



Short Communication

Evaluation of acute toxicity and intestinal transit time of *Olax scandens* Roxb. leaves

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Abstract

Introduction: *Olax scandens* Roxb. is a shrub or small tree found throughout tropical India. Fruits and leaves of this plant are used for medicinal and food purpose. Traditionally, leaves of *O. scandens* are used as vegetable in constipation. **Aim:** To evaluate the acute toxicity and intestinal transit time of *O. scandens* leaves on experimental animals. **Materials and Methods:** Acute oral toxicity study for sample was carried out following OECD guidelines. Evaluation of intestinal transit time was carried out in the dose of 1300 mg/kg by adopting Kaolin expulsion test and latency of the onset of kaolin expulsion in fecal matter in mice. **Results:** The results show that the test drug is not likely to produce any toxicity in higher dose. In kaolin expulsion test, the drug produced mild increase in intestinal motility in mice proved by fast clearance of kaolin pellet in comparison to control group. **Conclusion:** The leaves of *O. scandens* are safe at higher dose and showed mild laxative activity in the dose of 1300 mg/kg body weight of mice.

Key words: Acute toxicity, *Badru*, constipation, intestinal transit, *Olax scandens*

Introduction

India has a tribal population of 42 million, of which some 60% live in forest areas and depend on forest for various edible products.^[1] The evidence of man's dependency on plants for his survival can be demonstrated by palaeoethnobotanical demonstrations from prehistoric archaeological sites.^[2] Wild food plants found all over the world, represent inexpensive, locally available nutrition health quality. *Olax scandens* Roxb. (*Olacaceae*), an ethno medicinal plant, commonly known as "*Badru*", used for food and medicinal purposes.^[3] Tender leaves are taken as vegetable in constipation.^[4] Fresh young leaves chewed in mouth ulcer.^[5] Fomentation of boiled leaves are applied externally in headache.^[6] Though the plant has been widely used by the traditional healers, till now it has not been scientifically evaluated for its pharmacological studies. Scientific validation of such biological resources through experimental and clinical studies is need of the time. Hence, the present study was undertaken to evaluate the acute toxicity and intestinal transit time of *O. scandens* leaves.

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Materials and Methods

Test drug

Leaves of *O. scandens* were collected from its natural habitat, Balangir district, Odisha [Figure 1]. A sample specimen was preserved in Pharmacognosy laboratory of Institute for Postgraduate Teaching and Research in Ayurveda, Jamnagar (Specimen No - PHM 6062/21/09/2012), for future references. Collected leaves were washed, shade dried, powdered, sieved through mesh no. #100 and stored in air tight bottle.

Animals

Wistar strain albino rats and Swiss albino mice were used for the experiments. Animals were housed in each cage made up of polypropylene with stainless steel top grill.

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The dry wheat (posthulled) waste was used as bedding material and was changed every morning. The selected animals were kept under acclimatization for 1 week before experimentation.

The animals were exposed to 12 h light and 12 h dark cycle with the relative humidity of 50–70% and the ambient temperature was $22 \pm 03^\circ\text{C}$. Animals were fed with Amrut brand rat pellet feed supplied by Pranav Agro Mills Pvt. Limited. The drinking water was given *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC/13/2013/08).

Dose fixation

The general dose of *Churna* (powder) for the purpose of *Anulomana* in human is one *Karsha* according to Sharangadhara,^[7] which is about 10 g. Considering this, the dose for animal experimentation was calculated by extrapolating the human dose to animal dose based on the body surface area ratio following the table of Paget and Barnes, 1964.^[8]

The dose for mice for experimental study was as follows:

= Therapeutic human dose \times body surface area ratio (convertibility factor) for 20 g mice

= $10 \text{ g} \times 0.0026 = 26 \text{ mg}/20 \text{ g mice} = 1300 \text{ mg}/\text{kg}$ body weight of mice

The test drug was suspended in distilled water and administered in above mentioned doses by oral route with the help of an oral feeding cannula.

Acute toxicity study

Wistar strain albino rats weighing $200 \pm 20 \text{ g}$ of either sex were used for the evaluation of acute toxicity test. Acute oral toxicity study for sample was carried out following OECD guideline 425 with 2000 mg/kg as limit test.

Intestinal transit time (kaolin expulsion test)

The selected Swiss albino mice were divided into two groups of six each comprising three male and three females as follows:

- Group I - Normal Control received distilled water (10 ml/kg, po)
- Group II - *O. scandens* leaves powder (1300 mg/kg, po).



Figure 1: *Olax scandens* Roxb. plant in natural habitat

Leaf powder of *O. scandens* and vehicle (distilled water) were administered to overnight fasted animals. The effect of the test drug on intestinal transit time was carried out based on previous study.^[9] In short, 1 h after drug administration, 0.1 ml of 40% kaolin (Sigma-Aldrich, India) solution was administered with the help of oral catheter. The animals were placed in a transparent arena and were carefully observed for the beginning of the kaolin expulsion, which begins in the form of white colored fecal pellets.

Statistical analysis

The obtained data has been presented as mean \pm standard error mean, difference between the groups, statistically determined by Student's *t*-test for paired and unpaired data to assess the statistical significance between the groups. The value $P < 0.05$ is considered as statistically significant.

Results

No mortality was observed during the course of study (14 days) in drug treated group at dose level of 2000 mg/kg. Gross behavior of all the animals was found to be normal during the period of study. Test drug produced mild increase in kaolin expulsion time in mice when compared to control group [Table 1].

Discussion

No mortality was observed during the course of study (14 days). Gross behavior of all the animals was found to be normal during the period of study. From the above, it can be inferred that the LD_{50} value is much higher than 2000 mg/kg body weight in the rat. This level of LD_{50} value indicates that the test drug is not likely to produce drastic degenerative changes at the therapeutic doses administered in clinical conditions.

To assess the action of test drug on the intestinal motility, latency of onset of kaolin expulsion in fecal matter was selected as a parameter. It is well known fact that it is not an easy task to prove the *Anulomana* action of a drug in experimental animal model because of its broader meaning. It may be the reason why attempts were not made earlier. As explained by Acharya Sharangadhara, *Anulomana* is to break the bonds between *Mala* (fecal matter) and intestinal mucosa by completing the digestion process and brings quick excretion of flatus and fecal matter. In quick excretion, there is all the chances of increase in intestinal motility.^[10]

Kaolin was administered 1 h after drug administration to avoid possible interaction with test drug. While observing for the expulsion of pellets by keeping the animals in transparent arena, they were carefully observed for apparent toxic symptoms if any due to kaolin as well as symptoms of *Virechana* such as uneasiness, which may likely to be produced by test drug. Administration of leaf powder of *O. scandens* produced mild

Table 1: Effect of test drugs on intestinal transit time in mice

Groups	Kaolin pellet expulsion time (min)	Percentage change
Control	251.83 \pm 10.74	-
<i>Olax scandens</i>	245.50 \pm 09.22	2.52 \downarrow

Data: Mean \pm SEM, \downarrow : Decrease, SEM: Standard error of mean

increase in intestinal motility in mice proved by fast clearance of kaolin pellet in comparison to control group.

The mechanism of observed effect may be due to the interference with local stimulant effect on motility or acceleration of gastric emptying. The neural regulation of gastric motility involves stimulation by cholinergic neurons inhibition by adrenergic neurons. Antagonist of D2 and 5-HT₃ receptors as well as agonists of 5-HT₄ receptors can stimulate gastric motility.^[11,12] Some of the drugs increase the motility of intestine by modifying the fluid dynamics of the mucosal wall and may cause fluid accumulation in lumen.

In the light of the above, it can be suggested that the test drug may enhance the intestinal motility by cholinergic stimulation or stimulation of 5-HT₄ receptors; it is also possible that it may be antagonizing the effect of sympathetic system. Another probable mechanism is stimulation of the enteric nervous system. It may not be affecting the fluid dynamics because the test drug did not change the consistency of the expelled fecal matter to significant extent.^[13]

Conclusion

O. scandens at the dose of 2000 mg/kg in acute oral toxicity did not produce any mortality or adverse effects; hence drugs are classified as safe up to dose of 2000 mg/kg in rats. The drug mildly increases the intestinal motility in mice, which suggest the evacuation of gastrointestinal tract in the presence of *O. scandens*.

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Conflicts of interest

There are no conflicts of interest.

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हिन्दी सारांश

ओलेक्स स्केंडेन्स के पत्र चूर्ण की विषाक्तता और आंत्र पारगमन समय का मूल्यांकन

राघवेन्द्र नाईक, स्नेहा डी. बोरकर, रबिनारायण आचार्य, मुकेश बी. नारिया

ओलेक्स स्केंडेन्स रोकस्ब. समस्त भारत में पाये जाने वाला एक गुल्म है। इसके पत्तों एवं फलों का उपयोग औषधि और आहार हेतु किया जाता है। परंपरागत रूप से ओ. स्केंडेन्स का उपयोग कब्ज में सब्जी के रूप में किया जा रहा है। प्रस्तुत अध्ययन में ओ. स्केंडेन्स के पत्तों की तीव्र विषाक्तता का मूल्यांकन ओ.ई.सी.डी. निर्देशानुसार और आंत्र पारगमन समय का मूल्यांकन चूहों में ९३०० मिग्रा/किग्रा की मात्रा में केओलिन निष्कासन परीक्षण द्वारा किया गया। परिणाम से यह सिद्ध हुआ कि उच्चतम मात्रा में दवा का उपयोग करने पर किसी भी विषाक्तता के लक्षण नहीं पाया गया। केओलिन निष्कासन परीक्षण द्वारा आंत्र पारगमन समय में चूहों के आंत्रों की गतिशीलता में नियंत्रित समूह की अपेक्षा दवा द्वारा अल्प रूप से वृद्धि पायी गयी। ओ. स्केंडेन्स के पत्रों का उपयोग अधिक सुरक्षित पाया गया और ९३०० मिग्रा/किग्रा शारीरिक मात्रा पर चूहों में अल्प अनुलोमन गतिविधि पाया गया।