First report on laxative activity of *Citrullus lanatus*

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Summary

This study was aimed to assess the possible laxative effect of aqueous fruit pulp extract of *Citrullus lanatus* in albino's Wistar rats. Rats were divided in 5 groups of 6 animals each, first group as control, second group served as standard (sodium picosulfate) while group 3, 4 and 5 were treated with fruit pulp aqueous fruit pulp extract of *Citrullus lanatus* at doses of 250, 500 and 1000 mg/kg body weight (b.w.), per os respectively. The laxative activity was determined based on the weight of the faeces matter. The effects of the aqueous fruit pulp extract of *Citrullus lanatus* and reference standard on the gastro intestinal motility rate were also evaluated. Phytochemicals screening of the extract revealed the presence of flavonoids, tannins, polyphenols, sterols and polyterpenes. The aqueous fruit pulp extract of *Citrullus lanatus* administered orally at three different doses produced significant laxative activity and reduced loperamide induced constipation in dose dependant manner. The effect of the extract at 500 and 1000 mg/kg (p.o.) was similar to that of reference drug sodium picosulfate (5 mg/kg, p.o). The same doses of the extract (500 and 1000 mg/kg, p.o.) produced a significant increase ($p < 0.01$) of intestinal transit in comparison with castor oil (2 ml) ($p < 0.01$). The results showed that the aqueous fruit pulp extract of *Citrullus lanatus* has a significant laxative activity.

Keywords: Laxative, loperamide, constipation, gastro intestinal motility, intestinal transit.

Introduction

Constipation also known as costiveness refers to bowel movements that are infrequent and/or hard to pass.[1] Constipation is a symptom with many causes. These causes are of two types: obstructed defecation and colonic slow transit (or hypomobility). About 50% of patients evaluated for constipation at tertiary referral hospitals have obstructed defecation. This type of constipation has mechanical and functional causes. Causes of colonic slow transit constipation include diet, hormones, side effects of medications, and heavy metal toxicity. Laxatives are among the most widely used drugs. However, their consumption is limited due to insufficient efficacy or the side effects, especially when used continuously or with contraindications. Bloating, cramping, diarrhea, and metabolic disturbances such as hypercalcemia, hyperphosphatemia, hyponatremia, and hypokalemia are among the most common side effects[5] . Cardiotoxic and arrhythmogenic effects have been reported with magnesium purgatives and cisapride[3]. The use of stimulant laxatives such as senna compounds and bisacodyl may be associated with colonic neoplasia[4-6]. The search for novel safe laxative drugs seems, therefore, inevitable. Following the traditional use of watermelon in constipation we have attempted to evaluate the laxative effect of *C.lanatus.* The present work is the report on such activity and will provide the scientific ground on the use of laxative.

Watermelon (*Citrullus lanatus)* family Cucurbitaceae is an excellent source of vitamin A, B & C necessary for energy production. Pink watermelon is also a source of the arginine, carotenoids, lycopenes, carbohydrate, sodium, magnesium, potassium & water [(5)]. Traditionally *Citrullus lanatus* is in use as energy source, cleanse and purify the kidney and bladder, lowers high blood pressure, prevent erectile dysfunction, act as antioxidant and used to treat enlarge liver & jaundice[(7-8)].

Material and methods

Preparation of the crude extract

Fresh Watermelon (*Citrullus lanatus)* was purchased from the commercial market of Jaipur (Rajasthan, India) and botanical authentification was carried out at the Department of Botany, University of Rajasthan (voucher specimen no. RUBL20685).

Fresh fruit pulp was homogenized and dried under shade and thus mass obtained was powdered, weighed and subjected to the evaluation for its laxative potential. The yield of thick dark red-brown pasty like mass was 5% (wt/wt).

Animals

Male Swiss albino mice, weighing 20–25 g, and Wistar albino rats, weighing 120-150 g, were used for acute toxicity study and evaluation of pharmacological studies. Animals were housed in

standard environmental conditions and fed with standard rodent diet and water. The Institutional Animals Ethics Committee approved all the experimental protocols.

Acute toxicity studies

The test was carried out as suggested by Ganapaty et. al. [9]. Swiss albino mice of either sex weighing between 25–30 g were divided into different groups comprising six animals each. The control group received normal saline (2 ml/kg, p.o.). The other groups received 100, 200, 300, 600, 800, 1000, 2000, 3000 and 4000 mg/kg of the test extract respectively. Immediately after dosing, the animals were observed continuously for the first 4 hours for any behavioral changes. Thereafter, they were then kept under observation up to 14 days after drug administration to find out the mortality if any.

Laxative activity test

The method of Capasso et al. [10] was followed for this activity. Rats fasted for 12 h before the experiment were placed individually in cages lined with clean filter paper. Rats were divided in five groups with the first group acting as the control and administered saline (5 mL/kg, p. o.) that acted as the negative control. The second group received sodium picosulfate (5 mg/kg, p.o), this served as the positive control. The third, fourth and fifth groups received 250, 500 and 1000 mg/kg per os of the *Citrullus lanatus* aqueous fruit pulp extract. The administration was done using metal oropharyngeal cannula. The faeces production (total number of normal as well as wet faeces) in all five groups was monitored for 16 h.

Laxative activity on loperamide induced constipation in rats

This study was carried out, as earlier described by Takahura et al. [11] Rats were placed individually in cages lined with clean filter paper, allowed to fast for 18 hours and divided into five groups of five animals each. The first group received normal saline (5 mL/kg, p.o) and served as a negative control. The second group received per os the standard drug sodium picosulfate (5 mg/kg). The aqueous fruit pulp extract of *Citrullus lanatus* (250, 500, and 1000 mg/kg, p.o.) was administered per os to the last three groups of rats. After 1 h, all the animals received Loperamide (5 mg/kg, p.o.) by gavage. The faeces production (total number of normal as well as wet faeces) in all five groups was monitored for 8 h.

Gastrointestinal motility tests in rats

The method of Mascolo et al. [12] was used. Rats were divided into different groups of five rats each and fasted for 18 hours before the experiment. Three of the groups were then treated orally with three increasing doses (250, 500 and 1000 mg/kg) of the aqueous fruit pulp extract of *Citrullus lanatus* serving as the test groups. One group served as blank or negative control treated with saline (5 mL/kg, p.o.) and the remaining group was administered castor oil (2 mL/rat, p.o.), a laxative agent, as the positive control. After 30 min, the animals were given 1 mL

of freshly prepared charcoal meal (distilled water suspension containing 10% gum acacia, 10% vegetable charcoal). Following 30 min of charcoal administration, the rats were sacrificed by cervical dislocation and the abdomen immediately cut open, to excise the whole small intestine (pylorus region to caecum). The length of the small intestine and the distance between the pylorus region and the front of the charcoal meal was measured for obtaining the charcoal transport ration or percentage.

Results

Phytochemical screening

Phytochemical analysis of the crude fruit pulp extract of *Citrullus lanatus* was performed qualitatively for the presence of carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, tannins and polyphenols. [13]

Effect of the aqueous fruit pulp extract of *Citrullus lanatus* on Laxative activity

In this study, the different doses of the extract showed dose dependant increase in fecal output of rats when compared to the control group (table 1). There was no significant difference between the extract at the dose of 250 mg/kg (p.o.) and control group. The effects of ***Citrullus lanatus*** at doses of 500 and 1000 mg/kg (p.o.) increased significantly fecal output of rats compared to control group ($p < 0.05$-0.01). The effect of the extract at the doses 500 & 1000 mg/kg (p.o.) was comparable to that of the standard drug sodium picosulfate (5 mg/kg, p.o.).

Effect of the aqueous fruit pulp extract of *Citrullus lanatus* on loperamide induced constipation in rats

In the loperamide-induced constipation, the aqueous fruit pulp extract of *Citrullus lanatus* at the doses of 500 and 1000 mg/kg (p.o.), increased the total number of faeces in a dose dependent manner, and the results were statistically significant ($p < 0.05$-0.01) (Table 2). There was no significant effect with the dose of 250 mg/kg (p.o.) of the extract compared with control. The reduction of the loperamide induced constipation at 400 mg/kg (p.o.) of plant extract treatment was found to be almost comparable with that of treatment by 5 mg/kg of sodium picosulfate.

Effect of the aqueous fruit pulp extract of *Citrullus lanatus* on gastrointestinal motility

The results of gastrointestinal motility test are reported in Table 3. The aqueous fruit pulp extract of *Citrullus lanatus* increased propulsion of the charcoal meal through the gastrointestinal tract in a concentration dependant manner. No significant effect was observed at the dose of 250 mg/kg of the aqueous fruit pulp extract of *Citrullus lanatus*, but the doses of 500 mg/kg and 1000 mg/kg (p.o.) of the extract produced a significant increase in the propulsion of charcoal meal compared to control group (normal saline, 5 mL/kg, p.o.) ($p < 0.01$). Castor oil (2 mL/rat, p.o.) produced greater gastrointestinal motility effect than the highest dose of the extract (1000 mg/kg, p.o.) used.

Table 1: Laxative activity of aqueous extract of *C.lanatus* in rats

Group (Treatment)	Dose mg/kg	Faeces out put (g) 0-8h	8 h-16 h
Control (Saline)	5 mL/kg	1.862 ± 0.34	0.946 ± 0.12
Sodium picosulfate	5 mg/kg	5.522 ± 0.56**	5.190 ± 0.91**
C.lanatus	250 mg/kg	1.940 ± 0.58	0.892 ± 0.17
C.lanatus	500 mg/kg	2.873 ± 0.19*	2.170 ± 0.47*
C.lanatus	1000 mg/kg	4.648 ± 0.68**	4.239 ± 0.38**

Values are expressed as mean ± S.E.M (n = 6); * $p < 0.05$ compared to control group; and **$p < 0.01$ compared to control group.

Table 2: Effect of *C.lanatus* aqueous extract on loperamide induced constipation in rats

Group (Treatment)	Dose mg/kg	Weight of faeces (g)
Control	5 mL/kg	0.938 ± 0.45
Sodium picosulfate	5 mg/kg	4.14 ± 0.34**
C.lanatus	250 mg/kg	1.602 ± 0.36
C.lanatus	500 mg/kg	2.737 ± 0.28*
C.lanatus	1000 mg/kg	3.263 ± 0.25**

Values are expressed as mean ± S.E.M (n = 5); * $p < 0.05$ compared to control group; and **$p < 0.01$ compared to control group.

Table 3: Effects of *C.lanatus* aqueous extract on gastrointestinal motility in rats

Group (Treatment)	Dose mg/kg	Percentage of distance (%)
Control (Saline)	5 mL/kg	54.43 ± 3.94
Castor oil	5 ml/rat	92.61 ± 2.74***
C.lanatus	250 mg/kg	56.54 ± 4.48
C.lanatus	500 mg/kg	72.11 ± 1.74**
C.lanatus	1000 mg/kg	79.37 ± 1.88**

Values are expressed as mean ± S.E.M (n = 5); ** $p < 0.01$ compared to control group; and ***$p < 0.001$ compared to control group.

Statistical analysis

Data obtained are presented as means ± standard error of mean (S.E.M.) for the number of animals in each group (n = 6). The differences between the data obtained from 'test' animal groups and the data obtained from untreated animal groups, were subjected to one- way analysis of variance (ANOVA; 95% confidence interval), followed by Dunnett's test. Values with $p < 0.05$ compared with the control group were considered as being significantly different.

Discussion

The laxative activity of *C. lanatus* was studied in rats. The results showed that an oral administration of the fruit pulp aqueous extract of *C. lanatus* produced significant and dose dependant increase in faeces output of rats and the stimulation of gastrointestinal motility. These effects were comparable with that of castor oil (standard drug) at moderate dose of 500 mg/kg high dose of 1000 mg/ kg. Castor oil affects electrolyte transport and smooth muscle contractility in the intestine. Its cathartic action is due to water accumulation in the intestine [(14)]. Castor oil causes diarrhea due to its active metabolite ricinoleic acid [(15)] which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. The observed activities therefore suggest that laxative activity of the aqueous fruit pulp extract of *C. lanatus* may be mediated through this mechanism of action of ricinoleic acid. Adverse effects associated with use of castor oil are intestinal blockage, appendicitis, abdominal pain or cramping, nausea, bloating, vomiting, inflamed bowels or fecal impaction [(20)] etc. that can be overcome, possibly by replacing it with *C. lanatus* for treatment of constipation.

The results showed that the aqueous extract of *C.lanatus* increased the propulsion of charcoal meal. The propulsion of charcoal meal is probably due to the increasing of peristaltic movement in rat gastrointestinal tract resulting from the stimulation of cholinergic receptors by *C. lanatus*. The intestinal transit is controlled by both neural and myogenic mechanisms [(16)]. An increase of the contractile activity of the smooth layers in general is responsible for acceleration of intestinal propulsion. Several mediators and neurotransmitters govern these motor patterns. Acethylcholine is the main excitatory neurotransmitter in the enteric nervous system [(17)]. Thus the presence of cholinomimetic constituents in the plant extract can explain the usefulness of *C. lanatus* in constipation pointed out by the ethnobotanical informations [(18)].

The Presence of phytoconstituents like terpenoids, sterols, flavonoids, phenolic compounds [(19)] have been previously found to be responsible for laxative activities in plants. Phytochemical screening of the extract of *C. lanatus* revealed the carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, tannins and polyphenols. These constituents may be responsible for the laxative activity of *C. lanatus***.**

Conclusion

This study has shown that fruit pulp of *C. lanatus* has laxative effects in addition to the various physiological effects earlier reported by other authors. Further studies may be directed at characterizing the bioactive ingredients that are responsible for the observed activity in the plant.

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