

Original Article

ANTI-DIARRHOEAL ACTIVITY OF CARDIOSPERMUM HALICACABUM AND DODONEA VISCOSA

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ABSTRACT

Objective: The present study to carry out the anti-diarrhoeal activity of leaf extracts of *Cardiospermum halicacabum* and *Dodonea viscosa* in different experimental models of diarrhoea.

Methods: The acute toxicity of the extracts were determined, LD₅₀ was calculated according to the "Up and down method" following OECD guidelines No. 425 of CPCSEA. The anti-diarrhoeal potential was assessed by castor oil induced diarrhoea. Gastro-intestinal motility test and Prostaglandin-E₂ induced enteropooling in experimental animals.

Results: The alcoholic and aqueous extracts of *Cardiospermum halicacabum* and *Dodonea viscosa* have exhibited the dose dependant anti-diarrhoeal activity in all the three experimental models by reducing the frequency of defecation as well as total weight of wet faeces, decreased the propulsion of charcoal meal through the gastrointestinal tract and also decrease in the oedema volume in the intestine (intestinal secretion) of the drug treated animals with the respective models of diarrhoea.

Conclusion: *Cardiospermum halicacabum* and *Dodonea viscosa* shows to have a potential value for the development of a phytomedicine for diarrhoea. Further comprehensive chemical and pharmacological investigations are needed to elucidate the exact mechanism accountable for the exhibited activity.

Keywords: *Cardiospermum halicacabum*, *Dodonea viscosa*, Diarrhoea, Castor oil, Prostaglandin E₂, Charcoal meal.

INTRODUCTION

Diarrhoea is a very common and major national problem in many tropical countries which results 4-5 million deaths throughout the world annually [1]. During the past decade, oral dehydration therapy has reduced mortality from acute diarrhoeal disease, whereas chronic diarrhoea remains a life-threatening problem in those regions, in which malnutrition is a common co-existing and complication factor. Perpetuation of the diarrhoeal syndrome is said to be involved with the number of factors such as infective, immunological and nutritional components [2]. A diarrhoeal disease control program aimed at the holistic approach to include all aspects of traditional medical practices, evaluation of health education and preventive approaches had been constituted by the World Health Organisation (WHO) to combat the problem of diarrhoea in developing countries [1, 3-4]. In many Asian countries including India apart from modern medical therapy, the use of herbal drugs in the treatment of diarrhoeal diseases is a common practice. The plants *Cardiospermum halicacabum* and *Dodonea viscosa*, family sapindaceae, categorized under treating rheumatism, stiffness of the limbs, snake bite, nervous diseases, stomach disorders, skin rashes, teeth ache, fever and as astringent in the traditional system of medicines [5-6]. The number of pharmacological properties such as wound healing [7], insecticidal [8], anti-filarial [9], and antipyretic [10], antimicrobial [11], anti-inflammatory [12] and antidiabetic [13] activities has been reported with *Cardiospermum halicacabum* and *Dodonea viscosa* have been reported.

The crude leaf extracts of *Cardiospermum halicacabum* and *Dodonea viscosa* has been used traditionally by native practitioners for gastro intestinal problems. However, there are no systematic experimental reports on anti-diarrhoeal activity of these two plants in literature. The present study was undertaken to find out the scientific rationale behind the local use of *Cardiospermum halicacabum* and *Dodonea viscosa* in diarrhoea.

MATERIALS AND METHODOS

Extraction

The plants *Cardiospermum halicacabum* and *Dodonea viscosa* are collected from Hassan, Karnataka. Leaves were separated, washed

with water, shade dried, reduced to coarse powder and subjected to successive extraction with, alcohol using soxhlet apparatus and with distilled water by maceration and were subjected to preliminary phytochemical analysis [14].

Animals

Albino mice of either sex weighing 18-22 gms and albino rats of either sex weighing 180-220 gms were acclimatized for a period of seven days in the laboratory under standard husbandry conditions i.e. Room temperature 26±2°C, relative humidity 45-55% and light/dark cycle 12/12 hours. All the animals were fed with a standard diet (Gold Mohr, Lipton India Ltd., Bangalore) and water was supplied *ad libitum* under strict hygienic conditions. All the experimental protocols were approved by Institutional Animal Ethical Committee.

Acute toxicity studies

The acute toxicity of alcoholic and aqueous extracts of *Cardiospermum halicacabum* and *Dodonea viscosa* was determined in albino mice weighing 18-22 gms of either sex. After administration with different doses of these extracts, the number of animals survived with each extract was noted for acute (48 hours), and chronic (14 days) period of time. The animals were physically active and regularity in consumption of food and water was observed. The dose up to 2000mg/kg body weight did not produce any signs of toxicity or mortality. LD₅₀ was calculated according to the "Up and down method" following OECD guidelines No. 425 of CPCSEA.

Anti-diarrhoeal activity

Castor oil induced diarrhoea

Method: The method described by Awouters F. et. al [15], was followed here with some modifications.

In the present study albino rats of either sex weighing 160-190 gms were divided into ten groups of each comprising of six animals. They were fasted overnight prior to the test with free access to water all the time. Group 1 (served as control i. e. 0.2 ml of vehicle p. o.),

Group 2 (served as Standard drug i.e. loperamide 3mg/kg p. o.), Group 3-10 was treated with alcoholic and aqueous extracts of *Cardiospermum halicacabum* and *Dodonea viscosa* at the dose of 200mg/kg and 400mg/kg body weight respectively. After 30 minutes, each rat received 1 ml of castor oil orally and then was housed separately in the metabolic cages with special provision to separate urine and faeces. Then the diarrhoeal episode was observed for a period of 4 hours. During this period number and weight of diarrhoeal dropping were noted. Percentage of diarrhoea and percentage of inhibition was calculated by making use of mean weight of the stools. Anti-diarrhoeal activity was determined in terms of percentage of protection which was calculated by following formula:

$$\text{Percentage of protection (\%)} = \text{A-B/A}$$

Where, 'A' is the total weight of stools of control animals.

'B' is the total weight of stools of extracts treated animals.

The data of stools weight were expressed as mean \pm SEM.

Gastro-intestinal motility test

Method: The method described by Pazhani G. P. et. al. [16] was used in this study.

In the present study albino rats of the either sex weighing 160-200 gms were divided into ten groups of each comprising of six animals. They were fasted for 24 hours prior to the test with free access to water at all the time. Group 1 (served as control) received vehicle), Group 2 (serves as standard) received Atropine sulphate 5mg/kg i. p. Group 3-10 was treated with alcoholic and aqueous extracts of *Cardiospermum halicacabum* and *Dodonea viscosa* at the dose of 200mg/kg and 400mg/kg body weight respectively. After 30 minutes, 1 ml of charcoal meal (3% deactivated charcoal in 10% normal saline) was administered by oral route to all the animals. 30 minutes after this treatment, all rats were sacrificed and the distance travelled by the charcoal meal in each animal's intestine from pylorus to caecum was noted.

Percentage travelled and percentage of inhibition was calculated by the following formulae,

$$\% \text{ travelled} = (\text{A/B}) * 100.$$

$$\% \text{ of inhibition} = \{(\text{B-A})/\text{B}\} * 100.$$

Where 'A' is the distance travelled by the charcoal meal,

'B' is the total length of small intestine.

Prostaglandin-E₂ induced enteropooling

Method: The method described by Murugesan T et. al. [17] was used in this study.

Eleven groups of albino rats of either sex, (n=6) weighing between 16-200 gms were used. The rats were deprived of food and water 18 hours prior to the experiment. Group 1 (served as control) received 1 ml of 5% ethanol in normal saline i. p., then 0.2 ml of vehicle p. o, Group 2 (served as positive control) received 1 ml of 5% ethanol in normal saline i. p. Group 3 (served as standard) received Loperamide 3 mg/kg p. o., Group 4-10 were treated with alcoholic and aqueous extracts *Cardiospermum halicacabum* and *Dodonea viscosa* at the dose of 200mg/kg and 400mg/kg body weight respectively. Immediately after the above treatment each rat was treated with prostaglandin-E₂ (100µg/kg in 5% ethanol in normal saline orally). Group I served as control and was received vehicle only but not prostaglandin-E₂. After 30 minutes all the rats were sacrificed. The total length of the intestine from the pylorus to the caecum was dissected out and its contents were collected. Total fluid in each animal of each group was noted.

Percentage reduction or inhibition in oedema volume was calculated by using the formula:

$$\text{Percentage inhibition} = 100 - (\text{A/B}) * 100$$

Where "A" is the volume of intestinal fluid in the treated animals

and "B" is the volume of intestinal fluid in the prostaglandin E₂ treated group animals

Statistical analysis

Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Dunnett's test to calculate the significance difference among the groups.

RESULTS

The preliminary phytochemical studies confirmed the presence of sterols, saponins, carbohydrates, flavonoids tannins, fixed oils and triterpenoids with alcoholic extracts, while saponins, carbohydrates, flavonoids and tannins with the aqueous leaves extracts of *Cardiospermum halicacabum* and *Dodonea viscosa*. The Alcoholic and aqueous extracts of *Cardiospermum halicacabum* and *Dodonea viscosa* have exhibited the dose dependant anti-diarrhoeal activity in all the three experimental models. The extracts exhibited the most promising dose dependent activity against the castor oil induced diarrhea in rats by reducing the frequency of defecation as well as total weight of wet faeces. The extracts also significantly and dose dependently decreased the propulsion of charcoal meal through the gastrointestinal tract with respect to control group. The decrease in the oedema volume in the intestine (intestinal secretion) of the drug treated animals proved the inhibition of prostaglandin secretion in the intestine.

DISCUSSION

Inhibition of experimental diarrhoea and reduction in faecal output by a substance are the basis of the pharmacological evaluation of a potential anti-diarrhoeal agent. Reduction of gastrointestinal motility and secretions are the mechanism by which many anti-diarrhoeal agents can act. It is widely known that castor oil or its active component ricinoleic acid causes hypersecretory response and diarrhea due to permeability changes, induction of peristaltic changes and electrolyte transport in mucosal fluid. It is also reported to reduce active Na⁺ and K⁺ absorption and decreases Na⁺ and K⁺ ATPase activity in the small intestine and colon [18]. Experimental studies in rats demonstrated a significant increase in the portal venous PGE₂ concentration following oral administration of castor oil [18-19]. Ricinoleic acid markedly increased the PGE₂ content in the gut lumen and also caused an increase of the net secretion of water and electrolytes into the small intestine [20]. The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion [21]. It has been observed that prostaglandin attribute to the pathophysiological functions in gastrointestinal tract [22]. However, inhibitors of prostaglandin biosynthesis delayed castor oil induced diarrhoea. It has been shown that E type of prostaglandins causes diarrhoea in experimental animals as well as in human beings [15]. Their mechanism has been associated with dual effects on gastrointestinal motility as well as on water and electrolyte transport [23]. PGE₂ also inhibit the absorption of glucose, a major stimulus to intestinal absorption of water and electrolytes [24]. It was found that the flavonoids, saponins, tannins, steroids exhibited marked anti-bacterial activity [25] and anti-diarrhoeal activity [26] and since the phytoconstituents are present with our extracts might have contributed for the exhibited biological activity. The extracts have showed marked reduction of the peristaltic movement of the gut, reduction in the faecal output and intestinal secretion, supports the traditional use of these species in controlling diarrhoea in animals.

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CH AL 200: Alcoholic extracts of *Cardiospermum halicacabum* (200 mg)

CH AL 400: Alcoholic extracts of *Cardiospermum halicacabum* (400 mg)

CH AQ 200: Aqueous extracts of *Cardiospermum halicacabum* (200 mg)

CH AL 400: Aqueous extracts of *Cardiospermum halicacabum* (400 mg)

DV AL 200: Alcoholic extracts of *Dodonea Viscosa* (200 mg)

CH AL 400: Alcoholic extracts of *Dodonea Viscosa* (400 mg)

CH AQ 200: Aqueous extracts of *Dodonea Viscosa* (200 mg)

CH AL 400: Aqueous extracts of *Dodonea Viscosa* (400 mg).

Table 1: Anti-diarrhoeal activity of *Cardiospermum halicacabum* and *Dodonea viscosa* in castor oil induced diarrheal model

Group	Treatment	Mean weight of stools± SEM after 4 hours (gms)	Percentage Inhibition
1	Control	4.97±0.09	-
2	Standard	0	100.00
3	CH AL 200	2.65±0.05**	50.34
4	CH AL 400	1.40±0.04**	78.86
5	CH AQ 200	2.02±0.03**	59.40
6	CH AQ 400	0.77±0.01**	84.56
7	DV AL 200	2.47±0.06**	51.34
8	DV AL 400	1.40±0.04**	85.57
9	DV AQ 200	1.83±0.03**	63.09
10	DV AQ 400	0.62±0.02**	87.58

Significance at $p < 0.01^{**}$, $p < 0.05^{*}$, $n=6$

Table 2: Anti-diarrhoeal activity of *Cardiospermum halicacabum* and *Dodonea viscosa* in gastrointestinal motility test model

Groups	Treatment	Mean total length ± SEM (cms)	Mean distance traveled ± SEM (cms)	Mean percentage movement of charcoal ± SEM (cms)	Percentage Inhibition
1	Control	92.33±1.66	84.50±2.07	91.56±0.60	-
2	Standard	93.5±4.13	33.67±2.66	36.58±0.18**	63.54
3	CH AL 200	92.24±3.05	46.33±1.03	50.92±0.40**	49.28
4	CH AL 400	88.14±3.05	43.00±1.94	46.76±0.31**	53.43
5	CH AQ 200	89.06±3.05	42.83±1.41	46.58±0.35**	49.31
6	CH AQ 400	91.35±3.05	39.67±1.47	43.13±0.26**	53.06
7	DV AL 200	92.33±3.05	45.17±1.21	49.098±0.35**	51.08
8	DV AL 400	90.33±3.05	40.47±2.32	43.67±0.39**	56.50
9	DV AQ 200	89.67±3.36	38.83±1.47	42.24±0.37**	54.04
10	DV AQ 400	90.33±3.05	36.17±0.98	39.33±0.22**	57.20

Significance at $p < 0.01^{**}$, $p < 0.05^{*}$, $n=6$

CH AL 200: Alcoholic extracts of *Cardiospermum halicacabum* (200 mg), CH AL 400: Alcoholic extracts of *Cardiospermum halicacabum* (400 mg), CH AQ 200: Aqueous extracts of *Cardiospermum halicacabum* (200 mg), CH AL 400: Aqueous extracts of *Cardiospermum halicacabum* (400 mg), DV AL 200: Alcoholic extracts of *Dodonea Viscosa* (200 mg), CH AL 400: Alcoholic extracts of *Dodonea Viscosa* (400 mg), CH AQ 200: Aqueous extracts of *Dodonea Viscosa* (200 mg), CH AL 400: Aqueous extracts of *Dodonea Viscosa* (400 mg)

Table 3: Anti-diarrhoeal activity of *Cardiospermum halicacabum* and *Dodonea viscosa* in prostaglandin E₂ induced enteropooling

Group	Treatment	Mean volume of intestinal fluid ± SEM (ml)	Percentage inhibition
1	Control	0.65±0.04	-
2	Prostaglandin E ₂ in ethanol	2.97±0.05	-
3	Standard	0.82±0.04**	72.47
4	CH AL 200	1.85±0.03**	37.64
5	CH AL 400	1.02±0.01**	65.73
6	CH AQ 200	1.70±0.01**	42.70
7	CH AQ 400	0.85±0.02**	71.35
8	DV AL 200	1.78±0.02**	39.89
9	DV AL 400	0.95±0.02**	67.98
10	DV AQ 200	1.30±0.01**	56.18
11	DV AQ 400	0.83±0.02**	71.91

Significance at $p < 0.01^{**}$, $p < 0.05^{*}$, $n=6$.

CH AL 200: Alcoholic extracts of *Cardiospermum halicacabum* (200 mg), CH AL 400: Alcoholic extracts of *Cardiospermum halicacabum* (400 mg), CH AQ 200: Aqueous extracts of *Cardiospermum halicacabum* (200 mg), CH AL 400: Aqueous extracts of *Cardiospermum halicacabum* (400 mg), mDV AL 200: Alcoholic extracts of *Dodonea Viscosa* (200 mg), CH AL 400: Alcoholic extracts of *Dodonea Viscosa* (400 mg), CH AQ 200: Aqueous extracts of *Dodonea Viscosa* (200 mg), CH AL 400: Aqueous extracts of *Dodonea Viscosa* (400 mg)

CONFLICT OF INTERESTS

Declared None

CONCLUSION

Cardiospermum halicacabum and *Dodonea viscosa* shows to have a potential value for the development of a phytomedicine for diarrhoea. Further comprehensive chemical and pharmacological investigations are needed to elucidate the exact mechanism accountable for the exhibited activity.

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