Pol. J. Food Nutr. Sci., 2021, Vol. 71, No. 3, pp. 333–341
On-line ISSN: 2083-6007
Print ISSN: 1230-0322
DOI: 10.31883/pjfns/141560
http://journal.pan.olsztyn.pl

Original article
Section: Food Quality and Functionality

Protective Effect of the Ethanol Extract from *Hericium erinaceus* Against Ethanol-Induced Gastric Ulcers

Guoying Lv[1], *Xiaoya Song*[2], *Zuofa Zhang*[1]*

[1]*Institute of Horticulture, Zhejiang Academy of Agricultural Science, Hangzhou, Zhejiang 310021, China*
[2]*Institute of Fungi, Lishui Academy of Agricultural and Forestry Sciences, Lishui, Zhejiang 323000, China*

Key words: mushroom ethanol extract, gastroprotective activity, *Hericium erinaceus*, bioinformatics analysis

The fruiting bodies of *Hericium erinaceus* have been widely used for the treatment of dyspepsia, chronic gastric ulcers, and enervation. There remains a lack of data on the role of an ethanol extract from *H. erinaceus* (EEH) on ethanol-induced gastric ulcers. The ethanol-induced experimental gastric injury model was used to evaluate the gastroprotective activity of extracts. Ultra-high performance liquid chromatography-triple quadrupole--time of flight tandem mass spectrometry (UPLC-Triple-TOF-MS) analysis was used to identify the possible compounds present in EEH. Transcriptome sequencing(RNA-seq) and bioinformatics analyses were conducted to reveal the characteristics and molecular mechanism underlying EEH's protective effect of on gastric tissue injury. Administration of EEH at doses of 0.625, 1.25, and 2.5 g/kg body weight prior to ethanol ingestion dose--dependently inhibited gastric ulcers. EEH also significantly increased superoxide dismutase (SOD) activity and decreased malondialdehyde (MDA) content in the gastric tissue. Twelve compounds from EEH were identified including three diterpene compounds, two heteroterpene compounds, three isoindolinone compounds, one aromatic compound, *N*-(1-deoxy-D-fructos-1-yl)-L-valine, adenosine, and lumichrome. These compounds promote the inhibition of pathways involved in gastric ulcer formation. The RNA-seq results suggest that EEH indirectly protects the gastric tissue from injury by regulating the cell cycle and biological functions, up-regulating several signal molecules, or activating several proteasome functions. It was concluded that EEH represents a potential therapeutic option to reduce the risk of gastric ulceration.

INTRODUCTION

Gastric ulcers are a serious problem that affect more than 10% of individuals worldwide [O' Malley, 2003]. The process underlying their development is complex and multifactorial, and includes gastric mucosa ischemia, stress, smoking, *Helicobacter pylori* infection, alcohol, and poor dietary habits [Liu *et al.,* 2018]. In terms of conventional therapy, the drugs currently available for the treatment of gastric ulcers produce severe side effects [Chanda *et al*., 2011]. Thus, many scientists are currently evaluating natural products to identify an effective ulcer treatment with fewer side effects than the current conventional treatment. Of note, various edible plants are used in folk medicine to treat gastric ulcers with promising results [Sathish *et al*., 2011].

Hericium erinaceus, belonging to the Aphyllophorales, Hydnaecase and *Hercium* families [Friedman, 2015], is a precious medicinal and edible fungus in China. It serves a magnitude of physiological and health-promoting functions including immunity enhancement, antitumor, and antibacterial properties; it can also improve lipid metabolism and prevent gastrointestinal diseases [Kim *et al.*, 2011]. It has many active ingredients including phenolic compounds, erinacines, steroids, terpenoids, peptides, and polysaccharides, which are responsible for its bioactivity [Lee *et al*., 2016; Wu *et al*., 2018]. *H. erinaceus* is considered to have an outstanding value as a potential therapeutic option for gastrointestinal problems. Currently, its fruiting bodies are widely used for the treatment of dyspepsia, chronic gastric ulcers and enervation. A previous study has reported that the ethanol extract from *H. erinacues* elicits anti-inflammatory effects against ulcerative colitis [Qin *et al.*, 2016]. Furthermore, an aqueous extract and polysaccharides from *H. erinaceus* have been reported to eliminate ethanol-induced gastric damage [Wang *et al*., 2018a]. However, to date, there is no published data on the role of the ethanol extract from *H. erinaceus* (EEH) on ethanol-induced gastric ulcers.

Combined biological and analytical studies have the potential to increase our understanding of the use of *H. erinaceus* and its possible bioactivite effects. Therefore, the aim of the present study was to investigate the chemical composition and gastroprotective effect of EEH. Furthermore, transcriptome sequencing(RNA-seq) and bioinformatics analyses were conducted to reveal the characteristics and molecular mechanism underlying the protective effect of EEH on gastric tissue injury.

* Corresponding Author:
E-mail: zzf2050@163.com (Z. Zhang)

Submitted: 28 April 2021
Accepted: 24 August 2021
Published on-line: 3 September 2021

MATERIALS AND METHODS

Materials and preparation of extracts

Fruiting body of *H. erinaceus* was obtained from the Zhejiang Academy of Agricultural Science farm (Hangzhou, China). The samples were dried in a hot air oven at 60°C, ground, and then passed through a 120-mesh screen. The pretreated samples (100 g) were extracted with 3000 mL of ethanol at 25°C for 2 h. After sonication for 20 min, the extract was filtered through Whatman No. 4 paper. The residue was extracted again with 3000 mL of ethanol. The combined extracts were dried at 40°C under reduced pressure. A brown yellow paste-like extract was obtained.

Analysis of chemical compounds

The compounds in EEH were analyzed by ultra-high performance liquid chromatography-triple quadrupole-time of flight tandem mass spectrometry (UPLC-Triple-TOF-MS). An ACQUITY UPLC system (Waters Co., Milford, MA, USA) equipped with a ZORBAX SB C_{18} column (100×4.6 mm, Agilent, Santa Clara, CA, USA) and a Triple TOF 5600$^+$ time-of-flight mass spectrometer, equipped with an electrospray ionization source (AB SCIEX, Framingham, MA, USA), were applied for these analyses. The eluents and MS conditions were consistent with the method provided by Wang *et al.* [2018b] with some modifications.

The eluents were: A, 0.1% (*v/v*) methanoic acid in water; B, 0.1% (*v/v*) methanoic acid in acetonitrile. The elution conditions were: 0–22 min, linear gradient from 5% to 40% B; 22–33 min, linear gradient from 40% to 95% B; 33–36 min, 95% B; and 36–38 min, linear gradient from 95% to 5% B. The flow rate was 0.3 mL/min, and injection volume was 5 μL. The detection wavelength was 254 nm, and column temperature was 50°C. The MS conditions were listed as follows: positive ion scanning mode, scanning mass range: *m/z* 50–2000; pressure of gas 1 and gas 2: 55 psi; curtain gas pressure: 35 psi; temperature of the ion source: 550°C; voltage of the ion source: 4500 V; first order scanning: declustering potential: 100 V; collision energy: 40 V; second order scanning: TOF MS-Product Ion-IDA mode was used to collect MS data. CID energy was 20, 40, and 60 V.

Animals

The Institute of Cancer Research (ICR) male mice were obtained from the Experimental Animal Center of Zhejiang Province (certification No. SCXK (zhe) 2019–0002; Hangzhou, China). They were raised in cages in a room controlled at constant temperature of 23±2°C, relative humidity of 50–70%, and 12/12 h of light-dark periods. All experimental procedures were conducted in accordance with China legislation under No. 2019ZAASLA08 on the use and care of laboratory animals and within the guidelines established by the Institute for Experimental Animals of Zhejiang Academy of Agricultural Sciences.

Effect of EEH against ethanol-induced gastric ulcers in mice

Mice were randomized into six groups, each consisting of 10 animals. Groups 1 (normal group) and 2 (model group) received saline (0.9% NaCl) at a dose of 10 mL/kg body weight; and group 6 received ranitidine (0.108 g/kg) as the positive group. Other three groups (3–5, administration group) received EEH at doses of 0.625, 1.25, and 2.5 g/kg body weight, respectively. The corresponding saline, extracts, and ranitidine were administered once daily for eight days. On the last day of treatment, 2 h after administration, absolute ethanol (0.1 mL/animal) was administrated orally to mice of groups 2–6. Then, 1.5 h later, acute gastric mucosal injury model was established successfully, and the mice were sacrificed. The stomachs were removed and opened along the greater curvature; they were then washed several times with phosphate buffered saline (PBS, pH 7.4, 0.01 M) to remove dirt inside. The mice gastric mucosa was observed using a stereo microscope (Leica Micro Systems Imaging Solutions Ltd, Cambridge, UK). The ulcer inhibition was expressed in percent (%) as: ulcer inhibition (%) = [(ulcer index of model group – ulcer index of administration group) / ulcer index of model group]×100%.

Histopathological analysis

For histopathological analysis, gastric tissues were cut into 5-mm sections, then they were immersed in 4% paraformaldehyde for 24 h to fix the specimens. The most serious parts of the ulcer region obtained from stomach were chosen to produce paraffin wax tissue sections (4 μm). The tissue sections were then prepared for hematoxylin and eosin (H &E) staining.

Measurement of superoxide dismutase (SOD) activity and malondialdehyde (MDA) content

Gastric tissues were homogenized in ice-cold saline (1:9, *v/w*), and supernatant of the gastric tissues was harvested. SOD activity and MDA content of the supernatant were evaluated using commercially available kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

RNA-seq analysis of mice stomachs

Ten ICR mice, weighing 18–22 g, were randomly divided into a blank control group (0.9% saline at a dose of 10 mL/kg body weight) and an experimental group (EEH at 2.5 g/kg body weight), with five mice in each group. The corresponding saline and EEH were given by gavage once per day for eight consecutive days. Two hours after the last administration, three mice in each group were randomly selected for transcriptome experiments. The animals were sacrificed, and the stomachs were removed and opened along the greater curvature; they were then rinsed with RNase free water, dried with filter paper, put into a cryopreservation tube, and quickly placed into liquid nitrogen.

Trizol Regent (Invitrogen, Waltham, MA, USA) was used to extract total RNA. A total amount of 2 μg RNA per sample was used as the input material for RNA sample preparation. Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. In the first chain synthesis, divalent cations in NEBNext buffer (5X) were used for pyrolysis. Random hexamer primers and RNase H were used to synthesize the first strand cDNA. Then, the second strand cDNA was synthesized with buffer, triphosphate acid base deoxynucleotide (dNTPs), DNA polymerase I, and ribonuclease H

TABLE 1. Effect of the ethanol extract from *Hericium erinaceus* (EEH) on the ulcer index and oxidative markers in mice with ethanol-induced gastric ulcers.

Group	Ulcer Index	Ulcer inhibition (%)	SOD activity (U/μg protein)	MDA content (nmol/mg protein)
Normal group	–	–	3.57±0.35**	12.35±2.58**
Model group +ethanol	33.40±4.20	–	1.19±0.41	34.57±3.47
0.625 g/kg EEH +ethanol	22.50±2.87*	32.63	1.35±0.28	29.61±1.96
1.25 g/kg EEH +ethanol	21.64±3.21*	35.20	1.67±0.29	21.35±2.85*
2.5 g/kg EEH +ethanol	11.00±3.57**	67.07	2.14±0.31*	18.66±3.11*
0.108 g/kg ranitidine+ethanol	25.63±4.18*	23.26	2.25±0.45*	20.58±4.05*

Each value represents mean± standard deviation (n=10). SOD: superoxide dismutase; MDA: malondialdehyde. *significantly different from the model group ($p<0.05$). **significantly different from the model group ($p<0.01$).

(RNase H) (Sangon Biotech, Shanghai, China). The library fragments were purified by rapid polymerase chain reaction (PCR) kits and eluted with ethidium bromide (EB) buffer (Sangon Biotech, Shanghai, China). The library was constructed by PCR amplification. After clustering, the library was sequenced on Illumina platform, and 150 bp paired end reading was generated.

Statistical analysis

Results were expressed as mean±standard deviation. Statistical analysis was performed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) with ANOVA and Student's t-test. Differences between values were considered to be significant at $p<0.05$ or $p<0.01$.

The reference genomes and the annotation file were downloaded from the ENSEMBL database (http://www.ensembl.org/index.html). Bowtie2 v2.2.3 was used to build the genome index, and Clean Data was then aligned to the reference genome using HISAT2 v2.1.0. Genes with $q \leq 0.05$ and $|\log_2 \text{ratio}| \geq 1$ were identified as differentially expressed genes (DEGs).

RESULTS AND DISCUSSION

Effect of EEH on ethanol-induced gastric lesions

The gastric mucosa is a thin, fragile mucosal tissue, and it has a dynamic balance mechanism for self-repairs. The present study investigated the effects of EEH on ethanol--induced gastric lesions in mice. The ulcer index and ulceration inhibition rate were used to verify the gastroprotective effects of EEH (Table 1). The ulcer index of the ethanol-administered group was 33.40; however, the ulcer indices of the mice pre-treated with 0.625, 1.25 and 2.5 g/kg EEH were 22.50, 21.64 and 11.00, respectively, which were lower than the ulcer index of the ranitidine group (25.63), where the ulceration inhibition rates of the mice pre-treated with 0.625, 1.25 and 2.5 g/kg EEH were 32.63%, 35.20% and 67.07%, respectively (Table 1). The results indicate that the mice pre-treated with EEH and ranitidine had considerably reduced areas of gastric ulcer formation compared with the gastric ulcer model group.

The ethanol-induced experimental gastric injury model is a popular method to evaluate the gastroprotective activity of compounds or extracts [Sahin *et al*., 2019]. Previous studies have shown that ethanol directly damages gastric mucosa within 60 min [Ma & Liu, 2014]. It is generally accepted that ethanol disturbs the gastric secretory activity, alters cell permeability, and depletes the gastric mucus [Salim, 1990]. Changes in pro-inflammatory cytokines and gastric mucosal defensive factors are the pathogenic factors involved in ethanol-induced gastric ulcers. In this study, the EEH pre-treatment in mice with gastric ulcers significantly reduced the area of gastric ulcer formation and the ulcer index, suggesting that EEH is able to ameliorate the gastric ulcers.

Histopathological studies

Ethanol is an ulcerogenic agent that produces erosions, ulcerative lesions. Histological evaluations of the gastric walls of ethanol-induced ulcerated mice are depicted in Figure 1. Compared with the normal group, ethanol administration induced gastric mucosa edema, leucocyte infiltration of the submucosal layer, hemorrhage damage, and epithelial cell loss.

Histopathological studies were used to verify the gastric protective effects of EEH. Pre-treatment with EEH and ranitidine for eight days provided a protective effect on the gastric mucosa, reducing the ulcerated area, submucosal edema, and leucocyte infiltration.

Effects of EEH on oxidative markers

As shown in Table 1, the ethanol-treated mice exhibited an obvious reduction in gastric SOD activities as compared with the normal group, which was significantly reversed by ranitidine and EEH (2.5 g/kg). EEH at doses of 0.625 g/kg and 1.25 g/kg also increased the SOD activity when compared with the model group, though these differences were not statistically significant. As for lipid peroxidation, MDA content was significantly increased in the ethanol-stimulated gastric tissue as compared to the normal group. However, this was markedly reversed by 1.25 and 2.5 mg/kg EEH treatments when compared with the model group.

Reactive oxygen species (ROS) play a key role in gastric lesions induced by ethanol [Mei *et al*., 2012]. They suppress antioxidant enzyme activities and initiate lipid peroxidation, which is an important event in the toxicity mechanism of ethanol [Pan *et al*., 2008]. Lipid peroxidation destroys the integrity of the membrane structure, which is represented by an apparent increase in MDA content [El-Maraghy *et al*., 2015]. Therefore, controlling the formation of ROS is essential for

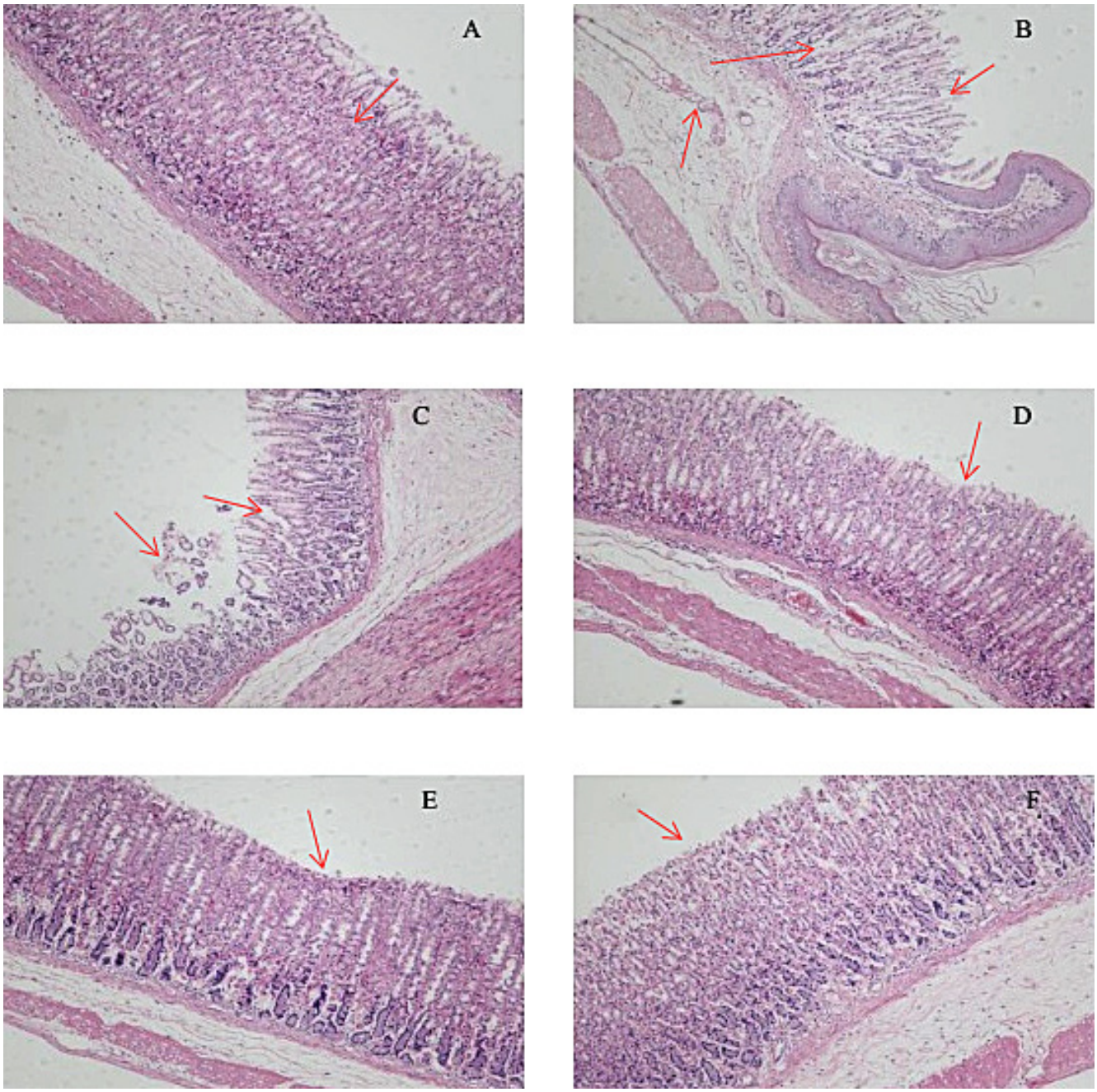

FIGURE 1. Photomicrograph of stomach of the mice from the normal group (A); the model group treated with ethanol (B); groups treated with *Hericium erinaceus* extract (EEH) and ethanol: 0.625 g/kg EEH+ethanol (C), 1.25 g/kg EEH+ethanol (D); 2.5 g/kg EEH+ethanol (E); and the group receiving ranitidine at dose of 0.108 g/kg and ethanol (F). Original magnification 100×.

the treatment of gastric lesions. The function of SOD is to remove the harmful superoxide anion radicals, and SOD forms the first line of defense in protection against the destructive action of ROS. The important role of SOD in protecting the stomach against mucosa damage has previously been described by Pan *et al*. [2008]. In the present study, pre-treatment with EEH produced a significant reduction in MDA content and an improvement in SOD activity as compared with the model group, indicating that EEH exerted potent gastric protection by alleviating oxidative stress.

The consumption of antioxidant-rich foods may also help to protect the stomach. The anti-ulcer drug – lansoprazole – has been reported to prevent the production of MDA [Agnihotri *et al*., 2007]. Likewise, lycopene (antioxidant compound) has been shown to exhibit protective effects in indomethacin-induced ulcers and the antioxidative extract from *Sphenodesme involucrate* to effectively decrease the ulcer index [Sreeja *et al*., 2018]. The antioxidant fraction of *Zingiber officinale* has been reported to confer protective actions against diclofenac sodium-induced gastric damage [Saiah *et al*., 2018]. In turn, Chen *et al*. [2019] reported that the ethanol extract from *H. erinaceus* produced antioxidant activity. The mentioned authors identified hericenone C, hericene B, ergosterol, and ergosterol peroxide in this extract. These results were supported

TABLE 2. Chemical composition of the ethanol extract from *Hericium ernaceus* determined using the UPLC-Triple-TOF-MS analysis.

Peak No.	Proposed compounds	Retention time (min)	$[M+H]^+$ (*m/z*)	MS/MS fragment ions (*m/z*)	References
1	*N*-(1-Deoxy-D-fructos-1-yl)-L-valine	1.34	280	130, 198, 216, 262	
2	Adenosine	1.83	268	119, 136	Hui *et al*. [2012]
3	Herierin IV	2.83	171	53, 101, 153	Miyazawa *et al*. [2012]
4	Lumichrome	12.83	243	103, 172, 198	Tsukamoto *et al*. [1999]
5	Erinaceolactam E	22.25	415	232, 370, 398	Wang *et al*. [2016]
6	Erinaceolactam A	23.00	330	83, 192, 248	Wang *et al*. [2016]
7	Erinacerin N	24.04	415	232, 260, 316, 370, 398	Tang *et al*. [2015]
8	Erinacerin M	24.35	344	83, 206, 262	Tang *et al*. [2015]
9	Hericenone I	26.24	331	83, 177, 233	Yaoita *et al*. [2005]
10	Erinacerin B	27.11	333	83, 177, 233, 315	Tang *et al*. [2015]
11	Hericenone A	27.68	331	85, 145, 193, 247	Kawagishi *et al*. [1990]
12	*N*-De(phenylethyl)isohericerin	27.82	316	164, 192, 232	Li *et al*. [2015]

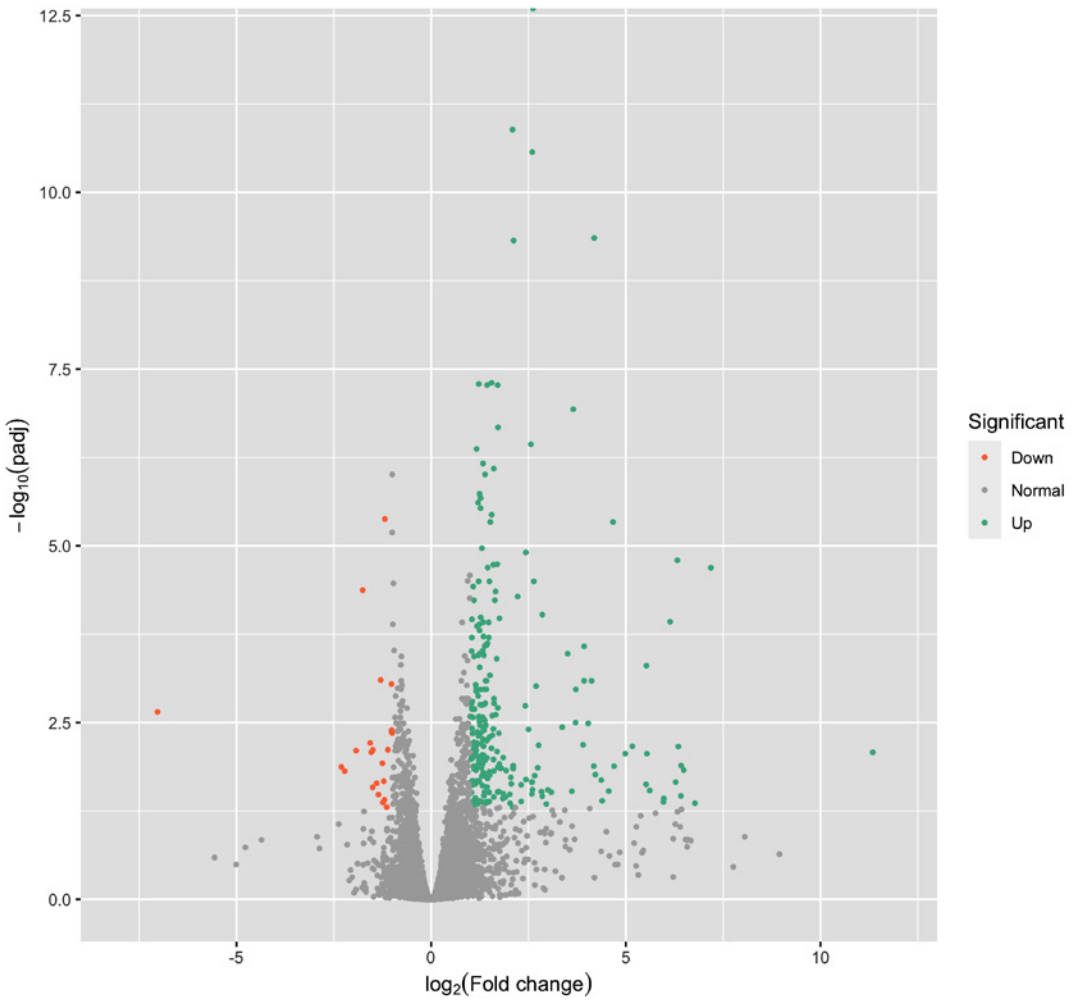

FIGURE 2. Comparison of operational taxonomic units (OTUs) of differentially expressed genes (DEGs) between blank control group and experimental group treated with the *Hericium erinaceus* extract (EEH).

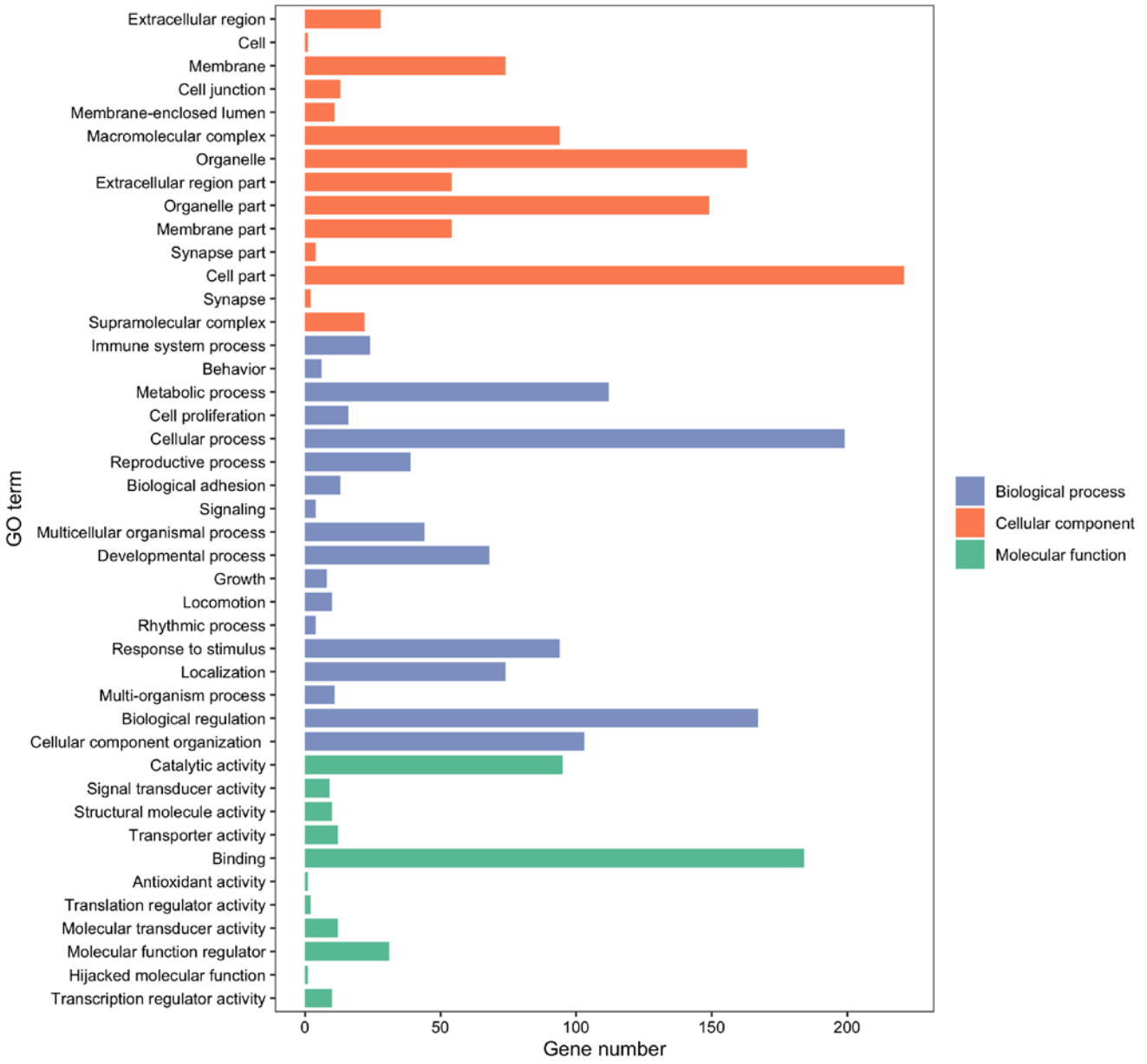

FIGURE 3. Gene Ontology (GO) function analysis of differentially expressed genes (DEGs) between the blank control group and the experimental group treated with the *Hericium erinaceus* extract (EEH).

by the research of Huang *et al.* [2017]. In addition, ethanol extracts from *H. erinaceus* have been found to exhibit the anti-*Helicobacter pylori* (an important factor in gastric disease) activity [Liu *et al*., 2016]. The mechanisms underlying the gastroprotective activity of EEH are complex, and the factors play leading roles in EEH effects require further investigation.

Identification of the EEH compounds

The UPLC-Triple-TOF-MS analysis was performed to identify the possible compounds present in EEH by considering data from the literature and MS databases. Table 2 shows the proposed chemical composition of EEH; the MS chromatograms of the identified compounds are presented in Supplementary file (S1). A total of 12 compounds were tentatively identified, including three diterpene compounds (erinacerin N, erinacerin M, and erinacerin B), two heteroterpene compounds (hericenone I and hericenone A), three isoindolinone compounds (erinaceolactam E, erinaceolactam A, and *N*-de(phenylethyl)isohericerin), one aromatic compound (hericerin IV), *N*-(1-deoxy-D-fructos-1-yl)-L-valine, adenosine, and lumichrome.

Previous research has demonstrated that adenosine can activate antioxidant enzymes *via* cell surface receptors [Ramkumar *et al*., 1995]. Lumichrome is a photodegradation product of riboflavin and riboflavin possesses distinct antioxidant activity [Masek *et al.*, 2012]. Extensive studies have confirmed the anti-inflammation effects of erinacines and hericenones [Lee *et al*., 2016]. Taken together, it appears that gastroprotective activity of EEH may be related to the synergistic effects of these compounds. The precise mechanism underlying the preventive effect of EEH on gastric ulcer and the active compounds responsible for this beneficial property need require further study.

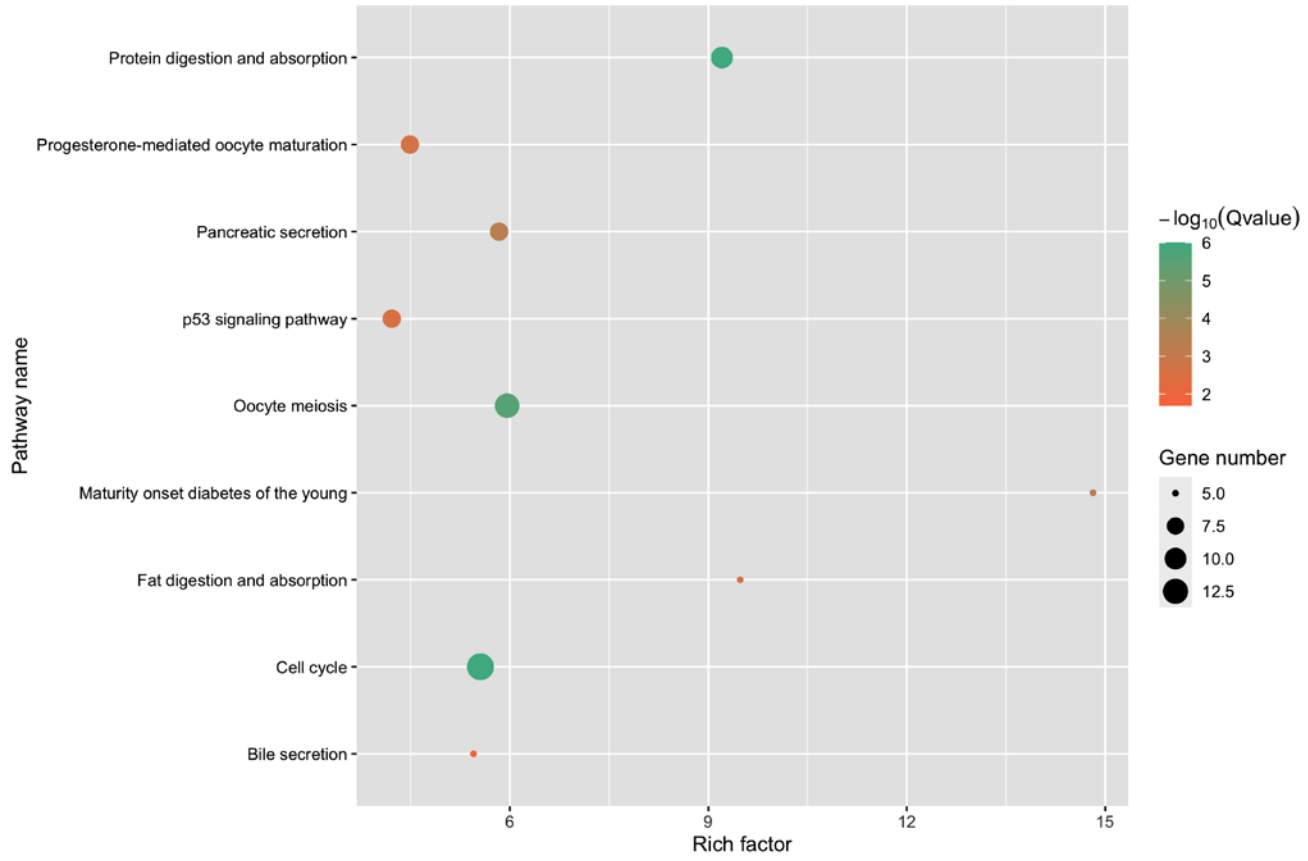

FIGURE 4. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of differentially expressed genes (DEGs) between the blank control group and the experimental group treated with the *Hericium erinaceus* extract (EEH).

RNA-seq analysis

The gastric tissues of blank control group and experimental group were analyzed by RNA-seq. Taking $|\log_2$ Fold change$|\geq 1$ and $q \leq 0.05$ as the thresholds, 278 DEGs were obtained, accounting for 1.0% of the total DEGs (Figure 2). The most differentially expressed genes were up- and down-regulated 11.3 and 7.03 times, respectively. The most significantly up-regulated genes were Ins2, reg1, serpini2, cel, and erp27, and the most significantly down-regulated genes were duoxa2, serpina7, Xlr3b, and Col4a3.

Reg1 is one of the genes that was significantly up-regulated. Reg and its related genes form a family belonging to the calcium-dependent lectin gene superfamily. This family expresses a group of small molecule secretory proteins. Asahara *et al*. [1996] performed *in situ* hybridization and immunohistochemical analyses and found that the expression of the reg1 protein in enterochromaffin-like (ECL) cells in mouse gastric mucosa was increased after stress stimulation by water immersion. In addition, the reg1 protein is expressed in the main cells and ECL cells of the human gastric fundus gland [Higham *et al*., 1999]. Reg protein is expressed in many organs and tissues, especially in digestive system [Zhao *et al*., 2013]. It promotes the proliferation and anti-apoptosis of gastric mucosal cells, while its overexpression can also reduce the chemosensitivity of gastric cancer patients.

The DEGs were counted and annotated using the NCBI, UniProt, Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases to obtain detailed descriptive information. Figure 3 shows the most significant entries in the three functional annotation categories (cellular component, biological process, and molecular function). In biological process, the most significantly enriched DEGs were cellular process, biological regulation, and response to stimulus. In cellular components, the most significantly enriched DEGs were cell part, organelle part, and membrane. In molecular function, the most significantly enriched DEGs were binding and catalytic activity. A large number of DEGs existed in the above GO functions, suggesting that these functions play important roles in EEH effects on gastric tissue.

The pathways with significant differences and a large number of genes are shown in Figure 4: cell cycle, protein digestion and absorption, pancreatic secretion and the p53 signalling pathway

CONCLUSIONS

Ethanol-induced model is widely used for the reproduction of gastric injury or ulcer. Twelve compounds from EEH were identified in this work. The results have demonstrated that EEH has gastroprotective activity and is a valuable source of compounds for the prevention of gastric mucosal injury induced by ethanol. This preventive effect may be related to the synergistic action of these compounds. This study results provide scientific support for the use of EEH in the treatment of gastric ulcers. The RNA-seq results suggest that EEH indirectly protects against gastric tissue injury by regulating cell

cycle and biological function, up-regulating several signal molecules, or activating several proteasome functions. This study clearly highlights the viability of using EEH in adjuvant therapy of gastric ulcers. Further clinical assays should be performed to verify these findings, and the main compounds should be investigated in relation to their gastroprotective activity in EEH.

SUPPLEMENTARY MATERIALS

The following are available online at http://journal.pan.olsztyn.pl/Protective-Effect-of-the-Ethanol-Extract-from-Hericium-erinaceus-Against-Ethanol,141560,0,2.html; MS and MS/MS spectra and structures of compounds 1 to 12.

RESEARCH FUNDING

This research was funded by the New Variety Breeding Project of Science Technology Department of Zhejiang Province (Grant number: 2021C02073).

CONFLICT OF INTERESTS

There is no conflict of interest to declare.

ORCID IDs

G. Lv https://orcid.org/0000-0002-9532-4838
Z. Zhang https://orcid.org/0000-0002-7228-0705

REFERENCES

1. Agnihotri, N., Kaur, H., Kaur, N., Sarotra, P. (2007). Role of oxidative stress in lansoprazole-mediated gastric and hepatic protection in Wistar rats. *Indian Journal of Gastroenterology, 26*(3), 118–121.
2. Asahara, M., Mushiake, S., Shimada, S., Fukui, H., Kinoshita, Y., Kawanami, C., Watanabe, T., Tanaka, S., Ichikawa, A., Uchiyama, Y., Narushima, Y., Takasawa, S., Okamoto, H., Tohyama, M., Chiba, T. (1996). Reg gene expression is increased in rat gastric enterochromaffin-like cells following water immersion stress. *Gastroenterology, 111*(1), 45–55. https://doi.org/10.1053/gast.1996.v111.pm8698224
3. Chanda, S., Baravalia, Y., Kaneria, M. (2011). Protective effect of *Polyalthia longifolia* var. pendula leaves on ethanol and ethanol/HCl induced ulcer in rats and its antimicrobial potency. *Asian Pacific Journal of Tropical Medicine, 4*(9), 673–679. https://doi.org/10.1016/S1995-7645(11)60172-7
4. Chen, Z.G., Bishop, K.S., Tanambell, H., Buchanan, P., Smith, C., Quek, S.Y. (2019). Characterization of the bioactivities of an ethanol extract and some of its constituents from the New Zealand native mushroom *Hericium novae-zealandiae*. *Food & Function, 10*, 6633–6643. https://doi.org/10.1039/C9FO01672D
5. El-Maraghy, S.A., Rizk, S.M., Shanin, N.N. (2015). Gastric protective effect of crocin in ethanol-induced gastric injury in rats. *Chemical-Biological Interactions, 229,* 26–35. https://doi.org/10.1016/j.cbi.2015.01.015
6. Friedman, M. (2015). Chemistry, nutrition and health-promoting properties of *Hericium erinaceus* (Lion's Mane) mushroom fruiting bodies and mycelia and their bioactive compounds. *Journal of Agricultural and Food Chemistry, 63*(32), 7108–7123. https://doi.org/10.1021/acs.jafc.5b02914
7. Higham, A.D., Bishop, L.A., Dimaline, R., Blackmore C.G., Dobbins, A.C., Varro, A., Thompson, D.G., Dockray, G.J. (1999). Mutations of RegIα are associated with enterochromaffin-like cell tumor development in patients with hypergastrinemia. *Gastroenterology*, *116*(6), 1310–1318. https://doi.org/10.1016/S0016-5085(99)70495-6
8. Huang, Y., Zhou, C.H., Dai, H.J., Huang, H.H. (2017). *In vitro* antioxidant activity of ethanol extract from *Hericium erinaceus* and its different polar fractions. *Science and Technology of Food Industry, 21*, 16–20.
9. Hui, Y., Zhao, S.S., Love, J.A., Ansley, D.M., Chen, D.D.Y. (2012). Development and application of a LC-MS/MS method to quantify basal adenosine concentration in human plasma from patients undergoing on-pump CABG surgery. *Journal of Chromatography B, 885–886*, 30–36. https://doi.org/10.1016/j.jchromb.2011.12.006
10. Kawagishi, H., Ando, M., Mizuno, T. (1990). Hericenone A and B as cytotoxic principles from the mushroom *Hericium erinaceum*. *Tetrahedron Letter, 31*(3), 373–376. https://doi.org/10.1016/S0040-4039(00)94558-1
11. Kim, S.P., Kang, M.Y., Kim, J.H., Nam, S.H., Friedman, M. (2011). Composition and mechanism of antitumor effects of *Hericium erinaceus* mushroom extracts in tumor-bearing mice. *Journal of Agricultural and Food Chemistry, 59*(18), 9861–9869. https://doi.org/10.1021/jf201944n
12. Lee, D.G., Kang, H.W., Park, C.G., Ahn, Y.S., Shin, Y. (2016). Isolation and identification of phytochemicals and biological activities of *Hericium ernaceus* and their contents in Hericium strains using HPLC/UV analysis. *Journal of Ethnopharmacology,* 184, 219–225. https://doi.org/10.1016/j.jep.2016.02.038
13. Li, W., Zhou, W., Kim, E.J., Shim, S.H., Kang, H.K., Kim, Y.H. (2015). Isolation and identification of aromatic compounds in Lion's Mane mushroom and their anticancer activities. *Food Chemistry, 170*, 336–342. https://doi.org/10.1016/j.foodchem.2014.08.078
14. Liu, B., Yili, A., Asia, H.A., Aikemu, M. (2018). Gastroprotective effect of the protease-rich extract from sheep abomasum against stress-induced gastric ulcers in rats. *Journal of Food Biochemistry, 42*(5), art. no. e12558. https://doi.org/10.1111/jfbc.12558
15. Liu, J.H., Li, L., Shang, X.D., Zhang, J.L., Tan, Q. (2016). Anti-*Helicobacter pylori* activity of bioactive components isolated from *Hericium erinaceus*. *Journal of Ethnopharmacology, 183*, 54–58. https://doi.org/10.1016/j.jep.2015.09.004
16. Ma, L., Liu, J.G. (2014). The protective activity of *Gonyza blinii* saponin against acute gastric ulcer induced by ethanol. *Journal of Ethnopharmacology, 158*, Part A, 358–363. https://doi.org/10.1016/j.jep.2014.10.052
17. Masek, A., Chrzescijanska, E., Zaborski, M., Maciejewska, M. (2012). Characterisation of the antioxidant activity of riboflavin in an elastomeric composite. *Comptes Rendus Chimie, 15*(6), 524–529. https://doi.org/10.1016/j.crci.2012.01.012
18. Mei, X., Xu, D., Xu, S., Zheng, Y., Xu, S. (2012). Novel role of Zn (II)-curcumin in enhancing cell proliferation and adjusting proinflammatory cytokine-mediated oxidative damage of etha-

nol-induced acute gastric ulcers. *Chemico-Biological Interactions, 197*(1), 31–39. https://doi.org/10.1016/j.cbi.2012.03.006

19. Miyazawa, M., Takahashi, T., Horibe, I., Ishikawa, R. (2012). Two new aromatic compounds and a new D-arabinitol ester from the mushroom *Hericium erinaceum*. *Tetrahedron, 68*(7), 2007–2010. https://doi.org/10.1016/j.tet.2011.11.068
20. O'Malley, P. (2003). Gastric ulcers and CERD: the new "plagues" of the 21st century update for the Clinical Nurse Specialist. *Clinical Nurse Specialist, 17*(6), 286–289. https://doi.org/10.1097/00002800-200311000-00008
21. Pan, J.S., He, S.Z., Xu, H.Z., Zhan, X.J., Yang, X.N., Xiao, H.M., Shi, H.X., Ren, J.L. (2008). Oxidative stress disturbs energy metabolism of mitochondria in ethanol-induced gastric mucosa injury. *World Journal of Gastroenterology, 14*(38), 5857–5867. https://doi.org/10.3748/wjg.14.5857
22. Qin, M., Geng, Y., Lu, Z., Xu, H., Shi, J., Xu, X., Xu, Z. (2016). Anti-inflammatory effects of ethanol extract of Lion's Mane medicinal mushroom, *Hericium erinaceus* (Agaricomycetes), in mice with ulcerative colitis. *International Journal of Medicinal Mushrooms, 18*(3), 227–234. https://doi.org/10.1615/IntJMedMushrooms.v18.i3.50
23. Ramkumar, V., Nie, Z., Rybak, L.P., Maggirwar, S.B. (1995). Adenosine, antioxidant enzymes and cytoprotection. *Trends in Pharmacological Sciences, 16*(9), 283–285. https://doi.org/10.1016/S0165-6147(00)89051-3
24. Sahin, H., Kaltalioglu, K., Erisgin, Z., Coskun-Cevher, S., Kolayli, S. (2019). Protective effects of aqueous extracts of some honeys against HCl/ethanol-induced gastric ulceration in rats. *Journal of Food Biochemistry, 43*(12), art. no. e 13054. https://doi.org/10.1111/jfbc.13054
25. Saiah, W., Halzoune, H., Djaziri, R., Tabani, K., Koceir, E.A., Omari, N. (2018). Antioxidant and gastroprotective actions of butanol fraction of *Zingiber officinale* against diclofenac sodium-induced gastric damage in rats. *Journal of Food Biochemistry, 42*(1), art. no. e12456. https://doi.org/10.1111/jfbc.1245
26. Salim, A.S. (1990). Removing oxygen-derived free radicals stimulates healing of ethanol-induced erosive gastritis in the rat. *Digestion, 47*(1), 24–28. https://doi.org/10.1159/000200472
27. Sathish, R., Vyawahare, B., Natarajan, K. (2011). Antiulcerogenic activity of *Lantana camara* leaves on gastric and duodenal ulcers in experimental rats. *Journal of Ethnopharmacology, 134*(1), 195–197. https://doi.org/10.1016/j.jep.2010.11.049
28. Sreeja, P.S., Arunachalam, K., Saikumar, S., Kasipandi, M., Dhivya, S., Murugan, R., Parimelazhagan, T. (2018). Gastroprotective effect and mode of action of methanol extract of *Sphenodesme involucrate* var. paniculate (C.B. Clarke) Munir (Lamiaceae) leaves on experimental gastric ulcer models. *Biomedicine & Pharmacotherapy, 97*, 1109–1118. https://doi.org/10.1016/j.biopha.2017.11.030
29. Tang, H.Y., Yin, X., Zhang, C.C., Jia, Q., Gao, J.M. (2015). Structure diversity, synthesis, and biological activity of cyathane diterpenoids in higher fungi. *Current Medicinal Chemistry, 22*(19), 2375–2391. https://doi.org/10.2174/0929867322666150521091333
30. Tsukamoto, S., Kato, H., Hirota, H., Fusetani, N. (1999). Lumichrome, a larval metamorphosis-inducing substance in the ascidian *Halocynthia roretzi*. *European Journal of Biochemistry, 264*(3), 785–789. https://doi.org/10.1046/j.1432-1327.1999.00642.x
31. Wang, X.L., Xu, K.P., Long, H.P., Zou, H., Cao, X.Z., Zhang, K., Hu, J.Z., He, S.J., Zhu, G.Z., He, X.A., Xu, P.S., Tan, G.S. (2016). New isoindolinones from the fruiting bodies of *Hericium ernaceum*. *Fitoterapia, 111*, 58–65. https://doi.org/10.1016/j.fitote.2016.04.010
32. Wang, X.Y., Yin, J.Y., Zhao, M.M., Liu, S.Y., Nie, S.P., Xie, M.Y. (2018a). Gastroprotective activity of polysaccharide from *Hericium erinaceus* against ethanol-induced gastric mucosal lesion and pylorus ligation-induced gastric ulcer, and its antioxidant activities. *Carbohydrate Polymers, 186*, 100–109. https://doi.org/10.1016/j.carbpol.2018.01.004
33. Wang, Y., Luan, G., Zhou, W., Meng, J., Wang, H., Hu, N., Suo, Y. (2018b). Subcritical water extraction, UPLC-Triple-TOF/MS analysis and antioxidant activity of anthocyanins from *Lucium ruhtenicum* Murr. *Food Chemistry, 249*, 119–126. https://doi.org/10.1016/j.foodchem.2017.12.078
34. Wu, F., Zhou, C., Zhou, D., Ou, S., Zhang, X., Huang, H. (2018). Structure characterization of a novel polysaccharide from *Hericium erinaceus* fruiting bodies and its immunomodulatory activities. *Food & Function, 9*(1), 294–306. https://doi.org/10.1039/C7FO01389B
35. Yaoita, Y., Danbara, K., Kikuchi, M. (2005). Two new aromatic compounds from *Hericium erinaceum* (Bull.:Fr.) Pers. *Chemical & Pharmceutical Bulletin, 53*(9), 1202–1203. https://doi.org/10.1248/cpb.53.1202
36. Zhao, J., Wang, J., Wang, H., Lai, M. (2013). Chapter Five – Reg proteins and their roles in inflammation and cancer of the human digestive system. *Advances in Clinical Chemistry*, *61*, 153–173. https://doi.org/10.1016/B978-0-12-407680-8.00006-3