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Original Research Article

EVALUATION OF ANTI-DIARRHOEAL ACTIVITY OF HYDRO-ALCOHOLIC LEAF EXTRACT OF *PHLOGACANTHUS THYRSIFLORUS* IN ALBINO WISTAR RATS.

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Abstract

Objective: In the present study Anti-diarrhoeal activity of Hydroalcoholic extracts of Leaves of *Phlogacanthus thyrsiflorus* were investigated. **Material & Method:** Anti-diarrhoeal activity of Hydroalcoholic extracts of Leaves of *Phlogacanthus thyrsiflorus* were evaluated by castor oil-induced-diarrhoea model & small intestine transit model in experimental rats. The Hydroalcoholic extracts of Leaves of *Phlogacanthus thyrsiflorus* (100 & 200 mg/kg) treat the Diarrhoea and produced significant reductions in fecal output and frequency of droppings. Standard drug Loperamide (3 mg/kg, p.o) was shown significant reductions in fecal output and frequency of droppings whereas HEPT at the doses of 100 and 200 mg/kg p.o significantly ($P<0.05$) reduced the castor-oil induced frequency and consistency of diarrhoea. **Conclusion :** The HEPT showed marked reduction in the number of diarrhoea stools as well as a modest reduction in intestinal transit. The results obtained establish the efficacy and substantiate the folklore claim as an anti- diarrheal agent. Further studies are needed to completely understand the mechanism of anti-diarrhoeal action of *Phlogacanthus thyrsiflorus* leaf.

Keywords: Antidiarrhoeal Activity, *Phlogacanthus thyrsiflorus* Traditional medicine, Castor oil induced diarrhoea, Small intestinal transit, charcoal

Introduction

Diarrhoea is a condition of having three or more loose or liquid stools per day. It is a common cause of death in Third World Countries and the second most known cause of children deaths worldwide. The loss of fluid and electrolytes through diarrhoea can cause dehydration and electrolyte imbalances. In 2009, diarrhoea was estimated to have caused 1.1million deaths in people aged 5 years and over, and 1.5 million deaths in children under the age of 5 years. Oral rehydration solutions are the treatment of choice and have been

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estimated to have saved 50 million children in the past 25 years [1-2].

Phlogacanthus thyrsiflorus Nees is found in the sub tropical Himalayas, upper Gangetic plain, Bihar, North Bengal and Assam. *Phlogacanthus thyrsiflorus* Nees is a medicinal herb which belongs to Acanthaceae family. It is known as Vasaka in Hindi. An evergreen shrub upto 2.4 m high, branchlets quadrangular, leaves are 13-35 cm long, oblanceolate, elliptic oblong, acute or acuminate, entire. Whole plant is used like *Adhatoda vasica* in Whooping cough and Menorrhagia. Fruits and leaves are burnt and it is prescribed for fever. The leaves are reported to contain diterpene lactone, Phlogantholide A. A decoction of leaves is also beneficial in liver and spleen diseases. Jaintia tribe of Meghalaya uses fruit and leaf ash of *Phlogacanthus thyrsiflorus* Nees and use it to treat fever. Ethanolic extract of *Phlogacanthus thyrsiflorus* Nees has analgesic activity on experimental mice. *Phlogacanthus thyrsiflorus* Nees has antimicrobial activity also. The generation of free radicals has been implicated in the causation of several diseases of known and unknown etiologies such as Rheumatoid Arthritis, Cancer, Diabetes etc., and compounds that can scavenge free radicals have great potential in ameliorating these disease processes. *Phlogacanthus thyrsiflora* Nees has prominent free radical scavenging property so it may prove as a very good medicinal herb. In the current literature there is not much data concerning the effect of *Phlogacanthus thyrsiflorus* on the GI Tract are abnormally altered in diarrhoea [3-6].

Material and Methods

Collection, identification and authentication of plant

The Plant *Phlogacanthus Thyrsiflorus Nees* (Leave) were collected from the Dibrugarh, Assam during the month of July-2012. The plant material was identified and authenticated by Prof. P. Jayaraman (Ph.D.), Director-Plant Anatomy Research Centre (PARC) Tambaram. The voucher specimen number is PARC/2012/1702 (b) and it was submitted to the laboratory of Department of Pharmaceutical Science, Shri Venkateshwara University Gajraula, Amroha (Uttar Pradesh) for future references.

Collection and maintenance of experimental animals

Wistar albino rats of either sex weighing between 150-250 gm of either sex were used. Institutional Animal Ethics Committee of Nagaji Institute of Pharmaceutical Science, Gwalior approved the experimental protocol; animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA Reg.No.-1498/PO/a/11/CPCSEA). The animals were housed in Poly propylene cages and maintained at 24˚C ± 2˚C under 12h light/ dark cycle and were feed *ad libitum* with standard pellet diet and had free access to water.

Acute Toxicity Studies

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD) received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). One-tenth of the median lethal dose (LD_{50}) was taken as an effective dose. Acute toxicity study was done as per OECD, 2006 Guidelines.Acute oral toxicity tests found the LD_{50} of the Plant extract to be >2,000 mg/kg. The animals were observed for signs of toxicity such as hyperactivity, grooming, convulsions, sedation, and hypothermia continuously for 2 hours, and for mortality up to 24 hours, after administration of the doses.

Extraction Method

The leaf of Phlogacanthus thyrsiflorus were shade dried and reduced to coarse powder in a mechanical grinder. The powdered material obtained was then subjected to successive extraction by Hot

Percolation Method using petroleum ether, chloroform, and Hydro-alcoholic solvents in a soxhelet extractor. The different extracts obtained were evaporated at 45˚C to get a semisolid mass. The extracts thus obtained were subjected to phytochemical analysis. The percentage yield of Hydro-alcoholic Extract of Phlogacanthus thyrsiflorus (HEPT) was found to be 9.5 w/w% [7].

Phytochemical Analysis of the Extracts

Preliminary phytochemical analysis of HEPT revealed that the presence of various phytoconstituents Cardiac glycosides, Steroids and Triterpenoids, Flavonoids and Tannin & Phenolic compound and Saponin in trace amount [8-9] .

Chemical and drug

Pet**.**ether, Chroform, Hydroalcoholic solvent (Methanol plus Aq. solvent), Anaesthetic ether, castor oil, sodium carboxy methyl cellulose, charcoal meal, Atropine sulfate, Loperamide.

Evaluation of Anti Diarrhoeal activity of *Phlogacanthus thyrsiflorus Nees* By

The antidiarrhoeal activity of *Phlogacanthus Thyrsiflorus Nees* was evaluated according to the method described by Teke *et al*., 2007. Rats were fasted for 18 hours and divided into four groups of five animals each group. Castor oil at a dose of 1 ml was given orally to all groups of animals for the induction of diarrhea. One hour prior to castor oil administration various treatments were given, Group I (control) received 0.5 % sodium carboxy methyl cellulose (Na CMC), Group II (standard) were treated with Loperamide (3 mg/kg, p.o.), a positive control. Group III-IV were administered Hydro-alcoholic Extract of *Phlogacanthus Thyrsiflorus Nees* (HEPT) in 100 mg/kg & 200 mg/kg doses respectively by oral route. Animals were placed separately in individual cages lined with filter paper. The filter papers were changed every hour and the severities of diarrhea were assessed hourly for 4 hours [10].

Gastrointestinal Motility Test

Wistar rats were fasted for 18 h and divided into three groups of five animals each, group I animals served as control and were treated orally with 0.5 % w/v sodium carboxymethyl cellulose in distilled water. Group II animals served as standard and treated with atropine (3 mg/kg, i.p.) a positive control. Animals of group III received Hydro-alcoholic Extract of *Phlogacanthus Thyrsiflorus Nees* (HEPT) in 200 mg/kg doses orally

After 1 h, each animal was administered orally with charcoal meal 0.25 ml (10% charcoal in 0.5 % w/v Sodium carboxymethy cellulose). Thirty minutes later, the animals were sacrificed. Total small intestine from pylorus to caceum was isolated and the total length and the length traveled by the charcoal meal were measured. This distance was expressed as a percentage of the length of the small intestine [10].

Castor Oil Induced Enteropooling

Wistar rats were fasted for 18 h and divided into three groups of five animals each. Group I received normal saline (2 ml, p. o.) served as the control group. Group II served as standard and received loperamide (3 mg/kg, p.o.). Group III animals received HEPT in 200 mg/kg doses orally, one hour before the oral administration of castor oil (2 ml). One hour later, the rats were sacrificed and the small intestine was removed after tying the ends with threads and weighed. The intestinal content was collected into a graduated cylinder and their volume was measured. The intestine was reweighed and the difference between the full and empty was calculated [11-12].

Statistical analysis

Results are expressed as mean ± SEM; n=5 in each group. Data was analyzed by one way ANOVA followed by Tukey-Kramer multiple comparisons test. $^{a}p<0.05$ when compared to control group, ^{b} $p<0.05$ when compared to standard group (atropine sulfate, 3 mg/kg).Graph Pad Prism Version was used for statistical calculations.

Result

Effect of Hydro-alcoholic Extract of *Phlogacanthus Thyrsiflorus Nees* on castor oil induced diarrhea

One hour after castor oil administration, all the rats in the control group of animals produced copious diarrhea. Pretreatment of rats with Hydro-alcoholic Extract of *Phlogacanthus Thyrsiflorus Nees* (100 & 200 mg/kg, p. o.) dose dependently and significantly ($p<0.05$) delayed the onset of diarrhea, reduced the frequency of defecation and the wetness of the fecal droppings (reduction in the no. of wet stool and the general diarrheal scores including the hard and copious stool. The standard antidiarrheal drug Loperamide (3 mg/kg, p.o.) produced a marked significantly greater ($p< 0.05$) inhibitory effects in all the diarrheal parameters.[Table-1 (a) & (b)]

Effect of HEPT on gastro intestinal motility test Compared with the control group, HEPT 200 mg/kg, p.o. significantly ($p<0.05$) decrease the propulsive movement and transit of charcoal meal to the gastrointestinal tract. The standard antidiarrheal drug atropine sulfate (3 mg/kg, p.o.) produced greater antimotility effect then the higher dose of HEPT 200 mg/kg, p. o. [Table-2 (a) & (b)].

Effect of HEPT on castor oil induced enteropooling

Oral administration of castor oil (2 ml, p. o.) produced a marked and significant ($p<0.05$) increase in the intestinal fluid volume of castor oil treated groups of rats compared to control group of animals treated with normal saline (2 ml, p. o.) only. Compared with the control group of rats, pretreatment of the test group of rats with HEPT (200 mg/kg, p. o.) dose dependently and significantly ($p<0.05$) inhibited castor oil induced enteropooling in rats (Table- 3). The standard drug, loperamide produced a marked and significant greater ($p<0.05$) inhibitory effects on castor oil induced fluid accumulation than the higher dose of HEPT (200 mg/kg, p. o.) used.[Table-3]

Table: 1 (a) Effect of HEPT on castor oil induced diarrhea

Treatment	Dose (mg/kg)	Total no. of feces	Total no. of diarrheal feces	Wt. of dry feces	Wt. of wet feces
Control (Castor oil)	1 ml	0.91 ± 0.01^{b}	3.71 ± 0.30^{b}	$0.06 \pm 0.02^{a, b}$	$0.70 \pm 0.09^{a, b}$
LOP+(castor oil)	3	0.18 ± 0.06^{a}	0.00 ± 0.00^{a}	0.22 ± 0.09^{a}	0.00 ± 0.00^{a}
HEPT-I+(castor oil)	100	0.72 ± 0.01^{b}	$2.06 \pm 0.90^{a, b}$	0.80 ± 0.09^{a}	0.08 ± 0.04^{a}
HEPT-II+ (castor oil)	200	0.46 ± 0.06^{a}	$0.86 \pm 0.00^{a, b}$	$0.61 \pm 0.01^{a, b}$	0.03 ± 0.01^{a}

Table: 1 (b) Effect of HEPT on castor oil induced diarrhea condition

Group	Treatment	Dose (mg/kg)	Mean defecation in 4 hrs	% inhibition of defecation
Group-I	Control (Castor oil)	1 ml	4	-----
Group-II	LOP+(castor oil)	3	1	75
Group-III	HEPT-I+(castor oil)	100	3	25
Group-IV	HEPT-II+ (castor oil)	200	2	50

Table: 2 (a) Effect of HEPT on gastro intestinal motility test

Treatment	Dose (mg/kg)	% movement by charcoal
Control (Castor oil)	1 ml	85.94 ± 8.11^{b}
Atropine sulfate + (Castor oil)	3	40.45 ± 2.49^{a}
HEPT-II+ (castor oil)	200	57.06 ± 7.12^{a}

Table: 2 (b) Effect of HEPT on small intestine transits in rats

Group	Treatment	Dose (mg/kg)	Total length of intestine	Distance travelled by marker	% of intestinal transit
G-I	Control (Castor oil)	1 ml	76	73	96
G-II	Atropine + (castor oil)	3	78	62	79
G-III	HEPT-I+ (castor oil)	100	76	66	86
G-IV	HEPT-II+ (castor oil)	200	72	59	81

Table: 3 EffectHEPT on castor oil induced enteropooling

Treatment	Dose (mg/kg)	Volume of fluid (ml)	Weight of intestinal content (gm)	Percentage of inhibition
Control (Castor oil)	1 ml	1.97 ± 0.18^{b}	2.91 ± 0.25^{b}	-----
LOP+(castor oil)	3	0.73 ± 0.90^{a}	0.80 ± 0.38^{a}	70.67
HEPT+(castor oil)	100	$1.50 \pm 0.01^{a, b}$	$1.09 \pm 0.45^{a, b}$	63.58

Discussion

From the experimental data obtained, it was observed that, the Hydro-alcoholic Extract of *Phlogacanthus Thyrsiflorus Nees* at the dose of 200mg/kg showed Maximum antidiarrhoeal activity and the activities were found to be dose dependent. In castor oil induced diarrheal model a significant reduction in frequency of defecation, weight of the wet stools was observed in extract treated groups compared to control. In gastrointestinal motility model, decrease propulsion of charcoal meal at the dose of 200 mg/kg was observed compared to control and the effect was found to be almost similar compared to the standard. The test dose of HEPT (200 mg /kg) by using ANOVAs test which was significant compared to control. These results suggest that the Hydro-alcoholic Extract of *Phlogacanthus thyrsiflorus* possess antidiarrhoeal activity and the drug is worth for further detailed photochemical investigations.

The HEPT significantly reduced the castor oil induced intestinal transit as compared with control group**.** In this study, atropine decreased intestinal transit possibly due to its anti-cholinergic effect. In castor oil induced diarrhoea, the liberation of ricinoleic acid results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion by prevents the reabsorption of NaCl and water. Probably HEPT increased the reabsorption of NaCl and water by decreasing intestinal motility as observed by the decrease in intestinal transit by charcoal meal.

The results indicated that HEPT possess the significant antidiarrhoeal activity. They inhibited the frequency of defecation and reduced greatly the wetness of fecal excretion. Moreover, the intestinal fluid secretion and gastrointestinal propulsion were inhibited. The HEPT also showed antimicrobial activities on some gastrointestinal microorganisms. These findings provided a scientific support for the utility of this plant in the treatment of diarrheal diseases. Further research is to be carried out to fraction and purify the extract, in order to find out the fractions and molecules responsible for the anti-diarrheal activity observed.

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Refernces

1. **Bodhankar S.L.,Vyawahare N.S.,** 2004 A Text book of Pathophysiology. Nirali prakashan 5th edition, p.62-65.
2. **Wilson M.E**. 2005. Diarrhoea in non travelers: risk and etiology. Clinical Infectious Diseases, 41: (Suppl. 8): S541–546.
3. **Mukharjee A, Chaliha M and Das S**.,2009 Study of analgesic activity of ethanol extract of *Phlogacanthus thyrsiflorus* on experimental animal models. Bangladesh Journal of Pharmacology; 4:147-49.
4. **Singh SA and Singh NR**., 2010 Antimicrobial Activity of *Cassia didymobotrya* and *Phlogacanthus thyrsiflorus*. Journal of Chemcal and. Pharmaceutical Research; 2:304-308.
5. **Chakravarthy sharmistha and Kalita jogen Chandra**., 2012 Preliminary phytochemical screening and acute oral toxicity study of the flower of *Phlogacanthus thyrsiflorus Nees* in albino mice. Internal national Research journal of Pharmacy (IRJP) ; 3 (4):293-295.
6. **Khare CP**., 2007 Indian medicinal plant, An illustrated dictionary.1st Vol: Springer publication;
7. **Mukaherjee PK,** 2002 Quality control of herbal drugs (an approach to evaluation of botanicals) New Delhi: Business Horizon's;: p.380-421.
8. **Ajayi IA, Ajibade O, Oderinde RA.,** 2011 Preliminary phytochemical analysis of some plant seeds. Res.J.Chem.Sci.;1(3):58-62.
9. **Kantamreddi VSSN, Lakshmi YN, Kasapu VVVS.,** 2010 Preliminary phytochemical analysis of some important indian plant species. International Journal of Pharma and Bio Sciences; 1(4): 351-357.
10. **Teke, G.N., J.R. Kuiate, O.B. Ngouateu and D. Gatsing,** 2007. Antidiarrheal and antimicrobial activities of *Emilia Coccinea* (Sims) G. Don Extracts. J. Ethnopharmacol., 112: 278-283.
11. **Inayathulla, W.R. Shariff, A.A. Karigar and M. Sikarwar,** 2010. Evaluation of anti-diarrhoeal activity of *Crataeva nurvala* root bark in experimental animals. Intl. J. Pharmacy and Pharmaceutical Sci., 2: 158-161.
12. **Robert, A., J.E. Nezamis, C. Lancaster, A.I. Hanchar and M.S. Kleppr,** 1976**.** Enteropooling assay: a test for diarrhea produced by prostaglandins. Prostaglandins, 11: 809-814.