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Research Paper

The protective activity of *Conyza blinii* saponin against acute gastric ulcer induced by ethanol

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ABSTRACT

Ethnopharmacological relevance: *Conyza blinii* H.Lév., is a type of natural plant. Its dried overground section is used to treat infections and inflammations in traditional Chinese medicine. Triterpenoidal saponins have a wide range of bioactivities, for instance, anti-cancer, anti-virus and anti-anaphylaxis. *Conyza blinii* saponin (CBS), mainly composed of triterpenoidal saponins, is the total saponin of *Conyza blinii* H.Lév. It has been reported that CBS also has gastric mucous membrane protection activity. This study aims to test CBS's protective activity of gastric's mucous membrane against ethanol. This investigation may lead to the development of novel drug from natural products as anti-ulcer agent, or as gastric mucous protective against chemical damage.

Materials and methods: CBS (*Conyza blinii* saponin) is the total saponin of *Conyza blinii* H.Lév., which was obtained as described previously. We tested the protective activity of CBS against ethanol-induced ulcer. Thirty six rats were grouped randomly as 'NORMAL', 'CONTROL', 'MODEL', 'LOW DOSE', 'MEDIUM DOSE' and 'HIGH DOSE'. The 'NORMAL' group were rats with no pathological model established within it. The 'CONTROL' group was administrated with colloidal bismuth subcitrate, while 'MODEL' group was not given any active agents apart from absolute ethanol in order to obtain gastric ulcer model. The three 'DOSE' groups were treated with different concentrations of CBS (5, 10, 20 mg/mL) before administration followed by absolute ethanol. All rats were sacrificed after the experiment to acquire the gastric tissue. The ulcer index (UI), malondialdehyde (MDA) and superoxide dismutase (SOD) were measured to monitor the activity of CBS. Besides, the rat gastric tissue was made to paraffin section and stained using the Hematoxylin–Eosin (HE) method. The histopathology examination was carried out to examine CBS efficacy in terms of gastric mucous protection.

Results: We found that CBS had a profound protection activity against acute gastric ulcer induced by ethanol and this activity displayed a concentration-dependent manner. The efficacy of 10 and 20 mg/mL CBS was comparable with colloidal bismuth subcitrate ($P<0.05$). All three level of CBS tested were able to significantly reduce UI, MDA and enhance SOD level ($P<0.05$).

Conclusions: It was deduced that the mechanism for such activity would be anti-lipid peroxidation, facilitating free radicals clearance. In addition , histopathology examination of the gastric mucous membrane supported the same conclusion, that CBS can efficiently suppress the inflammatory reactions, bleeding and protect the gastric mucosa.

1. Introduction

Conyza blinii H.Lév., is a native herbaceous plant in *Compositae*. It is spread in Sichuan and Yunnan provinces of China and is commonly called Jin Long Dan Cao. Its dried overground section is valuable in folk medicine for the treatment of chronic bronchitis, gastroenteritis and some other inflammatory diseases (Materia Medica, 1998; Pharmacopoeia of the People's Republic of China, 1977, 2010). Chemical profiles of this species have revealed many

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natural products including diterpenoids, flavonoids, triterpenoids and saponins (Xu et al., 1999; Su et al., 2003). CBS has 17 known components, whose chemical profiles were all listed in Table SI-1 and Fig. SI-1 (Su et al., 2000, 2001a, 2001b, 2003). It has been reported that the CBS has significant protective activity against the gastric mucosal damage. The possible mechanisms account for such activity is to reduce the secretion of gastric collection and gastric acid, as well as to prevent free radicals formation (Su et al., 2001a, 2001b, 2007a, 2007b). Recently, it has shown that CBS induces the apoptosis of Hela and SPC-A1 cells, indicating an antineoplastic activity (Liu et al., 2011).

Excessive consumption of ethanol-containing beverage exerts serious health problems in human. The direct damage of ethanol can lead to gastric lesion. Ethanol is destructive on gastric mucosa, which can be evidenced by gastric mucosa erosion and bleeding. This is caused by blood micro-circulation disorder in gastric wall, tissue necrosis, reduction in gastric mucosal blood flow and mucus secretion (Masuda et al., 1992; Morales et al., 1992; Ohta et al., 1997; Knoll et al., 1998; Kawano and Tsuji, 2000; Sibilia et al., 2003). This reinforces the need for developing new protective agents. Although CBS was originally shown to possess multiple anti-inflammatory and gastric mucosal protection activities, its effects on acute gastric ulcer has not been yet explored. The aim of the present study is to evaluate gastric mucosa protective properties and established the possible mechanisms.

2. Experimental and methods

2.1. Animals

Male and female Sprague & Dawley rats, 180–200 g, supplied by the Tianjin Medical University, PRC, were used throughout this study. All animal experimental procedures were performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals approved by the State Council of People's Republic of China. The animals were fed with a certified diet with free access to water, and placed in cages under a 12 h light/dark cycles for 4 days before experiments. The temperature was maintained at 24 ± 1 °C and the humidity at 70–75% in a controlled room.

2.2. The preparation of CBS from dried Conyza blinii H.Lév.

Conyza blinii H.Lév. the accepted name of a species in the genus *Conyza*, family *Compositae*, which has been checked against websites www.theplantlist.org (The Plant List), and www.ipni.org (The International Plant Names Index). A voucher specimen of *Conyza blinii* H.Lév. was identified by Dr. Yanfang Su, and deposited in the herbarium of the School of Pharmaceutical and Technology, Tianjin University. The method for CBS preparation was based on the protocols published before (Su et al., 2007a, 2007b). Simply, (1) the ground dried *Conyza blinii* H.Lév. was refluxed in 90% ethanol twice (each time for 2 h). The filter residue was then refluxed in 55–60% ethanol twice and filtered. The filtrates were combined and followed by evaporation under reduced pressure to completely remove the ethanol. (2) The distilled water was added to dilute the concentrated solution to make it 10–20 g per 100 mL. Ethyl acetate was added to fractionate the non-saponin components using separatory funnel. The ethyl acetate in the aqueous layer was totally evaporated under reduced pressure. Butanol was added to the resultant aqueous fraction using separatory funnel for three times until completion. Combined butanol fraction was concentrated until dried to obtain crude saponin. (3) The crude saponin was dissolved in water and columned using D101 macroporus resin chromatography. Water, 30%, 70% and 95% ethanol were subsequently used for column elution. The 70% ethanol elution was collected and concentrated to acquire CBS.

2.3. The grouping and administration plan

Thirty six Sprague & Dawley rats were divided into six groups randomly as follows: 'NORMAL', 'MODEL', 'CONTRL', 'LOW DOSE', 'MEDIUM DOSE' and 'HIGH DOSE'. Each group had six rats. All reagents were given using intragastric administration. For 'NORMAL' group, the rats were given 1 mL 0.5% carboxymethylcellulose sodium (CMC-Na) for consecutive two days. Before the first administration, all six rats were fasted for 24 h. Two hours after the last administration, all six rats were administered with 1 mL physiological saline, waited for another one hour before sacrification. 'MODEL' and 'CONTROL' groups were given 1 mL absolute ethanol and 14.4 mg/kg colloidal bismuth pectin respectively. For the other three 'DOSE' groups (LOW DOSE, MEDIUM DOSE and HIGH DOSE) designed to test CBS activity; 5, 10, 20 mg/mL CBS (dissolved in 0.5% CMC-Na) were given instead of physiological saline otherwise the same as 'NORMAL' group.

2.4. The preparation of rat gastric tissue homogenate

The rat gastric mucosa was removed by scraping to make 10% tissue homogenate in physiological saline using homogeniser on ice, followed by centrifugation at 2000 rpm for 10 min. The supernatant was collected for subsequent use.

2.5. The protein quantification in rat gastric tissue homogenate

Coomassie brilliant blue method was used to determine the protein concentration in gastric tissue homogenate as described previously (Sedmak and Grossberg, 1977).

2.6. The calculation of ulcer index

The gastric ulcer index (UI) was conducted as follow: stomachs were taken from the sacrificed rats immediately and rinsed by cold physiological saline to remove the remnant. Stomachs were incised along greater gastric curvature. The spot gastric lesion was counted 1 point; linear gastric lesion less than 1 mm was counted 1 point, adding upon 1 point when the length of the ulcer lesion increasing 1 mm. If the width > 1 mm, then the points were multiplied by factor 2.

2.7. Measurement of SOD

Xanthine-xanthine oxidase system, as shown previously (Peskin and Winterbourn, 2000), was used and modified for SOD measurement. Simply, 2% rat gastric tissue homogenate was made in physiological saline and different amounts of (from 10 to 50 μL) which were tested to work out the optimal suppression rate. The sample gave a suppression rate ranged 48–50%, whose amount was chosen as the optimal amount for the assay. The 'measured' tube and 'control' tube were both used in the assay. The 'measured' tubes were defined as the one which contained the samples subjected to quantification, while the 'control' tubes only contained distilled water otherwise were exactly the same as the 'measured' ones. The SOD concentration was calculated using the following equation:

$$SOD\ (U/\text{mg protein}) = \frac{2 \times OD_{control} - OD_{measured}/OD_{control} \times total\ reaction\ volume/measured\ sampling\ volume}{protein\ \ concentration\ in\ tissue}$$