Research Paper

Pharmacological investigation of *Cardiospermum halicacabum* (Linn) in different animal models of diarrhoea

N. Venkat Rao¹, K. Chandra Prakash², S.M. Shanta Kumar³

ABSTRACT

Objective: To evaluate the antidiarrhoeal activity of whole plant extracts of *Cardiospermum halicacabum* (Linn) in rats.

Materials and Methods: Petroleum ether (PeCH) and alcoholic (AlCH) extracts of whole plant of *Cardiospermum halicacabum* (Linn) were prepared, with successive extraction in soxhlet apparatus and aqueous (AqCH) extract, by the maceration process. LD₅₀ studies for all the three extracts were carried out up to the dose limit of 2000 mg/kg in albino mice. One-fifth of the maximum dose of LD₅₀ of each extract was selected to study the antidiarrhoeal activity in different experimental models such as castor oil-induced diarrhoea, prostaglandin E₂ (PGE₂)-induced enteropooling and charcoal meal test in rats.

Results: Preliminary phytochemical studies revealed the presence of sterols, carbohydrates, tannins and triterpenes in the PeCH extract; sterols, saponins, carbohydrates, flavonoids and tannins in the AlCH extract; sterols, saponins, carbohydrates, flavonoids and tannins in the AqCH extract. No mortality was observed with any of the three extracts up to the maximum dose of 2000 mg/kg. Further, all the three extracts at 400 mg/kg, p.o. had significantly (P<0.01) reduced the fecal output in castor oil-induced diarrhoea, intestinal secretions in PGE₂ -induced enteropooling and peristaltic movement in charcoal meal test, indicating antidiarrhoeal activity.

Conclusion: The present study revealed the antidiarrhoeal activity of the extracts of *Cardiospermum halicacabum*, which may be due to the presence of phytochemical constituents such as sterols, tannins, flavonoids and triterpenes.

KEY WORDS: Antidiarrhoeal, castor oil, charcoal meal, enteropooling.

Introduction

Diarrhoea, an important health problem worldwide, especially in developing countries, accounts for more than 5-8 millions deaths in infants and children under 5 years, each year.¹ In recent years, there has been a great interest in herbal remedies for the treatment of a number of ailments. Medicinal plants are promising source of antidiarrhoeal drugs.² Indigenous plants such as *Andrographis paniculata*, *Asparagus racemosus*, *Butea monosperma*, *Cassia auriculata* and others are widely used for the treatment of diarrhoea.³

*Cardiospermum halicacabum* (Linn), family Sapindaceae, is a deciduous, branching, herbaceous climber, which is distributed throughout the plains of India. The whole plant has been used for several centuries in the treatment of rheumatism, stiffness of limbs, snake bite;⁴ its leaves and stalks are used in the treatment of diarrhoea, dysentery and headache⁵ and as a poultice for swellings.⁶ Phytochemical constituents such as flavones, aglycones, triterpenoids, glycosides and a range of fatty acids and volatile ester have been reported from the various extracts of this plant.⁷-⁹

An infusion of whole plant is traditionally used in the treatment of diarrhoea by native medical practitioners in Gulbarga. However, the plant has not been experimentally tested for its antidiarrhoeal activity. Hence, an effort was made to investigate the same with whole plant extracts in experimentally-induced diarrhoea in rats.

Materials and Methods

Drugs and chemicals

Analytical grade petroleum ether and 95% ethanol (S.D. Fine Chemicals, Mumbai), glass distilled water and loperamide...
(Torrent Pharmaceuticals, Ahmedabad) were used for the study.

**Plant extraction**

The whole plant was collected from August to December, 2005 and identified by Professor Srivatsa, a botanist from LVD College, Raichur. A sample specimen was deposited, bearing voucher number C-2515. The shade-dried plant material was powdered. The coarse powder was subjected to successive extraction with petroleum ether and alcohol in soxhlet apparatus at (60-80°C) and the marc obtained after alcoholic extraction was macerated with water to obtain an aqueous extract.

**Phytochemical investigation**

The petroleum ether (PeCH), alcohol (AlCH) and aqueous (AqCH) extracts of *C. halicacabum* (Linn) were subjected to preliminary, qualitative phytochemical investigations. The percentage yield for PeCH, AlCH and AqCH were 2.26, 2.50 and 4.80, respectively.

**Experimental animals**

Swiss albino mice (18-22 g) and Wistar albino rats (150-200 g) of either sex were acclimatized for 7 days under standard husbandry conditions, i.e. room temperature 26±2°C, relative humidity 45-55% and light:dark cycle 12:12 h. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of V.L. College of Pharmacy, Raichur and conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

**Acute toxicity studies**

The acute toxicity of PeCH, AlCH and AqCH was determined in female albino mice (18-22 g). After administration with different doses of these extracts, the mortality with each dose was noted at 48 hours (acute) and at 14 days (chronic). LD50 was calculated as per OECD guidelines using AOT 425 software.

**Antidiarrhoeal activity**

**Castor oil induced diarrhoea**

The method described by Awouters et al. was followed. Healthy albino rats of the either sex (160-190 g) were divided into 5 groups of 6 animals each. They were fasted for 18 h prior to the test, with free access to water. Group I received the vehicle (0.2 ml of 5% Tween 80) and served as the control group. Groups II, III, IV and V were treated with standard drug (atropine sulphate 5 mg/kg, p.o.); Group III, IV and V with PeCH (400 mg/kg, p.o.), AlCH (400 mg/kg, p.o.) and AqCH (400 mg/kg, p.o.), respectively. After 30 min, 1 ml of charcoal meal (3% deactivated charcoal in normal saline) was administered orally to all and 30 min later, all the rats were sacrificed. The distance travelled by the charcoal meal from the pylorus to the caecum was noted.

**Gastrointestinal motility test**

Albino rats of either sex (160-200 g) were divided into 5 groups of 6 animals each. They were fasted for 24 h prior to the test, but allowed free access to water: Group I was treated with vehicle (0.2 ml of 5% Tween 80, p.o.), which served as control; Group II with standard drug (atropine sulphate 5 mg/kg, p.o.); Group III, IV and V with PeCH (400 mg/kg, p.o.), AlCH (400 mg/kg, p.o.) and AqCH (400 mg/kg, p.o.), respectively. Atropine sulphate (loperamide 3 mg/kg, p.o.) was used as the control.

**Statistical evaluation**

The groups were compared using one-way analysis of variance (ANOVA) followed by Dunnett’s test and P<0.05 was considered significant.

**Results**

It was found that the PeCH extract contained sterols, carbohydrates, tannins and triterpenes; the AlCH extract contained sterols, saponins, carbohydrates, flavonoids and tannins; and the AqCH extract had sterols, saponins, carbohydrates, flavonoids and tannins.

In acute toxicity, there was no mortality recorded in all the groups, i.e. PeCH-, AlCH- and AqCH-treated groups, up to the maximum dose of 2000 mg/kg. Hence 1/5th of the maximum dose tested was selected for the antidiarrhoeal studies.

In castor oil-induced diarrhoea, the PeCH, AlCH and AqCH significantly decreased the cumulative faecal mass when compared to the control group. The percentage inhibition of faecal weight with PeCH-, AlCH- and AqCH-treated groups were 49.06%, 62.49% and 85.30%, respectively [Table 1] when compared to the control group. All the three extracts have shown a significant reduction of intestinal fluid volume (enteropooling) in rats. The percentage reduction in intestinal fluid secretion with standard loperamide, PeCH, AlCH and AqCH were 72.86%, 42.82%, 51.60% and 67.11%, respectively. [Table 1] PeCH, AlCH and AqCH caused a decrease in propulsion of the charcoal meal through the gastrointestinal tract when compared to the control group. The percentage reduction of gastrointestinal motility with standard atropine sulphate, PeCH, AlCH and AqCH extracts were 69.66%, 31.66%, 40.98% and 44.77%, respectively when compared to the control group. [Table 1] The potency of antidiarrhoeal activity was in the following order: Loperamide > AqCH > AlCH > PeCH.

**Discussion**

In the present study, preliminary qualitative phytochemical
tests revealed the presence of sterols, carbohydrates, flavonoids, tannins, triterpenes and saponins in the extracts of *Cardiospermum halicacabum*. The inhibition of experimental diarrhoea and the reduction in faecal output by a substance are the basis of the pharmacological evaluation of a potential antidiarrhoeal agent. Many antidiarrhoeals act by reducing the gastrointestinal motility and/or the secretions. It is well-known that ricinoleic acid, an active component of castor oil, induces changes in mucosal permeability, electrolyte transport and intestinal peristalsis, leading to hypersecretory response and diarrhoea.\[13\] Ricinoleic acid causes irritation and inflammation of the intestinal mucosa, leading to prostaglandin release, which causes an increase in the net secretion of water and electrolytes into the small intestine.\[14,15\] Inhibitors of prostaglandin biosynthesis delay castor oil-induced diarrhoea.\[12\] It has been shown that E type of prostaglandins cause diarrhoea in experimental animals as well as in human beings. The mechanism has been associated with dual effects on gastrointestinal motility as well as on water and electrolyte transport.\[16\] PGE\(_2\) also inhibits the absorption of glucose, a major stimulus to the intestinal absorption of water and electrolytes.\[11\] The antidiarrhoeal activity of the extracts was comparable to the standard drugs. The activity might be due to tannins and flavonoids present in these extracts.

Tannins, flavonoids, alkaloids, sugars, sterols and triterpenes are reported for their antidiarrhoeal activity. Tannins can evoke an antidiarrhoeal effect and these substances may precipitate proteins of the enterocytes, reduce peristaltic movement and intestinal secretion.\[10\] The antidiarrhoeal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolyte secretion,\[22, 23\] which are known to be altered in this intestinal condition. *In vitro* and *in vivo* experiments have shown that flavonoids are able to inhibit the intestinal secretory response induced by prostaglandin E\(_2\).\[24\] In addition, flavonoids possess antioxidant properties,\[25\] which are presumed to be responsible for the inhibitory effects exerted upon several enzymes, including those involved in the arachidonic acid metabolism.\[26\] The whole plant extracts (i.e. petroleum ether, alcohol and aqueous) of *C. halicacabum* (Linn) contain tannins, flavonoids, saponins, sterols and triterpenes, which could have contributed to the antidiarrhoeal activity.

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Contact person:

Dr. S.C. Chopra
Professor & Head,
Department of Pharmacology, Dayanand Medical College & Hospital,
Ludhiana, Punjab-141001
Phone:0161-2441105 ext 724; Fax:01612; E-mail: sndpk1@yahoo.co.in

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