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Protective effects of friedelin isolated from *Azima tetracantha* Lam. against ethanol-induced gastric ulcer in rats and possible underlying mechanisms

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ABSTRACT

The current study was aimed to investigate the gastroprotective effects of friedelin isolated from the hexane extract of leaves of *Azima tetracantha*. Ethanol-induced gastric model was used to investigate the gastroprotective effects of friedelin. Antioxidant enzymes, lipid peroxidation, nitric oxide, gastric vascular permeability, pro and anti-inflammatory cytokines and apoptosis level have been investigated. Ethanol caused severe gastric damage and friedelin pretreatment protected against its deleterious role. Antioxidant enzyme activities, anti-inflammatory cytokines, prostaglandin E_2 (PGE_2), constitutive nitric oxide synthase (cNOS) and mucus weight have been increased significantly. However, the vascular permeability, pro-inflammatory cytokines, inducible nitric oxide synthase (iNOS), caspase-3 and apoptosis level have significantly been decreased after friedelin ingestion. The present study has clearly demonstrated the anti-ulcer potential of friedelin, these findings suggested that friedelin could be a new useful natural gastroprotective tool against gastric ulcer.

1. Introduction

Ethanol is considered as an agent that induces extreme gastric ulcers since it stimulates severe instabilities in the gastric mucosa (Hiruma-Lima et al., 2009). Ethanol performs its lethal effect on gastric epithelium and leads to the formation of typical necrotic injuries due to decrease of bicarbonate secretion and mucus production (Massignani et al., 2009). It also aggravates a reduction in gastric blood flow, induction of oxidative stress, increased activity of xanthine oxidase, an increase in malondialdehyde (MDA) level, solubilization of components of the mucus of the stomach and a decrease in glutathione level (Marrotta et al., 1999).

Azima tetracantha Lam. (Salvadoraceae) is known as 'Mulsangu' in Tamil and 'Kundali' in Sanskrit. Rheumatism has been cured by its leaves, root and root bark. It is a potent diuretic to treat rheumatism, dropsy, dyspepsia, chronic diarrhea; it is used as stimulant tonic after childbirth (Nadkarni, 1976). *A. tetracantha* is used to treat cough, phthisis, asthma, small pox and diarrhea. *A. tetracantha* leaf extracts showed in vitro free radical scavenging and antioxidant activities; the compounds such as azimine, azecarpin, carpine, isorhamnetin-3-O-rutinoside, friedelin, lupeol, glutinol and β-sitosterol are isolated from the leaves (Hepsibha et al., 2011). Friedelin is a triterpenoid isolated from the leaves of *Ageratum conyzoides* and *Aucuba japonica*. It showed good anti-inflammatory, analgesic and antipyretic properties on animal models (Antonisamy et al., 2011); friedelin isolated from *A. tetracantha* also exhibits liver defensive effects through free radical scavenging and antioxidant activities (Sunil et al., 2013).

Several terpenoids have been considered for their capability to defend the gastric mucosa against lesions induced by diverse agents, such as ethanol, hydrochloric acid (HCl), nonsteroidal anti-inflammatory drugs (NSAIDs). Since terpenoids displays significant gastroprotective activities against various experimental animal models, this study aimed to verify the gastroprotective

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effect of friedelin against ethanol-induced gastric ulceration in rats along with possible mechanisms of action.

2. Materials and methods

2.1. Animals

Male and female Wistar albino rats (200–220 g) were selected for this experiment. Animals were keeping up on a 12 h light/dark cycle at nearly 25 ± 1 °C, relative moisture 60–70%; they had access to diet and water ad libitum and were adapted minimum fourteen days (2 weeks) prior to the investigations. All studies were carried out using six animals in each group. All the animal studies have done based on Ethics Committee norms (permit number IAEC-ERI-LC-02) and CPCSEA guidelines.

2.2. Chemicals and drugs

Indomethacin, omeprazole, rofecoxib (COX-2 inhibitor), 5-(4-Chlorophenyl)-1-(4-methoxyphenyl)-3-trifluoromethyl pyrazole (SC560; COX-1 inhibitor), *N*-G-nitro-L-arginine methyl ester (L-NAME; non-specific nitric oxide synthase inhibitor), glibenclamide (K^{+}_{ATP} channel inhibitor) and Griess reagent were obtained from Sigma-Aldrich (USA). Carboxymethyl cellulose (CMC) was obtained from Himedia (India). Prostaglandin E_2 (PGE_2), tumor necrosis factor (TNF)-α, interleukin (IL)-6 and interleukin (IL)-10 enzyme-linked immunosorbent assay kits were purchased from eBioscience, San Diego, CA, USA; Cayman Chemical, Ann Arbor, MI, USA; GE Healthcare, Salt Lake City, UT, USA. Apoptosis assay kit was acquired from Boehringer Mannheim and Caspase-3 activity assays were conducted with using Quanti Zyme assay system Biomol Research Laboratories, Inc. (USA).

2.3. Friedelin identification and characterization

Isolation and description of friedelin have been formerly stated by Antonisamy et al. (2011). Chemical structure of friedelin is shown in Fig. 1.

2.4. Evaluation of acute toxicity study of friedelin

In order to assess the acute toxicity effects of fridelin, different doses (20, 40 and 80 mg/kg) were orally administered using 0.5% CMC as a vehicle solution, and evaluate the weight loss, respiratory distress, abnormal locomotion, uncoordinated muscle movements and mortality over a period of 24 h.

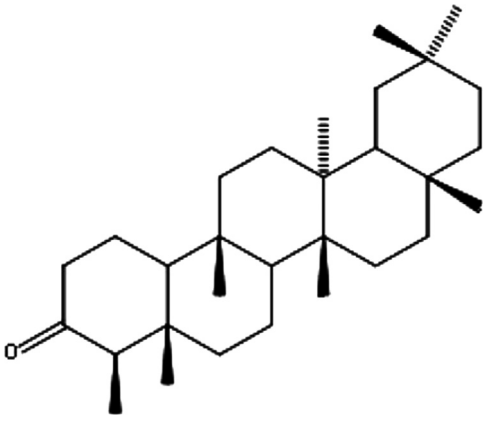

Fig. 1. Structure of friedelin.

2.5. Determination of doses

The lowest effective dose of friedelin was determined for ethanol-induced gastric ulcer according to the procedure by Robert et al. (1979). After having fasted for 24 h, rats were distributed into nine groups ($n=6$). The first group as a normal, received 0.5 ml of 0.5% CMC only, second group as a reference, received omeprazole (30 mg/kg), third group as an ethanol-induced ulcer control, received 0.5 ml of 0.5% CMC, remaining six groups received by different doses of friedelin (20, 25, 30, 35, 40 and 45 mg/kg). After 30 min each group of animals (except first group) received 96% ethanol (5 ml/kg) orally. After 1 h, animals were killed under ether anesthesia and ulcer score has been examined by Dekanski et al. (1975). The harshness of ulcer in each rat was assessed by an independent viewer blinded to the treatments.

2.6. Ethanol-induced gastric damage

Groups of rats fasted for 24 h and divided into nine groups (six rats per group) as follows: control group (normal control), ethanol group (negative control), both control and ethanol groups received 0.5 ml of 0.5% CMC as a vehicle, rest of the groups received friedelin (35 mg/kg, p.o.), omeprazole (30 mg/kg p.o.), friedelin+indomethacin (35 mg/kg p.o.+10 mg/kg p.o.), friedelin+SC560 (35 mg/kg p.o.+05 mg/kg p.o.), friedelin+rofecoxib (35 mg/kg p.o.+3.5 mg/kg p.o.), friedelin+L-NAME (35 mg/kg p.o.+50 mg/kg i.p.) and friedelin+glibenclamide (35 mg/kg p.o.+5 mg/kg p.o.). All the above drugs were administered using 0.5% CMC as a vehicle solution. After 30 min each group of animals received 96% ethanol (5 ml/kg) orally except normal+vehicle group (Robert et al., 1979). After 1 h, the animals were killed under ether anesthesia and the stomach was surgically removed, immersed in 5% formalin for 30 min, and then opened along the greater curvature to examine the lesions macroscopically according to the ulcer score described by Dekanski et al. (1975). The harshness of ulcer in each rat was assessed by an independent viewer blinded to the treatments.

No damage=0.
Blood at the lumen=1.
Pin-point erosions=2.
One to five small erosions (<2 mm)=3.
More than five small erosions (<2 mm)=4.
One to three large erosions (>2 mm)=5.
More than three large erosions (>2 mm)=6.

Ulcer index were expressed for each animal based on its mean ulcer score.

Percentage of ulcer inhibition calculated as follows:

$$[(UI_{nontreated} - UI_{treated})/UI_{nontreated}] \times 100.$$

2.7. Preparation of samples for biochemical assays

Instantaneously once the animals were killed, the mucosa of each stomach was scraped using glass slides, homogenized in a phosphate buffer (0.1 M, pH 7.4) and frozen at −80 °C until it could be assayed biochemically.

2.8. Determination of NO level

NO content was measured by quantifying nitrite/nitrate concentration spectrophotometrically at 540 nm using Griess assay (Miranda et al., 2001). The results were expressed as (micromoles/g tissue). Sodium nitrite was used as standard.

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