**Ganoderma lucidum** (Fr.) P. Karst occurring in South India attenuates gastric ulceration in rats

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The ethanol extract of *Ganoderma lucidum* (Fr.) P. Karst occurring in South India was examined for antiulcer activity against ethanol and Indomethacin induced ulcer in rats. Pretreatment of oral doses of 500 mg/kg and 1000 mg/kg showed significant reduction of ulcer index, with a percentage of inhibition of 55.17, 72.79 and 47 and 76.14% in ethanol and Indomethacin induced gastric ulceration, respectively. Administration of *G. lucidum* extract elevated the levels of Glutathione (GSH) as well as activities of Superoxide dismutase (SOD), Glutathione Peroxidase (GPx) and Catalase (CAT) and significantly lowered the levels of lipid peroxidation (LPO) in a dose dependent manner. The antiulcer property of the mushroom might be due to its profound free radical scavenging activity. The findings suggest the potential therapeutic use of *G. lucidum* as an effective non-toxic antiulcer agent.

**Keywords:** Ganoderma lucidum, Indomethacin, Ulcer, Ethanol, South India

**IPC code; Int. cl. (2011.01)—**A61K 36/00, A61P 1/04

**Introduction**

Gastric hyperacidity and gastroduodenal ulcer is a serious global problem today. Peptic ulcers have been described as an imbalance between the luminal acid peptic attacks versus the mucosal defenses. Acid and pepsin components form the aggressive factors, and the mucosal layer of mucin-bicarbonate secretion, phospholipid layer, tight junction’s cell proliferation, prostaglandins, and the urogastrone epidermal healing factors form the defensive factors. Enhancement of gastric mucus has been proposed to explain antiulcer activity. Stress, smoking, nutritional deficiencies and ingestion of nonsteroidal-anti-inflammatory drugs (NSAIDs) are the major factors, which increase the incidence of gastric ulcer.

Gastric ulcer therapy faces a major drawback in modern medicine due to the unpredictable side effects of the long-term use of commercially available drugs. As it affects 5% of the global population, the treatment of this painful disease and its prevention has become one of the challenging problems of today. Hence, the search is still on to find a drug, which will serve as a powerful therapeutic agent to prevent and cure gastric ulceration. The search now extends to the systematic screening of natural products.

Mushrooms represent a major and a yet largely untapped source of new pharmaceutical products. Medicinal mushrooms are traditionally used in China and Japan for the treatment of a variety of diseases. Many pharmaceutical substances with potent and unique valuable properties isolated recently from mushrooms are being used worldwide. *Ganoderma lucidum* (Fr.) P. Karst (Plate 1) commonly known as Reishi or Ling Zhi has been regarded as a panacea for all types of diseases in Traditional Chinese Medicines (TCM). *Ganoderma* belongs to the Polyporaceae family of Basidiomycota and are widely distributed in South India. *G. lucidum* contains a variety of chemical ingredients, including triterpenes, polysaccharides, nucleosides, fatty acids, alkaloids,
proteins, peptides, amino acids and inorganic elements. The mushroom has been used as a remedy for a wide range of ailments and chronic diseases, such as migraine, hypertension, arthritis, bronchitis, asthma, anorexia, gastritis, haemorrhoids, diabetes, hypercholesterolaemia, nephritis, dysmenorrhoea, constipation, lupus erythematosis, hepatitis and cardiovascular problems. Previous investigations carried out in our laboratory showed that *G. lucidum* occurring in South India possesses significant antioxidant, antitumor, anti-inflammatory, antinociceptive, nephroprotective and radioprotective properties. The current studies were undertaken to evaluate the gastroprotective effects of *G. lucidum* against ethanol and Indomethacin induced gastric injury in rats.

**Materials and Methods**

**Chemicals**

Nitroblue tetrazolium (NBT) and 5-5′ dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Sisco Research Laboratories, Mumbai, India. Deoxyribose and riboflavin were obtained from Merck, India. All other chemicals used were of analytical reagent grade.

**Animals**

Female albino rats of Wistar strain (180-200 g) were purchased from Small Animal Breeding Centre, Kerala Agricultural University, Mannuthy, Thrissur, Kerala, India and were kept for a week under environmentally controlled conditions with free access to standard food (Sai Durga Feeds and Foods, Bengaluru, India) and water ad libitum. All other chemicals used were of analytical reagent grade.

**Preparation of the extract**

Fruiting bodies of *G. lucidum* growing on the *Caesalpinia coriaria* (Jacq.) Willd. trees in the local area were collected. The voucher specimen was deposited in the herbarium of Centre for Advanced Studies in Botany, University of Madras, Chennai, India (HERB.MUBL.3175). The fruiting bodies of the mushroom were cut into small pieces, dried at 40-50°C for 48 h and powdered. 100 g samples of the powdered fruiting bodies of the mushroom materials were extracted with 70% (v/v) ethanol on a boiling water bath for 48 h. The extraction was repeated again. Extracts pooled and evaporated under vacuum and finally lyophilized. The residue (5%) was suspended in distilled water and employed for the experiments.

**Determination of the effect of *G. lucidum* extract on gastric ulcer induced by ethanol**

Animals were divided into 5 groups of 6 animals in each group. Group 1 was kept as normal without any treatment. All other groups were fasted for 36 h and administrated 5 mL/kg of 80% ethanol. Group II (Vehicle control) animals received ethanol alone. Groups III, IV, and V were treated orally with ranitidine (50 mg/kg body weight) and *G. lucidum* extract (500 and 1000 mg/kg body weight), respectively, 1 h prior to the administration of ethanol. Both *G. lucidum* extracts and ranitidine were suspended in distilled water and orally administrated to different groups using a gavage. Similarly control group was administrated with distilled water (vehicle) and animals were sacrificed after 4 h of the administration of ethanol. Subsequently stomachs were removed and examined.

**Determination of the effect of *G. lucidum* extract on gastric ulcer induced by Indomethacin**

Animals were divided into 5 groups of 6 animals in each group. Group 1 was kept as normal without any treatment. All other groups were fasted for 36 h and administered orally with Indomethacin (50 mg/kg body weight). Group II (control) animals received Indomethacin alone. Groups III, IV, and V were pretreated with ranitidine (50 mg/kg body weight) and *G. lucidum* extract (500 and 1000 mg/kg body weight), respectively, 1 hour prior to the administration of Indomethacin. Both *G. lucidum* extracts and ranitidine were suspended in distilled water and orally administrated to different groups using a gavage. Similarly control group was administrated with distilled water (vehicle). After 6 h, the animals were sacrificed and stomachs were removed and opened along the greater curvature and examined.

**Determination of ulcer index (UI)**

The ulcerative index (UI) was calculated by severity of gastric mucosal lesions and graded as follows:

<table>
<thead>
<tr>
<th>Erosions</th>
<th>Score</th>
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<tbody>
<tr>
<td>1 mm or less</td>
<td>1</td>
</tr>
<tr>
<td>1-2 mm</td>
<td>2</td>
</tr>
<tr>
<td>More than 2 mm</td>
<td>3</td>
</tr>
</tbody>
</table>

UI was calculated by using following formula:
UI = 1 × (number of lesions of grade 1) + 2 × (number of lesions of grade 2) + 3 × (number of lesions of grade 3).

The overall score was divided by a factor 10, which was designated as Ulcer index\(^\text{11}\).

**Biochemical analysis**

After the measurement of gastric lesions, the mucosa of the glandular stomach was removed by scraping with a blunt knife, and a 10% homogenate was prepared in Tris buffer (pH 7.0) and used for biochemical analysis by the following methods. The superoxide dismutase (SOD) was estimated by the riboflavin photoreduction method\(^\text{12}\), and catalase by measuring the rate of decomposition of H\(_2\)O\(_2\) at 240 nm\(^\text{13}\). The tissue lipid peroxidation was estimated by the thiobarbituric acid (TBA) method\(^\text{14}\) glutathione (GSH) by measuring its reduction with DTNB\(^\text{15}\). Glutathione peroxidase (GPx) was estimated by the method based on the degradation of H\(_2\)O\(_2\) in the presence of GSH\(^\text{16}\). The protein content of the homogenate was determined by Lowry’s method\(^\text{17}\).

**Histopathology**

After the termination of the experiments, animals were sacrificed, the stomach was excised and cut open through the ventral suture and a small portion was fixed in 10% formalin solution immediately after sacrifice, which was passed through ascending grades of alcohol, cleared in xylene and impregnated and embedded in paraffin-dehydrated specimens. The sections of 3-4 µm were made using a microtome and after staining with haematoxyline and eosine, the sections were molded in DPX and observed under light microscope.

**Statistical analysis**

All data were represented as mean ± S.D and it was statistically analyzed using one-way analysis of variance (ANOVA) (using the Graph Pad Instat3 software package). The significant difference between the normal, control, standard and treatment were further analyzed by Bonferroni’s t-test. P values less than 0.05 were considered as significant.

**Results**

The *G. lucidum* extract showed significant decrease in rat mucosal injury induced by ethanol and Indomethacin in a dose-dependent manner. Administration of 80% ethanol (1 mL) and Indomethacin (50 mg/kg body weight) to the animals resulted in severe gastric damage. The symptoms were visible from the outside of the stomach as thick reddish-black lines. After opening, the stomach lesions were found in the mucosa and consisted of elongated bands usually parallel to the long axis of the stomach. They were located mostly in the corpus (the portion of the stomach secreting acid and pepsin) whereas no gross lesions were developed in the fore-stomach (the non-secretory part of the stomach).

The effect of *G. lucidum* on gastric lesions induced by ethanol is presented in Table 1. Administration of 80% ethanol to rats produced severe gastric erosions with an ulcer index of 5.22 ± 0.74. Pretreatment with *G. lucidum* extract in doses of 500 mg/kg and 1000 mg/kg and standard drug ranitidine at 50 mg/kg significantly diminished the ulcer index and the percentage of lesion with no intra luminal bleeding compared with control group (*P* < 0.001). The percentage of ulcer inhibition was 55.17 and 72.79 for *G. lucidum* 500 mg/kg and 1000 mg/kg groups of animals, respectively (Table 1). The percentage reduction of ulcer lesion was compared with standard drug ranitidine. The group of positive control, using 50 mg/kg of ranitidine, presented a 57% reduction in lesions.

The activities of antioxidant enzymes such as SOD, Catalase and GPx were lowered approximately 2.14, 3.44 and 1.74 fold in the case of control group with respect to the normal. Ranitidine (50 mg/kg) showed 1.69, 2.66 and 1.41-fold increase for SOD, Catalase and GPx, respectively with respect to control group. Antioxidant activities were improved by the *G. lucidum* extract treatment. The increase in activity

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Ulcer index</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>—</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td>Vehicle Control (80% ethanol)</td>
<td>5 ml/kg</td>
<td>5.22 ± 0.74</td>
<td>0</td>
</tr>
<tr>
<td>Ranitidine + ethanol</td>
<td>50 mg/kg b.wt</td>
<td>2.2 ± 0.4***</td>
<td>57</td>
</tr>
<tr>
<td>GE + ethanol</td>
<td>500 mg/kg b.wt</td>
<td>2.3 ± 0.6***</td>
<td>55.17</td>
</tr>
<tr>
<td>GE + ethanol</td>
<td>1000 mg/kg b.wt</td>
<td>1.42 ± 0.68***</td>
<td>72.79</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6; *** *P* < 0.001 (Bonferroni test) with respect to control.
was 2.08, 2.52 and 1.44 fold (in the case of 1000 mg/kg treated group) and 1.64, 1.46 and 1.24-fold (in the case of 500 mg/kg treated group) for SOD, Catalase and GPx respectively than that of control group.

The level of tissue lipid peroxidation was found to be increased 3.03 fold in the case of control group than that of normal. Ranitidine (50 mg/kg) showed 1.55-fold decrease of lipid peroxidation. *G. lucidum* extract treatment decreased the level of lipid peroxidation and the decrease was approximately 2.14 and 1.44-fold in the case of 1000 mg/kg and 500 mg/kg treated group with respect to control (Figure 1).

The level of reduced glutathione was found to be decreased 1.83 fold in the case of control group with respect to normal. Ranitidine (50 mg/kg) showed 1.62 fold increase of GSH. *G. lucidum* extract improved the level of GSH and increase was approximately 1.65 and 1.19 fold in the case of 1000 mg/kg and 500 mg/kg treated group with respect to control (Figure 1).

Indomethacin administration to rats produced severe gastric damage in control group of animals with an ulcer index of $3.9 \pm 0.81$, whereas the treated groups of animals showed a significant decrease in ulcer index values in a dose-dependent manner (Table 2) with no intraluminal bleeding. The percentage of ulcer inhibition was 47 and 76.14 for *G. lucidum* 500 mg/kg and 1000 mg/kg groups of animals, respectively (Table 2). The group of positive control, using 50 mg/kg of ranitidine, presented a 51.28% reduction in lesions.

The activities of antioxidant enzymes such as SOD, Catalase and GPx were lowered approximately 3.68, 4.66 and 1.54-fold in the case of control group with respect to the normal. Ranitidine (50 mg/kg) showed effective antioxidant activity, 1.91, 2.23 and 1.24-fold increase for SOD, Catalase and GPx, respectively. Antioxidant activities were improved by the *G. lucidum* extract treatment. The increase in activity was 3.18, 4.39 and 1.44-fold (in the case of 1000 mg/kg treated group) and 1.96, 1.85 and 1.14-fold (in the case of 500 mg/kg treated group) for SOD, Catalase and GPx, respectively.

The level of tissue lipid peroxidation was found to be increased 2.92-fold in the case of control group than that of normal. Ranitidine (50 mg/kg) showed

![Graph showing effect of *G. lucidum* on antioxidant parameters](image)

**Fig. 1—Effect of *G. lucidum* on the antioxidant parameters in ethanol induced gastric ulcer: Values are mean ± SD, n = 6. Bars represent standard deviation; (a) $P<0.001$; (b) $P<0.01$ as compared to normal (Bonferroni test); *** $P<0.001$, ** $P< 0.01$, * $P< 0.05$ and (ns) $P>0.05$, non significant as compared to control group (Bonferroni test).**
1.41-fold decrease of lipid peroxidation. *G. lucidum* extract treatment decreased the level of lipid peroxidation and the decrease was approximately 2.23 and 1.63-fold in the case of 1000 mg/kg and 500 mg/kg treated group with respect to control (Figure 2).

The level of reduced glutathione was found to be decreased 1.59-fold in the case of control group with respect to normal. Ranitidine (50 mg/kg) showed 1.24-fold increase of GSH. *G. lucidum* extract improved the level of GSH in stomach. The increase was approximately 1.56 and 1.36 fold in the case of 1000 mg/kg and 500 mg/kg treated group with respect to control (Figure 2).

The histopathological observations of ethanol and Indomethacin induced ulceration models (Plates 2, 3) showed severe erosion of gastric mucosa with necrotic patches, sub-mucosal edema and neutrophil infiltration in control group animals. The control groups also showed the presence of necrotic debris in the lamina proper of the mucosa infiltrated with polymorphonuclear leukocytes. The depth of the injury extends up to the muscularis with RBC extravasations. All of these pathological alterations were inhibited to a greater extent by the treatment with *G. lucidum* extract.

**Discussion**

Reactive oxygen species are well known to play a major role in the etiology and pathophysiology of human diseases, in general and digestive system disorders in particular. A growing body of experimental and clinical evidence suggests that

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**Table 2—Effect of stabilization of gastric mucosa by *G. lucidum* (GE) extract in Indomethacin induced gastric ulcer**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Ulcer index</th>
<th>% of inhibition</th>
</tr>
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<tbody>
<tr>
<td>Normal</td>
<td>—</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td>Vehicle Control (Indomethacin)</td>
<td>50 mg/kg b.wt</td>
<td>3.9 ± 0.81</td>
<td>0</td>
</tr>
<tr>
<td>Ranitidine + Indomethacin</td>
<td>50 mg/kg b.wt</td>
<td>1.9 ± 0.61***</td>
<td>51.28</td>
</tr>
<tr>
<td>GE + Indomethacin</td>
<td>500 mg/kg b.wt</td>
<td>2.06 ± 0.45***</td>
<td>47</td>
</tr>
<tr>
<td>GE + Indomethacin</td>
<td>1000 mg/kg b.wt</td>
<td>0.93 ± 0.38***</td>
<td>76.14</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 6; *** P<0.001 (Bonferroni test) with respect to control

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**Fig. 2—Effect of *G. lucidum* on the antioxidant parameters in Indomethacin induced gastric ulcer: Values are mean ± SD, n = 6; Bars represent standard deviation; (a) P<0.001; (b) P<0.01 as compared to normal (Bonferroni test); *** P<0.001, ** P< 0.01, * P< 0.05 and (ns) P>0.05 non significant as compared to control group (Bonferroni test)**
Plate 2—Histopathological analysis of gastric mucosa of rats stained with hematoxylin and eosin: (a) Normal; (b) Vehicle Control (80% ethanol); (c) Ethanol + *G. lucidum* (500 mg/kg); (d) Ethanol + *G. lucidum* (1000 mg/kg); (e) Ethanol + Ranitidine (50 mg/kg).
Plate 3—Histopathological analysis of gastric mucosa of rats stained with hematoxylin and eosin: (a) Normal; (b) Vehicle Control (Indomethacin); (c) Indomethacin + *G. lucidum* (500 mg/kg); (d) Indomethacin + *G. lucidum* 1000 mg/kg (e) Indomethacin + Ranitidine (50 mg/kg).
The formation of gastric mucosal lesions by necrotizing agents, such as ethanol, has been reported to involve the depression of the gastric defensive mechanisms. Oral treatment with ethanol causes focal hyperemia, edema, necrosis, and submucosal hemorrhage, as well as circulatory disturbances. The formation of gastric mucosal lesion following ethanol administration involves several mechanisms which reduce the flow of gastric blood, thereby contributing to the development of hemorrhage and necrosis, and to the solubilization of mucus constituents in the stomach. These actions result in an increased flow of Na+ and K+, increased pepsin secretion, and a loss of H+ ions and histamine into the lumen. The control group treated orally with ethanol clearly produces the expected characteristic zone of necrotizing mucosal lesions. On the other hand, the treatments with *G. lucidum* significantly decreased the lesion index and percentage of lesion. These results indicate that *G. lucidum* possesses an antiulcerogenic effect related to cytoprotective activity.

Non-steroidal anti-inflammatory drugs (NSAIDs) such as Indomethacin, have the ability to cause gastroduodenal ulceration, and this effect is related to the ability of these agents to suppress prostaglandin synthesis. In the stomach, prostaglandins play a vital protective role, stimulating the secretion of bicarbonate and mucus, maintaining mucus blood flow, and regulating mucosal cell turnover and repair. Thus, the suppression of prostaglandin synthesis by NSAIDs results in increased susceptibility to mucosal injury and gastroduodenal ulceration. In this model, *G. lucidum* displayed a significant reduction in mucosal damage. These results suggest the possible involvement of prostaglandins and/or mucus in the antiulcer effect of the extract.

Histopathological studies showed that pretreatment with extract inhibited ethanol and Indomethacin induced ulcer, oedema, hemorrhage and necrosis in gastric mucosa. The protection against ulcerogenesis, as observed in the significant reduction in ulcer index as well as protection to the gastric mucosal GSH, GPx and antioxidant status of ulcerated animals clearly confirm the cytoprotective effect of *G. lucidum* extract against ethanol and Indomethacin induced gastric ulceration.

The preliminary phytochemical analysis of the extract of *G. lucidum* indicated the presence of terpenes and polysaccharides as major chemical constituents. *G. lucidum* polysaccharides and triterpenes/triterpenoids have been reported to possess significant antiulcerogenic effect related to cytoprotective activity.
biological activities. Several medicinal herbs have been found to have ulcer-healing effects, and they are traditionally used for the prevention and treatment of peptic ulcer disease. Poly saccharides isolated from *G. lucidum* have been reported to the potent healing effect on Indomethacin induced gastric lesions in the rats. Local direct effects of *G. lucidum* polysaccharides (GLPS) on gastric mucosa may play an important role. GLPS may directly bind to the infiltrated immune effector cells (e.g. macrophages), subsequently altering their activities. In addition intragastric administration of *G. lucidum* polysaccharides will form a layer of artificial mucus, which may provide a temporary protection for gastric mucosa against damaging factors. The presence of these constituents might be partially responsible for the exhibited activity both in vitro and in vivo.

**Conclusion**

*G. lucidum* occurring in South India has profound antiulcer property and is able to protect the gastric mucosa from ethanol and Indomethacin induced mucosal lesions. The antiulcer property of the mushroom might be mediated through its free radical scavenging activity. The findings suggest the potential therapeutic use of *G. lucidum* as an effective non-toxic antiulcer agent.

**References**