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Antidiarrhoeal and Antioxidant Effects of *Centella asiatica* Extract

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The objective of the study was to find out the antidiarrhoeal and antioxidant effects of *Centella asiatica* extract in rats. For antioxidant studies spectrophotometric method was used, while for antidiarrhoel studies castor oil induced diarrhoeal method was used. The results obtained indicate that the extracts of *Centella asiatica* have antidiarrhoeal and antioxident activities. The extract at graded doses (100, 200, 400 and 600 mg/kg body weight) was investigated for antidiarrhoeal activity in term of reduction in the rate of defecation in castor oil-induced diarrhoea. To understand the mechanism of its antidiarrhoeal activity, the gastro-intestinal transit and PGE2-induced intestinal fluid accumulation (entero-pooling) were further evaluated. At graded doses (100, 200, 400 and 600 mg/kg body weight), the extract showed a remarkable antidiarrhoeal activity evidenced by the reduction in the rate of defecation up to 78.68 % of control diarrhoeal animals at the dose of 600 mg/kg body weight. The results are comparable to that of a standard drug loperamide (3 mg/kg body weight). The extract produced profound decrease in intestinal transit (8.26-55.67 %) at selected doses comparable to that of single intraperitoneal injection of standard drug atropine sulphate at doses of 0.1 mg/kg body weight. It significantly inhibited PGE2-induced enteropooling (22.10-57.12 %). The results indicated that the ethanol extract of the *C. asiatica* possessed significant antidiarrhoeal effect and substantiated the use of this herbal remedy as a non-specific treatment for diarrhoea in folk medicine. *Centella asiatica* also possessed antioxidant activity that can be attributed to the presence of glycosides and triterpenes. The obtained result compared with that of standard antioxidant curcumin.

Key Words: Antidiarrhoeal and Antioxidant activities, *Centella asiatica* extract, Castor oil.

INTRODUCTION

Renewed interest in biological activities of medicinal plants emerged in early 1980's as the Council of Scientific and Industrial Research (New Delhi, India) have published the information on the screening of biological activities of many medicinal plants using experimental models[1]. Recently, the use of herbal preparations in remedies for various medical conditions have been rapidly increasing especially in India. It is believed that herbal preparations are safe although the ingredients have never been vigorously substantiated.

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In developing countries, the majority of people living in rural areas almost exclusively use traditional medicines to treat all sorts of diseases including diarrhoea. Many plants, namely *Andrographis paniculata, Acacia catechu, Acacia chandra, Terminalia chibula, Pterocarpus marsapium, Cassia auriculata, etc.* available in India are used in traditional folklore medicine for the treatment of diarrhoea[2].

Centella asiatica (family: Umbelliferae) is used as antibacterial and analgesic drug. There is a growing interest in co-relating phytochemical constituents of plants with its pharmacological activities. Recent studies on *Centella asiatica* indicate that it has different therapeutic uses like wound healing, anti allergic, antiinflammatory, antinociceptive, antidiabetic effects. Important active constituents presents in *Centella asiatica* are glycoside, triterpenoid saponins, including asiaticoside, centelloside, madecassoside and asiatic acid, rhanmanose. In addition, *Centella* contains other components, including volatile oils, flavonoids, tannins, phytosterols, amino acids and sugars.

EXPERIMENTAL

Aerial parts of *Centella asiatica* plant were collected freshly from in and around Trichur District, Kerala, India. The whole plant were dried under shade and made into coarse powder by grinding. The plant were identified and authenticated at Herbarium Tamil Nadu Agricultural University, Coimbatore, India (Ref: ID NCP/2006).

Atropine sulphate was purchased from Maxwell Pharma. Pvt. Ltd., Mumbai, India. Loperamide was procured from cetadel Pharma. Pvt. Ltd., Chennai, India. PGE_2 was purchased form Astra-IDL Ltd., India. Shimadzu spectorophotometer used for *in vitro* study of anti oxidant effect. pH-meter-manufactured by Control Dynamics Co., Instrumentation Pvt., Ltd., Banglore, India.

Reagents prepared: Phosphate buffer saline as per Indian Pharmacopoeia. 1,1-Diphenyl-2-picryl hydrazyl (DPPH) 3.9 mg in 10 mL ethanol.

Acute toxicity studies[3]: Animals were fed with increasing doses of 50, 100, 300, 600, 1000 and 3000 mg/kg of extract of cassia asiatica suspended in % w/v of gum acacia. The animals were observed continuously for 2 h for gross behavioural changes and then intermittently once every 2 h and finally at the end of 24 and 72 h to note any other toxic signs including death.

Preparation of plant extract

Aqueous/alcoholic extract: To 20 g of each dried plant powder form, 500 mL water/ethanol were added and contents of flask were mixed thoroughly by gentle shaking. Flasks were kept for 4 d with frequent shaking. After the completion of maceration process, the filtrates were obtained and water/ethanol evaporated to get the dried extract (evaporation by keeping flasks in electric mantle at 80 ºC). The residual extract was dissolved in water and used in the studies.

Groups and treatment

Antidiarrhoeal activity: Wistar albino rats (150-180 g) of either sex were used for the experiments. Animals were allowed to be acclimatized for a period of 2

weeks in the laboratory environment prior to the study. Animals were housed in polypro-pylene cages (4 animals per cage), maintained under standard laboratory conditions (*i.e.*, 12:12 h light and dark sequence; at an ambient temperature of 25 ± 2 ºC; 35-60 % humidity). The animals were fed with standard rat pellet diet (Hindustan Liver Ltd., Mumbai) and water *ad libitum*.

Antioxidant activity: For the study of antioxidant effects, the inhibition of lipid peroxidation, superoxide generation and the activities of superoxide or hydroxyl radical scavenging are usually evaluated.

Method

DPPH method: The equimixture of DPPH (10 ng/ethanol) and test compounds (10 ng/ethanol) kept for 20 min at room temperature. Then absorbance measured at 517 nm and curcumin used as standard drug to compare the activity.

Antidiarrhoeal activity

Castor-oil induced diarrhoea: Overnight fasted 36 rats were divided into 6 groups equally as follows. **Group I:** (Control group) rats of this group received 1 mL 2 % (v/v) aqueous Tween 80 orally. **Group II, III, IV and V:** (Extract treated groups) rats of these groups were treated with ethanol extract of *Centella asiatica* at the doses of 100, 200, 400 and 600 mg/kg body weight by oral route, respectively suspended in 2 % (v/v) aqueous Tween 80. **Group VI:** (Standard drug treated group). Rats of this group were treated orally with the reference drug, loperamide at the dose of 3 mg/kg body weight. After 1 h of administration, all the rats were treated with 1 mL of castor oil orally by gavage and observed for consistency of faecal material. The numbers of wet faecal droppings were measured for 4 h after castor oil administration. Characteristic diarrhoeal droppings were noted in transparent plastic dishes placed beneath the individual perforated rat cages[4,5]. The total number of diarrhoeal faeces of the control group was considered 100 %. The results were expressed as percentage of inhibition of diarrhoea.

Gastrointestinal motility tests: This experiment was done by using charcoal meal as a diet marker[6]. Albino rats were fasted for 18 h and divided into 6 groups containing 6 animals each. Each animal was administered with 1.0 mL of charcoal meal orally (3 % deactivated charcoal in 10 % aqueous Tween 80) and subsequent treatments were as follows **Group I:** (Control group) rats of this group received 1.0 mL 2 % (v/v) aqueous Tween 80 orally. **Group II, III, IV and V:** (Extract treated groups) rats of these groups were treated with ethanol extract of *Centella asiatica* (suspended in 2 % (v/v) aqueous Tween 80) at the doses of 100, 200, 400 and 600 mg/kg body weight by oral route, respectively. **Group VI:** (Standard drug treated group) rats of this group were treated with the reference drug, atropine sulphate at the dose of 0.1 mg/kg body weight, intraperitonealy. After 0.5 h, all rats were sacrificed under ether anesthesia. The peritoneal cavity was cut and the distance traversed by charcoal meal from the pylorus towards caecum was measured and expressed as a percentage of the distance from the pylorus to the caecum[7].

PGE_2-Induced enteropooling: In this method, rats were deprived of food and water for 18 h and divided into 6 groups of 6 animals each as follows. **Group I:** (Control group); rats of this group received 1.0 mL of 2 % (v/v) aqueous Tween 80 orally. **Group II, III, IV and V:** (Extract treated groups); rats of these groups were treated with ethanol extract of *Centella asiatica* at the doses of 100, 200, 400 and 600 mg/kg body weight by oral route, respectively suspended in 2 % (v/v) aqueous Tween 80. **Group VI:** (Standard drug treated group); rats of this group were treated with the reference drug, atropine sulphate at the dose of 3.0 mg/kg body weight, intraperitonealy. Immediately afterwards, PGE_2 was administered orally to each rat (100 µg/kg) in 5 % (v/v) ethanol in normal saline. After 0.5 h, each rat was killed and the whole length of the intestine from the pylorus to the caecum was dissected out and its contents were collected in a test tube and measured[8].

RESULTS AND DISCUSSION

Antidiarrhoeal activity

Inhibition of castor-oil induced diarrhoea: A single oral administration at various doses of *Centella asiatica* extracts produced a significant decrease in the severity of diarrhoea in terms of reduction in the rate of defecation in Wistar albino rats. The percentage inhibition for the number of wet faeces indicates the presence of antidiarrhoeal activity in extract as compared with that of control group. Experimental result reflects that the activity is more pronounced at the dose of 600 mg/kg body weight (Table-1). The percentage of inhibition of number of wet faeces was 78.68 %, $p < 0.01$ at the dose of 600 mg/kg body weight while that of standard drug loperamide (3 mg/kg) was 99.59 % control of castor oil-induced diarrhoea.

TABLE-1
EFFECT OF THE ETHANOL *Centella asiatica* EXTRACTS AT DIFFERENT DOSE LEVELS ON CASTOR OIL-INDUCED DIARRHOEA

Group	Treatment	Number of wet faeces	Inhibition (%)
I	2 % (v/v) aqueous Tween 80 (1 mL/kg) + Castor oil (1 mL)	4.10 ± 0.53	
II	Extract (100 mg/kg) + Castor oil (1 mL)	1.86 ± 0.23*	48.30
III	Extract (200 mg/kg) + Castor oil (1 mL)	1.89 ± 0.44*	53.12
IV	Extract (400 mg/kg) + Castor oil (1 mL)	1.18 ± 0.36*	65.24
V	Extract (600 mg/kg) + Castor oil (1 mL)	0.81 ± 0.29**	78.68
VI	Loperamide (3 mg/ kg) + Castor oil (1 mL)	0.03 ± 0.34**	99.59

Data are expressed as mean ± SEM, n = 6. *p < 0.05, **p < 0.01 when compared with vehicle-control.

Effects on gastrointestinal motility: The *Centella asiatica* extracts produced profound decrease in intestinal transit of 8.26-55.67 % at the dose range of 100, 600 mg/kg body weight (Table-2) and while atropine sulphate produced 54.40 % inhibition of intestinal transit at dose of 0.1 mg/kg body weight.

TABLE-2
EFFECT OF THE ETHANOL *Centella asiatica* EXTRACTS AT DIFFERENT DOSE LEVELS ON CHARCOAL-INDUCED GUT TRANSIT CHANGES

Group	Treatment	Distance travel by charcoal meal (%)	Inhibition (%)
I	Charcoal meal + 2 % (v/v) aqueous Tween 80 (1 mL/kg)	79.51 ± 2.38	–
II	Charcoal meal + Extract (100 mg/kg)	73.24 ± 1.88*	8.26
III	Charcoal meal + Extract (200 mg/kg)	66.62 ± 2.16*	17.00
IV	Charcoal meal + Extract (400 mg/kg)	57.40 ± 2.32*	29.10
V	Charcoal meal + Extract (600 mg/kg)	39.22 ± 2.76**	55.67
VI	Charcoal meal + Atropine sulphate (0.1 mg/kg)	35.75 ± 2.67**	54.40

Data are expressed as mean ± SEM, n = 6. *p < 0.05, **p < 0.01 when compared with vehicle-control.

Antienteropooling activity: The extract also inhibited significantly PGE_2-induced enteropooling in term of volume of intestinal content (Table-3). The extract at the dose range of 100-600 mg/kg body weight showed percentage inhibition ranging from 22.10 to 57.12 % while atropine sulphate at the dose of 3 mg/kg body weight produced 67.11 % inhibition of PGE_2-induced enteropooling.

TABLE-3
EFFECT OF THE ETHANOL EXTRACT OF *Centella asiatica* AT DIFFERENT DOSE LEVELS ON PGE2-INDUCED ENTEROPOOLING

Group	Treatment	Volume of intestinal fluid (mL)	Inhibition (%)
I	2 % (v/v) aqueous Tween 80 (1 mL/kg) + PGE_2 in ethanol (100 µg/kg)	2.60 ± 0.16*	–
II	Extract (100 mg/kg) + PGE_2 in ethanol (100 µg/kg)	2.12 ± 0.11*	22.10
III	Extract (200 mg/kg) + PGE_2 in ethanol (100 µg/kg)	2.02 ± 0.08**	30.96
IV	Extract (400 mg/kg) + PGE_2 in ethanol (100 µg/kg)	1.72± 0.14**	44.20
V	Extract (600 mg/kg) + PGE_2 in ethanol (100 µg/kg)	1.28 ± 0.09*	57.12
VI	Atropine sulphate (3.0 mg/kg) + PGE_2 in ethanol (100 µg/kg)	0.96 ± 0.46**	67.11

Data are expressed as mean ± SEM, n = 6. *p < 0.05, **p < 0.01 when compared with vehicle-control.

Antioxidant activity: As compared with the standard antioxidant curcumin, *Centella asiatica* extracts possess significant antioxidant activities (Table-4). Glycosides, triterpenes are the active principle may elicit antioxidant activity.

TABLE-4
ANTIOXIDANT ACTIVITY OF *C. asiatica* EXTRACTS

Treatment	Absorbance	Relative % activity, considering that of curcumin as 100 %
Curcumin	2.596	100
Alcoholic extract	1.642	64
Aqueous extract	1.382	54

In this study, the ethanol extract of *Centella asiatica* exhibited a significant dose-dependant antidiarrhoeal activity. The results were comparable to that of the standard drug loperamide (3 mg/kg) with regard to the severity of diarrhoea. The extract also significantly reduced intestinal transit as observed by the decrease in % of distance travel by charcoal meal. This may be due to the fact that the extract may increase the reabsorption of water by decreasing intestinal motility as observed in the decrease of intestinal transit by charcoal meal.

The extract also led to a marked reduction in the volume of the intestinal contents on PGE2-induced enteropooling. Thus the inhibiting effect of the extract justifies the use of the plant in folk medicine as a non-specific antidiarrhoeal agent. Preliminary phytochemical investigations indicated presence of glycosides and saponins present in *C. asiatica*. Saponins are known for their antidiarrhoeal activity[9]. Infact, saponins are responsible for the denaturation of proteins, which makes the intestinal mucosa more resistant and reduces secretion[10]. Thus, the antidiarrhoeal activity may be due to saponins present *Centella asiatica*.

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