Screening of intestinal transit time of *Euphorbia fusiformis* Buch.-Ham. ex D. Don in Swiss albino mice

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Effect of *Euphorbia fusiformis* Buch.-Ham. ex. D. Don root powder on intestinal transit time was carried out after standardizing the kaolin expulsion test by administering 40% kaolin to overnight fasted Swiss albino mice. Evaluation on intestinal transit time was carried out by using this standard procedure by administering the drug at 130 mg/kg and 260 mg/kg doses. *E. fusiformis* root powder significantly shortened the duration required for the expulsion of kaolin in dose dependant manner. The study shows that the root powder of *E. fusiformis* possesses significant intestinal motility enhancing effect, the exact mechanism of which needs to be elucidated.

**Keywords:** Albino mice, *Euphorbia fusiformis*, Intestinal transit, Kaolin, Purgative, *Virechana* drug, *Vishanika*.

**IPC code; Int. cl. (2011.01)** — A61K 36/47, A61K 125/00, A61P 1/10

**Introduction**

Plant based medicaments had served, from the earliest period of human civilization, as the most important therapeutic weapon available to man to fight various human and animal diseases. Herbal drug therapy is the most trusted system of medicine in countries like India where people strongly believe in Ayurveda as herbs are the part of rural Indian life style. *Euphorbia fusiformis* Buch.-Ham. ex D. Don (Family: Euphorbiaceae) is a rare medicinal plant found in Tropical Himalaya up to 450 m from Garhwal to Nepal and Wet Bengal. This plant is also rarely found in Konkan region and Deccan Hills¹⁴. In Gujarat state it is found in Dang, Rajippala and Chotaudaipur regions. The Bhagats (Folk physicians) of Dangs region use this drug in the name of *Ghate* for treatment of various abdominal disorders especially for tumors of abdomen. *Vishanika* is one of the important classical *Mulini Virechana* (roots with purgative action) drug of Ayurveda employed for purgation therapy. There are many plants which are used in the name of *Vishanika*, viz. *Vallaris solanacea* O. Ktze. (Apocynaceae), *Cryptolepis buchmani* Roem. & Schult. (Asclepidaeeae), *Euphorbia fusiformis* Buch.-Ham. ex D. Don (Euphorbiaceae) *Dolichandrone falcata* Seem (Bignoniaceae) and *Ervatamia heyneana* T. Cooke (Apocynaceae). Ashok *et al* (2005) critically analysed macroscopical, microscopical, phytochemical and pharmacological aspects and suggested⁵ that *E. fusiformis* is the possible source of classical *Vishanika*. Since it is the important potent classically described *Virechana* drug, it is expected to have purgative action which is suppose to decrease the intestinal transit time. Hence, the present study was undertaken to find out its action on intestinal transit time in experimental animals.

**Materials and Methods**

**Test drug**

The tuberous roots of *E. fusiformis* (Plate 1) were collected from Waghai forest, Gujarat in fully matured condition and the material was authenticated by qualified taxonomist of our institute. A voucher specimen has been preserved in the herbarium attached to the institute. The tuberous roots were made into slices and shade dried for 12 days and then pulverized to fine powder (mesh no 80) and stored in airtight container for experimental purposes.

**Animals**

Swiss albino mice of either sex of 8 weeks old weighing between 26 ± 4 g were procured from the...
animal house attached to pharmacology lab, IPGT & RA, Gujarat Ayurved University, Jamnagar, Gujarat. They were housed in large spacious polypropylene cages and fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water given ad libitum. The animals were acclimatized for at least one week in lab condition before commencement of the experiment in standard laboratory conditions 12 ± 01 hour day and night rhythm, maintained at 25 ± 3°C and 40 to 60% humidity. Institutional Animal Ethics Committee had approved the experimental protocol (Approval number: IAEC 04-05/01/MSc.01) and the care of animals was taken as per the CPCSEA guidelines.

Dose selection and schedule

The dose selection was done on the basis of body surface area ratio by referring to the standard table of Paget and Barnes\(^7\). On this basis the mouse dose was found to be 130 mg/kg. The test drug was suspended in distilled water with suitable concentration depending upon body weight of animals and administered orally to overnight fasted animals with the help of oral catheter. The study was carried out at two dose levels, viz. TED (Therapeutically Equivalent Dose (130 mg/kg) and TED × 02 (260 mg/kg).

Experimental design

**Standardization of Kaolin expulsion model to screen intestinal transit time**

Intestinal transit is usually quantified by measuring the movement of charcoal, dyes like phenol red meal, radio-opaque pellets or radioactive markers that have been instilled into the stomach or intestinal lumen of conscious animal to travel along the length of small intestine\(^8\). However many limitations of these methods like difficulty in screening, expensive and in almost all these methods animals are need to be killed. By considering these aspects, a simple non-invasive method called kaolin expulsion test was standardized prior to the proper experiment.

Six Swiss albino mice of either sex were kept on fasting for 12 h before the commencement of experiment, but drinking water was provided. Next day, 40% kaolin solution was administered to the fasted animals 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 coloured faecal pellets (Plate 2). The time required for the appearance of white coloured pellets was recorded and it was found to be 354.66 ± 6.88 minutes (n=6). This standardized method was used for the screening of test drug on intestinal transit time.

The selected animals were divided in to three groups. First group contained 8 animals and served as control. The second and third groups contained 6 animals each and were administered with test drugs at the dose of 130 and 260 mg/kg, respectively. One hour later 40% kaolin solution was administered with the help of oral catheter and was observed for the beginning of the kaolin expulsion as described above.
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Statistical analysis

Student’s t test for unpaired data has been used for analyzing the data generated during the study. P value less than 0.05 was considered as statistically significant.

Results and Discussion

Data pertaining to the effect of test drug on intestinal motility (Table 1) shows that administration of test drug at graded doses decreased intestinal transit time in dose dependant manner. The observed decrease in intestinal transit time is found to be statistically significant in comparison to control group.

Measurement of intestinal transit is a widely used technique for assessing the actions and mechanisms by which compounds influence intestinal motility. The evaluation of intestinal motility is helpful in, determining the therapeutic potential of new drugs in motility disorders, determining alteration in motility secondary to physiological or pharmacological stimuli and evaluating the effect of pathological condition on intestinal transit. It is also helpful in development of newer drugs in treatment of clinically known conditions of motility disorders such as acute intestinal ileus, chronic intestinal pseudoobstruction and generalized disorders of motility.

In this experiment to assess the action of test drug on the intestinal motility, latency of onset of kaolin expulsion in faecal matter was selected as a parameter. As it was difficult to assess in vivo movement of the drug, it was thought useful to administer a marker, which causes colour change of faecal matter and doesn’t alter the effect of drug.

Kaolin is a native aluminium silicate and has traditionally been used internally to control diarrhoea. It is reported that kaolin is insoluble and is not absorbed into the bloodstream. Instead it acts locally in the intestines, where it absorbs toxins and relieves mild diarrhoea. Therefore, it is not generally associated with toxicity. The kaolin used as a marker in this study in much lower dose (40% kaolin, 0.1 ml administered for 20 g mouse) than that of therapeutic human dose (26.2 g for every 6 hours) to avoid any significant action on intestine. Further, kaolin was administered one hour 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 like uneasiness, etc. which may likely to be produced by test drug. Conversely, all the animals were healthy with normal activities throughout the experiment.

It is well known fact that, it is not an easy task to prove the Virechana action of a drug in experimental animal model because of its broader meaning. It may be the reason why attempts were not made by researchers to provide experimental basis for Virechana effect of drug. As explained by Dalhanacharya, tikshna guna of Virechana Dravya is responsible to break the mala and helps in its quick excretion. In quick excretion, there is all the chance of increase in intestinal motility, based on these criteria the present study was designed to evaluate the effect of test drug on intestinal motility.

Administration of E. fusiformis root powder significantly shortened the duration required for the expulsion of kaolin in dose dependant manner. The mechanism of observed effect may be due to interference with local stimulant effect on motility or acceleration of gastric emptying. The neural regulation of gastric motility involves stimulation by

<table>
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<th>Group</th>
<th>Kaolin pellet expulsion time (Minutes)</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=8)</td>
<td>362.60 ± 10.30</td>
<td>-</td>
</tr>
<tr>
<td>Low dose (n=6)</td>
<td>250.00 ± 12.67*</td>
<td>32.0 ↓</td>
</tr>
<tr>
<td>High dose (n=6)</td>
<td>220.00 ± 16.14*</td>
<td>40.0 ↓</td>
</tr>
</tbody>
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Data : Mean ± SEM; ↓- Decrease *P < 0.001 (Compared with control group)

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Plate 2 — Rat faecal pellets: N-Normal coloured pellets, W-White coloured pellets

Table 1 — Effect of test drug on intestinal transit time

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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. 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.

It has been reported that the tuber of E. fusiformis contains phytoconstituents like diterpene lactone caudicifolin, methylellagic acid and euphol. It is possible that one or the combination of these phytoconstituents may be responsible for the observed effect. However, further detailed study is needed to identify the component responsible for this effect and to elucidate the probable mechanism(s) involved.

Conclusion

The root powder of E. fusiformis possesses significant intestinal motility enhancing effect, indicating towards some of the working mechanisms of a Virechana drug as described in Ayurveda. Based on the results obtained detailed study can be designed for future experiments.

References


