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Laxative activity of Cassia auriculata Pods in Rats

H. M. Suresh^{1*}, B. C. Hatapakki¹, S. I. Shivakumar¹, C. S. Hallikeri¹, B. M. Swamy², V. K. Chandur¹

1. K. L. E. Society's, College of Pharmacy, J.T.College Campus, Gadag - 582 101, Karnataka.

2. Rural College of Pharmacy, Devanahalli, Bangalore, Karnataka.

Abstract

<u>Objective</u>: To evaluate ethanol extract of pods of *Cassia auriculata* Linn at different doses (100, 200 mg / kg, p.o.) for laxative activity in rats. <u>Materials and Methods</u>: The ethanol extract of pods of *Cassia auriculata* was obtained by continuous soxhlet extraction and further assessed for laxative activity by charcoal meal test and feacal output in experimental rats and compared with standard senna. The extract was subjected to qualitative chemical analysis to identify the phytoconstituents. <u>Results</u>: Upon evaluation of Laxative activity on experimental animals, the ethanol extract at the dose 200 mg / kg, p.o. showed significant (P<0.001) laxative activity as observed from different evaluation parameters. However, the same at the dose 100 mg / kg, p.o. was less significant. The ethanol extract showed the presence of flavonoids, triterenoids, tannins, sterols and anthracene derivatives. <u>Conclusion</u>: From the results, it is revealed that, the active ethanol extract (200 mg / kg, p.o.) of *Cassia auriculata* pods is worthwhile to develop the bioactive principle for laxative activity and it is also concluded that, the anthracene derivatives present, could be attributed for the laxative activity.

Keywords: Cassia auriculata, Pods, Laxative, Anthracene Derivatives, Ethanol Extract.

1. Introduction

Constipation is a symptom of infrequent production of hard stools requiring strain to pass or a sense of incomplete evacuation caused by gastrointestinal disorders, inadequate intake of fiber and fluid, lack of exercise and modern lifestyles [1]. Various existing synthetic laxatives are associated with several therapeutic complications, which elicit the approach towards herbal laxatives that retain therapeutic efficacy and devoid of side effects. *Cassia auriculata* Linn. (Caesalpinacea), commonly known as Tanners Senna, is a common, highly branched shrub with large bright yellow flowers distributed wildly in dry regions of the central provinces and western peninsula of India [2]. The plant as a whole has been used as antidiabetic, anti-dysentric, anti-microbial and for various skin diseases from ancient times [3, 4]. In ayurveda the plant is used to treat various gastrointestinal disorders [5]. In our previous research work we have reported anti-diabetic activity of flowers of the plant [6]. However, no scientific study on laxative property of pods of *Cassia auriculata* plant has been reported.

^{*} Corresponding author

Email:klecpg@sancharnet.in

In the present study, we aimed at evaluating the under utilized and easily available plant for laxative activity in rats and also to establish Phytopharmacological profile to justify the traditional and folklore claim.

2. Materials and Methods

Dried pods of *Cassia auriculata* were collected from near by areas of Hubli and Gadag district between the month of October and November and were authenticated by Prof. Sasalhatti, Dept.of Botany, R.L. Science institute, Belgaum. A voucher specimen [CG - 06] has been deposited at the departmental herbarium.

2.1 Preparation of extracts

The dried plant pods approximately (500gm) were comminuted to coarse particle size no. (#) 40 and subjected to continuous hot extraction with 90 % ethanol in a soxhlet extractor for 48 h. The total ethanol extract was filtered and concentrated to dryness at 40°C under reduced pressure in a rota evaporator. The yield of ethanol extract was found to be 100 gm (20 % w/w). The extract was kept in a dessicator till the experiment.

2.1 Preliminary phytochemical studies

The preliminary phytochemical investigation of ethanol extract of the pods revealed the presence of flavonoids, triterpenoids, tannins, sterols and anthracene derivatives [7].

2.3 Acute Toxicity Evaluation (LD₅₀)

The acute toxicity of ethanol extract was studied in overnight fasted albino mice. Different groups containing 2 mice in each were orally administered with ethanol extract at 0.5, 1.0, 1.5, 2.0 gm / kg doses to the respective groups. Mortality and gross behavioral changes if any, were observed continuously for initial 4h and intermittently for next 6 h and then again at 24 h and 48 h after dosing. The parameters such as sedation, hyperactivity, grooming, loss of righting reflex, respiratory rate and convulsion were observed. 1/10 th of lethal dose was taken as the screening dose [8].

2.2 Evaluation of laxative activity

Laxative activity was evaluated by charcoal meal test and faecal output. The gastrointestinal transit rate was expressed as the percentage of the distance traversed by the charcoal divided by the total length of the small intestine. The ethical clearance was obtained by institutional animal ethics committee (Registration No.221 / CPCSEA) before the experiment.

Female Wister albino rats (150 -200 gm) were used for the study. The animals were housed in polypropylene cages and fed on standard laboratory diet (Lipton India Ltd) and water *ad libitum*, maintained at an ambient temperature of $25 \pm 2^{\circ}$ C and exposing them to 12 h light/ dark cycle.

2.5 Intestinal transit rate

The animals were divided into 4 groups of 6 animals each and were fasted for 12 hours prior to the experiment but permitted water ad libitum. Group 1 served as control received normal saline (25 ml / kg, p.o.). Group 2 and 3 were administered with 100 and 200 mg / kg, p.o. of ethanol extract respectively. Group 4 received reference drug senna (100 mg / kg, p.o.) Laxative activity was evaluated by orally administrating semisolid test charcoal meal (0.3 ml per rat) consisting of 10% charcoal and 5% gum acacia to all groups followed by test and reference drugs. The control group was maintained with only charcoal meal. After administration of the reference and test drugs, the animals were allowed to feed on standard laboratory animal diet for 50 min and thereafter sacrificed under light ether anesthesia. The abdomen was opened and the entire small intestine starting from the pyloric end was removed and placed on the blotting paper. The distance traveled by charcoal was measured and expressed as percent intestinal transit [9].

% Intestinal transit =
$$\frac{by \ charcoal}{Total \ length \ of} \times 100$$

small intestine

2.6 Feacal out put

The experimental animals were kept in individual cages for one week. Any rat producing wet feaces was rejected. The selected animals were divided into 4 groups of 6 in each. Group A

served as control received normal saline (25 ml/ kg, p.o.). Group B and C were administered with 100 and 200 mg / kg, p.o. of ethanol extract. Group D received reference drug senna (100 mg / kg, p.o.). All the animals were fasted for 12 h followed by administration of test compounds. After which, the animals were immediately placed in a separate wire meshed cage to enable the feaces to fall through onto blotting paper. The number of wet defecation were measured and weighed for 8 h by changing the paper for every 2 h [10, 11].

Table 1. Effect of the extracts of *Cassia auriculata* pods on gastrointestinal motility in rats

Treatment	Mean length of GIT	Distance traveled by	% intestinal
	(cm)	charcoal meal	transit
Control	60.57 ± 2.91	25.73 ± 0.31	42.47
(Vehicle)			
Ethanol extract	59.88 ± 2.01	$44.24 \pm 0.41 *$	73.88
(100 mg / kg, p.o)			
Ethanol extract	61.21 ± 1.76	50.90 ± 0.44 *	83.15
(200 mg / kg, p.o)			
Senna	60.09 ± 1.05	52.76 ± 0.23 *	87.80
(100 mg / kg , p.o)			

Each value represents mean \pm SE (n = 6) , * Indicates P< 0.001 v/s control group

Treatment	Mean no. defecation after treatment at				Faecal output (mg)
	2h	4h	6h	8h	
Control	0	0.50 ± 0.22	1.50 ± 0.22	1.83 ± 0.16	81.66 ± 0.25
(Vehicle)					
Ethanol extract	$0.83\pm0.21*$	$1.42\pm0.22*$	$3.17\pm0.25*$	$4.36\pm0.22*$	$294.66 \pm 1.47*$
(100 mg / kg, p.o)					
Ethanol extract	$1.50\pm0.22*$	$2.83\pm0.30^*$	$4.33\pm0.33*$	$5.33\pm0.21*$	$321.67 \pm 0.33*$
(200 mg / kg, p.o)					
Senna	$2.66\pm0.21*$	$3.50\pm0.22*$	$4.50\pm0.22*$	$6.00\pm0.36^*$	$424.33 \pm 1.96*$
(100 mg / kg , p.o)					

* Indicates P< 0.001 v/s control group at different time intervals

2.7 Statistical analysis

All the results are expressed as Mean \pm S.E. The statistical significance was analyzed by performing one-way ANOVA followed by Post-hoc Dunnett's test. P < 0.001 implies significance [12].

3. Results and discussion

The effect of different doses of ethanol extract of *Cassia auriculata* pods on intestinal transit and feacal out put are expressed in Table 1 and 2 respectively. The ethanol extract at the dose100 and 200 mg / kg, p.o. exhibited significant increase in gastrointestinal transit (P < 0.001) as compared to control. The ethanol extracts at the dose 100 and 200 mg / kg, p.o. and reference drug (senna 100 mg / kg , p.o.) increased intestinal transit rate significantly by 73.88 %, 83.15% and 87.80% respectively. An increase in the number, wetness and frequency of defecation was observed in the group treated with ethanol extracts, which was comparable with reference drug.

The acute toxicity study of *Cassia auriculata* pods extract revealed no mortality when administered orally up to a maximum dose of 2 g / kg body weight. At this dose there was no gross behavioral change.

The phytochemical investigation of ethanol extract has revealed to contain sterols, anthracene derivatives, triterpenoid and tannins. This study reports for the first time the Laxative activity of pods of *Cassia auriculata*, supporting its traditional and folklore claim.

Senna is a stimulant type of purgative, which induces purgation by irritating the intestinal mucosa and increase peristalsis. They also inhibit salt and water absorption in the colon and increase the fluid bulk enhancing the wet feacal evacuation [13]. With the observed significant increase in the intestinal transit rate and feacal out put by ethanol extract of the pods of Cassia auriculata, the mechanism of action could, possibly, be due to an increase in osmotic load within the intestine, excessive secretion of electrolytes and water into the intestinal lumen, exudation of fluid from the mucosa, intestinal promotility or by any other mechanism resulting in rapid transit.

The laxative activity of many medicinal plants has been suggested to be due to anthracene derivatives [11, 14]. The presence of anthracene derivatives in the pods of *Cassia auriculata* could be attributed for the prominent laxative activity. However, studies are in progress in our laboratory to trace the exact mechanism of action and to elucidate the structure of bioactive principle for laxative activity.

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