

## EVALUATION OF ANTI DIARRHEAL ACTIVITIES OF ETHANOLIC EXTRACT OF HIBISCUS PLATANIFOLIUS IN CASTOR OIL INDUCED DIARRHEA IN WISTAR ALBINO RATS

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### ABSTRACT

The plant *Hibiscus platanifolius* belongs to the family Malvaceae, which is being used traditionally for various disorders of hypertension, kidney and used as natural diuretic. *Hibiscus -platanifolius* has been proposed to contain anti diarrheal properties such as flavonoids, anti – oxidants, tannins, terpenes, alkaloids. This study was designed to evaluate anti diarrheal activity by castor oil induced diarrhoea and castor oil induced enteropooling in rats, for two methods have selected four groups individually in which group 3 & 4 received plant extract (200mg/kg, 400mg/kg) and first group received normal saline (5ml/kg) as disease control and second group received loperamide (5mg/kg) and one hour before the test and standard all the animals received castor oil (2ml/ rat) in both methods. In castor oil induced method rats were kept

in transparent metabolic cages and collected diarrheal and non diarrheal feces for 6 hours and assessed activity where as in castor oil induced enteropooling method rats were sacrificed and collected intestinal contents by milking into graduate tube and their volume measured.

**KEYWORDS:** Diarrhea, *Hibiscus platanifolius*, Castor oil, Diarrhea, loperamide.

### INTRODUCTION

Diarrhea is a major health worry and sources of malnutrition in developing countries.<sup>[1]</sup> It is an impaired absorption and hyper secretion syndrome of the gastrointestinal tract.<sup>[2]</sup> Diarrhea may be brought about by viruses, bacteria, fungi, protozoa, drugs and bacteria endotoxins.<sup>[3]</sup> In Nigeria, diarrhoea remains the major killer disease among children under 5 years, while 7-12 months-old-babies remain the most susceptible.<sup>[4]</sup> It is estimated that diarrhoea causes 4-5

million deaths annually throughout the world. Eighty percent of these deaths are reported in developing countries including Nigeria. To combat the problem of diarrhea in developing countries, the World health organization (WHO) has constituted a diarrhea disease control program aimed at the holistic approach to include all aspects of traditional medical practices, evaluation of health education and preventive approaches.<sup>[5-6]</sup>

**Types of Diarrhea:** Three clinical syndromes of diarrhea have been defined, each reflecting a different pathogenesis and requiring different approaches to treatment. These include the following: Acute watery diarrhea, dysentery diarrhea, persistent diarrhea, exudative diarrhea, inflammatory diarrhea.

**Routes of transmission:** The infectious agents that cause diarrhea are usually spread by the fecal-oral route, which includes the ingestion of fecal contaminated water or food, person-to-person transmission, and direct contact with infected feces. Examples of behaviors that help enteric pathogens to spread are: preparing food with hands that have been soiled during defecation and not washed; or allowing an infant to crawl, or a child to play in an area where human or animal feces are present.

**Causes of diarrhea:** Bacterial causes of diarrhea, viral causes of diarrhea, traveler's diarrhea, chronic diarrhea, diarrhea caused by medication, diarrhea caused by food intolerance, diarrhea caused by chronic conditions.

**Medications:** While antibiotics are beneficial in certain types of acute diarrhea, they are usually not used except in specific situations.<sup>[7][8]</sup> There are concerns that antibiotics may increase the risk of hemolytic uremic syndrome in people infected with *Escherichia coli* O157:H7.<sup>[9]</sup> In resource-poor countries, treatment with antibiotics may be beneficial. However, some bacteria are developing antibiotic resistance, particularly *Shigella*.<sup>[10]</sup> Antibiotics can also cause diarrhea, and antibiotic-associated diarrhea is the most common adverse effect of treatment with general antibiotics.

While bismuth compounds (Pepto-Bismol) decreased the number of bowel movements in those with travelers' diarrhea, they do not decrease the length of illness.<sup>[11]</sup> Anti-motility agents like loperamide are also effective at reducing the number of stools but not the duration of disease.<sup>[12]</sup> These agents should only be used if bloody diarrhea is not present.<sup>[12]</sup>

Diosmectite, natural aluminum magnesium silicate clay, is effective in alleviating symptoms of acute diarrhea in children,<sup>[13]</sup> and also has some effects in chronic functional diarrhea, radiation-induced diarrhea, and chemotherapy-induced diarrhea.

Bile acid sequestrants such as cholestyramine can be effective in chronic diarrhea due to bile acid malabsorption. Therapeutic trials of these drugs are indicated in chronic diarrhea if bile acid malabsorption cannot be diagnosed with a specific test, such as SeHCAT retention.<sup>[14]</sup>

## MATERIALS AND METHODS

**Plant material:** The plant *Hibiscus platanifolius* was from plant was collected during the march 2014 from Sri Venkateshwara University Tirupati, India. The plant was authenticated by Dr.Madhava Chetty, Department of Botany and voucher specimen of the plant were preserved at institute herbarium library.

**Preparation of plant extracts:** Fresh plants collected, were washed to remove adhered dirt, rinsed with distilled water, blotted and dried in shade. The shade-dried specimens were powdered in a mixer. This powder was used for solvent extraction. About 100 g of the powdered plant material was subjected to soxhlet extraction using 100 ml solvent ethanol. This cycle was repeated many times, over hours or a few days, until the colour of the solvent in the siphon of the soxhlet faded away. The extracts were concentrated under reduced pressure and preserved in refrigerator until further use at the end of the hot extraction process each extract was filtered. The filtrate was concentrated and the solvent was recovered using rotary evaporator. The extracts were then kept in desiccators to remove remaining moisture, if present and finally stored in air tight containers at 4°C for further use.

**Experimental animals:** Male albino rats (Wister strain) weighing between 150-200g are procured from nutritional society of India, Taranaka, Hyderabad. The animals were acclimatized for seven days under laboratory conditions. The animals were fed with commercially available rat pelleted diet. Water was allowed ad libitum under strict hygienic conditions. The study protocols were duly approved by the Institutional Animal Ethics Committee (IAEC). Studies were performed in accordance with CPCSEA guidelines.

### Determination of acute toxicity (LD50)

14 days single dose oral acute toxicity and gross behavioural study

Number of animals required: 6 rats (female)

Number of groups: 2 groups (3 animals each group)

Dose levels: 2000mg/kg body weight of the animals. Study duration: 14 days.

### **Effect of extract as anti diarrheal**

**(a) Castor oil induced diarrhea in rats:** Twenty (20) rats were fasted for 18 h and divided into four groups of four animals each. The plant extract (200, 400, and mg/kg body weight) were administered orally to groups 3 and 4 respectively. The second group received standard is loperamide (5mg/kg body weight) and the first group considered as disease control is received normal saline (5ml/kg).

One hour later, all the animals received 2 ml/rat of castor oil orally by gavage. The animals were kept in separate metabolic cages with a transparent plastic container beneath the cage to collect feces. The severity of diarrhea was assessed each hour for 6h. The total number of feces (both diarrheal and non-diarrheal) expelled were compared with the control group. The total score of diarrheal feces for the control group was considered as 100%. The results were expressed as a percentage of inhibition of diarrhea.

**(b) Castor oil-induced enteropooling:** Rats were divided into four groups of four animals each, one hour before oral administration of castor oil (2 ml/rat). Group 1 received normal saline orally (5 ml/kg body weight), and served as the control. Group 2 animals received loperamide (5mg/kg, oral) while groups 3, 4 received, by oral intubation, the extract of *Hibiscus platanifolius* doses of 200 and 400 mg/kg body weight, respectively. Two hours later, the rats were sacrificed and the small intestine from the pylorus to the caecum was isolated. The intestinal contents were collected by milking into a graduated tube and their volume measured.

**(c) Statistical analysis:** Data were analyzed by one-way ANOVA followed by Dennett's t-test using Instant<sup>®</sup> (Graph Pad software, U.S.A). At 95% confidence interval  $p < 0.05$  was considered statistically significant.

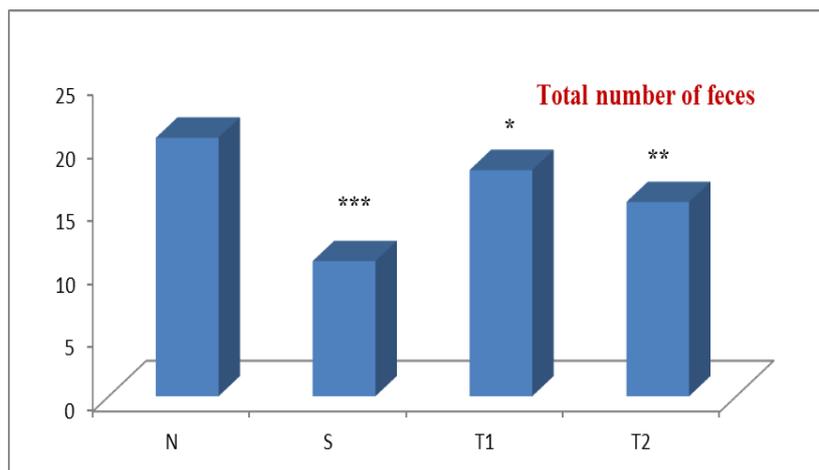
**RESULTS**

**Table. (1): Effect extract of ethanolic of whole plant of *Hibiscus Platanifolius* on castor-oil induced diarrhea in rats.**

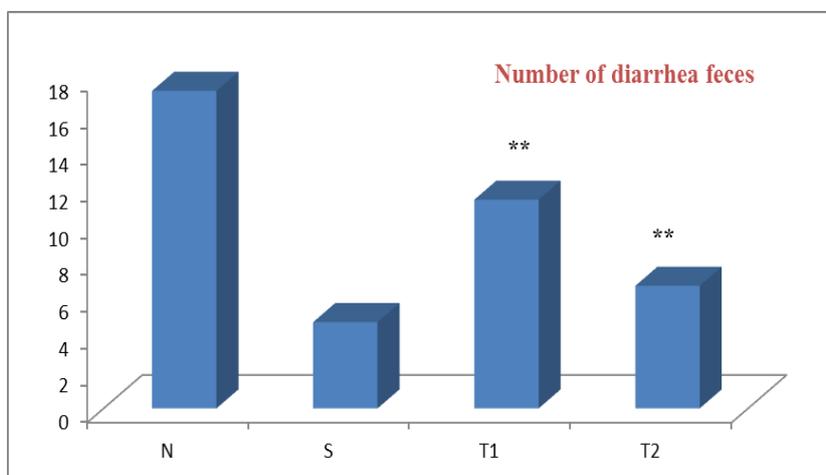
Treatment	Total number of feces	Number of diarrhea feces	Inhibition of diarrhea (%)
Normal saline (5mg/kg)+Castor oil(2ml)	20.46±0.894	17.28±0.797	
Loperamide(5mg/kg)+castor oil(2ml)	9.48±0.414***	4.56±0.743***	73.61
HP-EE(200mg/kg)+castor oil(2ml)	17.22±0.758*	11.84±0.693**	31.48

Values are mean ± SEM (n=5) one way ANOVA.

Where, \* represents significant at p<0.05, \*\* represents highly significant at p< 0.01, and \*\*\* represents very significant at p<0.001. All values are compared with toxicant.



**Fig. (1): Effect of ethanol extract of whole plant of *Hibiscus platanifolius* on Total number of feces levels in castor oil induced diarrhea rats.**



**Fig. (2): Effect of ethanol extract of whole plant of *Hibiscus platanifolius* on number of diarrhea feces levels in castor oil induced diarrhea rats.**

**Table. (2): Effect of ethanol extract of whole plant of *Hibiscus platanifolius* on castor-oil induced enteropooling in rats.**

Treatment	Volume of intestinal fluid	Inhibition of diarrhea (%)
Normal saline (5mg/kg)+Castor oil(2ml)	3.12±0.296	
Loperamide(5mg/kg)+castor oil(2ml)	1.56±0.143***	50
HP-EE(200mg/kg)+castor oil(2ml)	2.78±0.196**	34
HP-EE(400mg/kg)+castor oil(2ml)	1.73±0.143**	44.55

Values are mean ± SEM (n=5) one way ANOVA.

Where, \* represents significant at  $p < 0.05$ , \*\* represents highly significant at  $p < 0.01$ , and \*\*\* represents very significant at  $p < 0.001$ . All values are compared with toxicant.

## DISCUSSION

**Anti diarrheal activity:** Anti diarrheal properties of medicinal plants were found to be tannins, flavonoids, alkaloids, saponins, reducing sugar, sterols and terpenes. The anti diarrheal activity of flavonoids has been ascribed<sup>[15]</sup> to their ability to inhibit intestinal motility and hydro-electrolytic secretions which are altered in this intestinal condition. In vivo experiments have shown that flavonoids are able to inhibit the intestinal secretor response induced by prostaglandins E. In addition, flavonoids present antioxidant properties which are presumed to be responsible for the inhibitory effects exerted upon several enzymes including those involved in the arachidonic acid metabolism.<sup>[16]</sup> These constituents may be responsible for the anti diarrheal activity of the ethanolic extract of whole plant of *Hibiscus platanifolius*.

In the current study treatment of animals with ethanol extract whole plant of *Hibiscus platanifolius* significantly ( $p < 0.05$  in 200mg/kg b.w. and  $p < 0.01$  in 400mg/kg b.w) decrease the levels of total number of feces and number of diarrhea feces which indicates anti diarrheal activity, and which also decreases volume of intestinal fluid by inhibiting intestinal Na<sup>+</sup> K<sup>+</sup> ATP ase activity thus reduce absorption of intestinal fluids and shown significant effect ( $p < 0.01$  in 400mg/kg b.w). This indicates ethanol extract of whole plant of *Hibiscus platanifolius* possess anti diarrheal activity.

## CONCLUSION

**Anti diarrheal effect:** Anti diarrheal effect of ethanol extract of whole plant of *Hibiscus platanifolius* was confirmed by the following measures; In the present study, the ethanol extract of whole plant of *Hibiscus platanifolius* significantly reduced the total number of

feces elevated levels and reduced the number of diarrheal feces when compared with normal control and reduced the volume of the intestinal fluid.<sup>[15]</sup> Which can be determined by castor oil induced enteropooling, hence it may be concluded that the ethanolic extract of whole plant of *Hibiscus platanifolius*<sup>[16]</sup> possesses anti diarrheal activity.

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