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Gastroprotective activity of polysaccharide from *Hericium erinaceus* against ethanol-induced gastric mucosal lesion and pylorus ligation-induced gastric ulcer, and its antioxidant activities

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ABSTRACT

The gastroprotective activity of *Hericium erinaceus* polysaccharide was investigated in rats. The antioxidant activities were also evaluated. Pre-treatment of polysaccharide could reduce ethanol-induced gastric mucosal lesion and pylorus ligation-induced gastric ulcer. The polysaccharide exhibited scavenging activities of 1, 1-diphenyl-2-picryl-hydrozyl and hydroxyl radicals, and ferrous ion-chelating ability. In the pylorus ligation-induced model, gastric secretions (volume of gastric juice, gastric acid, pepsin and mucus) of ulcer rats administrated with polysaccharide were regulated. Levels of tumor necrosis factor-α and interleukins-1β in serum, and myeloperoxidase activity of gastric tissue were reduced, while antioxidant status of gastric tissue was improved. Defensive factors (nitric oxide, prostaglandin E2, epidermal growth factor) in gastric tissue were increased. These results indicate that *Hericium erinaceus* polysaccharide possess gastroprotective activity, and the possible mechanisms are related to its regulations of gastric secretions, improvements of anti-inflammatory and antioxidant status, as well as increments of defensive factors releases.

1. Introduction

Gastric ulcer is a common type of peptic ulcer, afflicting millions of individuals worldwide. Treatment of gastric ulcer mostly depends on the usage of synthetic drugs, while it triggers diverse side-effects (Kangwan, Park, Kim, & Hahm, 2014). In this regard, exploring more effective and safe anti-gastric ulcer products or seeking for materials with auxiliary protection function against gastric ulcer from natural resources has attracted much attention (Awaad, El-Meligy, & Soliman, 2012; Venkateswararao & Venkataramana, 2013). Currently, many edible and medicinal resources have been demonstrated to be conducive to the improvement of gastric ulcers in humans and many animal models with fewer adverse effects (Bi, Man, & Man, 2014; Gargano et al., 2017).

As an edible and medicinal mushroom, *Hericium erinaceus* is widely used in traditional Chinese medicine and cuisine (He et al., 2017). Its bioactive activity was recorded in “Ben Cao Gang Mu”, which showed that *Hericium erinaceus* could protect the five internal organs and improve digestion function. The cytoprotection of the freeze-dried fruiting body of *Hericium erinaceus* against ethanol-induced gastric ulcers has already been established (Abdulla, Noor, Wong, and Ali, 2008). Recent investigations have established that the aqueous extract of *Hericium erinaceus* fruiting body exerted anti-gastric ulcer activity in animal models (C. Wang et al., 2015; Wong et al., 2013). The polysaccharide isolated from the aqueous extract of mycelium culture of *Hericium erinaceus* has been ascertained to be the active component with anti-gastric ulcer activity in mice induced by ethanol and in cell experiments (Wang, Konishi, Gao, Xu, & Gao, 2015). However, it is not clear whether the polysaccharide is the bioactive compound or not. A study revealed that a purified polysaccharide isolated from *Hericium erinaceus* mycelium prevented the apoptosis of human gastric mucosal epithelial cell line (GES-1) induced by H_2O_2 through inhibiting activation of apoptotic cellular signals within mitochondria-dependent apoptotic pathways (Wang et al., 2017). In terms of polysaccharide obtained from the fruiting body of *Hericium erinaceus*, the action mechanism of anti-ulcerative effect has not been carried out.

Currently, ethanol-induced gastric mucosal lesion model has been adopted to investigate the protection of freeze-dried fruiting body,

Abbreviations: HECP, *Hericium erinaceus* crude polysaccharide; HERP, *Hericium erinaceus* refined polysaccharide; GES-1, human gastric mucosal epithelial cell line; DPPH, 1, 1-diphenyl-2-picryl hydrozyl; DPPH·, DPPH radical; ·OH, hydroxyl radical; Fe^{2+}, ferrous ion; Vc, ascorbic acid; EDTA, ethylenediamine tetraacetic acid disodium salt; SD, standard deviation; ANOVA, one-way analysis of variance; NC, normal control; LC, lesion control; UC, ulcer control; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-6, interleukin-6; MPO, myeloperoxidase; SOD, superoxide dismutase; GPx, glutathione peroxidase; NO, nitric oxide; PGE2, prostaglandin E2; EGF, epidermal growth factor

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aqueous extract or polysaccharide from *Hericium erinaceus* on gastric mucosa (Abdulla et al., 2008; C. Wang et al., 2015; M. Wang et al., 2015; Wong et al., 2013). On the other hand, pylorus ligation is a typical method for the uniform production of gastric ulceration in rat, thereby being commonly used in the assessment of antiulcer substance (Berté et al., 2014; Ribeiro et al., 2013; Zaghlool, Shehata, Abo-Seif, & El-Latif, 2015). Ethanol-induced and pylorus ligation-induced models have been widely applied together in the investigation of the gastroprotective activity of polysaccharides (Maria-Ferreira et al., 2014; Sun, Matsumoto, & Yamada, 1991). Accordingly, the gastroprotective effect of polysaccharide from *Hericium erinaceus* fruiting body was investigated in the present study using ethanol-induced and pylorus ligation-induced models in *Sprague-Dawley* rats. The action mechanism was systematically explored in the pylorus ligation-induced model. Besides, the *in vitro* antioxidant activities of this polysaccharide was evaluated, including scavenging activities of 1, 1-diphenyl-2-picryl hydrozyl (DPPH) and hydroxyl radicals along with the ability of ferrous ion-chelating.

2. Materials and methods

2.1. Materials and chemicals

Hericium erinaceus fruiting body from Changbai Mountains was obtained from Jiangxi NanHua Medicine Co. Ltd. (Jiangxi, China). Pentobarbital sodium salt and DPPH were gained from Sigma Chemical Corp. (St. Louis, USA). ELISA kits including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and epidermal growth factor (EGF) were bought from BOSTER (Wuhan, China). Gastrin, prostaglandin E2 and histamine ELISA kits, and pepsin, myeloperoxidase, α-amylase, trypsin and chymotrypsin assay kits were purchased from Nanjing jiancheng Bioengineering institute (Nanjing, China). Superoxide dismutase (SOD), glutathione peroxidase (GPx) and nitric oxide (NO) assay kits were obtained from Beyotime Institute of Biotechnology (Shanghai, China). Ferrozine and pyrogallic acid were attained from Aladdin Industrial Corp. (Shanghai, China). All other chemicals used in this study were of analytical grade.

2.2. Animals

Male adult *Sprague-Dawley* rats (180–220 g) purchased from Beijing HFK Bioscience (certificate SCXK (jing) 2014-0004) were used to investigate the protective effect of polysaccharide on ethanol-induced gastric mucosal lesion. Rats (160–180 g) bought from Hunan Slac Jingda Laboratory Animal Co. Ltd. (certificate SCXK (xiang) 2011-0003) were applied to evaluate the effect of polysaccharide against pylorus ligation-induced gastric ulcer. All of them were handled in accordance with the Guide for Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996). The rats were raised in cages in a room with controlled temperature (25.0 ± 0.5 °C), relative humidity (50 ± 5%), 12/12 h of light-dark periods with *ad libitum* food and water before starting the experiments.

2.3. Polysaccharide preparation

Hericium erinaceus crude polysaccharide (HECP) and *Hericium erinaceus* refined polysaccharide (HERP) were prepared from the fruiting body using water-extraction and ethanol precipitation methods according to the previous procedures (Wang, Yin, Nie, & Xie, 2018).

2.4. Effect of polysaccharide against ethanol-induced gastric mucosal lesion

Protective effects of HECP and HERP on gastric mucosa were evaluated using an ethanol-induced gastric mucosal lesion model recommended by China Food and Drug Administration (CFDA Publication No.107, revised 2012). Briefly, rats were randomly assigned into eight groups (n = 12). Group 1 and 2 received normal saline (0.9% NaCl), and other six groups received HERP or HECP at doses of 100, 200 and 400 mg/kg bw for 2 weeks. After the last intragastric administration, they were fasted and freely accessed to water for 24 h, and then received 1.0 mL of absolute ethanol except the rats in the normal control (NC) group. One hour later, rats were sacrificed and their stomachs were removed. The cardia of each rat was ligated with thin line and 5.0 mL of 10% formalin was injected into the stomach. Stomach of each animal was subjected to fixation with 10% formalin solution for 20 min after pylorus ligation. Formalin-fixed stomach was opened along the greater curvature and washed with normal saline. After that, flattened stomach was viewed and its lesion stripes were measured with a vernier caliper to evaluate the gastric mucosal lesion as: stripe length was recorded as the lesion score, and the score would double if the stripe width was over 1 mm. The mean lesion score for each group was expressed as lesion index and the lesion inhibition rate was calculated by formula (1):

$$\text{Lesion inhibition (\%)} = [(A_0 - A_1)/A_0] \times 100 \quad (1)$$

Where A_0 is the lesion index of lesion control (LC) group, A_1 is the lesion index of polysaccharide group.

2.5. Effect of polysaccharide against pylorus ligation-induced gastric ulcer

2.5.1. Animal experiment design

Effect of *Hericium erinaceus* polysaccharide against pylorus ligation-induced ulcer was assessed adopting the model described by Kamarolzaman et al. (2014) with slight modifications. Briefly, rats were randomly divided into eight groups (12 rats per group). Group 1 (normal control group, NC) and group 2 (ulcer control group, UC) received normal saline (0.9% NaCl). Other six groups received HERP or HECP at dosages of 100, 200 and 400 mg/kg bw. On the 14th day, animals were fasted for 24 h with free access to water after the last orally administration. Then, they were in narcotism by injecting pentobarbital sodium salt solution (12 mg/mL, 2.0 mL) into cavum abdominis, and abdomens of them were incised an osculum of 2–3 cm without damaging any blood supply to allow pylorus ligation. The pylorus of each rat was ligated with silk thread except the NC group. And their duodenums were injected into equivalent volume as oral administration of the corresponding solution. Abdomen of each animal was sutured and allowed to recuperate for 5 h in the cage. Subsequently, blood of each rat was collected and animal was sacrificed by cervical dislocation. Finally, gastric juice, stomach, duodenum content and colonic content were collected for following measurements.

2.5.2. Gastric ulcer evaluation

Stomach of rat was opened along the greater curvature and cleaned with normal saline. Ulcer occurrence in the gastric mucosal layer was carefully observed. Meanwhile, the number of hemorrhagic spot was recorded and the length and width of ulcer stripes were measured with a vernier caliper. Ulcer index was calculated applying GUTH method (Park et al., 2015), and the scoring criteria was followed Table 1. The ulcer inhibition rate was computed using Eq. (2):

$$\text{Ulcer inhibition (\%)} = [(A_0 - A_1)/A_0] \times 100 \quad (2)$$

Table 1
The scoring criteria of pylorus ligation-induced ulcer model.

Ulcer morphology		Score
Each Hemorrhagic spot		1
Erosion		2
Length of ulcer stripe	< 1 mm	3
	1–2 mm	4
	> 2 mm	5

The score would double if the stripe width was > 1 mm.