Mushrooms represent a major and as yet, largely untapped source of potent pharmaceutical products. The antioxidant, antiinflammatory, antinociceptive, antimutagenic, anticarcinogenic, antitumour, hepatoprotective, nephroprotective and cardioprotective activities of the methanolic extract of *Ganoderma lucidum* (Fr.) P. Karst. collected from tropical South India were evaluated for the revalidation of its utilization in Chinese folklore medicine. Review of results is presented in this paper.

**Keywords** : Medicinal properties, Mushroom, *Ganoderma lucidum*, Reishi, Ling Zhi.

**IPC code; Int. cl.** — A61K 35/84, A61P 1/16, A61P 9/00, A61P 29/00, A61P 35/00, A61P 39/06

**Introduction**

Mushrooms represent a major and as yet, largely untapped source of potent pharmaceutical products. Of the approximately 10,000 known species of mushrooms, 2000 are safe for people's health and about 300 of them possess medicinal properties. *Ganoderma lucidum* (Fr.) P. Karst., which is commonly known as *Reishi or Ling Zhi* has been used in folk-medicine in China and Japan for 2000 years for a wide range of ailments. In Chinese folklore, fruiting bodies of *Ganoderma P. Karst.* have been regarded as a panacea for all types of diseases. This is probably due to the demonstrated efficacy of it as a popular remedy to treat several disease conditions, namely chronic hepatitis, arthritis, hypertension, hyperlipidemia, insomnia, bronchitis, neoplasia, asthma, gastric ulcer, atherosclerosis, diabetes, debility due to prolonged illness, etc. Almost all magical effects have been attributed to *Ganoderma P. Karst.* and the mushroom is called as *Mushroom of Immortality* in China, Japan and Korea. The extensive range of traditional medical treatments with *Ganoderma* has not been fully substantiated by modern scientific standards.

*Ganoderma* species were classified into several types, including black, red, purple, light black, yellow and white. Each type of *Ganoderma* has its own characteristic biological properties. The commonly used medicinal *Ganoderma* include, *G. lucidum, G. tsugae* Murrill, *G. capense* Junhua & Ronglan and *G. applanatum* (Pers.) Pat. Some of the physiological effects and distinctive properties of *Ganoderma* are strain dependent. Polysaccharides and triterpenes of *Ganoderma* are the major source of its pharmacological active constituents. Currently more than 100 types of polysaccharides and 130 triterpenoids are known from this mushroom.

Species of the genus, *Ganoderma* have been reported to occur throughout the world. Over 250 species of this mushroom are known. *G. lucidum* has been found to occur widely in India, particularly in the tropical area. No attempt has been made to evaluate the medicinal properties of *Ganoderma* mushrooms occurring in India. Investigations carried out in our laboratory revealed that *G. lucidum* occurring in tropical South India possessed significant antioxidant, antiinflammatory, antinociceptive, antimutagenic, anti-carcinogenic, antitumour, hepatoprotective,
nephroprotective and cardioprotective activities.

**Preparation of mushroom extract**

During experiments the fruiting bodies of *G. lucidum* were collected from the foot hills of Thrissur District, Kerala. They were cut into small pieces and dried at 40-50°C for 48 hours and then powdered. The powdered samples were first extracted with petroleum ether using a Soxhlet apparatus for 8 hours. The defatted material was then extracted with ethyl acetate and finally with aqueous-methanol (30:70 v/v) for 8 hours. The extracts were concentrated at low temperature using a rotary vacuum evaporator till the solvents were completely removed. The residues thus obtained were used for the experiments.

**Medicinal properties**

*Antioxidant* — The antioxidant activity of the methanolic extract of *Reishi* was assayed by FRAP (Ferric reducing power), DPPH (1,1-diphenyl-2-picryl hydrazyl) assay, and ABTS (2,2-azobis-3-ethylbenzthiazoline-6-sulfonic acid) spectrophotometric assay using TEAC (Trolox equivalent antioxidant capacity) and AEAC (Ascorbic acid equivalent antioxidant capacity) as standards. Superoxide radical, and hydroxyl radical scavenging and lipid peroxidation inhibiting activities of ethyl acetate, methanolic and aqueous extracts were also determined using quercetin and catechin as standards. The FRAP, DPPH and ABTS assays indicated that the methanolic extract showed significant antioxidant activity. The antioxidant assays also showed that ethyl acetate, methanolic and aqueous extracts of the mushroom possessed significant superoxide radical and hydroxyl radical scavenging and lipid peroxidation inhibiting activities. The FRAP assay indicated the first line defense (preventive antioxidant) activity of the extract which suppressed the formation of the free radicals. The ABTS assay showed the second line defense activity of the extract against free radicals (suppression of chain initiation and/or break of chain propagation reactions). The DPPH assay showed significant antioxidant activity of the extract to scavenge the primary free radicals. Our results also reveal that the sample of *G. lucidum* from South Indian tropics has greater antioxidant activity than *G. lucidum, G. formosanum* Chang et Chen and *G. neojaponicum* Imazeki occurring in Taiwan.

*Antiinflammatory* — Acute and chronic antiinflammatory activities of ethyl acetate and methanolic extracts were determined by carrageenan-induced acute and formalin-induced chronic inflammatory models in mice. The ethyl acetate and methanolic extracts showed significant effect on carrageenan-induced acute and formalin-induced chronic inflammation in mice. The activity of the extract at a concentration of 1000mg/kg body wt was remarkably high and was comparable with that of reference drug, Diclofenac.

*Antinociceptive* — The antinociceptive activity of ethyl acetate and methanolic extracts was determined by writhing test by intraperitonial injection of 0.2ml acetic acid (0.6%) in mice. Animals were treated with the extracts

<table>
<thead>
<tr>
<th>Activities</th>
<th>Ethyl acetate extract</th>
<th>Methanol extract</th>
<th>Aqueous extract</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide radical scavenging</td>
<td>213 ± 2.12</td>
<td>61 ± 2.5</td>
<td>475 ± 2.5</td>
<td>3.7 ± 0.16 (Quercetin)</td>
</tr>
<tr>
<td>Hydroxyl radical scavenging</td>
<td>185 ± 25</td>
<td>159 ± 3.6</td>
<td>140 ± 2</td>
<td>850 ± 20</td>
</tr>
<tr>
<td>Lipid peroxide inhibiting</td>
<td>205 ± 25</td>
<td>615 ± 4.08</td>
<td>-</td>
<td>418 ± 28.6 (Catechin)</td>
</tr>
</tbody>
</table>

All values are represented as mean ± S D, n = 3

Table 1: *In vitro* antioxidant activity of *Ganoderma lucidum* extracts (IC<sub>50</sub> µg/ml)
(500 and 1000mg/kg) orally one hour prior to acetic acid injection. Results showed that ethyl acetate and methanolic extracts inhibited acetic acid-induced abdominal constriction response in mice. The methanolic extract possessed higher activity than ethyl acetate extract in a dose-dependent manner. The activity of the extract is higher than the commercial strain available in international market.

**Antimutagenic** — The antimutagenic activity of the methanolic extract of *G. lucidum* was assayed by Ames Salmonella mutagenecity test using histidine mutants of *Salmonella typhimurium* tester strains, TA 98, TA 100 and TA 102. The extract significantly inhibited \( P<0.001 \) the in vitro sodium azide (Na\( _3 \)), N-methyl-N-nitro-N-nitrosoguanidine (MNNG), 4-nitro-O-phenylenediamine (NPD), 2-aminofluorine (2-AF) and benzo[a] pyrene (B[a]P) induced his+ revertants in a dose dependent manner. The in vivo antimutagenic activity of the extract was also determined by the mutagenicity test of the urine of rats administered with B[a]P as mutagen. The administration of the extract at a dose of 500mg/kg body wt inhibited 59.4% mutagenicity induced by B[a]P.

**Anticarcinogenic** — The anticarcinogenic activity of the methanolic extract of *G. lucidum* was determined by N-nitrosodietylamine (NDEA) induced hepatocarcinoma (HCC) and 7,12-dimethylbenz[a]anthracene (DMBA) induced rat mammary tumour models. HCC was induced in rats by the administration of NDEA (94mg/kg body wt) orally for 5 days/week for 20 weeks. The extract (500mg/kg body wt) was given one hour prior to each NDEA administration. Twelve weeks after the last dose of NDEA, animals were sacrificed and biochemical parameters were evaluated for determining the effect of the extract on HCC. Mammary tumour was induced in 40-50 days old female rats by the administration of 10mg DMBA/animal in olive oil once a week for 75 weeks. The extract (500mg/kg body wt) was administered orally twice weekly for 2 weeks and DMBA was administered as mentioned above. Animals were observed for tumour development and tumour latency period was recorded. The animals were sacrificed after 160 days, blood was collected, serum separated and alkaline phosphate (ALP) activity determined.

Experimental results showed that all animals in the NDEA treated group developed liver tumours by the end of 32 weeks. The number of tumours and percent of incidence was reduced significantly in the group of animals administered with *G. lucidum* extract. The NDEA treatment drastically elevated gamma glutamyl transpeptidase (GGT), glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT), alkaline phosphate (ALP) activities and lipid peroxidation. The treatment of the animals with the extract significantly reduced the enhanced activities of GGT, GPT, GOT and ALP and the melondialdehyde (MDA) level (lipid peroxidation). This indicated the preventive effect of *G. lucidum* extract against hepatocarcinoma is caused by NDEA.

Mammary tumours were first observed in animals 73 days after the administration of DMBA. The treatment with *G. lucidum* extract delayed the induction of the tumour by 25 days. The incidence of tumour was 100% in animals treated with DMBA alone while treatment with the extract (1000mg/kg) reduced the incidence by 33.3%. An average of four mammary tumours were observed in animals treated with DMBA alone while the animals treated with 1000mg/kg and 500mg/kg extract showed an average of one and two tumours per animal. DMBA treatment enhanced ALP level and the mushroom extract significantly reduced the activity of this enzyme.

**Antitumour** — Antitumour activity of the aqueous and methanolic extracts of the mushroom was determined by implanted tumour model in mice. Ehrlich’s ascites carcinoma cells were implanted subcutaneously on the dorsal side of the right hind limb of mice. Twenty-four hours after the implantation of tumour cells, the animals were administered with either 125, 250 and 500mg/kg aqueous or methanolic extract of *G. lucidum* once daily for a period of 10 days. The tumour development was monitored for a period of one month and tumour volumes were measured. At the end of the experiment animals were sacrificed, tumours were excised and weighed. From these data tumour inhibition was calculated. Both aqueous and methanolic extracts of the mushroom at concentrations of 125, 250 and 500mg/kg body wt showed significant inhibitory effect of solid tumour induced by EAC cells. The extracts inhibited >80% tumour volume and weight at 500mg/kg body wt (Table 2). The results of the investigation thus indicate significant antitumour property.

**Prevention of anticancer drug toxicity** — Cisplatin (cisplatinum II), diamine dichloride and Doxorubicin are extensively used as anticancer drugs.
However, Cisplatin chemotherapy is found to manifest dose-dependent nephrotoxicity and long term administration of Doxorubicin results in cumulative dose related cardiotoxicity in cancer patients. The effect of methanolic extract of G. lucidum was examined to ameliorate the toxicity associated with these drugs. Cisplatin induced nephrotoxicity was assessed by determining the serum creatinine and urea levels and renal antioxidant status in mice after Cisplatin administration (16mg/kg body wt i.p). Methanolic extract of G. lucidum (250 and 500mg/kg body wt) was administered orally 1 hour before Cisplatin administration. The results indicated that the extract significantly reduced the elevated serum creatinine and urea levels. Renal antioxidant defense systems, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) activities and reduced glutathione (GSH) level depleted by Cisplatin therapy were restored to normal with the treatment of the mushroom extract. Cisplatin-induced increased lipid peroxidation was also found markedly reduced by the treatment with the extract. The experimental findings indicated that the extract rendered significant protection against Cisplatin-induced nephrotoxicity.10.

The protective effect of G. lucidum was also examined against Doxorubicin-induced cardiotoxicity. Administration of 3 doses of Doxorubicin (6mg/kg body wt, i.p) for each dose on alternate days showed clear signs of cardiotoxicity in rats. Doxorubicin drastically enhanced serum creatin kinase (CK) and lipid peroxidation activities in tissue and also caused significant decrease in GSH levels and activities of CAT, SOD and GPX. Administration of 3 doses of methanolic extract (500 and 1000mg/kg body wt) orally 1 hour before Doxorubicin administration significantly lowered the activity of CK and lipid peroxidation in a dose dependent manner. The mushroom extract also significantly increased the level of GSH and activities of SOD, CAT and GPX. The findings reveal the therapeutic use of G. lucidum extract for the amelioration nephrotoxicity and cardiotoxicity due to oxidative stress caused by chemotherapy. The findings also suggest the uses of this mushroom extract in adjuvant therapy.

**Hepatoprotective —**

Hepatoprotective effect of the methanolic extract of G. lucidum was evaluated using carbon tetrachloride (CC14) induced chronic hepatotoxicity in rats. Hepatotoxicity was induced by the administration of 1.5ml CC14 (CC14/paraffin oil 1:5 v/v i.p.) 3 times in a week for 5 weeks (total 15 dozes). Two groups of animals were administered with the extract (500/1000 mg/kg) orally once daily for 5 weeks (1 hour before each CC14 injection). Twenty four hours after the last dose of the treatments, animals were sacrificed, blood was collected and serum glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT) and alkaline phosphate (ALP) were determined. Histopathological examination of the liver sections was also made to

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dosage (mg/kg)</th>
<th>Tumour volume (cm³)</th>
<th>Tumour weight (g)</th>
<th>% Decrease in tumour volume</th>
<th>% Decrease in tumour weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>-</td>
<td>1.053 ± 0.09</td>
<td>5.3 ± 0.76</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard (Cisplatin)</td>
<td>4</td>
<td>0.046 ± 0.001</td>
<td>0.381 ± 0.060</td>
<td>95</td>
<td>93</td>
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<tr>
<td>Aqueous extract</td>
<td>125</td>
<td>0.313 ± 0.010*</td>
<td>1.705 ± 0.090*</td>
<td>70</td>
<td>67</td>
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<tr>
<td></td>
<td>250</td>
<td>0.182 ± 0.020*</td>
<td>1.248 ± 0.051*</td>
<td>80</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.160 ± 0.010*</td>
<td>0.925 ± 0.004*</td>
<td>84.8</td>
<td>82.6</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>125</td>
<td>0.375 ± 0.060*</td>
<td>1.960 ± 0.060*</td>
<td>64</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.167 ± 0.010*</td>
<td>0.921 ± 0.007*</td>
<td>84</td>
<td>82.6</td>
</tr>
<tr>
<td>Ethyl acetate extract</td>
<td>500</td>
<td>0.273 ± 0.030*</td>
<td>1.432 ± 0.028*</td>
<td>74.1</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0.166 ± 0.034*</td>
<td>0.930 ± 0.035*</td>
<td>84.3</td>
<td>82.5</td>
</tr>
</tbody>
</table>

Values are mean ± S D; n=6; *P < 0.001
evaluate necrosis, fatty infiltration, fibrosis, lymphocyte infiltration, etc. CC1₄ injection caused drastic increase in the activities of GPT, GOT and ALP indicating hepatic damages. The administration of the methanolic extract at doses of 500 and 1000mg/kg lowered the elevated levels of these enzymes in a dose dependent manner. Histopathological examination of the liver of animals challenged with CC1₄ showed centrilobular necrosis, degeneration, infiltration of lymphocytes and fatty changes. The liver sections of rats treated with the mushroom extract showed well-preserved architecture.

**Conclusion**

The experimental findings reveal that *G. lucidum* occurring in South India possesses antioxidant, antiinflammatory, antinociceptive, antimutagenic, anticarcinogenic, antitumour, hepatoprotective, nephroprotective and cardioprotective activities. In Oriental traditional medicine it has been considered as a panacea for several chronic diseases. A number of products prepared from it are sold throughout the world as dietary supplements. The global production of this mushroom was about 4900-5000 tonnes in 2002 of which 3800 tonnes were produced in China. At least one hundred brands of this mushroom products are sold in the market. The estimated global turnover of Ganoderma products is approximately $ 2.16 billion². Modern researches on this mushroom’s biology, biochemistry, pharmacology and therapeutics have provided a firm basis for the market of Ganoderma products. The tropical South India is gifted with a rich flora of this mushroom, hence exploitation of its therapeutic potential would be highly beneficial for health care. The product developments from this mushroom would also be commercially rewarding.

**References**


**Cheese whey substrate for cultivating Ganoderma lucidum**

A novel approach to utilize cheese whey for cultivating *Ganoderma lucidum* whey as a substrate, was introduced. Response surface analysis (RSA) with central composite in cube design was successfully applied to determine the optimal conditions where the maximum mycelial production occurred, which was at pH 4.2 and 28.3 °C. The high extract ratio as well as high content of polysaccharide (i.e., 1.2 g/l) indicated that the whey could be an alternative substrate for the mycelial production. Soluble chemical oxygen demand (SCOD) removal ranged from 80.7 to 93.1% within the design boundary. Therefore, cultivation of this mushroom mycelia using cheese whey can provide a unique solution to solve the dual problems of an alternative utilization of the whey and waste management [Hwanyoung Lee, Minkyung Song, Youngseo Yu and Seokhwan Hwang, Production of *Ganoderma lucidum* mycelium using cheese whey as an alternative substrate: response surface analysis and biokinetics, *Biochem Eng J*, 2003, 15(2), 93-99].