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

Raghavendra Naik, Rabinarayan Acharya, Mukesh B. Nariya, Sneha D. Borkar

Department of Dravyaguna and Pharmacology Laboratory, Institute for Post Graduate Teaching and Research in Ayurveda, Jamnagar, Gujarat, India. (Department of Agada Tantra, Mahatma Jyotiba Fule Medical College of Ayurveda, Chomu, Rajasthan, India)

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.

**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 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 ± 03°C. Animals were fed with Anrutt 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/15/2013/08).

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.[10]

The dose for mice for experimental study was as follows:

\[ \text{Dose for mice} = \text{Therapeutic human dose} \times \text{body surface area ratio (convertibility factor)} \times \text{body weight of mice} \]

\[ = 10 \text{ g} \times 0.0026 = 26 \text{ mg/20 g mice} = 1300 \text{ mg/kg} \]

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 ± 20 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 (1500 mg/kg, po).

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 ± standard error, 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 LD₅₀ value is much higher than 2000 mg/kg body weight in the rat. This level of LD₅₀ 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 ± 10.74                     | -                 |
| Olax scandens | 245.50 ± 09.22                     | 2.52              |

Data: Mean±SEM, ↓: 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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