

ORIGINAL RESEARCH



LAXATIVE ACTIVITY OF *CLEDRODENDRUM SERRATUM* AND *ORYZA SATIVA* IN COMBINATION

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ABSTRACT:

Constipation refers to bowel movements that are infrequent or hard to pass and severe constipation can progress to bowel obstruction and become life threatening. Laxatives are agents that increase the movement of feces along the colon and facilitate bowel movement. The present study was carried out to evaluate the laxative effect of *Cledrodendrum serratum* leaves and *Oryza sativa* root individually and in combination using mouse models. Methanolic extract of *Cledrodendrum serratum* and root of *Oryza sativa* at the doses 50, 100 and 200 mg/kg was tested on experimental animals individually and in combination. Results showed that all the doses administered increased the total number of fecal pellets in a dose dependent manner showing significant laxative properties. For the first time, we report the laxative activity of leaves of *Cledrodendrum serratum* and root of *Oryza sativa* which might be a potential natural laxative with good safety profile.

KEY WORDS: *Cledrodendrum serratum*, *Oryza sativa*, Constipation, Laxative activity.

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INTRODUCTION:

Plant and plant products are being used as a source of medicine for a long time in human history. According to world health organization, 80% of world population in developed countries depends on traditional medicines for their primary healthcare. Medicinal plants can be significant source of undiscovered chemical substance with potential therapeutic effects. Constipation is a common, often chronic gastrointestinal condition in which a person has fewer than three bowel movement a week or has a bowel movement with stool that are hard, dry and small making them painful or difficult to pass and which affect the quality of life^{2,3}. *Cledrodendrum serratum* Linn. Belonging to family Verbenaceae is a shrub widely distributed in tropical and subtropical region of the world. It is commonly known as Bharangi in Sanskrit, Blue glory in English. Medicinal importance of plant reported in various indigenous system of medicines such as Ayurveda, Unani and Siddha for the treatment of various life threatening diseases⁴. *Oryza sativa* belonging to the family Gramineae is the second most important cereal crop and staple food for more than half of world's population. Health benefits of rice include providing fast and instant energy, good bowel movement, stabilizing blood sugar level and providing essential source of Irregular meal Vitamin B1 to human body. Literature survey revealed that the plant extract has not yet been screened for its traditional laxative effect in experimental animals. Therefore, the present study was carried out to provide pharmacological evidence for the medicinal consideration of leaves extract of *Cledrodendrum serratum* and root extract of *Oryza sativa* as laxative.

MATERIAL AND METHODS:**Plant material**

The leaves of *C. serratum* were collected from forest of Khed district Ratnagiri in the month of August and the root of *O. sativa* were collected from Goregaon district Raigad in the month of July. Both drug sample submitted for authentication to St. Xavier's College, Mumbai and authenticated by Dr. Rajendra D. Shinde, Botanist, Associate Professor at St. Xavier's College, Mumbai-400001. The Specimen matches with Blatter Herbarium specimen number 11824 of H. Santapu & K. V. S.4917 of K. V. Shenoy respectively for *C. serratum* & *O. sativa*.

Preparation of extract

Methanolic leaf extract of *Cledrodendrum serratum* was extracted by the Soxhlet apparatus by continuous cycle collection of the extract. The leaves of the plant were washed and dried at room temperature and crushed by the mechanical grinder to fine powder. The powder (500 gm) was then extracted with 2.5 litre of 90% methanol in a Soxhlet apparatus at 65°C, until the powder became exhausted totally. The resulting extract was filtered & dried. The extract was stored in a desiccator for administration orally to mice in three increasing graded dose⁶.

Aqueous suspension of root extract of *Oryza sativa* was made in 2% CMC. The root of plant were washed and dried in sun and crushed by the mechanical grinder. Freshly prepared suspension administration orally to mice in three increasing graded dose.

Animals

The animals used for experiments were healthy adult female Swiss-Albino mice 25-30 g were procured from Bharat Serum and Vaccines Pvt. Ltd. Thane, Mumbai. The animals were group-housed in standard polypropylene cages (6 mice/cages) under good hygienic condition in the registered animal house 1464/PO/a/CPCSEA dated 25/05/2011 and maintained under controlled room temperature (22±2°) and humidity (55±5%) with 12-h light-dark cycle (Light ON from 7:00 am to 7:00 pm), with food and water available *ad libitum*. All animals experiments were conducted in accordance with the Council of IAEC Efforts were made to minimize animal suffering and to use only the numbers of animals necessary to produce reliable scientific data. The Institutional animal ethics committee India approved the experimental protocols (Proposal No: OCP/IAEC/2015-2016/05).

LAXATIVE ACTIVITY^{7,8}

Mice were fasted for 12 hours. Fasted mice were placed individually in cages. Mice were divided into five groups with the first group acting as a control and administered distilled water that act as negative control. The second group were received sodium picosulphate / Dulcolax this were serve as positive control. The Third group (A) were received extract of *Cledrodendrum serratum*, Fourth group (B) were received extract of *Oryza*

sativa & Fifth group (C) were received combination extract of *Cledrodendrum serratum* & *Oryza sativa*. Immediately after dosing, the animals were examined for laxation for 5h with the withdrawal of food and water. The animals were kept for washing for a period of 10 days & that they were reused for Charcoal meal gut transit test.

CHARCOAL MEAL GUT TRANSIT TEST^{9,10}

18 hr. prior to the experimentation food was withdrawn but not water. The drugs were administered 60 min before administration of charcoal meal. All the animals were sacrificed after various time interval i.e. nine animals after 60 min, nine animals after 120 min in test groups and three animals after 60 min and three animals after 120 min in standard and control groups. Entire intestine were removed immediately and immersed in 5% formalin. Then distance between pylorus region and the front of charcoal meal were measured and evaluated by student t-test.

STATISTICAL ANALYSIS

All the data expressed are Mean \pm Standard Error of Mean (S.E.M.) with 95% confidence intervals.

One way analysis of variance (ANOVA) followed by Dunnett's test was used to assess the laxative activity & Charcoal meal transit test. All the graphs, calculations and statistical analysis were performed using Graph Pad Prism 6 for windows (Graph Pad Software, San Diego, California, USA).

RESULT:

Effect on extracts on laxative activity

The methanolic extract of *Cledrodendrum serratum* (A), aqueous extract of *Oryza sativa* (B) and combination of both extract (C) was studied for its laxative activity in mice. The laxative activity was assessed by measuring the wet faeces in all test groups. The statistical significance of the difference between the data of test groups was calculated and values are presented in table 1. Extract A, B and C showed increase in fecal output in mice when compared to control group. The effect of these three extracts at a dose of 100, 200 and 400 mg/kg increased significantly fecal output of mice as compared to control group. The effect of combination extract of *Cledrodendrum serratum* & *Oryza sativa* (C) at dose 400mg/kg was comparable to that of standard drug dulcolax.

Table 1: Faeces output in mice

Sr no.	Groups	Drug Treatment	Faeces out put
1.	Control	Distilled Water (2ml/kg)	3.42 \pm 0.026
2.	Standard	Dulcolax (5mg/kg)	12.00 \pm 0.011***
3.	A 1	E. Cs (50mg/kg)	10.57 \pm 0.013***
4.	A 2	E. Cs (100mg/kg)	10.77 \pm 0.013***
5.	A 3	E. Cs (200mg/kg)	11.88 \pm 0.015***
6.	B 1	E. Os (50mg/kg)	10.56 \pm 0.013***
7.	B 2	E. Os (100mg/kg)	10.78 \pm 0.016**
8.	B 3	E. Os (200mg/kg)	11.78 \pm 0.014***
9.	C 1	Combination E. Cs + E. Os (50mg/kg)	10.89 \pm 0.013**
10.	C 2	Combination E. Cs + E. Os (100mg/kg)	10.87 \pm 0.013***
11.	C 3	Combination E. Cs + E. Os (200mg/kg)	11.90 \pm 0.015***

Values are in Mean \pm SEM (n=6); *P<0.05, **P<0.01, ***P<0.001 Vs Control

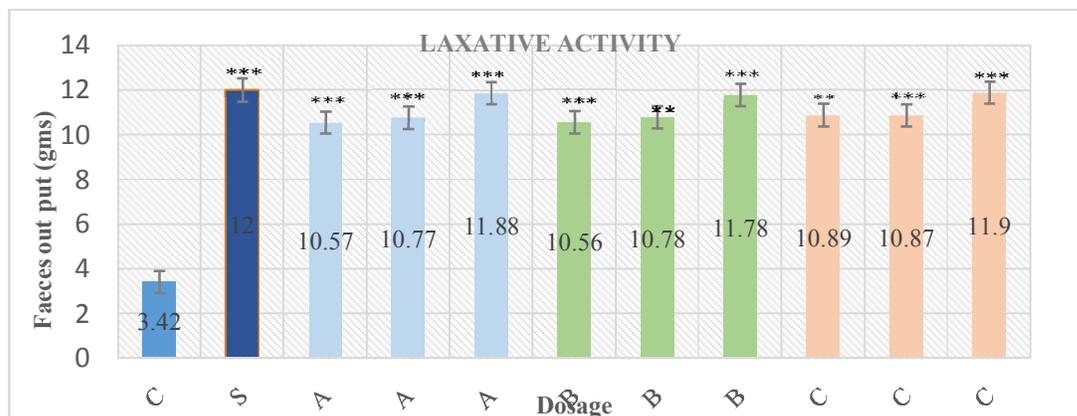


Fig 1: Laxative Activity in Mice

Bar diagram showing the Dose dependent effect of E. Cs, E.Os, Combination of E.Cs+E.Os on the faeces output in mice. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ show a comparison of group 2,3,4,5,6,7,8,9,10,11 vs group 1 (One way ANOVA followed by Dunnett's test). Each bar shown represents mean \pm SEM of 6 animals per group.

Effect on extracts on gastro intestinal motility

The passage of charcoal meal through the gastrointestinal tract in mice is used as parameter for intestinal motility and to study the effect of laxatives. The effect of methanolic extract of *Cledrodendrum serratum* (A), aqueous extract of *Oryza sativa* (B) and combination of both extract (C) on gastrointestinal motility was tested on mice as per the protocol motioned in materials and

methods. The distance travelled by charcoal meal from the pylorus to the caecum was measured, and expressed as the percent of the total length of the distance. The dose of 400mg/kg combination extract of methanolic extract of *Cledrodendrum serratum* and *Oryza sativa* (C₃) significantly increased in the propulsion of charcoal meal as compared to control group. The result of motility test is reported in table 2.

Table 2: Gut motility in mice

Groups	Drug Treatment	Charcoal Dose	% length of charcoal moved		P Value
			60 min	120 min	
1. Control	Distilled Water (2ml/kg)	0.2ml	14 \pm 0.00	15 \pm 0.00	
2. Standard	Dulcolax (5mg/kg)	0.2ml	86.33 \pm 0.88	95.33 \pm 0.33	0.0001***
3. A 1	E. Cs (50mg/kg)	0.2ml	80.67 \pm 0.66	85.00 \pm 0.57	0.0080**
4. A 2	E. Cs (100mg/kg)	0.2ml	82.33 \pm 1.20	89.00 \pm 0.57	0.0075**
5. A 3	E. Cs (200mg/kg)	0.2ml	84 \pm 0.577	94.00 \pm 0.57	0.0003***
6. B 1	E. Os (50mg/kg)	0.2ml	78.67 \pm 0.33	87.33 \pm 0.66	0.0003***
7. B 2	E. Os (100mg/kg)	0.2ml	81.33 \pm 0.66	86.76 \pm 0.88	0.0085**
8. B 3	E. Os (200mg/kg)	0.2ml	82.67 \pm 0.88	92.33 \pm 0.66	0.0014**
9. C 1	E. Cs+E. Os(50mg/kg)	0.2ml	82.33 \pm 0.33	88.00 \pm 1.00	0.0058**
10. C 2	E. Cs+E. Os(100mg/kg)	0.2ml	84.33 \pm 0.88	90.33 \pm 0.88	0.0086**
11. C 3	E. Cs+E. Os(200mg/kg)	0.2ml	85.33 \pm 0.33	94.33 \pm 0.66	0.0003***

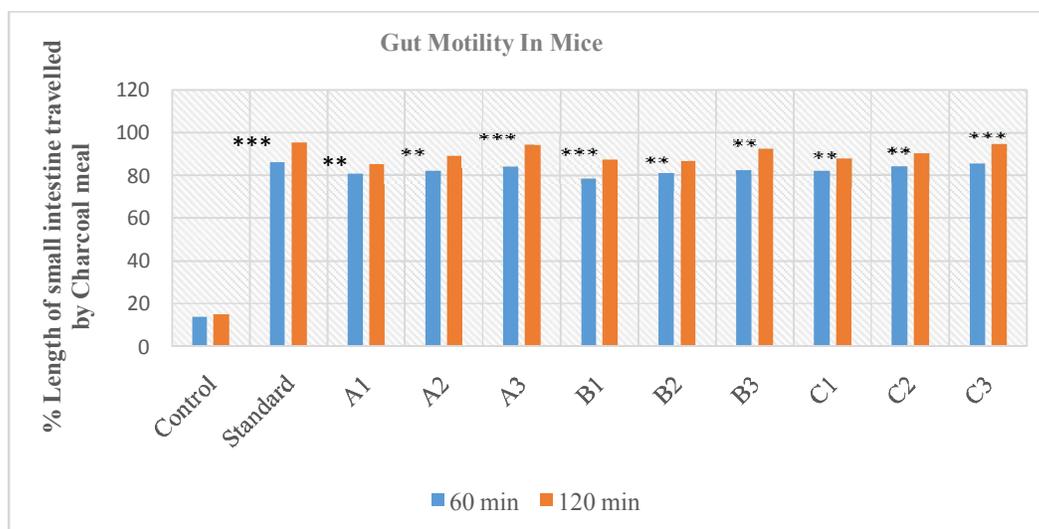


Fig 2: Percentage Length of Small Intestine Travelled By Charcoal Meal

Bar diagram showing the Dose dependent effect of E. Cs , E.Os, Combination of E.Cs+E.Os on the travel of charcoal meal through small intestine of mice. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ show a comparison of group 2,3,4,5,6,7,8,9,10,11 vs group 1(One way ANOVA followed by Dunnett's test). Each bar shown represents mean \pm SEM of 6 animals per group

DISCUSSION:

The laxative activity of *Cledrodendrum serratum* & *Oryza sativa* was studied in mice. The result showed that an oral administration of the leaves extract of *Cledrodendrum serratum* & root extract of *Oryza sativa* produced significant & dose dependant increase in stool count in mice, it also shows dose dependant effect through distance travelled by charcoal meal. Bisacodyl is widely used stimulant laxative in the management of constipation. The therapeutic effect of bisacodyl is believed to be due to its motility and secretory properties¹¹. *Cledrodendrum serratum* shows presence of Anthraquinone compounds which are famous for their laxative property. Their laxative effects are caused by two independent mechanisms:

1. Changing in colonic motility which leads to accelerated large intestinal transit
2. Alteration in colonic absorption and Secretion, resulting in fluid accumulation which cause diarrhea.

Oryza sativa contain insoluble fiber that act like a soft sponge that may be pushed through intestinal tract quickly and easily. The result suggest that the methanolic extract of *Cledrodendrum serratum* & aqueous extract of *Oryza sativa* individually and

with combination produced laxative activity by reducing the water reabsorption in the colon which might soften the stool. Combination extract is more significant as compare to individual extract that is due to different mechanism of two different plants i.e. *Cledrodendrum serratum* & *Oryza sativa* which act by their different mechanism depending on the phytochemicals present in them.

CONCLUSION:

From the result it could be concluded that oral administration of methanolic extract of *Cledrodendrum serratum* & aqueous extract of *Oryza sativa* show significant laxative activity in mice through faeces output and percentage length of small intestine travelled by charcoal meal. It is also concluded that combination of both extract (methanolic extract of *Cledrodendrum serratum* & aqueous extract of *Oryza sativa*) shows more faeces output and percentage length of small intestine travelled by charcoal meal than individual extracts.

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CONFLICT OF INTEREST REPORTED: NIL; SOURCE OF FUNDING: NONE REPORTED