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Laxative activity of *Cassia auriculata* Pods in Rats

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Abstract

Objective: To evaluate ethanol extract of pods of *Cassia auriculata* Linn at different doses (100, 200 mg / kg, p.o.) for laxative activity in rats. Materials and Methods: 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. Results: 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. Conclusion: 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 anti-diabetic, 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.

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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 (LD_{50})

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 ± 2°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].

$$\% \text{ Intestinal transit} = \frac{\text{Distance traveled by charcoal}}{\text{Total length of small intestine}} \times 100$$

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

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

Table 2. Effect of extracts of *Cassia auriculata* pods on feacal out put in rats

Treatment	**Mean no. defecation after treatment at**				**Faecal output (mg)**
	2h	**4h**	**6h**	**8h**	
Control (Vehicle)	0	0.50 ± 0.22	1.50 ± 0.22	1.83 ± 0.16	81.66 ± 0.25
Ethanol extract (100 mg / kg, p.o)	0.83 ± 0.21*	1.42 ± 0.22*	3.17 ± 0.25*	4.36 ± 0.22*	294.66 ± 1.47*
Ethanol extract (200 mg / kg, p.o)	1.50 ± 0.22*	2.83 ± 0.30*	4.33 ± 0.33*	5.33 ± 0.21*	321.67 ± 0.33*
Senna (100 mg / kg , p.o)	2.66 ± 0.21*	3.50 ± 0.22*	4.50 ± 0.22*	6.00 ± 0.36*	424.33 ± 1.96*

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

2.7 Statistical analysis

All the results are expressed as Mean ± 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.

4. Acknowledgement

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Effect of aqueous alcoholic extract of *Hibiscus sabdariffa* L. and *Ocimum sanctum* L. on plasma triglycerides in wistar rats

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Abstract

Objective: To study the hypolipidaemic effect of aqueous alcoholic extract of *Hibiscus sabdariffa* L. and *Ocimum sanctum* L. in Triton induced hypercholesterolaemic Albino rats. Materials and Methods: Aqueous alcoholic extracts of fresh leaves and flowers of *Hibiscus sabdariffa* L., fresh leaves of *Ocimum sanctum* L. were assessed for hypolipidaemic activity by estimating plasma cholesterol and plasma triglyceride levels in Triton induced hypercholesterolaemic rats. Result: Significant reduction ($P<0.01$) in plasma triglyceride was observed in aqueous alcoholic extract of *Hibiscus sabdariffa* L. treated animals at 6 h and 24 h and by 24 h in aqueous alcoholic extract of *Ocimum sanctum* L. treated animals. Conclusion: From the results, it is revealed that aqueous alcoholic extract of *Hibiscus sabdariffa* L. and *Ocimum sanctum* L. can effectively reduce plasma triglyceride levels in laboratory animals and may be useful as adjuvant in effectively controlling plasma triglyceride levels in susceptible patients.

Keywords: *Hibiscus sabdariffa, Ocimum sanctum,* Hypolipidaemia.

1. Introduction

Lipoproteins transport lipids and cholesterol through the blood stream and is essential to life, but excessive concentration in the plasma increase the risk of ischaemic heart disease [1]. Elevated lipoprotein concentration is now a known risk factor for several cardiovascular diseases and the beneficial effect of lowering elevated levels of cholesterol in preventing coronary heart disease is well established [2]. Several synthetic and herbal drugs are used, which are claimed to reduce elevated cholesterol levels, hence useful in preventing coronary heart disease.

Hibiscus sabdariffa L. (Family: Malvaceae) is reported to have wide variety of pharmacological activities and its phytochemical, pharmacological and toxicological aspects has been reviewed [3]. Some of the activities include it's hepatoprotective effect against azothioprine

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induced hepatotoxicity [4], membrane stabilizing activity in anemia [5], time and dose dependent apoptosis in human leukaemic cells by DP3- SAM, an anthrocyanin [6, 7], antioxidant effect [8], antihypertensive effect that is comparable to captopril in mild to moderate hypertension [9] and inhibition of development of Atherosclerosis in cholesterol fed rabbits [10].

Ocimum sanctum is a popular folklore medicine in India and Asian countries is recently reviewed by Prakash and Gupta [11] and has been reported to have antibacterial activity [12], antitussive activity [13], ability to augment cardiac endogenous antioxidants [14] and *in vitro* antilipidperoxidative property [15].

The present study was taken up to investigate the effect of aqueous alcoholic extract of *Hibiscus sabdariffa* L. and *Ocimum sanctum* L.on plasma triglyceride and cholesterol in Triton induced hypercholesterolaemia in Albino Wistar rats.

2. Materials and Methods

Healthy, male, adult Albino Wistar rats weighing between 190–250 g, maintained in our animal house facility under standard animal house conditions were used. The difference between animals in various groups did not exceed 20g. CPCSEA guidelines were adhered to during the maintenance and experiment. Experiment protocol was submitted to Institutional animal ethics committee and approval was taken (Reg. No. 626/02/a/CPCSEA).

Fresh leaves and flowers of *Hibiscus sabdariffa* L. were collected during October at Pondicherry and were Sun dried. Fresh leaves of *Ocimum sanctum* L. were collected during October at Cuddalore, TamilNadu and Sun-dried. Both the plants were authenticated by Society for Health, Education and Research on biodiversity, Pondicherry.

2.1 Preparation of Plant Extract

2.1.1 Aqueous alcoholic extract of Hibiscus sabdariffa L. (HS I)

Sun-dried leaves and flowers were extracted with aqueous alcohol three times. Extracts were combined, alcohol was removed and spray dried to get extract powder.

2.1.2 Defatted aqueous alcoholic extract of Hibiscus sabdariffa L. (HS II)

Shade dried leaves and flowers were extracted with aqueous alcohol three times. The extracts were combined and distilled to remove alcohol. Further, it was extracted with hexane several times. The hexane washed aqueous layer was spray dried to get extract in powder form.

2.1.3 Aqueous alcoholic extract of Ocimum sanctum L. (OS)

Sun-dried leaves were extracted with aqueous alcohol by boiling it for few minutes. It was repeated three times. Combined extracts were filtered, concentrated to dryness under vacuum.

2.2 Acute toxicity studies

Acute toxicity study was carried out for all three extracts following OECD guidelines [16]. Overnight fasted, healthy Wistar Albino rats (n=3) were administered orally extract dissolved in water in the dose of 2000 mg/Kg body weight and observed continuously for 2 h and 24 h for mortality. No visible change was observed in any test animal and all animals survived beyond 24 h.

2.3 Estimation of antihypercholesterolemic activity

The method described by Tamasi *et al*. [17] was used for estimation of antihypercholesterolemic activity. 24 healthy, male Wistar Albino rats weighing 190 – 250 g were randomly assigned to 4 groups of 6 animals each. Such animals were fasted for 16 h prior to experiment, but water was given *ad libitum*.

On the day of experiment, HS I, HS II and OS at 200 mg/Kg dose level and saline 2 ml/animal were administered orally to test groups and control group respectively. Simultaneously all the animals received a single intraperitoneal injection of Triton WR-1339 (isooctyl-polyoxyethylene phenol) at 100 mg/Kg body weight. Serum cholesterol and triglyceride were estimated at 6, 24 and 48 h after Triton injection by employing enzymatic method [18]. Blood sample were withdrawn from retroorbital puncture under light ether anesthesia. Blood samples of each group were pooled, centrifuged for 2 min at 1500 rpm and serum separated. The serum cholesterol and triglyceride were determined for each bloodsample using autoanalyser.

For the estimation of serum cholesterol and triglyceride, serum separated from blood within 30 minutes of collection were used. Reagents used for the estimation of cholesterol were mixture of reagent 1 and 1A. Reagent 1 contains cholesterol esterase (200U/l), cholesterol oxidase (250 U/l), peroxidase (1000 U/l) and 4-amino antipyrine (0.5 mM). Reagent 1A contains Pipes buffer pH 6.90 (50 mM), phenol (24 nm) and sodium cholate (0.5 mM). Equal volumes of reagent 1 and 1A were mixed, kept aside for 5 minutes before using. The samples and the reconstituted reagents were brought to room temperature prior to use and incubated for 5 min at 37°C, mixed and read using autoanalyser. Reagents used for the estimation of triglycerides contain buffer, pH 7.2 (50mM), lipase (2000 IU/l), glycerol kinase (300 IU/l), glycerol phosphate oxidase (1000 IU/l), peroxidase (500 IU/l), ATP (1 mM) and chromogens (2mM). This assay mixture was incubated for 10 minutes at 37°C and read using autoanalyser.

2.4 Statistical Analysis

Experimental data were subjected to analysis of One way ANOVA and difference at $P<0.01$ were considered significant.

3. Results and Discussion

Neither mortality nor any visible changes were observed during the acute toxicity studies. Hence the extracts in the dose up to 2000 mg/Kg body weight were found to be safe in laboratory animals.

Concentrations of plasma cholesterol and triglycerides are as shown in the Table1. Significant ($P<0.01$) reduction in plasma triglyceride was seen in 6 h and continued up to 24 h, in HS I and HS II treated animals. In OS treated animals, significant reduction ($P<0.01$) in plasma triglyceride level was observed by 24 h. However, there was no significant fall in plasma cholesterol during the entire course of experiment in any group of drug treated animals.

Table 1. Effect of aqueous alcoholic extracts of *Hibiscus sabdariffa* L. and *Ocimum sanctum* L. on Triton induced hypercholesterolaemic Wistar rats.

Treatment	Dose	Plasma Cholesterol (mg/dl)			Plasma Triglycerides (mg/dl)		
	(mg/kg)	6 h	24 h	48 h	6 h	24 h	48 h
Control	-	86.66±3.48	65.33±2.72	76.9±6.16	72.00±1.50	56.67±0.66	56.03±7.24
HS I	200	87.00±1.72	62.0±0.58	81.2±0.64	49.6±5.48*	38.33±0.32*	41.53±1.06
HS II	200	94.66±2.40	65.0±0.58	92.66±8.20	52.6±1.76*	36.33±0.32*	66.26±2.80
OS	200	98.00±1.16	84.0±1.16	93.5±2.02	60.33±0.33	51.33±0.66*	61.7±0.34

Values are mean ± SEM of six animals in each group. One way ANOVA followed by Dunnett's '*t*' test.
*$P<0.01$ when compared to control.

Triton induced hypercholesterolaemia in Phase I is thought to be due to increased hepatic synthesis of cholesterol through the ability of Triton to interfere with the uptake of plasma lipids by the tissues. Drugs interfering with cholesterol biosynthesis were shown to be active in Phase I, while drugs interfering with cholesterol excretion and metabolism were active in Phase II [19].

Interestingly enough, significant reduction in plasma triglyceride levels was observed in HS I, II and OS treated animals and the recent studies have shown that triglycerides are independently related with coronary heart disease [20, 21]. Most of the hypolipidaemic drugs do not decrease serum triglyceride level and these extracts exhibited significant reducing effect. Several fibric acid derivatives (fibrates) cause marked reduction in circulating VLDL and hence triglyceride, with a modest reduction in LDL and an approximately 10% increase in HDL. The mechanism of action of fibrates is incompletely understood, but they stimulate lipoprotein lipase, hence increasing the hydrolysis of triglycerides in chylomicrons and VLDL particles [1]. These results demonstrate hypolipidaemic effect of extracts of *Hibiscus sabdariffa* L. and *Ocimum sanctum* L. probably with fibrate like mechanism.

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