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Gastroprotective effect of aucubin against ethanol-induced gastric mucosal injury in mice

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ABSTRACT

Aims: Aucubin, an iridoid glycoside, was isolated from seeds of *Eucommia ulmoides* Oliver. This study was aimed to evaluate the protective effect of aucubin against ethanol-induced gastric mucosal injury in mice.
Materials and methods: Mice were orally administrated with aucubin (20, 40 and 80 mg/kg) for 3 consecutive days. On the 3rd day, the mice of gastric mucosal injury were induced with 70% ethanol after the last administration of aucubin. Gastric tissue of mice were submitted for evaluating the severity of gastric mucosal injury. The protective effect of aucubin was evaluated by the gastric ulcer index and histological examinations and determining the levels of inflammatory cytokines, oxidative stress and some gastric mucosal protection factors.
Key findings: Prophylactic oral administration of aucubin decreased gastric ulcer indexes and histological scores. A significant decrease of myeloperoxidase (MPO) activity and the levels of malondialdehyde (MDA), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were observed in aucubin administrated groups. In addition, mice administrated with aucubin increased glutathione (GSH) and heat shock protein-70 (HSP-70) levels and superoxide dismutase (SOD) activity, as well as normalized the levels of epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and cyclooxygenase-1 (COX-1) in gastric tissue of mice.
Significance: The findings of this study demonstrated that aucubin shows protective effect against ethanol-induced acute gastric mucosal injury through its anti-inflammatory and anti-oxidant effects. Furthermore, aucubin enhanced gastric mucosal protection by up-regulation of HSP-70 level and normalization of EGF, VEGF and COX-1 levels.

1. Introduction

Gastric ulcer is a common digestive disease. Multiple pathogenic factors can cause the disease, including *Helicobacter pylori* [1], medicine such as aspirin [2,3] and stress [4]. Although the pathogenic factors of gastric ulcer are complex, the formation of the disease is closely associated with the changes in gastric mucosal defensive factors and pro-inflammatory cytokines. Studies have found that these pathogenic factors induce gastric mucosal injury, resulting in the increase of pro-inflammatory cytokines levels and reduction of some gastric mucosal defensive factors levels. Elevated levels of local pro-inflammatory cytokines mediates the development of local inflammation. Continuous inflammation is a risk for gastric mucosal injury. In addition, decrease of gastric mucosal defensive factors such as anti-oxidants can aggravate gastric mucosal damage [5,6]. Therefore, increasing gastric mucosal defensive factors and reducing pro-inflammatory cytokines in gastric tissue may help prevent gastric ulcer [7].

At present day, drugs such as cimetidine, ranitidine and roxatidine have been widely used in anti-gastric ulcer. Cimetidine, as a strong H2 receptor antagonist, is an anti-gastric ulcer agent as it inhibit the secretion of gastric acid, and also can improve gastric mucosal protection function and inhibit the increase of pro-inflammatory cytokines levels [8]. However, side effects such as hepatic injury limit its applications.

Eucommia ulmoides Oliver. is a deciduous leaf arbor. Its bark was a traditional Chinese medicine [9]. Because seeds of *Eucommia ulmoides* Oliver. contains abundant polyunsaturated fatty acids [10], it attracts many nutritionists to pay attention. In recent years, aucubin isolated from seeds of *Eucommia ulmoides* Oliver. has been found to show a

Abbreviations: GSH, glutathione; HSP-70, heat shock protein-70; SOD, superoxide dismutase; MDA, malondialdehyde; MPO, myeloperoxidase; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; EGF, epidermal growth factor; VEGF, vascular endothelial growth factor; COX-1, cyclooxygenase-1

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variety of pharmacological activities [11,12]. Previously, toxicity, distribution and pharmacokinetics of aucubin have been investigated, and we found that aucubin exhibits good anti-osteoporosis and anti-oxidant effects [13,14]. In addition, other researchers indicated that aucubin can inhibit increase of pro-inflammatory cytokines levels such as TNF-α and IL-6, and can improve anti-oxidant function by reducing MDA level and increasing SOD and glutathione peroxidase activities [15,16]. Aucubin with good anti-inflammatory and anti-oxidant effects has been proved to show many benefits in streptozotocin-induced diabetes rats [16], liver protection [17,18], skin fibroblasts and neuroprotection protections [19,20]. However, whether aucubin can protect against gastric mucosal injury through involvement of anti-inflammatory and anti-oxidant effects is still unclear.

Therefore, the aim of the present study was to assess the protective effect of aucubin against ethanol-induced gastric mucosal injury in mice. The protective effect of aucubin was evaluated by the ulcer index, histological examinations and determining the levels of inflammatory cytokines, oxidative stress and some gastric mucosal protection factors.

2. Materials and methods

2.1. Animals

Sixty healthy adult male kunming mice (28 ± 3 g), provided by the Experimental Animal Center, Xi'an Jiaotong University (Shaanxi, China), were housed at a standard conditions: 25 ± 3 °C, 30–70% humidity and normal light/dark (12 h/12 h) cyclic conditions. The experiments were carried out from beginning to end under Specific Pathogen Free (SPF) Animal Lab at Biomedicine Key Laboratory of Shaanxi Province, Northwest University (Shaanxi, China). All mice were allowed free access to standard laboratory water and food and acclimated to housing conditions for one week before the experiment. The animal experimental procedures were conducted in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

2.2. Chemicals and drugs

Aucubin (Purity ≥ 98%, Pub Chem CID: 479-98-1) was prepared from seeds of *Eucommia ulmoides* Oliver. by the method reported previously [13], and its molecular structure was shown in Fig.1 [21]. Ethanol was provided by Tianjin TianLi Chemical Reagent *Co., Ltd.* (Tianjin, China). Cimetidine was purchased from Shanxi TongDa pharmaceutical *Co., Ltd.* (Shanxi, China). Water was secondary distilled water. Other reagents were of analytical grade.

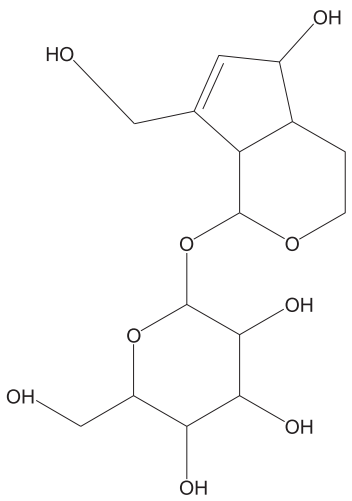

Fig. 1. Chemical structure of aucubin.

Mouse HSP-70 chemiluminescent immunoassay (CLIA) kit was obtained from ALEXIS Biochemicals (Switzerland). The kit for biochemical analysis of MPO activity was obtained from Elabscience (Wuhan, Hubei, China). Enzyme-linked immunosorbent assay (ELISA) kits for mouse EGF, VEGF and COX-1, TNF-α and IL-6 were all commercially available from Sigma-Aldrich (USA).

2.3. Ethanol-induced gastric mucosal injury

Gastric mucosal injury in mice was induced with 70% ethanol [22]. Mice were randomly divided into 6 groups with 10 mice each: Normal control group and ethanol control group (negative control) received saline (0.9%); Cimetidine control group (positive control) received cimetidine at a dose of 100 mg/kg [16]; Three aucubin investigated groups received 20, 40 and 80 mg/kg of aucubin respectively. Saline and drugs were all given by intragastric administration once a day for 3 consecutive days. On the 3rd day, 1 h after the last administration of drugs, mice from cimetidine control group and aucubin investigated groups received 70% ethanol (0.01 ml/g) by intragastric administration, while the normal control group received saline. 1 h later, all animals were sacrificed under anesthesia, removed and cut the stomach longitudinally. Immediately the stomach were opened along the greater curvature, rinsed slightly with ice-cold saline to remove the gastric contents and unfold above the ice, and then the gastric ulcer indexes were calculated according to the method reported with slight modifications as follows [23]: 0, no damage; 1, mucosal erythemas; 2, small erosions (< 1 mm); 3, medium erosions (1 mm ~ 2 mm); 4, large erosions (2 mm ~ 3 mm); 5, large area erosions (3 mm ≤). Subsequently, the gastric tissues were collected and submitted for histological examinations and biochemical analyses.

2.4. Histological evaluations

The damaged gastric tissue samples were fixed in 4% paraformaldehyde solution for 24 h, dehydrated with 95% ethanol and embedded in paraffin. Subsequently, the gastric tissue were cut at 5 μm, mounted on clean glass slides, deparaffinized and rehydrated, and then dyed with hematoxylin and eosin (H & E). Photographs taken from tissue sections were digitized using CMOS camera. The analyses of all figures were performed by the GMS image analysis system (Shanghai optical instrument factory, China). The histological changes were evaluated by an experienced pathologist according to the method with slight modifications [24]: (1) epithelial cell loss (score: 0–3), (2) hemorrhage (score: 0–4), (3) inflammatory cell infiltration (score: 0–2) and (4) lamina propria mucosae erosions (score: 0–4).

2.5. Biochemical analysis

The stomach tissue samples were homogenized in potassium phosphate buffer (pH 7.4). The tissue homogenates were centrifuged at 2000 × *g* at 4 °C for 10 min to get supernatant for the determination of SOD activity, MPO activity, and GSH and MDA levels. Aliquots of supernatant were frozen at − 80 °C for later ELISA and CLIA.

2.6. Determination of MDA

MDA level in gastric tissue of mice was determined by measuring thiobarbituric acidre active substances according to the previous reported method [25]. The concentration of thiobarbituric acid reactive substances was measured using a standard curve of malondialdehyde at 532 nm. The result was expressed as nmol/mg protein.

2.7. Determination of GSH

GSH level in gastric tissue of mice was determined with the method described [26]. Aliquots of the supernatant were added into 12.5%