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Original article

Antidiarrhoeal activity of eriosematin E isolated from the roots of *Eriosema chinense* Vogel

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ABSTRACT

Background: Roots of the plant *Eriosema chinense* Vogel (Fabaceae) is distributed mainly over the Eastern Himalayan region of India and China. The roots of the plant are used as a vegetable by the people of Northern Australia, China and North East India and are used traditionally by the tribal people of Meghalaya (India) for the treatment of diarrhoea. It has been reported to have significant antidiarrhoeal, cytotoxic and antimycobacterial activity.

Purpose/Objective: The present investigation was undertaken to isolate a lead molecule responsible for the observed antidiarrhoeal activity.

Methods: Eriosematin E, a prenylated flavanone, was isolated using column chromatography and was characterized by comparing its melting point and spectroscopic data (UV, IR, ^{1}H NMR, ^{13}C NMR, Mass Spectra) from literature. Eriosematin E (2.5, 5 and 10 mg/kg p.o.) was then screened for normal faecal excretion rate and castor oil-induced diarrhoea models in rats. Further, it was examined for small intestinal transit, intestinal fluid accumulation and PGE_2 induced enteropooling models in rats. Biochemical estimations and Na^+ and K^+ concentration in intestinal fluid were also determined along with colonic histopathological studies.

Results: The results illustrated a significant ($P < 0.05$) reduction in normal faecal output at 10 mg/kg p.o. after 5th and 7th h of treatment and also showed maximum protection of 69.43% from diarrhoea in the castor oil-induced diarrhoea model. Significant results were also observed at the maximum effective dose of eriosematin E (10 mg/kg p.o.) in inhibiting peristaltic index (small intestinal transit) and reducing intestinal fluid volume of castor oil induced and PGE_2 induced enteropooling models. Further, eriosematin E restored all the alterations in biochemical parameters such as nitric oxide, protein, DNA, superoxide dismutase, catalase and lipid peroxidation. It also significantly recovered Na^+ and K^+ loss from body and confirmed its protective nature through the histopathological studies.

Conclusion: The study corroborates the antidiarrhoeal potential of eriosematin E which may be attributed to its antisecretory and antioxidant potential.

Introduction

Diarrhoea may be defined as a disorder with an increase of the volume or fluidity of stools, with a frequence of at least three times in a 24 h period resulting from a rapid movement of the faecal matter through the large intestine (Yakubu and Salimon, 2015). Recent studies suggest that the number of deaths due to diarrhoea in developing countries is close to 5–6 million mainly in children younger than 5 years, contributing to 15% of total mortality. This situation can be attributed to malnutrition, inadequacy of safe drinking water, sanitation, and hygiene, which is one of the major problems in developing countries like India (Rahman et al., 2015; Prasad et al., 2013; Thapar and Sanderson, 2004).

In order to overcome the threat of diarrhoea in developing countries, the World Health Organisation (WHO) has promoted the use of traditional herbal medicines, due to their economic viability, accessibility, ancestral experience and perceived efficacy

Abbreviations: CAT, catalase; CMC, carboxy methyl cellulose; ECM, Eriosematin E; ESI-MS, electrospray ionisation mass spectroscopy; FT/IR, Fourier transform infrared spectroscopy; KBr, potassium bromide; NMR, nuclear magnetic resonance spectroscopy; PGE_2, prostaglandin E2; PI, Peristaltic Index; SOD, superoxide dismutase; TBRAS, thiobarbituric acid reactive substances; TLC, thin layer chromatography.

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(Njume and Goduka, 2012). *Eriosema chinense* Vogel, a plant belonging to the family Fabaceae, is distributed mainly over the Eastern Himalayan region of India and China. It is also found in countries like Thailand, Myanmar and Australia. The roots of the plant are used as a vegetable by the people of Northern Australia, China and North East India (Neogi et al., 1989; Martin and Ryan, 2004) and are traditionally used by the tribal people of Meghalaya (India) as a decoction prepared from its grains, along with powdered pepper, for the treatment of diarrhea. Eight new prenylated flavonoids i.e. khonklonginols A-H, along with six known compounds including the five flavonoids lupinifolinol, dehydrolupinifolinol, flemichin D, eriosemaone A, lupinifolin, and one lignan yangambin have been isolated from the root of the plant. The roots of the plant have been reported for its cytotoxic and antimycobacterial activity (Sutthivaiyakit et al., 2009). Recently, we have evaluated the antidiarrhoeal activity of the roots extract, its bioactive fraction and lupinifolin isolated from the roots. Lupinifolin, did showed antidiarrhoeal activity; however; it was moderate as compared to the bioactive chloroform fraction (Prasad et al., 2013). Therefore, in the search of a potent antidiarrhoeal agent, we were successful in isolating a prenylated flavanone eriosematin E for the first time from the roots of this plant. The compound has been reported to have a potent antibacterial and antifungal activity (Ma et al., 1995). Thus, the present study is a valid attempt to evaluate the antidiarrhoeal potential of eriosematin E through different animal models and to investigate some biochemical parameters.

Material and methods

Plant material and extraction procedure

E. chinense roots were obtained from Jowai Area, Jaintia Hills District of Meghalaya (India) in May-June 2013 and were authenticated by Dr. B.K. Sinha (Scientist C, in charge), Botanical Survey of India, Shillong, India. For future reference, a voucher specimen (COG/EC/14) of the plant has been deposited in Department of Pharmaceutics, Indian Institute of Technology (Banaras Hindu University), Varanasi, India. The roots of the plant (500 g) were ground and extracted with ethanol (1.5 l) using a Soxhlet apparatus until the powder was exhausted. The extract was then concentrated and evaporated in a Rota evaporator (IKA India Private Limited, Bangalore, India) to obtain a brown extract (13.5% w/w) which was kept in a desiccator until use.

Isolation

The extract was fractionated using different solvents such as hexane: (2.75% w/w), chloroform: (24.32% w/w), ethyl acetate: (10.14% w/w) and based upon the yield of the fractions and previous reports (Prasad et al., 2013), chloroform fraction was subjected to column chromatography. Chloroform fraction (15 g) was loaded on a silica gel column and was eluted with hexane with increasing amounts of ethyl acetate. Thirty fractions of 500 ml each were eluted and, of them, the fraction eluted with 7.5% ethyl acetate was evaporated, dried and further purified with preparative TLC using hexane: ethyl acetate (70: 30 v/v) as mobile phase. A yellow, needle-shaped crystalline compound (ECM) with 5.4% w/w yield was obtained from chloroform fraction. Further, it was characterized by IR, ^{1}H NMR ^{13}C NMR and mass spectroscopy. Melting point was recorded by using Sonar melting point apparatus, ISO 9001–2000 in open capillary tubes (Associated Scientific Technologies, New Delhi, India). The IR spectral study was performed on SHIMADZU FT/IR 8400 infrared spectrophotometer, Japan using KBr disc method for sample preparation. ^{1}H NMR and ^{13}C NMR spectroscopical analysis was performed on DDR X – 500 m/z Bruker Deltonics NMR spectrophotometer, Germany. The ESI-MS was recorded on a MICROMASS QUATTRO II triple quadrupole MASS spectrometer, Waters, USA. Further the purity of the isolated compound was checked using high performance liquid chromatography (HPLC) with mobile phase, that consisted of a gradient mixture prepared from 0.5% glacial acetic acid (component A) and acetonitrile (component B) at different proportion. The result showed a single sharp peak of ECM with 60% of component B between 50–60 min (55 min), thus confirming its purity. Also, the quantity of ECM in the chloroform fraction was determined by HPLC analysis, which was found to be 5.82% w/w (Details available as Supplementary document).

Experimental animals

Charles foster albino strain rats (150 to 200 g in weight) of either sex were obtained from the Central Animal House (Reg. No. 542/02/ab/CPCSEA), Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. The animals were kept in polypropylene cages and maintained under standard conditions (12 h light and dark cycle at an ambient temperature of 25 ± 1 °C and 45–55% relative humidity). The rats were fed with commercially available rat feed (Amrut Rat & Mice Feed Pvt. Ltd., Sangli, Maharashtra, India) and water *ad libitum*. The animals were allowed to acclimatize to the environment for 7 days before the commencement of experiments. The experimental protocols were approval by the Central Animal Ethical Committee of Banaras Hindu University (No. Dean 10-11/60 dated 07/01/2011) and were conducted in accordance with accepted standard guidelines of National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985).

Acute oral toxicity study

Acute oral toxicity study of the compound was performed following Irwin test. The compound was administered orally to overnight fasted rats at a dose range of 100–500 mg/kg p.o. The rats were then individually kept under observation for 1 h first and then hourly for 4 h finally after every 24 h up to 14 days for any physical or neurological signs of toxicity viz. drowsiness, sedation, diarrhoea, sleep, lacrimation, tremors, convulsions, salivation, writhing, gasping, palpitation and decreased respiratory rate or mortality. In addition, differences between the organ weights of the treated and untreated rats such as lungs, kidney, heart, liver, stomach and brain were also determined (Christudas et al., 2013).

Normal faecal excretion rate

For determining faecal excretion rate in normal rats, they were divided into five groups ($n = 6$), where Group 1 was served as control and was given 0.5% carboxy methyl cellulose (CMC). Rats in Group 2 to 4 received the compound ECM, suspended in CMC at a dose level of 2.5, 5 and 10 mg/kg p.o. (Dose selection was done based upon results of acute oral toxicity study). Finally, rats of Group 5 were administered with loperamide as a reference drug at dose level of 2 mg/kg p.o. (Torrent Pharmaceuticals India Ltd., Ahmadabad, India). Food was withdrawn from the cages 3 h before commencement of the experiment and the pellets discharged by the rats after 1st, 3rd, 5th and 7th h of treatment were collected and immediately weighed followed by taking its dry weight, after drying them for 24 h at 50 °C (Tangpu and Yadav, 2004).

Castor oil-induced diarrhoea

Overnight fasted rats were divided into six groups ($n = 6$), where Group 1 and 2 were kept as normal control and castor oil control administered with 0.5% CMC p.o. Group 3–5 were treated