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EVALUATION OF THE ANTI-DIARRHEAL ACTIVITY OF ETHANOLIC LEAF EXTRACTS OF *LINDENBERGIA MACROSTACHYA* BENTH. IN SWISS ALBINO MICE

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ABSTRACT

Lindenbergia macrostachya Benth, a perennial herb commonly found in moist places and shady fissured walls, was collected to determine antidiarrheal activity of ethanolic leaf extracts by using Soxhlet apparatus. The phytochemical profile was conducted by performing the qualitative tests and the antidiarrheal activity was investigated by three different methods, i.e. impact on castor oil-induced diarrhoea, enterpooling by inducing castor oil and gastrointestinal motility test. Alkaloids, carbohydrates, flavonoids, saponins and tannins were present in leaves of *L. macrostachya*. Ethanolic extract showed significant results in the castor oil-induced diarrhoeal method. The 300 mg/kg^{-1} leaves extract and loperamide showed the most significant result (P = 0.001). In enterpooling by inducing castor oil, the leaves extract displayed better results. The leaf extract of loperamide plus 300 mg/kg^{-1} revealed significant results in the gastrointestinal motility test. All the results were compared with standard drug loperamide and control group. The present study revealed the presence of significant phytochemicals in ethanolic leaf extract of *L. macrostachya* that effectively control induced diarrhoea in Swiss albino mice.

Key words: antidiarrheal activity, gastrointestinal motility, *Lindenbergia macrostachya*, phytochemicals.

INTRODUCTION

It has been estimated by the World Health Organization (WHO) that herbal medicines are used in more than 80% of the world population in their routine healthcare intakes (Shinwari *et al*., 2006). The role of plants in human's life is more important than animals considering the variety of benefits that human's receive from them (Arif *et al.*, 2022). Plants contains a large number of compounds of diverse in nature possessing various biological activities including antifungal (Javed *et al.*, 2021; Jabeen *et al.*, 2022), antibacterial (Ferdosi *et al.*, 2020, 2021), antioxidant, anticancer, antioxidant (Khan and Javaid, 2019, 2020), anti-inflammatory and analgesic (Javaid *et al.*, 2021). The production, conservation and processing of medicinal plants in Pakistan and Azad Jammu & Kashmir is not very well known and studied, which blurs the actual potential of the subject (Ajaib *et al.*, 2022). The size, production area, trade potential and other specificities related to the subject can be a vital area of research in future (Ajaib *et al.*, 2011, 2015).

Diarrhoea is that the section of surprising fluid or unformed stool at an expanded recurrence. Irresistible operators, certain prescriptions, plant and creature poisons, gastrointestinal issues, and substances that expand digestive tract emissions may cause it (Meite *et al*., 2009).

Diarrhoeal sickness treatment is generally incorporated within the substitution of fluids and electrolytes utilizing oral rehydration arrangements. Although the treatment is effective, diarrhoea mortality and bleakness rates are still significant (Thiagarajah *et al*., 2014).

Lindenbergia macrostachya Benth. (Orobanchaceae) is commonly found in moist and shady places, on walls (Ajaib, 2012). It is mostly present in Africa, Burma, Afghanistan, Bangladesh, China, Egypt, Ethiopia, Iran, Philippines, Nepal, Israel, Jordan, Malaysia, Nepal, India, Sudan and Pakistan. *L. macrostachya* is a perennial herb found in the lower Himalayas, up to 1800 m (Rifai and Reksodihardjo, 1969). Keeping in view the local medicinal use of *L. macrostachya* in treatment of diarrhoea, the present study was carried out.

MATERIALS AND METHODS

Plant preparation

L. macrostachya Benth. (Family Orobanchaceae) was collected during the spring of 2019 (March) from Peer Gali, Mirpur, Azad Jammu & Kashmir (AJK). The plant was identified, dried, mounted on a dry pane and afterwards, it was verified from herbarium in the Department of Botany (Mirpur University of Science and Technology), with a valid voucher number of specified sample MUH-315. The leaves of *L. macrostachya* were separated. Cleared with all dirt and soil by washing with tap water and then dispersed evenly to dry under the shade at room temperature. Completely dried leaves were ground to powder form by using an electric grinder and stored in an airtight container for further procedure.

Powder of (250g) leaves was weighed and soaked in ethanol (1500 mL) for 7 days and shaken after every 24 hrs. After 7 days, filtration of the extract was made through Whatman filter paper. The filtrate, i.e. the crude extracts was stored in the conical flask. This procedure was repeated twice to completely exhaust the plant material. The extract thus obtained was concentrated and then dried through a rotatory evaporator (Handa *et al.,* 2008).

Phytochemical analysis

The ethanolic extract was tested for the presence of various phytochemicals using the procedure of Chandrashekar and Rao (2013).

Animals

Swiss albino mice with good health were used. Swiss albino mice weight between 19-23 g. Mice were got from the animal house of Botany Department, GC University, Lahore. Mice were placed in polyethylene glycol cages (6 animals per crate). Softwood shavings were utilized as bedding. Standard conditions of relative humidity i.e. 44-56 %, the temperature at 25 ± 2°C, and a day and night cycle of 12 h each was maintained. Proper feeding was ensured during the experiment period. Mice were ensured to provide the best care and ensured adaptability with the environment by procuring the mice 7 days before the start of the experiment. The methodology of experimental work was approved by the Animal Ethical committee of Punjab University College of Pharmacy (AEC/UCP/1042/4313).

Grouping

Every group contained five mice (n=6).

Group 1: received as normal saline and castor oil 0.5mL given orally.
Group 2: was administered Loperamide 5 mg/kg and castor oil 0.5mL given orally.
Group 3: ethanolic extract of leaves of 300 mg/kg and 0.5 mL of castor oil was fed.
Group 4: ethanolic extract of leaves of 500 mg/kg and 0.5 mL of castor oil was fed.
Group 5: ethanolic extract of leaves of 300 mg/kg, loperamide 5 mg/kg and 0.5 mL of castor oil was fed.

Dose preparation

The relative doses of ethanolic leaves extract of *L. macrostachya* were designed by saturated it in distilled water.

Castor oil induced diarrhoea

The technique depicted by Awouters *et al.* (1978) and slightly modified by Abdela (2019) was employed in this examination. In this technique, Swiss pale mice were utilized. Mice have fasted for 18h. Mice were isolated into five gatherings with six creatures in each gathering. Following 60 min, each group got 0.5 mL/kg of castor oil orally. Swiss pale mice were kept in a different straightforward confine and then the white paper was used for the floor. The paper was changed, after 4h regularly. In the measurement session, the beginning of diarrhoea, weight and number of wet stools, all-out weight and the all-out number of faecal yield was noted. At long last, the level of faecal yield and diarrhoeal restraint (% hindrance of defecation) was determined by the following method,

$$\text{Percentage inhibition} = \frac{\text{Aver. no. of WFC-Aver. no. of WFT}}{\text{Aver. no. of WFC}} \times 100$$

where, WFC & T wet faecal in the control and treatment group

$$\text{Percentage of wet faecal output} = \frac{\text{Mean wet faecal Wt. of each group treatment}}{\text{Mean faecal wt. control group}} \times 100$$

$$\text{Percentage of total faecal output} = \frac{\text{Mean total faecal Wt. of each group treatment}}{\text{Mean faecal wt. control group}} \times 100$$

Castor oil induced enteropooling

The technique depicted by Robert *et al*. (1976) were utilized to decide the impact of the concentrate on intraluminal liquid collection. Mice were fasted for this examination about 18 h and partitioned into five gatherings with six creatures in each gathering. 60 min. after given castor oil organization. By cervical dislocation, the mice were sacrificed. Individually mice were opened by the abdomen and the entire length of the small intestine, analysed. The small digestion tracts were gauged and the intestinal substance was gathered by draining into a graduated cylinder to quantify the volume. The unfilled digestive organs have reweighed the contrast between the two loads were determined.

$$\text{Percentage of reserve by using MVSIC} = \frac{\text{MVICC-MVICT}}{\text{MVICC}} \times 100$$

Where MVSIC is the mean volume of the little intestinal substance, MVICC is the mean volume of the intestinal substance of the benchmark group, and MVICT is the mean volume of the intestinal substance of the experimental groups.

$$\text{percentage of inhibition by using MWSIC} = \frac{\text{MWICC-MWICT}}{\text{MWICC}} \times 100$$

Where MWSIC represent by mean load of the little intestinal substance, MWICC = mean load of the intestinal substance of the control, and MWICT = mean load of the intestinal substance of the experimental group.

Gastrointestinal motility test

Mice were fasting about 18 h and gathered. After 1 hour of castor oil organization, mice were gotten 1 mL of 5% enacted charcoal mixture. The creatures were then relinquished by cervical disengagement after half-hour of controlling castor oil and the whole length of the digestive tract (from the pylorus to the cecum) was expelled and put the long way on a paper. The separation went by the charcoal supper and the complete length of the digestive tract was then estimated. The peristaltic record and level of hindrance were determined through utilizing the accompanying recipe (Ruwart *et al*., 1980);

$$\text{Peristalsis index} = \frac{\text{Distance travelled by charcoal meal}}{\text{Whole Length of small intestine}} \times 100$$

$$\text{Percentage inhibition} = \frac{\text{Dc-Dt}}{\text{Dc}} \times 100$$

Where,
Dc represents whole distance moved charcoal by the negative, and
Dt represents whole distance moved charcoal by the treatment set.

***In vivo* antidiarrheal index (ADI)**

In vivo antidiarrheal index of loperamide plus concentrate treated gathering was dictated by utilizing various information from the above experiments utilizing the recipe created via Hussain *et al.* (2009) and Then *et al.* (1989).

$$\text{ADI} = \sqrt[3]{\text{D freq} \times \text{G meq} \times \text{P freq}}$$

Wherever:
G freq = gut meal travel reduction (in % of control)
Pfreq= purging frequency as the number of wet stool reduction (in % of control)
Dfreq = Delay in crap time or diarrheal beginning (in % of control);

$$\text{Dfreq} = \frac{\text{MODTG-MODCG}}{\text{MODCG}} \times 100$$

Wherever,
MODTG & CG = Mean onset of diarrhoea in the test group & control group

Statistical Analysis

Values are showed as Mean Standard Error Mean (n=6), investigation was implemented with One way-ANOVA by using SPSS 21. version.

RESULTS

Phytochemicals

Flavonoids, tannins and alkaloids were strongly present in plant, they are responsible for curing as antidiarrheal activity. Proteins, saponins, carbohydrates and resins were also visible (Table 1).

Table 1. Phytochemicals analysis of the ethanolic extract of leaves of *Lindenbergia macrostachya*.

Constituents	Leaves extract of *L. macrostachya*
Carbohydrate	+ +
Protein	+
Glycosides	-
Saponin	+ +
Flavonoids	+ + +
Steroids	+ +
Tannin	+ ++
Resin	+ +
Alkaloids	+ + +

- nothing, ± doubtful, + Slightly observed, ++ Obvious, +++ visible

Impact on castor oil-induced diarrhoea

Extreme impact recognized both the concentrate and medicine was blended (82.19%) and loperamide showed 63%. Oral pre-treatment of mice with various portions of the concentrate demonstrated a critical ($p < 0.01$) reduced diarrhoea on the start, with the upper portion of the concentrate showing the higher impact (Table 2). Also, the concentrate essentially diminished the recurrence of crap. Yet, the best gathering was that wherein both the concentrate and drugs was blended. During this gathering, the extent of faecal yield was likewise decreased by various portions of the concentrate, within which both the concentrate and drugs were blended delivering a superior impact contrasted with any of the gatherings as portrayed.

Table 2. Effect of the ethanolic leaves extract of *Lindenbergia macrostachya* on castor oil induced diarrhoea in mice.

Treatment	Dose (mg/kg)	Onset of diarrhoea (min)	Total no. of faecal	Total no. of wt of faecal (g)	Total wt of faecal (g)	Total wt of wet faecal (g)	% inhibition of defecation	%WFO	%TWFO
NS	10 mL/kg	50.65 ± 8.23	19.4 ± 1.07	14.6 ± 0.4	0.57 ± 0.007	0.44 ± 0.016	-		
LM	5 mg/kg	154.5 ± 9.82	6.6 ± 0.51	5.4 ± 0.41	0.27 ± 0.006	0.15 ± 0.004	63	-	-
ELLM	300 mg/kg	98.34 ± 7.68	11.4 ± 0.5	7.5 ± 0.52	0.37 ± 0.004	0.21 ± 0.01	48.63	34	47
ELLM	500 mg/kg	145.21 ± 10.61	7.2 ± 0.58	5.7 ± 0.53	0.30 ± 0.003	0.16 ± 0.009	60.95	36.3	52.63
LM + ELLM	5+300 mg/kg	205.8 ± 14.25	3.8 ± 0.37	2.6 ± 0.24	0.16 ± 0.011	0.04 ± 0.009	82.19	9.09	28.07

Where NS = normal saline, LM = Loperamide, ELLM = ethanolic leaves extract of *L. macrostachya*, WWFO = weight of wet faecal output and TWFO = total weight of faecal output.

Impact on castor oil-induced enteropooling

The ethanolic extract of leaves of *L. macrostachya* showed greater impact by using loperamide plus 300 mg/kg on enteropooling development (84.37%) (Table 3).

Table 3. Impact of leaves extract of *Lindenbergia macrostachya* on anti-enteropooling in mice.

Group	Dose controlled	AWSIC (g)	% inhibition	AVISC (mL)	% inhibition
1	NS	0.83 ± 0.043	-	0.57 ± 0.021	-
2	ELLM 300	0.42 ± 0.024	49.4	0.24 ± 0.003	57.89
3	ELLM 500	0.26 ± 0.009	68.67	0.15 ± 0.004	73.68
4	LM	0.2 ± 0.009	75.9	0.1 ± 0.01	82.45
5	LM + 300 ELLM	0.1 ± 0.01	87.9	0.049 ± 0.004	91

Where NS = Normal Saline, ELLM = ethanolic leaves extract of *L. macrostachya*, LM = Loperamide, AWSIC = Average Weight of Small Intestinal Contents and AVISC = Average Volume of Small Intestinal Contents.

Effect on gastrointestinal motility

The ethanolic extract of leaves of *L. macrostachya* showed extreme impact by using loperamide plus 300 mg/kg on typical gastrointestinal development (84.37%) (Table 4).

Table 4. Impact of the leaves extract of *Lindenbergia macrostachya* on castor oil induced gastrointestinal transit in mice.

Group	Dose controlled	TLSI (cm)	DTC (cm)	PF	% inhibition
1	NS	41.02 ± 0.92	33.92±1.26	82.69	-
2	ELLM 300	43.28 ± 1.39	18.28±0.66	42.23	46
3	ELLM 500	43.28 ± 1.39	14.26±0.73	34.44	57.95
4	LM	43.34 ± 1.28	8.98±0.5	20.71	73.52
5	LM + 300 ELLM	41.72 ± 1.37	5.3±0.6	12.7	84.37

Where NS = Normal Saline, ELLM = ethanolic leaves extract of *L. macrostachya*, LM = Loperamide, TLSI = Total length of small intestine, DTC = Distance travelled by charcoal and PF = Peristaltic File.

***In vivo* anti-diarrhoeal index**

Leaves extract of *L. macrostachya* most elevated enemy of diarrhoeal record was seen at the greatest portion loperamide plus 300 mg/kg, of the concentrate as appeared in Table 5.

Table 5. *In vivo* antidiarrheal index value of the leaves extract of *Lindenbergia macrostachya*.

Group	Dose controlled	Dfreq	Gmeq	Pfreq	ADI
1	NS				
2	LM	205	73.52	63	98.28
3	ELLM 300 mg/kg	94	46.1	48.63	59.49
4	ELLM 500 mg/kg	186.69	57.95	60.95	87
5	LM + ELLM 300 mg/kg	306	84.37	82.19	128.5

Where, ELLM = ethanolic leaves extract of *L. macrostachya*, G freq = gut meal travel reduction (in % of control), Pfreq= purging frequency as the number of wet stool reduction (in % of control), Dfreq = Delay in crap time or diarrheal beginning (in % of control), ADI= Antidiarrheal Index.

DISCUSION

The ethanolic extract concentrate showed flavonoids, alkaloids, tannins and saponins in the leaves of a *L. macrostachya* (Table 1). The result of phytochemical was closely related to Ajaib *et al*. (2017) while working on phytonutrient test and anthelmintic profiles of *Andrachne cordifolia*.

For antidiarrheal investigation ethanolic extract of leaves was used which was evaluated by using different methods which showed that plant part had antidiarrheal. In antidiarrheal activity plant extract of *L. macrostachya* given significant results at the concentration of 300mg/kg extract and loperamide 5mg/kg (Table 2). Diarrhoea was induced in mice by castor oil given orally and significant results were obtained. The beginning of diarrhoea, number of dry faecal, weight of dry faecal. The same effect was reported by Degu *et al.*, (2020) during the investigation of the antidiarrheal profile of crude hydromethanol extracts of *Vernonia amygdalina* and *Ruta chalepensis* in mice.

Leaves extract showed significant outcomes in the castor oil method. The leaves sample at 300 mg/kg exhibited promising results as compared to normal saline, while the leaves sample at 500 mg/kg displayed significant results at 0.16 ± 0.009 ($P < 0.001$) weight (Table 1). The 300 mg/kg leaves extract plus loperamide showed the most significant results at 0.04 ± 0.009 ($P < 0.001$) weight. All these results were similar to the work of Aslam and Janbaz (2019) studies on ethanol-aqueous extract of *Asphodelus tenuifolius* laxative activities and antidiarrheal.

Concentrates on castor oil-actuated intestinal liquid aggregation indicated that the concentrate diminished together with the volume and weight of the intraluminal substance. These impacts, which are immediate outcomes of decreased electrolysis and water emission into the small digestive system, propose that the concentrate can improve electrolysis retention from the intestinal lumen reliable with the restraint of hypersecretion. Li *et al*. (2019) were worked on *Sophora tonkinensis* of the methanolic extract against diarrheal activity in mice. They also work in rabbits of spasmolytic effect on smooth muscle concentration.

Tannins in the plant denature proteins in the mucosa intestinal shaping protein tannate complex and lessens emission according to Israili and Lyoussi, (2009). Pandey *et al*. (2012) reported the similar results while working on Ethnomedicinal plants.

Enteropooling diarrhoeal model and castor oil incited greatest impact (Table 3) were seen with the furthermost elevated portion of the concentrate as opposed to the standard medication in charcoal feast test (Table 4). The same result was reported by Yacob *et al.* (2016) by an investigation of methanol aerial part extract of *Ajuga remota* Benth. which belongs to family Lamiaceae was studied antidiarrheal profile in mice.

The antidiarrheal file (ADI) is a proportion of the consolidated impacts of these various segments of diarrhoea, for example, cleansing recurrence, the beginning of diarrhoeal stools, and intestinal liquid gathering. The same results were obtained from the work of Sathish *et al*. (2011) on *Lantana camara.* The most elevated chosen portion of the concentrate, with the most elevated ADI esteem, is enriched with the best antidiarrheal movement when contrasted and other chose dosages as appeared on the above outcomes (Table 5). The same results were obtained from the work of Tadesse *et al*. (2017) on analysis of the anti-diarrhoeal profile of *Lantana camara* Linn. Tannins lessen diarrhoea by decreasing discharge intervened by the degradation of conceal proteins and repressing the flux of the digestive tract through modifying the intracellular Ca^{2+} level. A similar result was reported by Belemtougri *et al*. (2006) on ethnomedicinal plants leaf extracts of *Diospyros mespiliformis* L. (Ebenaceae) and *Psidium guajava* L. (Myrtaceae) applied on skeletal muscle cells in rats. Aleem and Janbaz, (2019) and Mora *et al*. (1990) reported the same results as the studies on the double technique of Ca^{2+} antagonistic and anti-muscarinic activities to legalize the traditional usages of *Cyperus niveus* Retz. as antidiarrheal and antispasmodic

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