Int J Pharm Bio Sci 2013 Jan; 4(1): (P)63 – 69

Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

PHYTOHEMICAL SCREENING AND GUT MOTILITY ACTIVITY OF *PONGAMIA PINNATA* BARK IN EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

The present study was carried out with the bark of *Pongamia pinnata* Linn, belonging to the family *Fabaceae*. It is one of the fast growing, glabrous, deciduous tree and has recently gained importance as a commercial source of furanoflavones like kaempferol, karanjin. Methanolic extract of *Pongamia pinnata* was subjected to phytochemical screening. Gut motility activity was produced by oral administration of castor oil. Various models used are gut motility in isolated rat intestine, propulsive gut motility in mice and laxative activity in rats. In pharmacological screening, the effect of methanolic extract of bark of *Pongamia pinnata* Linn was evaluated in Wistar Albino Rats of either sex (150-200g) for Gut motility activity at a dose of 200 mg/kg and 400 mg/kg (p.o). The extract reduced the laxative activity as well as distance travelled by the charcoal meal. The presence of anthraquinone glycosides in the plant extract is responsible for the gut motility effect. Thus from the study and literature, it can be concluded that *Pongamia pinnata1* Linn have potent gut motility activity.

KEYWORDS: ***Pongamia pinnata*, Screening methods, Castor oil, Anthraquinone glycosides**.

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INTRODUCTION

Laxatives are drugs that either accelerate faecal passage or decrease faecal consistency. They work by promoting one or more of the mechanisms that cause diarrhoea. Because of the wide availability and marketing of OTC laxatives, there is a potential that an appropriate diagnosis will not be sought[1]. Rarely in medicine there is an absolute indication for the use of laxatives. A high fibre, well balanced diet rich in fruits and vegetables supplemented by bran should be enough to normalize bowel function. The fear of autointoxication and the constant concern of many patients regarding the frequency and quality of bowel movement make laxatives one of the most popular over – the – counter drugs in the market with serious potential for user abuse. Accepted indications for laxatives and stool softners include preparation for diagnostic colonic examination (Barium enema, colonoscopy: treatment of anorectal disorders) and prevention of hepatic encephalopathy[2]. Fibre is defined as the undigested residue of fruits, vegetables, and other foods of plant origin after digestion by the human GI enzymes. Fibre's water holding capacity is the ability of fibre to hold water and make bulking of faecal materials possible. Fibre's stool bulking capacity is the ability of the fibre to increase the volume of intestinal content because it can absorb and holds water. Bacterial growth in the colon provides additional bulking. Insoluble fibre's speeds GI transit time[3].

Cholinergic mechanisms are also responsible for modulating motor phenomena in the gut; thus it is not surprising that cholinomimetic agents are effective in promoting gastrointestinal motility. It also has cholinomimetic properties, apparently sensitizing intestinal smooth muscle cells to the action of Acetylcholine rather than acting on acetylcholine receptors. The drugs acts to hasten esophageal clearance, raise lower esophageal sphincter pressure, accelerate gastric emptying, and shorten small bowel transit time. Laxative acts by their hydrophilic or osmotic nature, laxative can cause retention of fluids. In the colonic content, as well as increase the mass. They may inhibit electrolyte and water absorption from the colon by direct or indirect mechanisms e.g., activation of adenylate cyclase within the colonic mucosa or by enhancing secretion of hormones such as cholecystokinin by Mg^{2+} salts, laxatives may enhance the motility of the colon, thereby reducing the time. Available for absorption of electrolytes and water. Hence, the fecal mass presenting at the rectum is more fluid. Senna is obtained from the leaves and pods of *Cassia augustifolia* and contains the anthraquinone glycosides called *emodins*. In oral dose the sennosides is poorly absorbed, but after removal of the sugar and reduction to *anthrol* by colonic bacteria, they are absorbed into circulation – excreted in bile to act on small intestine. It takes 6-7 hrs to produce action. The active principle is believed to act on the myentric plexus to increase peristalsis and decrease segmentation. They also inhibit salt and water absorption in the colon. In India, sennosides are usually marketed in combination with stool softeners such as docusates. Side effects observed are nausea, vomiting, diarrhoea, colic, urine discoloration (yellowish brown to red) and melanosis (colonic atony and mucosal pigmentation after a regular use of the drug). It should be used cautiously in women and children bellow 6 years of age, and after abdominal surgery[4].

Pongamia pinnata (L) Pierre (*Fabaceae*) synonym (*Pongamia glabra* Vent.) popularly known as 'Karanja' in hindi, is a medium sized glabrous tree, found throughout India and further distributed eastwards, mainly in the littoral regions of south eastern Asia and Australia. In the Ayurvedic literature of India, different parts of this plant have been used in the treatment of several ailments.

Traditionally used in treatment of bronchitis, whooping cough, rheumatic joints, diabetes. The seeds and oil used for treating various inflammatory and infectious diseases such as leucoderma, leprosy, lumbago and muscular and articular rheumatism. The leaves are hot, digestive, laxative, anthelmintic and cure piles, wounds and other inflammations. A hot infusion of leaves is used as a medicated bath for relieving rheumatic pains and for cleaning ulcers in gonorrhea and scrofulous enlargement[5,6,7]. The roots, seeds, fruits, flowers have been reported in treatment of various diseases. In addition, phytochemical examination of this plant has indicated the presence of furanoflavones, furanoflavonols, chromenoflavones, furanodiketones, flavonoid and glycosides[8,9,10]. Based on the presence of glycosides in the plant extract, the objective of the present study was to investigate the gut motility activity of methanolic extract of *Pongamia pinnata* bark.

MATERIALS AND METHODS

Plant material

The bark of *Pongamia pinnata* was collected from Erode (TN) during September, 2010. It was authenticated by Botanical survey of India, Coimbatore under herbarium no. BSI/SRC/5/23/09-10/Tech-815.

Preparation of extract and phytochemical screening

Shade dried and powdered bark (1kg) was extracted with 70% methanol in Soxhlet apparatus for 7 days. Solvent evaporation under reduced pressure yielded the semisolid extract. Chemicals used were either of analytical grade or were freshly prepared. Methanolic extract of *Pongamia pinnata* was subjected to qualitative analytical test for the detection of various chemical constituents viz. Alkaloids, steroids, carbohydrates, fixed oils, glycosides, tannins, proteins, saponin and flavonoids. And alkaloids, anthraquinone glycosides, flavonoids, carbohydrates, saponins and tannins were found to be present.

Animal used

Wistar Albino rats of either sex weighing between 180-250g and Swiss Albino mice (15-20g) were used. Animals were obtained from IRC Perundurai, Erode, Tamil Nadu. Animals were housed under standard conditions of temperature (24 ± 2^0C) and relative humidity (30-70%) with a 12:12 (light: dark) cycle. The animals were given standard diet and water *ad libitum*. All procedures involving animals were carried out under the Institute ethics committee approval 688/02/C-CPCSEA) of NCP.

Toxicity studies

Acute toxicity studies of the bark extract was carried out according to OECD guidelines in Swiss Albino mice of either sex weighing between 20 and 25 g. The LD_{50} of the bark extract was found to be safe till 2000 mg/kg (p.o).

Chemicals and drugs

Glaxenna collected from market, is a laxative containing Senna as active constituent manufactured and marketed by Glaxo Lab. Acetyl Choline, Castor Oil purchased was marketed from Nice pharmaceuticals Ltd, Other Chemical used were of Analytical Grade and reagents were freshly prepared.

GUT MOTILITY ACTIVITY
Isolated rat intestine[11]

Wistar Albino rats (150-200g) of either sex were obtained from the Animal House. Each rat was starved for about 12 hours prior to the experiment, but was allowed to have free access to water. This was to ensure that the intestines were free of faecal materials. The rat was killed by cervical dislocation and the intestines were quickly dissected out and free from other connective tissues. They were placed inside a beaker containing aerated Tyrode solution: NaCl 136; KCl 2.7; $MgCl_2$ 1.8; $CaCl_2$1.8;

$NaHPO_4$ 0.3; $NaHCO_3$ 12.0 and Glucose 5.6mM which was maintained at 37°C. Effects of Extracts and agonists on the Tissues: 2 – 3 cm of the required segment of the intestines was cut and mounted vertically inside a 20ml organ bath containing Tyrode solution which was maintained at 37^0C by a thermostat – bearing heater and aerated with air from an aerator. The tension on the tissue was adjusted to 1 g in order to maintain its muscle tone. The tissue was allowed to equilibrate for one hour during which the Tyrode solution was replaced at a ten minute interval. Graded doses (0.25 – 20.0 mg/ml) of the methanol extract or infusion of the plant material were applied to the tissue and its responses were recorded by a kymograph. Contact time of study was between 30 and 45 seconds, because the responses of the tissues in some cases were not immediate. The tissue was washed at least thrice with fresh Tyrode solution after each dose to ensure that the tissue was free of the drug. The tissue was allowed to rest for between 10-15 minutes before the next dose of the extract or infusion was applied. The dose of the extract which produced the maximum response was taken as the Working Dose against which graded doses of Acetylcholine was tested. The tissue was always pretreated with the required dose of the Acetylcholine for 3 minutes before the application of the Working Dose of the extracts or infusions. Each agonist was used until the dose which produced the maximum reduction in tissue response to the drugs was obtained. This dose was tested against the Working Dose in quadruplicate.

PROPULSIVE GUT MOTILITY IN MICE[12]

Five groups of six mice were selected. Group I received normal saline, Group II received solvent, Group III received standard, Group IV and V were administered the extract in the dose of 200 and 400 mg/kg by means of a feeding canula. Half an hour later charcoal meal consisting wheat, flour and water in a ratio 1:2:6 respectively were given orally. After 20 min of the meal, the animals were sacrificed by cervical dislocation and the intestinal transit of charcoal meal was measured in mm from pylorus to caecum.

LAXATIVE ACTIVITY IN RAT[13]

Five groups of six rats weighing 150-200 gm of either sex were fed on standard diet for 3 days, with water *ad libitum.* In this model, Group I served as normal receiving normal saline, Group II served as solvent control, Group III receiving castor oil and Glaxenna, Group IV and Group V animals received methanolic extract of *Pongamia pinnata* (200 mg/kg and 400 mg/kg, p.o.) respectively. Then each rat was kept for observation under a transparent cage, the floor of each was lined with blotting paper and observed for 5 hrs. The parameters observed were number of wet faecal pellets, total number of faecal pellets output. Then calculations were made for the correspondent percentages and later by comparison with respective control group.

Statistical analysis

All the results were expressed as mean ± standard error. The data was analysed statistically using ANOVA followed by Dunnett's T test.

RESULTS

Phytochemical screening of Pongamia pinnata

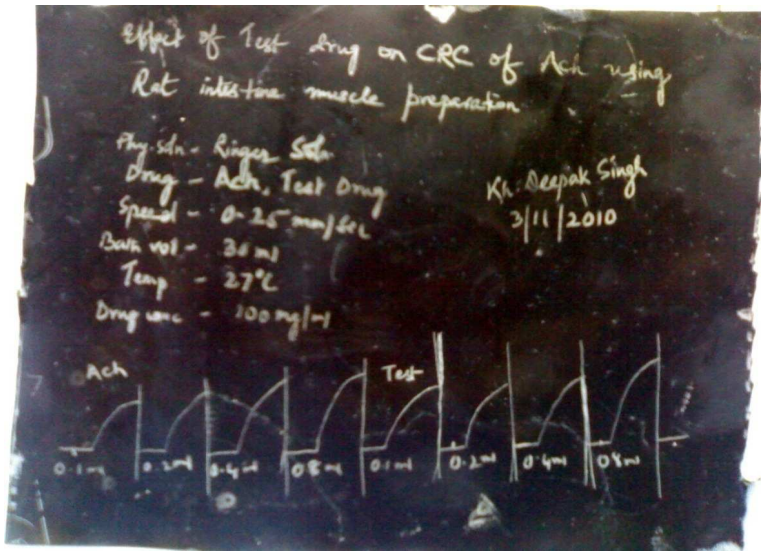
The phytochemical screening of the plant extract shows the presence of alkaloids, carbohydrates,saponins, tannins, flavonoids, anthraquinones and triterpenes(Table 1).

Table: 1
PHYTOCHEMICAL EVALUATION OF Pongamia pinnata.

Chemical constituents	Present(+ve)/ Absent(-ve)
Qualitative Analysis:	**+**ve
a. Alkaloid	+ve
b. Carbohydrates	+ve
c. Glycosides	-ve
d. Sterols	+ve
e. Saponins	+ve
f. Tannins	**+**ve
f. Flavonoids	**+**ve
g. Anthraquinones	-ve
h. Triterpenes	

Gut Motility Activity in Isolated Rat Intestine
Effect of *Pongamia pinnata* on isolated rat intestine was studied (Figure1). Concentration response curve of acetylcholine was recorded on isolated rat intestine. *Pongamia pinnata* increases the Concentration response curve of acetylcholine on isolated rat intestine. It indicates that *Pongamia pinnata* potentiates the CRC induced by acetylcholine on isolated rat intestine.

Figure1.
Isolated rat intestine



Propulsive Gut Motility in Pongamia pinnata treated Animal Model
Results suggest that *Pongamia pinnata* extract at the dose level of 200 mg/kg and 400 mg/kg produced a significant gut motility (P value <0.01), which is also evidenced by significant increase in % motility at the dose of 200 mg/kg and 400 mg/kg (52.60 and 62.73) respectively(Table 2). The activity at both the doses levels were comparable and equipotent as that of standard treated group (P value <0.01).

Table: 2
Propulsive Gut motility in Pongamia pinnata treated Animal Model.

Sl. No.	Treatment	After 1 Hr Intestinal Transmission (%)
1.	Normal Transit (Normal Saline, 1 ml/kg)	38.34±0.99
2.	Solvent control (1% CMC, 1 ml/kg)	43.15±1.39
3.	Standard (Castor oil, 0.2 ml/mice)	63.08±0.72**
4.	*Pongamia pinnata* (200 mg/kg)	52.60±0.78**
5.	*Pongamia pinnata* (400 mg/kg)	62.73±1.59**

Values are mean ± SEM; No. of animals in each group = 6
***** P value <0.01 compared with the corresponding control*.

Laxative Activity in Rat

Result suggests that *Pongamia pinnata* extract at the dose level of 200 mg/kg and 400 mg/kg produce a significant laxative activity (P value <0.01), which is also evidenced by significant increase in laxative effect at the dose of 200 mg/kg and 400 mg/kg (7.50 and 9.16) respectively(Table 3). The activity at both the dose levels were comparable and equipotent as that of Glaxenna treated group (P value <0.01).

Table: 3
Laxative Activity in Pongamia pinnata treated animal model.

Sl. No.	Treatment	Total number of Faeces	Total number of wet Faeces
1.	Normal Transit (Normal Saline, 1 ml/kg)	16.50±0.56	6.50±0.22
2.	Solvent control (1% CMC, 1ml/rat)	17.67±0.88*	6.17±0.33**
3.	Standard (Glaxenna, 1ml/rat)	26.66±0.66**	11.33±0.49**
4.	*Pongamia pinnata* (200 mg/kg)	24.50±0.76**	7.50±.0.34
5.	*Pongamia pinnata* (400 mg/kg)	26.16±0.87**	9.16±0.16**

***Values are mean ± SEM; No. of animals in each group = 6** P value <0.01 compared with the corresponding control.*

DISCUSSION

Gut motility activity was also performed by using the models such as, Gut motility activity in rat intestine, propulsive gut motility in mice and Laxative activity in rat. Concentration response curve was recorded by using Acetylcholine and the *Pongamia pinnata* extract. This shows the increased in contraction of the rat intestine. Propulsive gut motility in mice was conducted by administering the charcoal meals. The distance travelled by charcoal from pylorus to caecum was measured for each group and found to be increased in *Pongamia pinnata* treated animals. Laxative activity was observed by administering castor oil. The extract increased the intestinal transit. This may be due to the presence of Anthraquinone glycosides.

CONCLUSION

Finally from the study we can state that *Pongamia pinnata* bark has significant Gut Motility activity which may be due to the presence of Anthraquinone Glycosides and can be further explored to give it a shape of Laxative Formulation.

ACKNOWLEDGEMENT

The Authors are very much thankful to Mr.S.Sengottuvelu of Nandha College of Pharmacy and Research Institute, Erode, TN for his continuous help during the Project and also to the Management of Nandha Institutions, Erode for supplying the facilities, reagents and chemicals required for the work.

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