

Full Length Research Paper

Evaluation of antitumour action of *Ganoderma lucidum* extract in hepatocarcinoma mice

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The present study shows the protective effects of the *Ganoderma lucidum* aqueous crude extract (GLE) against mice liver cancer. The mice were randomized into four groups, namely, controls and tumor bearers that were *G. lucidum* extract-treated or untreated. Tumor bearers were inoculated intraperitoneally (ip) with 108 Yoshida AH-130 ascites hepatoma cells, whereas the controls received saline alone. The treatment began 1 day after tumor cell inoculation, and continued for 14 days. Administration of *G. lucidum* extract reduced the tumour weight of *G. lucidum*-treated groups in a dose dependent way (1.627 ± 0.135 , 1.142 ± 0.144 versus 2.163 ± 0.316) as compared to model control group. The inhibition rate was 24.84, and 47.31 by *G. lucidum* extract administration at concentrations of 200 and 400 mg/kg body weight, respectively. Results of this investigation justify continuing with further studies of *G. lucidum* extract components to develop chemoprevention strategies as an option in the treatment of cancer.

Key words: Liver cancer, mice, *Ganoderma lucidum*, interleukin-2, antitumour.

INTRODUCTION

Ganoderma lucidum (Fr.) Karst. (Polyporaceae) is a medicinal mushroom known to the Chinese as 'G. lucidum'. Its fruiting bodies have been used for their medicinal properties in traditional Chinese medicine for over 2000 years. The oriental fungus, *G. lucidum* (Leyss.ex Fr.) Karst. (*G. lucidum*), has been widely used as a remedy to promote health and longevity in China and other Asian countries (Shiao et al., 1994; Lin, 2001). *G. lucidum* has long been known to present a wide spectrum of biological effects including prevention of chronic diseases (Kwok et al., 2005; Tang et al., 2005; Chen et al., 2006; He et al., 2006; Li et al., 2007), immunoregulatory and antitumor activities (Hong et al., 2004; Cao and Lin, 2006; Tang et al., 2006), and sedative effects (Chu et al., 2007). Increasing lines of evidence have shown that *G. lucidum* has immunoregulatory (Hong et al., 2004; Chen et al., 2006) and anti-tumor activities (Sliva et al, 2002; Jiang et al., 2004a, b; Sliva, 2006). The *G. lucidum* extract can promote the healing of acetic acid-

induced ulcers in rats (Gao et al., 2004). Also, it can reduce plasma cholesterol levels (Berger et al., 2004). While *G. lucidum* has long been known to exhibit a wide spectrum of biological effects, its effect on the nervous system has not been sufficiently explored and even less is known about its effects in aging related neurodegenerative diseases. Primary liver cancer (or hepatocellular carcinoma, HCC) is the sixth most common cancer worldwide in terms of numbers of cases of 626,000, and the third most common cause of death from cancer (598,000 deaths annually) (Parkin et al., 2005). Since over 80% of deaths are in developing countries, liver cancer has been a major public health problem in these parts of the world. China is the area of the world most affected by liver cancer, with an age-standardized incidence rate of 37.9 per 100,000 for men, and of 14.2 per 100,000 for women (Parkin et al, 2005). Although, liver cancer is a relatively uncommon malignancy, owing to a very high case-fatality, it is the third most lethal malignancy worldwide. With treatments being largely ineffective, identification of environmental risk factors is the key for successful primary prevention. A recent, comprehensive systematic review of prospective cohort studies reported an almost doubling of liver cancer

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Table 1. Effect of *G. lucidum* extract on tumor weight.

Group	Tumour weight	Inhibition rate (%)
NC	-	-
MC	2.163 ± 0.316	-
GLAE I	1.627 ± 0.135	24.84
GLAE II	1.142 ± 0.144	47.31

GLAE: *G. lucidum* aqueous extract; NC: normal control; MC: model control.

risk in obese relative to normal weight persons (Larsson and Wolk, 2007).

In this study, we investigated the antitumour effects of water soluble extract isolated from *G. lucidum* on liver cancer rats model, and to explore the possible underlying mechanisms.

MATERIALS AND METHODS

Preparation of *G. lucidum* extract

Fresh *G. lucidum* were first air dried without exposure to sunlight, kept in large plastic bags and stored in a cool and dry place. The dry material (300 g) was then added to 1.5 L of tap water at boiling point (100°C) and allowed to stand for 50 min. The mixture was cooled to room temperature (25°C), and filtered (0.45 mm nylon filter). This extract was prepared freshly every day.

Liver cancer model

The mice were randomized into four groups, namely, controls and tumor bearers that were *G. lucidum* extract-treated or untreated. Tumor bearers were inoculated intraperitoneally (ip) with 10⁸ Yoshida AH-130 ascites hepatoma cells, whereas the controls received saline alone.

The treatment began 1 day after tumor cell inoculation, and continued for 14 days. The *G. lucidum* extract was dissolved daily in distilled water and administered by gavage in doses of 200 or 400 mg/kg (n = 10). Animals of the control group (n = 10) received a similar volume of distilled water. After 14 days of treatment, all animals were anesthetized with ketamine (Dopalen®, Vetbrands, Paulinia), in a dose of 60 mg/kg, and xylazine (Anasedan, Vetbrands, Paulinia), in a dose of 7.5 mg/kg; and blood samples from the inferior cava vein were obtained for biochemical assays. Both controls and tumor hosts, either treated or untreated, were sacrificed by cervical dislocation under light ether anesthesia 7 days after tumor transplantation. The tumors were harvested from the peritoneal cavity, and volume and cellularity were evaluated. The project was approved by the Ethics committee in research.

Measurement of spleen index, thymus index and IL-2 level

The following formulae can be used for the calculation of spleen index, thymus index and IL-2:

Spleen index = spleen weight/body weight; Thymus index = thymus weight/body weight; and IL-2 level was measured by using commercially available kits.

Vascular endothelial growth factors (VEGFs)

All steps were carried out at room temperature in a humidified chamber. After deparaffinization and hydration, endogenous peroxidase was blocked with 1% hydrogen peroxide for 5 min. After blocking with 1% goat serum in phosphate buffered saline (PBS) for 30 min, the sections were incubated with commercially available monoclonal mouse anti-VEGF antibodies (SantaCruz Biotechnologies, Inc., USA) for 2 h. For negative controls, the primary antibody was omitted. The sections were rinsed with PBS and were subsequently reacted with biotinylated goat anti-mouse IgG for 30 min. They were incubated in 3, 3'-diaminobenzidine tetrachloride solution (DAB). Finally, the sections were counterstained with haematoxylin and mounted.

Statistical analysis

Data are expressed as the means ± standard deviation (SD). The significance of differences from the saline control was determined by Student t test. Differences were considered significant at p < 0.05.

RESULTS AND DISCUSSION

The water-soluble extract of *G. lucidum* (Reishi or Ling-Zhi) has been used in traditional Chinese medicine (TCM) as anti-tumor and immuno-modulating agent. It also exhibits liver protective, hypoglycemic and platelet aggregation-inhibiting activities (Lien, 1990). The active constituents responsible for each of these activities have been qualitatively described, but the molecular basis of their action has not been elucidated. Its immuno-modulating and anti-tumor activities are of particular significance among these functions is (Sone et al., 1985; Wang et al., 1997).

Administration of *G. lucidum* extract reduced the tumour weight of *G. lucidum*-treated groups in a dose dependent way (1.627 ± 0.135, 1.142 ± 0.144 versus 2.163 ± 0.316) as compared to model control group. The inhibition rate was 24.84, and 47.31 by *G. lucidum* extract administration at concentrations of 200 and 400 mg/kg body weight, respectively (Table 1).

The spleen is the largest organ in the reticuloendothelial system. It has been a standard practice, for many years, to use splenic size as an indicator of disease activity in a variety of disorders of the reticuloendothelial system. The thymus plays an important role in the development of the immune system, being the primary site of T cell maturation. The organ is most active between the late stages of gestation and early puberty. With the onset of puberty, the organ atrophies, gradually shrinks in size and function (Sutherland, 2005). In various stressful conditions, the thymus is subjected to incidental involution, mostly due to the thymocytolytic effect of secreted glucocorticosteroids. Lymphocyte depletion in the cortex, due to apoptosis and phagocytosis by macrophages, results in mass reduction and thymus shrinkage. This progressive but reversible process takes place within few days of stress

Table 2. Effect of *G. lucidum* extract on spleen index, thymus index and white blood cells count.

Group	Spleen index	Thymus index	White blood cells ($10^9/L$)
NC	5.826 ± 0.177	1.986 ± 0.207	7.18 ± 1.06
MC	6.029 ± 0.163	1.287 ± 0.173	6.97 ± 1.21
GLAE I	7.972 ± 0.197	3.161 ± 0.231	8.94 ± 1.09
GLAE II	8.893 ± 0.154	4.288 ± 0.184	9.55 ± 1.19

GLAE, *G. lucidum* aqueous extract; NC, normal control; MC, model control.

Table 3. Effect of *G. lucidum* extract on serum IL-2 and VEGF levels.

Group	IL-2 (pg/ml)	VEGF (pg/ml)
NC	0.17 ± 0.01	27.83 ± 1.72
MC	0.31 ± 0.02	49.28 ± 2.81
GLAE I	0.45 ± 0.03	39.16 ± 1.57
GLAE II	0.51 ± 0.03	33.05 ± 1.43

GLAE: *G. lucidum* aqueous extract; NC, normal control; MC, model control.

(Glavina-Durdov et al., 2003). White blood cells, or leukocytes (also spelled "leucocytes"; from the Greek word leuko- meaning "white"), are cells of the immune system involved in defending the body against both infectious disease and foreign materials. Five (LaFleur-Brooks, 2008) different and diverse types of leukocytes exist, but they are all produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. They live for about 3 to 4 days in the average human body. Leukocytes are found throughout the body, including the blood and lymphatic system (Minors, 2004). An increase in the number of leukocytes over the upper limits is called leukocytosis, and a decrease below the lower limit is called leukopenia. The physical properties of leukocytes, such as volume, conductivity, and granularity, may change due to activation, the presence of immature cells, or the presence of malignant leukocytes in leukemia.

Table 2 shows the values of spleen index, thymus index and white blood cells count for all experiment groups after 2 weeks of treatment. The spleen index and white blood cells count in model group did not significantly differ (6.029 ± 0.163 versus 5.826 ± 0.177; 6.97 ± 1.21 versus 7.18 ± 1.06), whereas thymus index was significantly decreased as compared to normal control group. It was found that *G. lucidum* extract administration significantly elevated the spleen index, thymus index and white blood cells count in *G. lucidum*-treated group as compared to model control group. This indicated that *G. lucidum* extract may improve immunity function in liver cancer model mice.

Interleukin-2 (IL-2) is a cytokine produced endogenously by activated T cells and is commercially

available as aldesleukin (Proleukin), a human recombinant product (El mir et al., 2001). IL-2 is effective in the treatment of a variety of malignancies, including renal cell carcinoma and melanoma, because it has both immune-modulating and antitumor properties (Casana et al., 2002). A variety of IL-2 doses and schedules have been studied; however, high dose IL-2 administered as a single agent has proven to be one of the most effective regimens for metastatic renal cell carcinoma and melanoma to date. Vascular endothelial growth factors (VEGFs) are crucial regulators of vascular development during embryogenesis (vasculogenesis) as well as blood-vessel formation (angiogenesis) in the adult. In mammals, five VEGF ligands, which occur in several different splice variants and processed forms, have been identified so far (Morales-Gutiérrez et al., 2011; Small et al., 2008; Mahjoub et al., 2012; Wang et al., 2012; Sher et al., 2012). The ability of tumours to induce new blood-vessel formation has been a major focus of cancer research over the past few decades, and vascular endothelial growth factor (VEGF) is now known to be central to this process. The quest for VEGF and other factors that promote tumour angiogenesis was initiated many decades ago, and a long and complicated path has led to the development of inhibitors of these molecules as anticancer agents. How did this field begin, and how have we arrived at our present understanding of the role of VEGF in tumour progression (Raskopf et al., 2008).

Levels of serum IL-2 and VEGF in model control group was significantly different from rats in normal control group (0.31 ± 0.02 versus 0.17 ± 0.01; 49.28 ± 2.81 versus 27.83 ± 1.72, $p < 0.01$). Treatment with 200 and 400 mg/kg of *G. lucidum* extract in *G. lucidum* extract-treated rats significantly enhanced the level of serum IL-2, and decreased serum VEGF level, as compared to the untreated model control rat. This indicated that *G. lucidum* extract may play its antitumour activity partly by enhancing serum IL-2 level and decreasing VEGF level (Table 3).

Conclusion

G. lucidum extract can significantly inhibit tumour growth in liver cancer model mice. In addition, *G. lucidum* extract can significantly enhance spleen index, thymus index,

white blood cells count and IL-2 level, and decrease VEGF level in liver cancer model mice. These results indicate that *G. lucidum* extract plays its antitumor activities by regulating spleen, thymus index, white blood cells count, IL-2 level, and VEGF level.

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