mcg/ml) and graded concentrations of the methanol extract on acetylcholine induced contraction of the tissue were then recorded on a smoked kymograph paper through an isotonic frontal writing lever.

Statistical analysis

The data were expressed as mean ± S.E. and compared with the control group.

Results and Discussion

A. Anti-motility Activity

Charcoal meal test

Methanol extract inhibited charcoal movement on intestine of mice in a dose dependent manner. The results are tabulated below in Table 1.

Table 1: Effect of methanol extract on charcoal meal movement

Treatment	Dose (mg/kg)	No. of animals used (n)	% charcoal movement ± SE
Control	-	3	97.87± 5.10
Extract	100	3	33.33± 3.51*
Extract	200	3	18.82± 4.09*
Atropine	5	3	20.45 ± 0.32*

*P<0.05 versus control

There was significant dose dependent reduction in propulsion of charcoal meal through the gastrointestinal tract by methanol extract as compared to the control group. The reduction of gastrointestinal motility by 200mg/kg i.p. was found to be comparable to that of the atropine at dose of 5mg/kg i.p. This activity indicates that it can be used to treat diarrhea as an antimotility agent. As methanol extract of *C. caesia* contains alkaloids and also anthraquinone glycoside is absent, these may be responsible for antisecretory activity to reduce gastrointestinal motility and to have an anti-diarrhoeal activity[9,10] justifying its use for the treatment of diarrhea as reported in various literatures.[3]

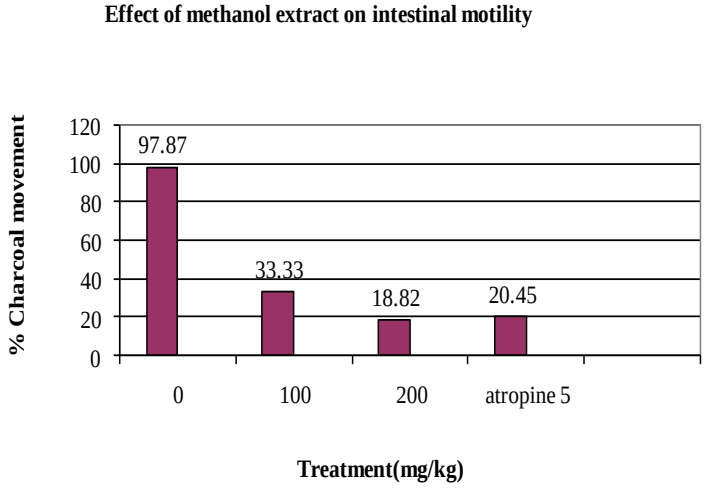

Figure 1: Effect of methanol extract on intestinal motility

B. CNS Depressant and Muscle Relaxant Activity

Locomotor activity

Methanol extract inhibited locomotor activity of mice in a dose dependent manner indicating its CNS depressant property. The results are tabulated below in Table 2.

Table 2: **Effect of methanol extract on locomotor activity**

Treatment	Dose (mg/kg)	No. of animals used (n)	Mean no. of square cross ± SE	% Inhibition
Control	-	3	88 ± 1.63	-
Extract	50	3	68 ± 1.56	22.72*
Extract	100	3	46 ± 0.89	47.72*
Extract	200	3	20 ± 0.92	77.27*

*P<0.05 versus control

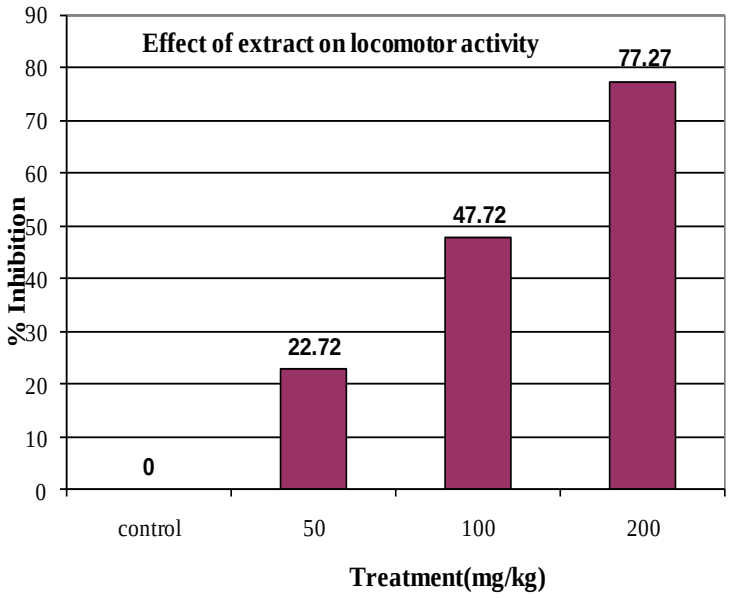

Figure 2: Effect of methanol extract on locomotion of mice

The methanol extract was found to inhibit the locomotor activity of mice in a dose dependent manner and this activity was significant as compared to the control. This result suggests that the extract possesses CNS depressant and muscle relaxant properties as revealed by locomotor activity test.

C. Anti-muscarinic Activity

Effect of methanol extract on ileum of rat

Methanol extract inhibited acetylcholine induced contraction of intestine of rat in a dose dependent manner. The inhibition was reversible type. The results are tabulated below in Table 3.The methanol extract significantly inhibited the acetylcholine induced contraction of ileum of a rat in a dose dependent manner indicating a possibility of muscarinic receptor blocking action of the extract.The plant was found to possess antimuscarinic activity which justifies its traditional use in diarhhoea[3].

Table 3: Effect of methanol extract on acetylcholine (0.2mcg/ml) induced contraction of ileum of rat

Treatment	Dose (µg)	No. of animals used (n)	% inhibition ± SE
Extract	10µg	3	20.85± 0.37*
Extract	20µg	3	41.55± 0.19*
Extract	40 µg	3	61.03± 0.10*
Extract	80 µg	3	93.56± 0.08*

*P<0.05 versus control

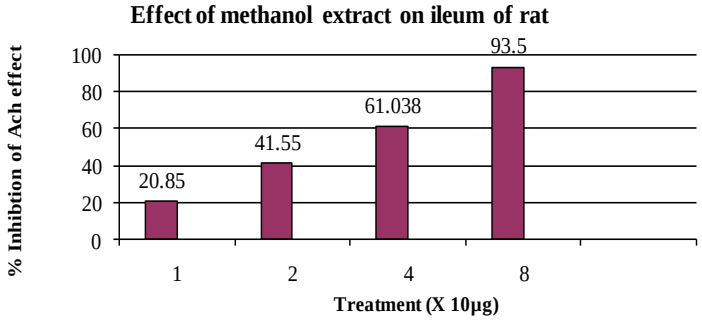

Figure 3: Effect of methanol extract on ileum of rat

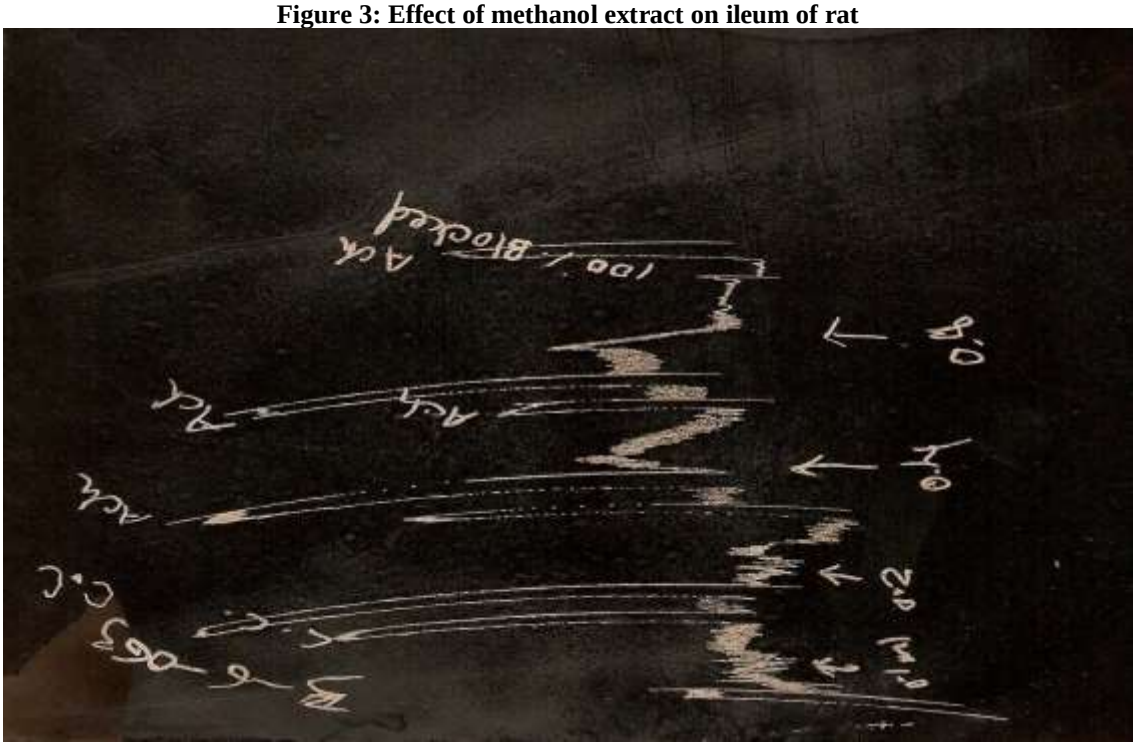

Figure 4: Effect of methanol extract on ileum of rat showing inhibition of acetylcholine induced contraction in a dose dependent manner

Abbreviations: *C.c. Curcuma caesia;* Ach. Acetylcholine

Conclusion

The preliminary pharmacological investigation of methanolic extract (of plant, *Curcuma caesia*) on mice and rat revealed its significant anti-motility, anti-muscaranic, muscle relaxant and CNS depressant activities. A detailed phytochemical and biological study for isolation, purification, identification, and characterization of the compound with exact mechanism of action responsible for the particular biological activity is highly recommended to find out a new molecule which may be developed as a drug.

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