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Studies on laxative effect of extract of dried fruit pulp of *Cassia fistula*

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Abstract

Cassia fistula (CF) is known as Aragvadha (disease killer) in Ayurvedic classics and its sun-dried (SD) fruit pulp has been advocated for the treatment of constipation, fever, leprosy, diabetes, intestinal disorders and wounds. Present study includes the evaluation of SD and non-sun dried (NSD) fruit pulp of CF for purgative action in rat and acute toxicity in mouse. Aqueous suspension of SD and NSD were administered orally 60 min before experiment in rats and SD just before toxicity study in mice. Both SD and NSD in the dose of 1.0 g/kg showed an increase in the number of defecations and fecal output during 4 hour after treatment but stool was semisolid with SD and semisolid and watery with NSD. Both SD and NSD treated rats showed increase in the intestinal intraluminal fluid (ILF) accumulation and motility but the accumulation of ILF was less marked in SD group compared to NSD group. The stimulatory effect of SD on ILF accumulation and intestinal motility could be due to its predominant action on NO formation as only L-NAME a NOS inhibitor blocked both ILF accumulation and intestinal motility *per se* and in SD-treated rats while atropine (anti-cholinergic), loperamide (μ and k receptor inhibitor) and indomethacin (PGs synthesis blocker) partially blocked them. 10 g/kg oral dose (10 times of optimal effective dose) of SD did not show any acute toxic effect in mice. The result confirms the indigenous use of sun-dried fruit pulp of *C. fistula* in constipation.

Keywords: *Cassia fistula*, purgative, nitric oxide, PGs, opioids, cholinergic

1. Introduction

Cassia fistula Linn (CF) (Hindi-Amaltas, English-Golden shower, Indian labrum), is known as Aragvadha (disease killer) in Ayurveda [1]. It is a flowering plant, in the family Fabaceae, native to southern Asia, from southern Pakistan east through India to Myanmar and south to Sri Lanka. The tree is a medium sized deciduous tree, 6-9 meters tall with straight trunk and spreading branches. The plant has got golden yellow coloured flower and long stick like fruit with a pungent odor and containing several seeds [2]. CF fruit has been advocated in indigenous system of Medicine for purgative (Mridu virecana), fever (Jwara), leucoderma (Kustha), diabetes (Prameha), intestinal disorders (Udara), and wounds (Vrana) [3] dating back to Sushruta Samhita and Charaka Samhita [4]. Pharmacological screening of CF

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plant was reported to have analgesic, antipyretic [5], sedative [6] and anti-hypercholesterolemic potential [7], anti-inflammatory [8], hypoglycemic activity [9], antipyretic [10], anti-rheumatic [8], anti-tumor [11, 12], hepatoprotective [13], antioxidant [14, 15], anti-fungal and anti-bacterial activities [16, 17]. Extensive studies have been carried out during the past few decades on isolation and characterization of chemical constituents of various parts of *C. fistula*. Rhein, glucose, sucrose and fructose were isolated from the fruit pulp [18] while, fistulic acid and kaempferol and a leucopelargonidin tetramer having free glycol unit were isolated from the pods and flowers respectively [19]. CF Pod was reported to have very low level of toxicity and no pathological effects were seen on liver, kidney and rat's testis [20]. Both the leaves and pods were widely used in traditional medicine as purgatives and laxatives [1, 21].The purgative action of fruit pulp of CF is due to presence of anthraquinone, known to have purgative action [22]. Though the fruit pulp of CF has been well known for its mild purgative action since ancient times as found in Charaka Samhita (1000 BC to 4th Centuary), the most important information regarding its use has been described in Kalpa sthana of Charaka Samhita (K.8) [23], where it has been advised that the fruit grown in time, ripe and possessed with good qualities should be collected and kept within sand for a week. Thereafter they should be taken out and dried in the sun and then their pulp should be taken out and stored in a clean container for purgative use.

The present study is therefore, planned to find i) the purgative action of both sun-dried (SD) and non-sun dried (NSD) powder of fruit pulp of *C. fistula*, ii) any difference in the purgative effect of SD and NSD powder taking into consideration the use of SD fruit pulp of this plant for purgative action in Kalpa sthana of Charaka Samhita (K.8) [23] and iii) the role of intrinsic intestinal stimulators of secretion and motility like nitric oxide (NO), cholinergic (M_2 receptor), opiods (μ and k receptors), prostaglandin (Es and Fs) in the purgative effects of SD. Selective NO synthesis blocker, N^G- Nitro-L-arginine methyl ester (L-NAME); cholinergic-blocker, atropine; μ and k receptor blocker, loperamide and indomethacin (PGs synthesis blockers) were used to find the possible mechanism of stimulatory effects of SD on intestinal secretion and motility.

2. Materials and Methods

2.1 Plant material

Authentic fruits of *C. fistula* were collected from campus of Banaras Hindu University, Varanasi, India in the month of June, verified and confirmed with the authentic samples kept in herbarium in the Department of Dravyaguna, Faculty of Ayurveda, I.M.S., B.H.U. The sun dried (SD) and non sun dried (NSD) fruit pulp were powdered in Ayurvedic Pharmacy of B.H.U. and Packed in air tight container for experimental use.

2.2 Animals

Adult Charles Foster strain albino rats and mice of either sex, weighing between 130 to 150 g and 20 to 25g respectively were obtained from the Central Animal House of the Institute of Medical Sciences, B.H.U Varanasi. All animals were kept in colony cages under ideal housing conditions at an ambient temperature of 25^0C ± 2^0C and 45-55% relative humidity with a 12 hour light-dark cycle in the animal house of pharmacology department and fed on standard pellet diet. Animals were acclimatized for a week before use. The ethical permission for the investigation of animals used in experiments was taken from the Animal Ethics Committee of the Institute (Notification No. Dean/2010-11/90 dated 05.05. 2010).

2.3 Drugs and chemicals

L-NAME (Sigma Aldrich, U.S.A.), Atropine (Neon Laboratories, India), Loperamide (Torrent Pharmaceuticals, India), Indomethacin (Jagsonpal Pharmaceuticals, India) were used.

2.4 Experimental design

2.4.1 Treatment protocol

Sun-dried (SD) and non sun-dried (NSD) powder of fruit pulp of *C. fistula* was suspended in distilled water (DW). They were given orally with the help of an orogastric tube in the volume of 1ml/100 g rat. The rats were fasted overnight and the experiments were conducted next day. A dose-dependent effect of SD (0.5, 1.0 and 1.5 g/kg) was observed on number of defecations, fecal output and characteristics of stools till 4 hour after the treatment. SD showed a dose-dependent increase in the number of defecation as well as fecal output in 4 hour of study indicating the purgative activity and an optimal effective dose of SD (1.0 g/kg) was then selected for further studies.

2.4.2 Effect of SD and NSD

Four groups containing six animals each were used for the study. 1st Control group received DW (negative control), 2nd group received castor oil (CO, 5 ml/g, positive control) and 3rd and 4th groups received 1.0 g/kg of SD and NSD powders. They were studied for their effects on number of defecation and fecal output and characteristics of stool till 4 hr after the treatment in overnight fasted rats.

2.4.3 Effect of SD and NSD on intestinal intraluminal fluid (ILF) accumulation and motility

2.4.3.1 Intestinal ILF accumulation

Four groups containing six animals each were used for the study. SD (1 g/kg), NSD (1 g/kg), CO (5 ml/kg) and DW (control group) were administered orally 60 min before sacrificing the animals. Small intestine from pyloric end to ileocaecal junction was taken out separating them carefully from the mesentery without damaging the intestine. They were weighed and the results were calculated on the basis of weight of small intestine as g/100 g body weight of the animal. The change in the amount of fluid of the test drug-treated group was calculated from the difference in the weights of small intestine from drug-treated group to that of DW group.

2.4.3.2 Intestinal motility [24]

Four groups containing six overnight fasted rats each were used for the study. SD (1 g/kg), NSD (1 g/kg), CO (5 ml/kg) and DW (10 ml/kg, Control) were administered orally 60 min before sacrifice/experiment. Charcoal (0.2 ml, 3% in DW) was administered to rats after 30 min of above test drugs treatment and the animals were sacrificed after 30 min of charcoal administration with over dose of ether. Small intestine from pyloric end to ileocaecal junction was taken out separating them carefully from the mesentery without damaging the intestine. The distance travelled by charcoal with reference to the total length of small intestine was calculated and expressed as percentage of distance travelled.

2.4.4 Effects of anti-secretory and anti-motility drugs on SD-induced intestinal intraluminal fluid accumulation and motility

To find the mode/mechanism of purgative action of SD, four anti-secretory and anti-motility drugs like nitric oxide synthase inhibitor, L-NAME; prostaglandin synthesis inhibitor, indomethacin; opiods receptors (μ and k) antagonist, loperamide and anti-cholinergic, atropine were studied against SD-induced stimulatory effects on intestinal motility and secretion.

2.4.4.1 Intraluminal fluid accumulation

Ten groups of overnight fasted rats having 6 rats each were employed. DW (10ml/kg, po) was given to rats of 1st and 6th groups, Loperamide (1 mg/kg, po) to 2nd and 7th groups, atropine (5 mg/kg, im) to 3rd and 8th groups, L-NAME (25 mg/kg, i.p.) to 4th and 9th groups and indomethacin (20 mg/kg, po) to 5th and 10th groups respectively 90 minutes before experiment. 30 min after the above treatments, the rats of 1st to 5th groups received DW (10 ml/kg, po) while, rats of 6th to 10th groups received SD (1 g/kg, po). Rats of all the above ten groups were then sacrificed after 60 min of DW/SD administration with an over dose of ether. Small intestine from pyloric end to ileocaecal junction was taken out in each rat separating them carefully from the mesentery without damaging the intestine. They were weighed and the results were calculated on the basis of weight of small intestine as g/100 g body weight of the animal. The change in the amount of fluid of the treated group was calculated from the difference in the weight of small intestine from respective control (DW/SD) from that of the treated groups.

2.5.4.2 Intestinal motility

Ten groups of overnight fasted rats having 6 rats each were employed. Rats of 1st to 5th groups received DW (10 ml/kg, po), Loperamide (1 mg/kg, po), atropine (5 mg/kg, im), L-NAME (25 mg/kg, i.p.) and indomethacin (20 mg/kg, po) respectively 90 min before experiment and DW was then given orally to all groups of rats 30 min after the above DW/drug treatments. Rats of 6th to 10th groups received DW, loperamide, atropine, L-NAME and indomethacin respectively in the same dose as mentioned above in 1st to 5th groups 90 min before and then after 30 min all the rats of the above 6th to 10th groups received SD (1 g/kg) orally. Charcoal (0.2 ml, 3% in DW) was then administered to rats of all the above 10 groups after 30 min of SD/DW treatment and the animals were sacrificed with over dose of ether after 30 min i.e. at the completion of 90 min of the experiment. Small intestine from pyloric end to ileocaecal junction was taken out separating them carefully from the mesentery without damaging the intestine. The distance travelled by charcoal with reference to the total length of small intestine was calculated and expressed as percentage of distance travelled.

2.4.5 Acute toxicity study [25]

Adult Charles Foster strain albino mice of either sex, weighing between 20 to 25g fasted overnight, were used for toxicity study. Aqueous suspension of SD was orally administered at 10 g/kg stat dose (10 times of the optimal effective dose of 1 g/kg) to mice. Subsequent to SD administration, animals were observed closely for first three hours, for any toxicity manifestation, like increased motor activity, salivation, colonic convulsion, coma and death. Subsequently observations were made at regular intervals for 24 h. The animals were under further investigation up to a period of 1 week.

2.5 Statistical analysis

Statistical comparison was performed using one way analysis of variance (ANOVA) and for multiple comparisons versus control group was done by Dunnett's test.

3. Results

3.1 Effect on number of defecation and fecal output and characteristics of stool

Effect of SD, NSD and CO was seen on number of defecation, fecal output and characteristics of stool in albino rats during 4 hr study period. Result showed SD-treated rats having mild purgative effect compared with NSD and CO groups (Table 1).

3.2. Effect of SD and NSD on intraluminal fluid accumulation and intestinal motility

3.2.1 Effect on intraluminal fluid accumulation

SD, NSD and CO caused a significant increase in the accumulation of intraluminal fluid. However, the accumulation of intraluminal fluid (ILF) was less marked in SD compared to NSD group (Table 2).

3.2.2 Effect on intestinal motility

SD, NSD and CO pretreatments led to an increase in intestinal motility as observed with an increase in % distance travelled by charcoal after a charcoal meal (Table 2). However, SD-treated rats showed an equal intestinal motility compared to NSD group.

3.3 Effects of anti-secretory and anti-motility drugs on SD-induced intestinal motility and intraluminal fluid accumulation

3.3.1Effect on intraluminal fluid accumulation

There was significant decrease in the accumulation of ILF *per se* in atropine, L-NAME and indomethacin treated rats (10.4 to 21.2% decrease, P<0.05 to P<0.01) while, rats treated with loperamide showed little decrease in ILF (4.8% decrease) accumulation. However, the stimulatory effect of SD on ILF was maximally inhibited by L-NAME (28.1% decrease, P<0.001) but less with loperamide, atropine and indomethacin (6.8 to 12.5% decrease, P<0.05 to P<0.01) pretreatments (Table 3).

3.3.2 Effect on intestinal motility

There was significant decrease in intestinal motility by loperamide (21.9%), atropine (22.0%), L-NAME (43.2%) and indomethacin (25.3%) *per se* but decrease was more in L-NAME-treated rats. SD-treated rats showed significant increase (20.9%, P<0.001) in intestinal motility which was maximally blocked in L-NAME-treated rats (48.8%, P<0.001) but less by others (18.8 to 21.4 decrease, P<0.05) where motility was reversed near to the DW control value (Table 4).

3.4 Acute toxicity study

SD even at 10 g/kg oral dose did not show any acute toxicity manifestation like increased motor activity, salivation, colonic convulsion, coma and death, observed up to a period of 1 week.

Table 1: Effect of castor oil (CO, 0.5 ml/100 g, 1 hr), Sundried (SD, 1 g/kg, 1 hr) and Non-sundried (NSD, 1 g/kg, 1 hr) powder of fruit pulp of *Cassia fistula* on number of defecation and fecal output and characteristics of stool in albino rats

Oral treatment	No. of Defecations/4 hr	Fecal output/4 hr (g/100 g bw)	Characteristics of fecal matter
Control (DW)	5.00 ± 0.58	2.62 ± 0.12	Formed stool
CO	12.2 ± 0.60 [c]	4.21 ± 0.21 [c]	Semisolid, watery stool
SD	8.50 ± 0.43 [c]	3.72 ± 0.11 [c]	Semisolid
NSD	9.50 ± 0.43 [c]	4.05 ± 0.17 [c]	Semisolid, slight watery

Values are expressed as mean ± SE (n=6). [c] P<0.01 as compared to respective control group.

Table 2: Effect of castor oil (CO, 0.5 ml/100 g, 1 hr), SD (1 g/kg, 1 hr) and NSD (1 g/kg, 1 hr) on intestinal intraluminal fluid (ILF) accumulation and motility in rats

Oral treatment	Intestinal ILF accumulation		Intestinal motility	
	Intestinal weight (g/100 g bw)	IL (mg)	% Intestinal length travelled by charcoal	% Control
Control (DW)	6.173 ± 0.18	–	73.1 ± 1.56	100.0 ± 2.13
CO	7.713 ± 0.36[b]	1540	98.7 ± 0.71[c]	135.0 ± 0.97[c]
SD	6.802 ± 0.15[a]	629[a]	88.4 ± 1.22[c]	120.9 ± 1.67[c]
NSD	7.093 ± 0.12[b]	920[b]	85.4 ± 2.57[c]	116.8 ± 3.52[c]

Values are expressed as mean ± SE (n=6). [a] P<0.05, [b] P<0.01, [c] P<0.001 as compared to respective control group.

Table 3: Effect of loperamide (1mg/kg, oral, 90 min), atropine (5 mg/kg, intramuscular, 90 min), L-NAME (25 mg/kg, intraperitoneal, 90 min) and indomethacin (20 mg/kg, oral, 90min) *per se* and on SD (1 g/kg, 60 min)-induced intestinal ILF accumulation in rats

ORAL TREATMENT	INTESTINAL ILF ACCUMULATION		
	Intestinal weight (g/100 g bw)	% Control (weight)	ILF accumulation (mg)
	***per se* effect**		
Control (DW)	6.173 ± 0.18	100.0 ± 2.92	–
Loperamide	5.875 ± 0.09	95.2 ± 1.46	- 298
Atropine	5.533 ± 0.17[a]	89.6 ± 2.75[a]	- 640[a]
L-NAME	4.863 ± 0.25[b]	78.8 ± 4.05[b]	- 1310[b]
Indomethacin	5.503 ± 0.10[b]	89.1 ± 1.62[b]	- 670[b]
	Effect on SD-induced ILF		
SD	6.802 ± 0.15[a]	110.2 ± 2.43[a]	+ 629
Loperamide + SD	6.385 ± 0.09[*]	103.4 ± 1.46[*]	+ 212[*]
Atropine + SD	6.060 ± 0.10[**]	98.2 ± 1.62[*]	- 113[**]
L-NAME +SD	5.066 0.19[***]	82.1 ± 3.08[***]	- 1107[***]
Indomethacin + SD	6.033 ± 0.15[**]	97.7 ± 2.43[**]	- 140[**]

Values are expressed as mean ± SE (n=6). [a] P<0.05, [b]P<0.01 as compared to respective control group and [*] P<0.05, [**] P<0.01, [***]P<0.001 compared to respective SD-treated group.

[@] Intraluminal fluid weight has been calculated on the basis of change in small intestine weight from control rats per 100 g body weight. (+) indicated increase and (-) indicated decrease in intestinal fluid levels from control (DW) intestinal value.

Table 4: Effect of loperamide (1mg/kg, oral, 90 min), atropine (5 mg/kg, intramuscular, 90 min), L-NAME (25 mg/kg, intraperitoneal, 90 min) and indomethacin (20 mg/kg, oral, 90 min) *per se* and on SD (1 g/kg, 60 min)-induced intestinal motility in rats

ORAL TREATMENT	INTESTINAL MOTILITY	
	% Length travelled	% Control (DW)
	***per se* effect**	
Control (DW)	73.1 ± 1.56	100.0 ± 2.13
Loperamide	57.1 ± 2.05 ^{c}	78.1 ± 2.80 ^{c}
Atropine	57.0 ± 0.17 ^{c}	78.0 ± 3.24 ^{c}
L-NAME	41.5 ± 4.62 ^{c}	56.8 ± 6.32 ^{c}
Indomethacin	54.6 ± 4.48 ^{c}	74.7 ± 5.58 ^{c}
	Effect on SD-induced Motility	
SD88.	4 ± 1.22 ^{c}	120.9 ± 1.67 ^{c}
Loperamide + SD	72.7 ± 3.01***	99.5 ± 4.12***
Atropine + SD	74.3 ± 2.29***	101.6 ± 3.13***
L-NAME + SD	52.7 ± 4.38***	72.1 ± 5.99***
Indomethacin + SD	74.6 ± 2.53***	102.1 ± 3.46***

Values are expressed as mean ± SE (n=6). ^{c} P<0.001 as compared to respective control group and ***P<0.001 as compared to the respective SD-treated group

4. Discussion

Àragvadha (*Cassia fistula*) grows abundantly in regions of Asia where both hot and cold temperature extremes are common. The fruit pulp of this plant has been reported to possess laxative action and has been in use since ancient times. Biological variables like temperature, cultivation (soil), maturity (ripe/unripe) and method of preparation of the herbal drug play an important role [26]. It has been mentioned in Kalpa sthana of Charaka Samhita (K.8) [23], that the good quality fruits grown in time and ripe indicating maturity should be collected and kept within sand for a week. Thereafter they should be taken out and dried in the sun. Then their pulp should be taken out and stored in a clean container for use. Therefore, attempts have been made to do a comparative study using both sun-dried (SD) and non-sun-dried (NSD) for any difference in their purgative action in terms of their effects on intestinal motility and secretions. The result of the present study indicated that though intestinal motility and secretion were increased by both SD and NSD but SD showed equal effect on motility but less effect on intestinal secretion indicating mild action compared to NSD. Further the above action led to semisolid stool in SD while NSD-treated rats showed slight watery diarrhea in rats. As the fruit pulp powder has been in common use for children, so NSD powder may led to increased loss of water and minerals and might show cramps compared to SD powder treatment. This action corroborates the use of the sun-dried fruit pulp of *C. fistula* as referred in ancient original scripture of Ayurveda i.e., Charaka Samhita.

Anthraquinone are stimulant laxatives and derivatives of plants such as *aloe*, *cascara* and *senna*. The phytochemical review of *C. fistula* showed that the fruit pulp contained a major anthraquinone [27] and fistulic acid (anthraquinone) was also reported from the pods of *C. fistula* [19]. Stimulant laxatives (anthraquinones) have direct effects on enterocytes, enteric neurons, and GI smooth muscle and probably induce a limited low-grade inflammation in the small and large bowel to promote accumulation of water and electrolytes and stimulate intestinal motility. Mechanisms include activation of prostaglandin-cyclic AMP and NO-cyclic GMP pathways, platelet-activating factor production and perhaps inhibition of Na^+,K^+-ATPase [28]. Motility is a complex phenomenon affected both by local reflex (distension of GIT with food material or waste product) either through stretch receptors and the involvement of local hormones like NO, PGs, kinins, cholecystokinin, ATP, vasoactive protein, intestinal peptides etc or ENS stimulation via Aurbach's and Meissner's plexeses. In our present study Sun-dried fruit pulp of *C. fistula* showed mild laxative action with an increase in intestinal fluid accumulation and intestinal motility.

Acetylcholine, prostaglandins, opioid receptors and nitric oxide all play an important role in intestinal secretion and motility. Acetylcholine increases both secretion and motility of small intestine thereby increasing the passage of chyme through gut while, both PGEs & PGFs stimulate the movement of water and electrolyte into the intestinal lumen and increase the peristaltic activity secondary to increase in intraluminal fluid content [29]. Opioid receptors, μ and k affect intestinal motility and secretion respectively while nitric oxide (NO) plays a critical role in several of major physiologic processes of gastrointestinal tract like motility, secretion, digestion, absorption and elimination [30]. Therefore, attempts were made to study the effects of loperamide (an opioid, μ and k receptor blockers), atropine (anti-cholinergic), indomethacin (PGs synthesis inhibitor) and N^G-Nitro-L-arginine methyl ester (L-NAME, nitric oxide synthesis inhibitor) both *per se* as well on SD-induced purgation.

Loperamide has got little effect on ILF accumulation while atropine and indomethacin significantly reduced it. They *per se*, decreased the intestinal motility but could partially block the motility increased by SD indicating complexity of involvement of Cholinergic, ì opioid receptor and PGs in SD-induced motility. L-NAME decreased the ILF accumulation and motility equally *per se* as well as in SD-treated rats, and the decrease was more pronounced on the above parameters as compared to atropine, loperamide and indomethacin indicating the role played by NO in the actions of SD on intestinal secretion and motility. NO has also been reported to be one of the mediators of the intestinal secretion and laxative-induced diarrhea induced by castor oil [31], magnesium sulfate [32] and other anthraquinone containing laxatives such as senna and cascara [33]. Our findings with L-NAME on intestinal secretion and motility are in concordance with that of Perner and associates (2001) [34], who demonstrated that topical administration of the NOS inhibitor N-monomethyl-L-arginine reduced fluid secretion in patients with collagenous colitis.

Many medicinal activities have been attributed to the presence of alkaloids, triterpenes, anthraquinones and polyphenolics, comprising flavanoids, catechines and proanthocyanidins etc. [10,19,35]. The laxative effect of Sun-dried fruit pulp of *C. fistula* may be due to presence of anthraquinone constituent with predominant action on intestinal NO formation with cholinergic, opioids and PGs playing accessory

role. The use of the sun-dried fruit pulp of *C. fistula* as referred in ancient original scripture of Ayurveda i.e., Charaka Samhita is thus, authenticated.

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