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Study of Antidiarrheal Activity of *Hydrocotyle javanica* in Mice

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The effect of the methanolic leaf extract of *Hydrocotyle javanica* (family Hydrocharitaceae) was investigated in mice to evaluate its antidiarrheal activity on magnesium sulfate ($MgSO_4$) induced diarrhea and gastrointestinal motility. *Hydrocotyle javanica,* at doses of 50 mg/kg and 100 mg/kg, was tested for its effect on fecal output in magnesium sulfate induced diarrhea in albino mice and the results (percentage of animals showing diarrhea) were compared with those of diphenoxylate hydrochloride, a standard allopathic antidiarrheal drug. *Hydrocotyle javanica* (at a dose of 100 mg/kg) inhibited the magnesium sulfate induced diarrhea and also reduced gastrointestinal motility in mice. The results indicate that the leaf extract significantly reduced diarrhea in mice with a reduction in weight of stools.

Keywords: *Hydrocotyle javanica,* antidiarrheal, magnesium sulphate, diphenoxylate hydrochloride, gastrointestinal motility, methanolic leaf extract.

INTRODUCTION

Diarrhea has long been recognized as one of the most important health problems in the developing world [1]. The highest mortality rates have been reported in children under five years of age [2]. A number of factors have been involved in the diarrhea syndrome, of which malnutrition [3-4] and prolonged injury to the intestinal mucosa [5] are the common co-existing and complicating factors. Diarrhea is a leading cause of mortality in developing countries.

In view of this, the World Health Organization has given due importance to studies on traditional medicinal practices in the management of diarrhea. There are several potent antidiarrheal drugs in the modern system of medicine [6] that, however, on prolonged use do have some adverse effects [7]. A phenomenal increase in the use of herbal medicine for various ailments has been observed recently.

Hydrocotyle javanica is a prostrate herb found throughout the Himalayas and Assam Hills at altitudes of 2,000 to 8,000 ft. It is a local stimulant and diuretic that used especially in cutaneous diseases; the leaves are used in blood purification, indigestion, nervousness and dysentery [8-9]. The aim of the present paper is to evaluate the effect of the methanolic extract of *Hydrocotyle javanica* on experimentally induced diarrhea, based on the ethnobotanical lead.

EXPERIMENTAL

Preparation of Extract

Leaves of *Hydrocotyle javanica* were collected in bulk from Kolli Hills, Namakkal District, Tamilnadu, India and their identity was confirmed at the Department of Botany, St Joseph's College, Tiruchirappalli. The collected material was washed with distilled water to remove dirt and soil. The leaves were further shade dried and then coarsely powdered. The dried leaves of *Hydrocotyle javanica* were subjected to Soxhlet extraction. The methanolic extract (5.92 % w/w) was vacuum-dried and processed further for evaluation of anti-diarrheal activity in albino mice. The extract *Hydrocotyle javanica* was suspended in 1 % carboxy methylcellose (CMC) in water for administration.

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Effect of *Hydrocotyle javanica* on Fecal Output in Magnesium Sulfate induced Diarrhea [10-11]

Swiss albino mice of either sex weighing between 20-25 g were used for these experiments. They were housed in polypropylene cages in an air-conditioned area at 25 ± 2 °C with 10:14 hours light and dark cycle. Animals were fasted for 24 h before the study with free access to water *(ad libitum)*. The mice were divided into four groups of six mice each. The animals in group I served as the control and were treated with magnesium sulphate (2 mg/kg orally). The group II and group III mice served as experimental groups and were treated orally with *Hydrocotyle javanica* at doses of 50 mg/kg and 100 mg/kg. The animals in group IV served as the positive control and were given diphenoxylate hydrochloride orally (5 mg/kg). The treatment to various groups of mice was given 45 min prior to magnesium sulfate adminstration. Immediately after carthartic agent challenge, the animals were kept in polypropylene cages lined with filter paper at the bottom. They were observed for parameters such as time of occurrence of diarrhea, number of total defecations and the number of wet defecations which were recorded for up to 4 hours.

Effect of Extract on Gastrointestinal Transit [11]

The effect of *Hydrocotyle javanica* on small intestinal transit was studied in three groups of overnight fasted mice. Thirty minutes after treatment with *Hydrocotyle javanica* (100 mg/kg orally) or atropine (5 mg/kg intramuscularly) or vehicle (0.2 ml), the mice were administered with 0.2 ml of charcoal meal (3 % charcoal in 5 % gum acacia) orally. All animals were sacrificed after 20 min and the distance traveled by charcoal was expressed as a percentage of the total length of the small intestine for each mouse according to the equation given below [12] and the results were tabulated in Table 2.

STATISTICAL ANALYSIS

The results of all experiments were reported as mean ± SEM. These results were further analyzed using the students't' test to calculate the significance.

RESULTS

Effect of *Hydrocotyle javanica* on Fecal Output

Hydrocotyle javanica when administered at doses of 50 mg/kg and 100 mg/kg significantly reduced diarrheal episodes in the mice. *Hydrocotyle javanica* significantly delayed onset of diarrhea as well as causing a reduction in number of wet fecal dropping when compared to untreated control rats. These effects were comparable to those of the standard antidiarrheal agent, diphenoxylate hydrochloride (5 mg/kg). The methanolic extracts of *Hydrocotyle javanica* (50 mg/kg and 100 mg/kg) and diphenoxylate hydrochloride (5 mg/kg) offered percentage protection against magnesium sulphate induced diarrhea of 74 %, 81 % and 91 % respectively. The extract showed dose dependent inhibition of magnesium sulphate induced diarrhea in mice. This effect was significant when compared to the control but was less when compared to that of diphenoxylate hydrochloride (Table 1).

Effect of *Hydrocotyle javanica* on Gastrointestinal Motility

The methanolic extract also showed a significant reduction in propulsion of charcoal meal through the gastrointestional tract as compared to the vehicle treated group. The results of the present study revealed that *Hydrocotyle javanica* (100 mg/kg) and atropine sulfate (5 mg/kg), significantly ($p < 0.001$) reduced the gastrointestinal transit of charcoal meal in mice by 49.4 % and 66.9 % respectively as compared to the control group (Table 2).

$$\text{Distance Travelled (\%)} = \frac{\text{Distance travelled by charcoal in small intestine}}{\text{Total length of small intestine}}$$

DISCUSSION

One of the main causes of diarrhea is increased and altered intestinal motility and fluid accumulation, which reduces the time for absorption [13]. Many antidiarrheal drugs act by reducing the motility. Magnesium sulphate induced diarrhea is presumed to be due to a combination of its osmotic properties and stimulation of cholecystokinin production [14].

Table 1: Effect of Methanolic Extract of *Hydrocotyle javanica* on Magnesium Sulphate induced diarrhea.

Treatment Groups	Time of Occurrence of diarrhea (min)	Total number of faeces	Total number of wet faeces	Total weight of faeces (mg)	Percent protection (%)
Control (magnesium sulphate, 2mg/kg, p.o)	49.5±3.01	22.1±1.01	18.7±0.77	211.5±3.4	
Hydrocotyle javanica (50mg/kg, p.o)	67.6±1.28*	12.3±0.99*	7.3±0.7*	54.0±2.18*	74.46
Hydrocotyle javanica (100mg/kg,p.o)	89.5±2.92*	9.4±0.92*	3.1±0.59*	39.5±3.4*	81.32
Diphenoxylate hydrochloride (5mg/kg,p.o)	123.5±1.54*	6.0±0.59*	2.3±0.28*	19.8±1.97*	90.63

Number of animals used N=6; *P< 0.001 Vs control; Results are analyzed by students 't' test and expressed as ± SEM.

Table 2: Effect of *Hydrocolyte javanica* on gastrointestinal motility after charcoal meal in mice.

Treatment Groups	Mean distance traveled by charcoal (% of total length of small intestine)	Percentage inhibition (%)
Control (0.2 ml of charcoal meal p.o.)	85.7 ± 1.6	
Hydrocotyle javanica (100 mg/kg,p.o)	43.3 ± 1.9*	49.4 %
Atropine sulfate (5 mg/kg,i.m)	28.3 ± 1.43*	66.9 %

Number of animals used n = 6; * P <0.001 Vs control; Results are expressed as mean ±SEM analyzed by students 't' test.

The cathartic action of magnesium sulphate is believed to result from osmotically mediated water retention, which then stimulates peristalsis [15]. The antidiarrheal activity of *Hydrocotyle javanica* may be attributed to a reduction in peristaltic activity.

In the present study, the antidiarrheal activity of *Hydrocotyle javanica* has been evaluated in terms of percentage protection using the model of magnesium sulphate induced diarrhea in mice. *Hydrocotyle javanica* leaf extract offered significant percentage protection and showed satisfactory antidiarrheal activity. In conclusion *Hydrocotyle javanica* dose dependently inhibited magnesium sulphate induced diarrhea and increased gastrointestional transit time in mice. The present investigation supports the use of medicinal plants in traditional antidiarrheal formulations [16]. The mechanism of the antidiarrheal action of *Hydrocotyle javanica* is unknown, and further studies are required to elucidate it.

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