Research Article
Nutritional Composition, Antioxidant Activities, and Antiulcer Potential of \textit{Lentinus squarrosulus} (Mont.) Mycelia Extract

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Water extract of \textit{Lentinus squarrosulus} mycelia was analysed for nutritional content, antioxidant capacity, and antiulcer ability. The extract contains high protein (57.6 g/100 g) and low total fat (0.5 g/100 g) and is rich in magnesium (0.4 g/100 g), potassium (3.8 g/100 g), vitamins B\textsubscript{1} (1.42 mg/100 g), and B\textsubscript{3} (194.29 mg/100 g) with total phenolic content of 39.16 mg/100 g. The cupric reducing antioxidant capacity and 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity of the extract were $A_{450}$ of $0.20 \pm 0.03$ at 0.5 mg/ml and IC\textsubscript{50} of 14.29 mg/ml, respectively. Oral feeding of \textit{L. squarrosulus} extract (250 mg/kg) offered significant gastric mucosal protection of Sprague-Dawley rats compared to cimetidine (50 mg/kg). The ulcer healing rate of ulcerated rats after 24, 48, and 72 hours of treatment was 82%, 90%, and 100%, respectively. The IL-1\textbeta level in the serum and the NF-kB level in the tissues indicate that the healing potential was associated with attenuation of proinflammatory cytokines.

1. Introduction

Peptic ulcer disease is common, affecting millions of people yearly. The principal causes of peptic ulcer are infection by \textit{Helicobacter pylori} and administration of NSAIDs (nonsteroidal anti-inflammatory drugs). \textit{Helicobacter pylori} is the commonest cause of peptic ulceration, but only 15% of infected people develop an ulcer in their lifetime. Peptic ulcers developed due to an imbalance between aggressive factors (\textit{H. pylori}, NSAIDs, gastric acid) and protective factors (mucin, bicarbonate, prostaglandins) leading to an interruption in the mucosal integrity \cite{1}. NSAIDs are used worldwide for the treatment of pain, rheumatic, and cardiovascular diseases, and more recently, for the prevention of colon cancer and Alzheimer’s disease \cite{2}. Different NSAIDs confer different levels of risk, but even aspirin 75 mg/day, may occasionally cause serious ulceration \cite{1}. It is known that stress, alcohol, and steroidal and nonsteroidal anti-inflammatory drugs are some of the factors that increased ulcer risk \cite{3}.

Most modern antiulcer drugs, which are currently available in the market, show limited efficacy against gastric diseases and are associated with severe side effects \cite{4}. A drug with multiple mechanism of protective action including antioxidant activity may be highly effective in minimizing tissue injury in human diseases. It has been demonstrated that many drugs and formulations possess potent antioxidant activity and are effective in healing experimentally induced gastric ulcers \cite{5}. Reactive oxygen species (ROS), which cause tissue damage, are decreased by antioxidant enzymes such as endogenous glutathione (GSH), superoxide dismutase (SOD), glutathione S-transferase (GST), and catalase (CAT).

The ulcerated tissue damage is also accompanied by upregulation in nitric oxide synthase (NOS), epithelial cell apoptosis, and the induction of proinflammatory cytokines interleukin-1\textbeta (IL-1\textbeta) and tumor necrosis factor-alpha (TNF-\alpha) that triggers nuclear factor-kB (NF-kB) activation. IL-1\textbeta has a wide spectrum of inflammatory, metabolic, and immunological properties. IL-1\textbeta plays a significant role in hippocampal synaptic function and is a potential genetic marker as indicator of gastric cancer risk. The prolongation of the healing was associated with an increase in gastric mucosal expression and the release of TNF-\alpha and IL-1\textbeta.
It has been reported that NF-κB plays an important role in gastric ulcer healing in rats. NF-κB was activated in ulcerated tissue but not in normal mucosa, and the level of the activation was decreased with ulcer healing. The results demonstrate that NF-κB activated in ulcerated tissue might upregulate the expression of healing-promoting factors responsible for gastric ulcer healing in rats [7].

Mushrooms have been part of the human diet for thousands of years. Most mushrooms are very important nutritionally and rich in proteins, minerals, and vitamins. Mushrooms have been discovered to have therapeutic values, like anticancer, antitumoral, anti-cholesterol and antihemorrhagic effects. Most bioactive compounds that play essential roles in human and animal physiology have been found in many mushrooms. Okwulehie and Oduenze reported that Auricularia auricularia judae, Pleurotus squarrosulus and Russula sp. were found to contain appreciable amounts of alkaloids, phenols, saponins, and flavonoids [8]. Hericium erinaceus contains many biologically active compounds that have shown interesting biological activities, such as promotion of the synthesis of nerve growth factor, providing remedies for gastric ulcer and chronic gastricism, antitumor, antioxidant, and antimicrobial effects [8].

Lentinus squarrosulus is an edible mushroom commonly found in the wild and has not been cultivated on a large scale for the production of fruit bodies. The tough fruit body is rich in proteins, sugars, lipid, amino acids, vitamin B, C, and D, and minerals [9]. It has been reported that liquid fermentation of mushroom produces high amounts of uniform mycelial biomass as a source of bioactive compounds.

Mushroom mycelia have been reported to have high antioxidant properties. Hot water extract from Agrocybe cylindracea mycelia showed high 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability and high reducing power [10]. Antrodia cinnamomea had potent antioxidant activity both in vitro and in vivo and showed protection of normal erythrocytes against oxidative damage [11]. Daker et al. also demonstrated that mycelia extract of Marasmellus sp. possesses high antioxidant activity by the inhibition of lipid peroxidation [12].

Presently, there is no data available regarding the nutritional content, antioxidant capacity, and antiulcerogenic activity of L. squarrosulus mycelia extract. In this study, the antiulcer activity was assessed via prevention and treatment of gastric ulcers. The roles of proinflammatory cytokine, IL-1β, and the activation of NF-κB in a model of ethanol-induced gastric ulcer in rats were also investigated.

2. Materials and Methods

2.1. Mushroom Mycelia. Mycelia of L. squarrosulus (KUM 50016) were obtained from Mycology Laboratory, Institute of Biological Sciences, University of Malaya and maintained on glucose (1.5%), yeast (0.8%), malt extract (0.8%), and peptone (0.8%) agar medium (GYMP). Seven days old L. squarrosulus mycelia grown on GYMP agar media at 25°C was used as inoculum. Five plugs cut from the periphery of the colony were transferred into 500 mL Erlenmeyer flasks containing sterile liquid GYMP media and incubated for two weeks at 25°C under static condition.

2.2. Preparation of Lentinus squarrosulus Extract. The extract was obtained by water extraction of L. squarrosulus mycelial broth. Mycelia broth was homogenized in water at a ratio of 1 : 1 and boiled for 30 minutes. The broth was centrifuged at 3000 g for 15 minutes and the supernatant was filtered using Whatman no.1 filter paper. The water extract was freeze-dried.

2.3. Nutritional Content of the Extract. Fifty grams sample of L. squarrosulus mycelia extract was analysed for nutritional components by Consolidated Laboratory (M) Sdn. Bhd.

2.4. In Vitro Antioxidant Capacity and Total Phenolic Content of the Extract. Antioxidant activity of L. squarrosulus extract was analyzed using DPPH, according to the method by Brand-Williams et al. [13]. Briefly, DPPH in methanol was prepared and 3.9 mL of this solution was added to 100 μl extract dissolved in methanol at different concentrations (5, 10, 15, 20, and 25 mg/mL). The mixture was shaken vigorously, and the absorbance was measured at 515 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity which was expressed as IC50.

The cupric reducing antioxidant capacity (CUPRAC) of the L. squarrosulus mycelia extract was determined according to the method of Apak et al., based on utilizing the copper (II)-neocuproine reagent as the chromogenic oxidizing agent [14]. The mixture of 1 ml of copper (II), neocuproine, and ammonium acetate buffer solution and extracts was added to make up a final volume of 4 mL. The absorbance at 450 nm was recorded against a reagent blank. The results of antioxidant activity were expressed in absorbance at 450 nm and compared with ascorbic acid as a positive control.

Total phenolic content was measured using Folin-Ciocalteu method according to Singleton and Rossi and using gallic acid as a standard phenolic compound [15]. Briefly, 250 μl of L. squarrosulus mycelia extract was added to 250 μl of 10% Folin-Ciocalteu and incubated for 3 minutes. After 3 minutes, 500 μl of 10% Na2CO3 was added and then allowed to stand for 1 hour in the dark. The absorbance was measured at 750 nm in a spectrophotometer. Phenolic content in the extract was expressed as gallic acid equivalents (GAE).

2.5. Antiulcer Potential of Extract

2.5.1. Animals. Adult male and female Sprague-Dawley (SD) rats aged 6–8 weeks and weighed between 180 and 200 g were purchased from Animal Science Centre, University of Malaya, Kuala Lumpur, Malaysia. The animals were housed at 27 ± 2°C temperature, fed with standard laboratory pellet, and provided water ad libitum. Experimental protocols were approved by the ethical committee (which follows the guidelines of Animal Care and Use Committee), Laboratory
2.5.2. Acute Toxicity Study. Acute toxicity (if any) of the extract was assessed based on the method by Cadirci et al. [16]. A total of 9 male and 9 female rats were divided randomly into 3 groups (n = 6), namely, control, low-dose, and high-dose groups. The rats were administered orally with *L. squarrosulus* mycelia extract at dose levels of 2 g/kg (low dose) and 5 g/kg (high dose) equivalent to a volume of 5 mL/kg body weight. Normal control rats received the same amount of vehicle (distilled water) only. Animals were observed carefully for 24 hours after extract administration and then for the next 14 days. At the end of this experimental period, the rats were observed for signs of toxicity, morphological behavior, and mortality. Acute toxicity was evaluated based on the number of deaths (if any).

2.5.3. Ulcer Prevention Property. A total of 30 (15 males and females each) of SD rats were divided randomly into five groups of six rats in each group. All groups were deprived of food for 24 hours before the experiment. The experiment began with pretreatments according to the assigned group. Group 1 (ulcer control) received the vehicle (distilled water) only; Groups 2, 3, and 4 received 125, 250, and 500 mg/kg of extract, respectively, while Group 5 (positive control) received the same amount of vehicle (distilled water) only. The ulcerated groups (2, 3, and 4) were prefasted for 24 hours before inducing ulcer using absolute ethanol and then for the next 14 days. At the end of this period, the stomachs were removed and kept immersed in 10% of buffered formalin before the analysis of gastric lesions.

2.5.4. Ulcer Healing Property. A total of 24 (12 males and females each) of SD rats were divided randomly into four groups comprising six rats in each group. Group 1 animals which served as normal control received vehicle (distilled water) only; Groups 2, 3, and 4 received 125, 250, and 500 mg/kg of extract, respectively, while Group 5 (positive control) received 50 mg/kg of cimetidine, an H2-receptor blocker. All animals were administered with absolute ethanol after thirty minutes of the pretreatment. After additional thirty minutes, all animals were sacrificed and their stomachs were removed and kept immersed in 10% of buffered formalin before the analysis of gastric lesions.

2.5.5. Gross Evaluation of Gastric Lesions. Each stomach was incised along a greater curvature and rinsed with tap water to remove gastric contents. The stomach was examined under a dissecting microscope (1.8x) with a square grid eyepiece (big square: length × width = 10 × 10 mm² = ulcer area) to access the formation of ulcer area (hemorrhagic lesions). The sum of all lesions, in mm², for each stomach was expressed as the ulcer area (mm²) [17]. The percentage of inhibition (%) was calculated by the following formula:

\[
% \text{ inhibition} = \left( \frac{UA_{\text{control}} - UA_{\text{treated}}}{UA_{\text{control}}} \right) \times 100. \quad (1)
\]

2.7. Determination of IL-1β. IL-1β was determined by using AssayMax Human Interleukin-1β ELISA Kit (Catalog no. E12200-1, St. Charles, MO 63304). A murine monoclonal antibody specific for IL-1β has been precoated onto a microplate. IL-1β in standards and samples was sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for IL-1β, which was recognized by a streptavidin-peroxidase conjugate. All unbound materials were then rinsed off, and a peroxidase enzyme substrate was added. The colour development was stopped, and the intensity of the color was measured immediately at a wavelength of 450 nm.

2.8. Determination of Total NF-kB. The NF-kB/p65 was measured by using the NF-kB/p65 ActivELISA Kit (Catalog no. IMK-503, from IMGENEX Corporation, San Diego, CA 92121). The anti-p65-antibody-coated plate captures free...
The nutritional components of *L. squarrosulus* mycelia extract are shown in Table 1. The crude extract contains high protein (57.6 g/100 g) and low total fat (0.5 g/100 g) and is rich in magnesium (0.4 g/100 g) and potassium (3.8 g/100 g) minerals, vitamins B₁ (1.42 mg/100 g), and B₃ (194.29 mg/100 g).

3. Results

3.1. Nutritional Content of the *L. squarrosulus* Extract. The nutritional components of *L. squarrosulus* mycelia extract are shown in Table 1. The crude extract contains high protein (57.6 g/100 g) and low total fat (0.5 g/100 g) and is rich in magnesium (0.4 g/100 g) and potassium (3.8 g/100 g) minerals, vitamins B₁ (1.42 mg/100 g), and B₃ (194.29 mg/100 g).

3.2. In Vitro Antioxidant Capacity of Extract. Table 2 shows the DPPH radical scavenging activity and cupric reducing power of *L. squarrosulus* mycelia extract compared to the positive control, ascorbic acid. DPPH radical was used as a stable free radical to determine antioxidant activity of natural compounds. Antioxidant activity was defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% (IC50; units = mg extract/mL methanol). The lower IC50 indicates the stronger ability of the extracts to act as DPPH scavengers. The IC₅₀ of the extract was 14.29 mg/mL compared to ascorbic acid of 0.11 mg/mL.

CUPRAC was estimated by the method described by Apak et al. [15]. Table 2 shows the absorbance of *L. squarrosulus* extract at 0.50 mg/mL (100 times higher concentration than positive control) was 0.20 ± 0.03, whereas ascorbic acid at 5 × 10⁻³ g/mL was 0.17 ± 0.04.

Phenolics are important constituents with scavenging ability due to their hydroxyl groups and may contribute directly to the antioxidative action. The amount of total phenolic contents in *L. squarrosulus* mycelia extract was 39.16 mg/GAE g (Table 2).

3.3. Acute Toxicity Study. Rats that received oral doses of 2 g/kg and 5 g/kg did not manifest any clinical signs of toxicity. None of the doses tested could produce mortality in rats during the treatment period indicating that LD₅₀, if any, should be higher than this dose. The present results showed that *L. squarrosulus* mycelia extract even at higher doses (2.5–5 g/kg) was well tolerated by rats.

3.4. Gross Evaluation of Gastric Lesions

3.4.1. Prevention of Ulcer. The groups orally pretreated with 50 mg/kg cimetidine, 125, 250, and 500 mg/kg of *L. squarrosulus* mycelia extract showed significantly (P < 0.05) reduced formation of gastric ulcers induced by ethanol. The inhibition percentage of ulcer area pretreated with 125, 250 and 500 mg/kg was 24%, 85%, and 18%, respectively, compared to cimetidine which shows a decrease in defective area in the ulcerated region by 60%, 90%, and 100% at 24, 48, and 72 hours of treatment, respectively (Table 3, Figure 1). However, the best concentration of extract to prevent gastric ulcer was 250 mg/kg (Table 3, Figure 1(c)).

3.4.2. Healing of Ulcer. Administration of 250 mg/kg of *L. squarrosulus* mycelia extract for 24, 48, and 72 days markedly accelerated the healing of gastric ulcer in ethanol-induced rats. *Lentinus squarrosulus* mycelia extract decreased the ulcer area by 82%, 90%, and 100% at 24, 48, and 72 hours of treatment, respectively, compared to cimetidine which shows a decrease in defective area in the ulcerated region by 60%, 82%, and 100%, respectively (Table 4).

All values were expressed as mean ± standard error mean of six replicates of animals. Means with different superscripts was significantly different (P < .05), adjusted to the nearest mm.

### Table 2: Prevention of gastric ulcer by *L. squarrosulus* mycelia extract.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Ulcer area (mm²)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>—</td>
<td>841 ± 59a</td>
<td>—</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>50</td>
<td>376 ± 25b</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>640 ± 32c</td>
<td>24</td>
</tr>
<tr>
<td><em>L. squarrosulus</em> extract</td>
<td>250</td>
<td>130 ± 13d</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>686 ± 24e</td>
<td>18</td>
</tr>
</tbody>
</table>

### Table 3: The total phenolic contents and antioxidant capacity of *L. squarrosulus* extract compared with ascorbic acid.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total phenolic content GAE (mg/100 g)</th>
<th>DPPH (IC₅₀ mg/mL)</th>
<th>Concentration (μg/mL)</th>
<th>CUPRAC (A₄₅₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. squarrosulus</em> mycelia extract (0.05 μg/ml)</td>
<td>39.16</td>
<td>14.29</td>
<td>500</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td>Gallic acid (0.005 μg/ml)</td>
<td>710.55</td>
<td>0.11</td>
<td>5</td>
<td>0.17 ± 0.04</td>
</tr>
</tbody>
</table>

1,1-diphenyl-2-picrylhydrazil radical (DPPH) results are expressed as inhibition concentration, (IC₅₀). Cupric reducing antioxidant capacity (CUPRAC) results are expressed as mean ± standard deviation.

3.5. Determination of IL-1β Status in Serum. The concentration of IL-1β in serum after treatment with *L. squarrosulus* mycelia extract and untreated rats is depicted in Table 4. The
concentrations of IL-1β were significantly increased after the administration of ethanol than the intact region of control rats. Treatment with extract attenuated these changes by decreasing the level of IL-1β after 72 hours of treatment (Table 4). The results suggest that there was an inhibition of neutrophil influx in the gastric mucosa caused by ulcerogen toxicity upon treatment with L. squarrosulus mycelia extract.

3.4.4. Determination of NF-κB in Gastric Tissues. The activation of NF-κB level after 24, 48, and 72 hours of treatments is shown in Table 4. A significantly decreased activation of NF-κB was observed between groups after 24, 48, and 72 hours of treatment.

4. Discussion

It has been reported that tropical mushrooms are rich in protein, minerals, and vitamins. Protein content of mushrooms is twice that of vegetables, four times that of oranges and significantly higher than that of wheat [18]. Lentinus squarrosulus mycelia extract studied possessed high protein (57.6 g/100 g) which contributes to 100% of RDA (50 g) and low total fat (0.5 g/100 g) and was rich in magnesium (0.4 g/100 g) which contributes to 100% of RDA (0.4 g) and potassium (3.8 g/100 g) which contributes to 100% of RDA (3.5 g), vitamin B₁ (1.42 mg/100 g) which contributes almost 100% of RDA (1.5 mg), and vitamin B₃ (194.29 g/100 g) which contributes to 100% of RDA (20 g).

Mushrooms contain relatively large amounts of minerals, and vitamins. Protein content of Lentinus edodes mycelia extract significantly (P > .05) reduced the ulcer index and afforded significant protection against ethanol-induced ulcer. Ethanol causes vascular damage and necrosis of fruits and vegetables higher in potassium can lower blood pressure. Mushrooms also contain relatively large amounts of carbohydrate and fiber, ranging from 51% to 88% and from 4% to 20% (dry weight), respectively, for the major cultivated species [19].

In this study, water extract of L. squarrosulus mycelia having total phenolic content of 39.16 mg/100 g GAE exhibited DPPH scavenging activity with IC₅₀ value of 14.29 mg/mL, and CUPRAC value at 0.5 mg/mL was 0.20 ± 0.03 absorbance (Table 2). Cheung et al. reported that water extract of Lentinus edodes fruiting bodies showed the most potent radical scavenging activity, 55.4% in DPPH radical scavenging (at 6 mg/mL) compared to methanol extract [20]. According to Tsai et al., scavenging abilities of DPPH radicals were higher in Agrocybe cylindracea mycelia water extract, 1.66 mg/mL compared to extract of fruit bodies, 0.82 mg/mL [10]. Mycelia of Marasmiellus sp. fermented on maize were able to produce bioactive compounds with enhanced DPPH radical-scavenging ability, having IC₅₀ value of 1.88 mg/mL [12]. Tsai et al. reported that the antioxidant activity from mycelia was higher than fruiting bodies of Agrocybe cylindracea, G. tsugae, Lentinula edodes, and Pleurotus spp. [10]. Previous study showed that the CUPRAC assay of stem root extracts of Rhubarb (Rheum ribes) at 50 µg/mL was 1.25 [21].

Pihan et al. demonstrated that oxygen-derived free radicals are implicated in the mechanism of acute and chronic ulceration in the gastric mucosa, and scavenging these free radicals can play an appreciable role in healing these ulcers [22]. This study shows that L. squarrosulus mycelia extract significantly (P > .05) reduced the ulcer index and afforded significant protection against ethanol-induced ulcer.

### Table 4: Healing of ethanol-induced ulcers by L. squarrosulus and the levels of IL-1β and NF-κB after 24, 48, and 72 hours of treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration of treatment (hour)</th>
<th>Dose (mg/kg)</th>
<th>Ulcer area (mm²)</th>
<th>Inhibition (%)</th>
<th>IL-1β (pg/ml)</th>
<th>NF-κB (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without treatment</td>
<td>24</td>
<td>—</td>
<td>0 ± 0²</td>
<td>—</td>
<td>20.6 ± 1.2ᵃ</td>
<td>3.92 ± 0.71ᵃ</td>
</tr>
<tr>
<td>Distilled water</td>
<td>48</td>
<td>—</td>
<td>962 ± 11ᵇ</td>
<td>—</td>
<td>48.9 ± 2.7ᵇ</td>
<td>5.49 ± 0.52ᵃ</td>
</tr>
<tr>
<td>L. squarrosulus extract</td>
<td>250</td>
<td>172 ± 3ᶜ</td>
<td>82</td>
<td>65.8 ± 3.2ᶜ</td>
<td>4.51 ± 0.39ᵇ</td>
<td></td>
</tr>
<tr>
<td>Cimetidine</td>
<td>50</td>
<td>386 ± 3ᵈ</td>
<td>60</td>
<td>30.8 ± 1.5ᵈ</td>
<td>5.29 ± 0.68ᵇ</td>
<td></td>
</tr>
<tr>
<td>Without treatment</td>
<td>48</td>
<td>—</td>
<td>0 ± 0²</td>
<td>—</td>
<td>20.6 ± 1.2ᵃ</td>
<td>3.92 ± 0.71ᵃ</td>
</tr>
<tr>
<td>Distilled water</td>
<td>72</td>
<td>—</td>
<td>333 ± 7ᵇ</td>
<td>65</td>
<td>39.1 ± 0.7ᵇ</td>
<td>0.98 ± 0.20ᵇ</td>
</tr>
<tr>
<td>L. squarrosulus extract</td>
<td>250</td>
<td>95 ± 1ᶜ</td>
<td>90</td>
<td>47.2 ± 1.2ᶜ</td>
<td>0.78 ± 0.34ᵇ</td>
<td></td>
</tr>
<tr>
<td>Cimetidine</td>
<td>50</td>
<td>173 ± 16ᵈ</td>
<td>82</td>
<td>29.1 ± 0.8ᵈ</td>
<td>2.35 ± 0.00ᵇ</td>
<td></td>
</tr>
<tr>
<td>Without treatment</td>
<td>72</td>
<td>—</td>
<td>0 ± 0²</td>
<td>—</td>
<td>20.6 ± 1.2ᵃ</td>
<td>3.92 ± 0.71ᵃ</td>
</tr>
<tr>
<td>Distilled water</td>
<td>—</td>
<td>82 ± 3ᵃ</td>
<td>92</td>
<td>37.8 ± 1.3ᵇ</td>
<td>0.78 ± 0.20ᵇ</td>
<td></td>
</tr>
<tr>
<td>L. squarrosulus extract</td>
<td>—</td>
<td>0 ± 0ᵇ</td>
<td>100</td>
<td>44.0 ± 1.3ᶜ</td>
<td>0.59 ± 0.00ᵇ</td>
<td></td>
</tr>
<tr>
<td>Cimetidine</td>
<td>50</td>
<td>0 ± 0ᵇ</td>
<td>100</td>
<td>28.2 ± 0.7ᵈ</td>
<td>1.57 ± 0.39ᵇ</td>
<td></td>
</tr>
</tbody>
</table>

The group “without treatment” refers to normal control group, “distilled water” refers to ulcer control group, “L. squarrosulus extract” refers to treated group and “cimetidine” refers to positive control group. All values were expressed as mean ± standard error mean. Means with different superscripts were significantly different (P < .05). Six replicates animals were used. Concentration of extract tested was 250 mg/kg, while cimetidine was 50 mg/kg.
on mucosa by increasing the release of vasoactive products from mast cells, macrophages, and other blood cells. Tissue damage begins with the formation of lipid radicals in cell membranes and continues with the conversion of these radicals to lipid hydroperoxides and finally to toxic products such as aldehydes, alkenes, and monoaldehydes [23]. It is feasible to speculate that the antioxidant potential of the extract could play an important role in the prevention and healing of gastric ulcer.

Macroscopic examinations showed the occurrence of stomach damage in all ethanol-induced gastric ulcer rats. Groups treated with *L. squarrosulus* mycelia extract and commercial drug (cimetidine) showed minor lesions. The gastric lesions were significantly reduced by the administration of *L. squarrosulus* mycelia extract at 125, 250, and 500 mg/kg (Figures 1(b), 1(c), and 1(d)). All doses of *L. squarrosulus* mycelia extract showed better gastroprotective effect in comparison to cimetidine, which is an H2-receptor blocker. However, the best concentration of extract to prevent gastric ulcer was 250 mg/kg. Damaged stomachs showed lesions in various forms and sizes dispersed on all stomach surfaces. Remarkable hyperemia and the surrounding blisters were observed in damaged stomachs. Hyperemia was at the highest degree in the ulcer control group. The severity of hyperemia paralleled with the extent of damage. As shown in Table 3, the percentage of the inhibition of ulcer treated with *L. squarrosulus* mycelia extract at concentration of 250 mg/kg was higher compared to other groups.
**5. Conclusions**

This study shows that *L. squarrosulus* mycelia extract can prevent ethanol-induced ulcers at a wide dose, range but a dose of 250 mg/kg of extract was the most effective. Toxicity studies of *L. squarrosulus* mycelia extract carried out in rats indicate no lethal effect at least up to an oral dose of 5 g/kg body weight indicating that LD50 of *L. squarrosulus* mycelia extract will be higher than this dose. Besides ulcer prevention, *L. squarrosulus* mycelia extract was also able to heal ulcer and this was associated with the attenuation of proinflammatory cytokines IL-1β and the inhibition of NF-κB in ulcerated rats. Hence, *L. squarrosulus* mycelia extract being nontoxic and having a variety of nutritional value may potentially serve as nutraceutical ingredient for antiulcer prevention and treatment.

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