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Research Article

ANTIDIARRHOEAL ACTIVITY OF *Sebastiania chamaelea* Muell. Arg

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ABSTRACT

Objective: There is an immense need in the herbal remedies still in the treatment of diarrhea. The juice of the *Sebastiania chamealea* is used as astringent and used as a remedy for diarrhea by the herbalists. Hence antidiarrhoel activity of leaf extracts was carried out.

Methods: Acute toxicity studies were carried out as per OECD guidelines up to 3000mg/kg b.w, observed for 14 days. Antidiarrhoel studies by castor oil induced fecal defecation effect up to 4 hrs, and also eneropooling of intestinal fluid content was observed by the methods of Awoters *et al*; Zaval *et al* and Robert *et al*.

Results: There was no toxicity up to maximum of 3000 mg/kg b.w was observed without any mortality of rats. 89-90% of inhibition of fecal defecation was observed with the methanol extracts at 200 mg/kg b.w, and 63.85% inhibition of intestinal fluid content on castor oil induced rats compared with atropine 3 mg/kg at an 35% inhibition of fecal defecation and 52.55% of intestinal content inhibition.

Conclusion: Aqueous and methanolic leaf extracts of *S.chamaelea* at 100-200 mg/kg b.w showed 98% effective antidiarrhoel activity to that of *Securinega virosa* at low concentrations with 90% of inhibitory activities. Hence there is a need to isolate related tannins which may be the potential drugs to control diarrhoea and its possible causative pathogens as *Bacillus* and *Vibrio* strains.

Keywords: Acute toxicity, Castor oil, Fecal defecation, Enteropooling, Intestinal fluid.

INTRODUCTION

Oral Rehydration Therapy (ORT) against diarrhoea has not been much applauded by the scientists. Reports showed that the herbal remedies are still relevant in the treatment of diarrhoea by mothers in Mexico[**1**]. However the effectiveness of these anti-diarrhoeal herbal drugs has not been scientifically evaluated **[2].** The herbal medicinal plant *Sebastiania chamaelea* (Euphorbiaceae) whole plant decoction in ghee is given as tonic and applied to the head in vertigo. The juice of the plant is astringent and is used as a remedy for syphilis and diarrhea **[3-4]**. *S.chamaelea* accounted for 77.5% of human necessary amino acids, among which argenine stands highest with 60% of free amino acids may promoted the role of its medicament activity **[5].** *S. chamaelea* leaf methanolic and aqueous extracts reported the presence of flavonoids, phenols, tannins, glycosides and sterols. Qualitative analysis of phenolic acids reported the presence of caffeic acid, melilotic acid, aesculetin, p-hydroxy benzoic acid, coumarin, cinnamic acid, salicylic acid and scopoletin along with five flavonoids myrecetin, quercetin, kaempferol, luteolin, and apigenin **[6].** Hence it was aimed to investigate further the leaf aqueous and methanolic extracts for their antidiarrhoeal activity.

Alkaloid extract of *E.hirta* showed significant antibacterial activity against *Enterobacter aerogens, Bacillus subtilis, Klebsiella pneumonia, Raoultella planticola and Agrobacterium tumefaciens.* MIC values are 0.039 mg/ml **[7]**. In Orissa tribes of the Kennojhar and Mayurbhanj districts *Jatropha curcas* (Baigaba, Dhala Jahaji) latex with ripen banana given orally once or twice a day to check dysentery in adults. *J. gossypifolia* (Nali) stem bark aqueous extract given orally twice a day to cure blood dysentery. And also *Phyllanthus fraternus* (Bhuin amla) root paste is administered children (below 2 years) twice a day for three days to control diarrhea **[8]**. *Mallotus philippensis Var philippensis* stem extracts of hexane, chloroform and ethanol at 100 mg/ml concentration expressed effective on gram negative like *Vibrio pharahaemolytius* and *E.coli* strains than on gram positive bacterial and fungal strains only with ethanol extracts to that of the standard drugs antimicrobial activity *Vibrio* and *Bacillus* strains which causes diarrhoel infections reveals its herbal activity against diarrhea **[9].** *S.chamaelea* leaf methanolic extracts also showed effective inhibition on the growth of *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* than the standard drug. [6]

MATERIALS AND METHODS

Collection and identification of Plant material

The medicinal herb *Sebastiania chamaelea (Euphorbiaceae)* was collected from the fields of S.V.Veterinary College, Tirupati, Andhra Pradesh, India during the month of June.2010 and preserved as per the standard method **[10].** The taxonomic identity was confirmed by Prof. N. Yasodamma, Department of Botany, Sri Venkateswara University, Tirupati. The voucher specimen KS711 was preserved in the Herbarium, Department of Botany. Leaves were thoroughly washed and dried under shade for one week. Dried leaves were ground in a mixer grinder and sieved. Powder was stored in air sealed polythene bags at room temperature until further use.

Preparation of aqueous and methanol extracts

Dried leaf powder (70 g) was soaked in water, after 72 hrs the filtrate was dried on water bath. Simultaneously the dried powder (40 g) was extracted in a Soxhlet apparatus using 200 ml of methanol then the filtrate was concentrated on rotavapour and dried. Both the extracts were stored at 4^0C in refrigerator until further use.

Animal selection

For acute toxicity and antidiarrhoeal activity male Wistar albino rats weighing between 150-200 gms were selected. The animals were acclimatized to standard laboratory conditions (temperature 25±2oC) and maintained on 12 hours light; 12 hours dark cycle. They were fed with *adlibitum*. The experiment was conducted according to the ethical norms approved by CPCSEA, Ministry of social justice and empowerment, Government of India and ethical clearance was granted by institutional ethical committee resolution no.11/2011-2012/(i)/ a/CP CSEA/ IAEC/ SVU/NY-KSS/dt:14/11/2011.

Acute toxicity study

Acute toxicity studies of aqueous and methanol extracts were carried out as per the 423 guidelines set by OECD (Organization for Economic Co- operation and development). Albino rats (n=5) of either sex were selected by random sampling technique, were used for the study. Aqueous and methanol extracts were orally administered by using a gavage, at the dose level of 10, 100, 1000, 1500 and 3000mg/kg b.w and observed for 14 days.

Antidiarrhoeal activity

Castor oil induced diarrheoa

Antidiarrhoeal activity of aqueous and methanol extracts were studied by castor oil induced diarrheoa method **[11].** The male wistar albino rats weighing 150-200 g were fasted for 18 hrs before the test and divided into six groups of five rats each. Control group I received vehicle (1% Tween 80 in water) at a dose of 10 ml/kg orally. The positive control group II received atropine at the dose of 3 mg/kg, test groups III and IV received aqueous extract and test groups V and VI received methanol extracts oral doses of 100 and 200 mg/kg b.w. Each animal was placed in an individual cage, the floor of which was lined with blotting paper and was changed by every hour. Animals in each group treated with castor oil at the dose of 10 ml/kg b.w, after 30 min of the plant extracts treatments. All animals were observed for defecation up to 4 hrs. The frequency of defecation and number of diarrhoeal feces excreted in the recorded time were scored and compared with control group. The results were expressed in percentage of inhibition**[12].**

Castor oil- induced enteropooling

Intra-luminal fluid accumulation was determined by the castor oil induced enteropooling method **[13]**. Overnight fasted rats were divided into six groups of five animals each. Control group-I received vehicle (1% Tween 80 in water) at a dose of 10 ml/kg orally. The positive control group-II received atropine at the dose of 3 mg/kg, test groups III and IV received aqueous extract and test groups V and VI received methanol extracts of oral doses 100 and 200 mg/kg b.w. Castor oil was administered orally after 30 min of drug administration. Two hours later rats were sacrificed, and the small intestine was removed after tying the ends with thread and weighed. The intestinal contents were collected by milking into a graduated tube and their volume was measured. The intestine was reweighed and the difference between full and empty intestine was calculated.

Statistical Analysis

The results were analyzed for statistical significance using one way Anova followed by Dunnet, s test. The p< 0.01 was considered significant.

RESULTS

Acute toxicity study

Acute toxicity studies with aqueous and methanol extracts of leaf did not show any significant toxicity signs when observed for first four hours and followed by daily observations for 14 days also no mortality was observed. The drug was found to be safe at all test doses of 10, 100, 1000, 1500 and 3000 mg/kg b.w.

Castor oil induced diarrhoea (Table.1)

Both aqueous and methanol extracts at different dose levels significantly reduced the number of defecation and wet fecal matter in comparison to control drug atropine. Percentage inhibition of diarrhoea at 100 and 200 mg/kg 72.42%, 75.87% and 75.87%, 89.66% respectively compared to that of the standard drug atropine 34.49%; It was observed that methanol extract at 200 mg/kg b.w showed more inhibitory activity on fecal defecation than aqueous extract.

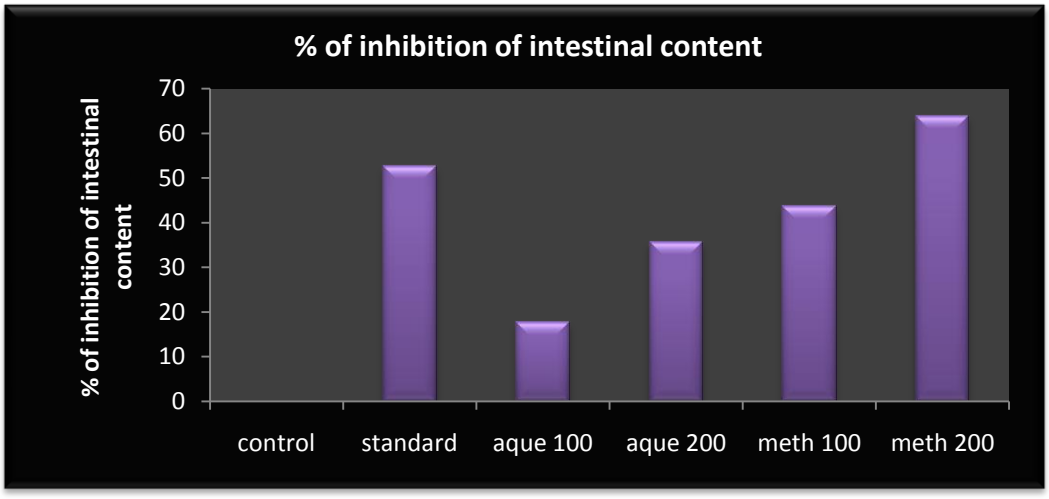
Castor oil induced enteropooling (Graph. 1)

Castor oil caused accumulation of water and electrolytes in intestinal loop. Aqueous and methanol extracts at 100 and 200 mg/kg showed 17.52%, 35.60% and 43.51%, 63.85% of inhibition of weight of intestinal content respectively. Positive control, atropine 3 mg/kg, po showed 52.55% of inhibition of intestinal content can be compared to the activity of methanol extracts at 200mg/kg and was more effective than aqueous extracts.

Table 1: Effect of inhibition in faecal defecation

Treatment Groups	Dose (mg/kg) b.w	Mean frequency Defecation in 4 hours.	Mean fecal defecation in 4 hours.	% of activity
I. Control (castor oil)	10 ml/kg	7.8±0.20	5.8±0.20	-----
II. Atropine	3	6.4±0.24**	3.8±0.20**	34.49
III. Aqueous extract	100	7.2±0.20	1.6±0.24**	72.42
IV. Aqueous extract	200	5.6±0.24**	1.4±0.24**	75.87
V. Methanolic extract	100	6.4±0.24**	1.4±0.40**	75.87
VI. Methanolic extract	200	6.8±0.48	0.6±0.24**	89.66

All the data are expressed as mean ±SEM: **indicate *p*<0. 01 as compared to control group, n=5: One -way ANOVA followed by Dunnett's test.



Graph 1: Percentage of inhibition of intestinal fluid content

Control: Castor oil; standard: Atropine; aque: Aqueous; meth: methanol.

DISCUSSION

An indigenous drug formulation (IDF-16) containing 1.4 % of *Jatropha multifida* stem found to possess antidiarrhoeal index of 77% on rat and mice on gastro intestinal motility of castor oil induced purgation[**14]**. Red sap of *Croton urucurana* (Dragon's blood) bark oral dose at 600 mg/kg on rats **[15];** *Alchornea cordifolia* leaf extracts at 800 mg/kg b.w on mice showed effective antidiarrhoeal **[16]**. *Euphorbia paralias* aerial part methanol extracts at 400 mg/kg on rats **[17]**. *Emblica officinalis* methanolic extracts **[18]**. Aqueous extracts of *E. hirta* **[19].** *Securinega virosa* methanolic root bark extracts produced 100% protection at 100mg/kg to that of 5mg/kg loperamide control drug [**20]**. *E.indica* aerial part petroleum ether, benzene, chloroform and ethanol extracts showed antimotility with 68.61% to that of control drug morphine sulphate 30mg/kg **[21]**. *Manniophyton africanum* leaf methanolic extracts inhibited the fecal droppings and charcoal plug at 600 mg /kg in rats **[22]**. *J. curcas* petroleum ether and methanol extracts at 50-400mg/kg in mice inhibited fluid accumulation **[23]**.

The Aqueous and methanolic leaf extracts of *S.chamaelea* at the doses of 100 and 200 mg/kg exhibited significant anti-diarrhoeal activity upto 90% may be due to the presence of tannins, which may form protein tannate cause an astringent action and may resulted effective antidiarrhoeal activity and also due to the presence of caffeic acid may act as anti inflammatory activity **[24]** Presence of cinnamic acid acts as antifungal, anthelmintic, natural protection against pathogenic infections[6,**25]**.

CONCLUSION

Aqueous and methanolic leaf extracts of *S.chamaelea* at 100-200 mg/kg b.w showed most effective antidiarrhoel activity to that of the *Securinega virosa* at low concentrations 90% of diarrhoeal inhibitory activities. Hence there is a need to isolate related tannins which may be the potential drugs to control diarrhoea and its possible causative pathogens as *Bacillus* and *Vibrio* species.

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