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Acute Toxicity Study and Faecal Dropping Capability of Ethanolic Extract of *Tecoma stans* in Albino Rats

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ABSTRACT

Objective: Diarrhea is one of the widespread wellbeing situation upsetting people in less developed countries. This study was done to appraise anti-diarrheal influence of the ethanolic flower extract of *Tecoma stans* (EETS) using Wistar albino rats to determine the acute toxicity in mice. **Material and Methods:** The flowers were collected and extracted with 70% ethanol. The dry extract was reconstituted using normal saline for the oral administration to diverse groups of rats at doses of 200, 400 and 600 mg kg^{-1}. Anti-diarrheal activity was resulted using the percentage fall in the incidence of defecation in rats with castor oil-induced diarrhea. Loperamide (1 mg kg^{-1}) was worn as positive control. Acute toxicity was evaluated by fortitude of LD_{50} and observations of toxic signs. **Results:** EETS showed momentous ($p<0.05$) antidiarrheal activity evidenced by the lessening in rate of defecation by up to 78.33% at 600 mg kg^{-1} b.wt. analogous to loperamide (100%). This activity could be accredited to the phytochemicals such as flavonoids and tannins in *Tecoma stans* that were present in high levels and have been reported to demonstrate antidiarrheal activity through denaturing protein hence forming protein tannates which curtail the intestinal mucosa permeability. The LD_{50} of the crude EETS was 10,715 mg kg^{-1}. **Conclusion:** The findings of this study illustrate that the flower of *Tecoma stans* have a very significant antidiarrheal commotion and are not dangerous to use as indicated by the high LD_{50} value. This ropes the conventional use of the EETS as herbal therapy for treatment of diarrhea.

Key words: *Tecoma stans*, diarrhea, castor oil, permeability, flavanoids

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INTRODUCTION

Diarrhoeal diseases are one of the foremost causes of childhood morbidity and mortality in developing countries. An anticipated 1000 million episodes crop up each year in children under 5 years of age. Diarrhoeal causes a projected 5 million death in children fewer than 4 years of age per year[1]. Commonness of diarrhoeal diseases still relics high despite intervention of government agencies and international organization to halt the drift. The use of herb drugs in the management of diarrhoeal diseases is a frequent practice in many African countries[2]. Despite immense technology advancement in medicine, many people in budding countries still rely on traditional healing practices and medicinal plant\for their day after day health care need[3]. The World Health Organization (WHO) optimistic studies for the treatment and preclusion of diarrhoeal diseases depending on traditional medical practices[4]. There is an imperative necessitate for the intensification of research into medicinal plant claim to be effective in the management of diarrhoeal diseases.

The majority of populace living in rural areas roughly always utilizes traditional medicines to treat all types of diseases counting diarrhea. Many plants available in Uganda, namely *Tecoma stans*, *Pseudathri hookeri*, *Capiscus frutescens*, *Cannabis sativa*, *Hibiscus aponeurus*, *Toddalia asiatica*, *Bidens pilosa*, *Vernonia amygladina*, *Rhus vulgaris*, *Hypericum peplidifolium*, *Paullia pinnata*, *Canthiium gueinxii*, *Maesa lanceolata*, *Leonotis mollissima*, *Annona senegalnesis*, *Ehretia cymosa*, *Lippia javanica*, are used in traditional folklore medicine for treatment of diarrhea[5].

Tecoma stans (common name yellow bell) also known as yellow trumpet bush belongs o the family Bignoniaceae. It is an ornamental plant. It is an erect, branched, sparingly hairy or nearly smooth shrub

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two to four meters in height. The flowers are opposite, odd-pinnate, Up to 20 cm in length with 5 to 7 leaflets. The leaflets are lanceolate to oblong-lanceolate, 6 to 13 cm long, pointed at both ends and toothed on the margins. Trumpet shaped flowers are yellow faintly scented and borne in short, dense, terminal clusters. The calyx is green 5 to 7 mL long and 5 toothed. Flowering can begin as early as April and continue in to fall. The flowers are followed by 6 inch long, tan pods that are filled with small, papery winged seeds[6].

Flowers of *Tecoma stans* contain the alkaloids tecomin and tecostamine are potent hypoglycaemic agent when given intravenously. Anthranilic acid is responsible for the anti diabetic activity. Roots are powerful diuretic and vermifuge[7]. *Tecoma* is not toxic because this plant is used in latine America as a remedy for diabetes and moreover for feeding cattle and goats in Mexico[8]. The aim of the present study, to evaluate anti diarrheal activity of ethanolic extract of *Tecoma stans* in castor oil induced diarrhea in albino rats.

MATERIALS AND METHODS

Collection and extraction of plant: The flowers of *Tecoma stans* were collected in the month of May 2011 from Rasipuram (Namakkal District) Tamil Nadu. A herbarium specimen of the plant was deposited in the Department of Pharmacognosy. The plant was identified by Dr. G.V.S. Murthy, Joint Director of the Botanical Survey of India, Southern circle, TNAU Campus, Coimbatore, who authenticated the plant from information available in the literature. The flower petals were dried in the shade for 10-12 days. After complete drying, the flower petals were pulverized to a coarse powder of 40 mesh size in a mechanical grinder. The powdered material was subjected to soxhlet extraction using ethanol for 18 h at 50-55°C. The extract was thereafter concentrated under vacuum and air-dried[9,10,11].

Dosage formulation: For the formation of the dosage EETS was reconstituted by suspending crude extract (4 g) in 20 mL of distilled water to acquire a stock solution of 200 mg mL^{-1}. The quantity of the extract to be administered was calculated[12] using the formula:

$$\text{Volume of the extract (mL)} = \frac{\text{Weight of the rat (kg)} \times \text{Dose rate (mg kg}^{-1}\text{)}}{\text{Stock concentration (mg mL}^{-1}\text{)}}$$

Animals: Wistar albino rats (30Nos) of either sex weighing between 100-150 g. The rats were used for this activity. The animals were allowed to acclimatize for 2 weeks prior to the study. They were housed in clean cages and maintained under standard laboratory condition of 12 h natural light and 12 h darkness at ambient room temperature. The rats were fed with standard pellets and water was made available *ad libitum*.

Acute toxicity test of *Tecoma stans* extract: Albino mice were fasted for 8 h but provided with water *ad-libitum* and were divided into five groups of 6 animals in each group. Group 1 served as negative control group, received 0.5 mL of normal saline orally animals of the group 2, 3, 4 and 5 were given with EETS at the doses of 5000, 10000, 15000 and 20000 mg kg^{-1} correspondingly prepared from a stock of 400 mg mL^{-1} of EETS. The total numeral of dead mice per group within 24 h was noted and percent mortality deliberated. The signs of the toxicity were also observed and recorded.

Evaluation of antidiarrheal activity: Wistar albino rats were fasted for 18 h prior to the experiment but given water ad-libitum and divided into five groups of 6 rats each. The rats in group 1 (negative control group), received 1 mL of distilled water orally. The rats in groups 2, 3 and 4 were treated with the ethanolic flower extract of *Tecoma stans* at doses of 150, 300 and 500 mg kg^{-1} b.wt. by oral route, respectively. The rats in group 5 (positive control group) were treated with loperamide at the dose of 1 mg kg^{-1} b.wt. orally. After an hour of dosing, all the rats were treated with 1.5 mL of castor oil orally using a gavage tube to induce diarrhea. The rats were then observed for frequency and consistency of fecal material. The numbers of wet fecal droppings were counted within 4 h after castor oil administration[13,14].

Statistical analysis: The experimental results were expressed as the Mean±SEM of five determinations. The ANOVA test was used to assess for any statistically significant differences between the treatment and control groups while the Dunnet method was used to test mean differences. p-values less than 0.05 were considered significant. For LD_{50} determination, the percent mortality at each dose was transformed into probit[15,16,17] and presented as mortality probit versus log dose plot. Correlation was done between mortality probits and log dose and the correlation coefficient (R2) determined.

RESULTS

Acute toxicity: The most dominant signs of acute toxicity effects were: laboured breathing, irritation, hind limb paralysis, convulsions and ataxia. The severity of the signs of toxicity improved with enlarges in the dose. There was no observed sign of toxicity in the group of mice that received normal saline. The Lowest Observed Effect Level (LOEL) recorded was at a dose rate of 5000 mg kg^{-1}. The number of death reported at LOEL was one mice out of six (16.7%) and the corresponding toxic effects included hypoactivity and laboured

Table 1: Mortality data for ethanolic flower extract of *T. stans*

Dose (mg kg^{-1})	Log dose	Dead/total	Dead (%)	Corrected dead (%)	Probits
0.5 mL normal saline	-	0	0.0	0.0	0.00
5,000	3.70	1/6	16.7	16.7	3.51
10,000	4.00	2/6	33.3	33.3	4.59
15,000	4.18	4/6	66.7	66.7	5.43
20,000	4.30	6/6	100.0	95.8	6.73

N = 6 mice per group

Table 2: Effect of EETS on loperamide induced diarrhea

Treatment	No. of wet faeces	Inhibition of (%)	DF	p-value
1.5 mL normal saline + castor oil	6.1±0.8100	0.000	5	-
1 mg kg^{-1} Loperamide + castor oil	0.00±0.000	100.000	5	0.011
150 mg kg^{-1} EETS + castor oil	4.66±0.940	21.669	5	0.390
300 mg kg^{-1}EETS + castor oil	3.32±0.0.48	45.001	5	0.390
500 mg kg^{-1}EETS + castor oil	1.32±0.600	78.329*	5	0.041

*p<0.05, when compared with the negative control, Data was expressed as Mean±SEM, n = 6, DF: Degree of freedom

breathing. At the highest dose level, all mice in the group (100%) died within 3 h post-administration and signs of toxicity included ataxia, convulsion, laboured breathing and paralysis of the hind limb (Table 1).

The dose of the extract that killed all the mice in a group was 20,000 mg kg^{-1}. The calculated LD50 of *T. stans* was 10714 mg kg^{-1}.

Anti-diarrheal activity of *Tecoma stans*: The mean frequency of wet faecal droppings decreased with increase in dose of the ethanolic flower extract of *Tecoma stans*, with the mean frequency of defecation being lower in the group that was treated with 500 mg kg^{-1} and higher in the groups that was treated with 300 mg kg^{-1} and 150 mg kg^{-1}, respectively as shown in Table 2. The mean frequency of wet faecal droppings for the rats in the loperamide-treated group was 0.0; hence it totally inhibited diarrhea. The negative control group treated with 1.5 mL distilled water had the highest mean frequency of faeces (Table 2), hence there was no inhibition of diarrhea in this group. The extract exhibited significant anti-diarrheal activity against castor oil induced diarrhea in laboratory albino rats. There was statistically significant reduction ($p<0.05$) in the number of wet faeces by 78.329% at 500 mg kg^{-1} when compared to negative control rats. There was no significant difference ($p>0.05$) in percent reduction of wet faeces for the 150 and 300 mg kg^{-1} groups when compared with the negative control group. The extract thus showed dose dependent inhibition of diarrhea as shown in Table 2.

The activity of the extract at 500 mg kg^{-1} was comparable to that of loperamide since there was no significant difference (p-value, 0.082) in the mean number of faecal droppings between these two groups.

DISCUSSION

This study involved costing of the anti-diarrheal activity of the ethanolic flower extract of *Tecoma stans* in castor oil induced diarrhea in albino rats using loperamide as positive control, resolute its acute toxicity in mice and its phytochemical composition. The extract exhibited noteworthy anti-diarrheal activity in laboratory albino rats with statistically momentous decline in the number of wet faeces by 78.329% at 500 mg kg^{-1} when compared to negative control rats ($p<0.05$). The activity of the extract at 500 mg kg^{-1} was analogous to that of loperamide since there was no significant diversity in the mean number of fecal droppings between these two groups ($p>0.05$). The anti-diarrheal activity was dose-dependent.

Phytochemical screening of the extract showed towering levels of tannins and flavonoids and these phytochemicals could be accountable for the anti-diarrheal activity observed in this study through shyness of peristaltic movement. Tannins and tannic acids also denature proteins forming tannates which decrease the intestinal mucosa permeability[18]. Other studies point toward that flavonoids[19] and alkaloids[20] possess antidiarrheal activity. The antidiarrheal activity of flavonoids has been endorsed to their ability to inhibit peristaltic activity and hydroelectrolyte secretion[21,22], which increase in diarrhea. *In-vitro* and *in-vivo* experiments by Sanchez *et al.*[23], have shown that flavonoids are able to inhibit the intestinal secretory response and induce prostaglandin E2. In addition, flavonoids hold antioxidant properties[24] which are presumed to be accountable for inhibitory effects exerted upon several enzymes counting those involved in the arachidonic acid metabolism[25]. Therefore it is possible that the antisecretory, anti-inflammatory and antioxidant properties of flavonoids could be dependable for the antidiarrheal activity of *Tecoma stans*. Therefore a mishmash of tannins and flavonoids presumably led to a synergistic anti-diarrheal activity in albino mice.

The LD_{50} of the ethanolic leaf extract of *Tecoma stans* in mice was determined as 10,715 mg kg^{-1} indicating that the leaves of plant have stumpy toxicity as compared to the value of 5000 mg kg^{-1} which is painstaking to be practically safe. In toting up to the antidiarrheal activity of this plant confirmed in this study, other studies have shown other medicinal effects such as antiinflammatory, antihemostatic[26,27], antimala-rial[28], antimicrobial[29], antifungal[30,31] and anti-diabetic effects.

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