Studies on the Medicinal Properties of Local Ganoderma sp.

Tong Chow Chin

Faculty of Science and Environmental Studies
Universiti Putra Malaysia
43400 UPM, Serdang, Selangor
Malaysia

Telephone Number of Corresponding Author: 03- 89466716
E-mail of Corresponding Authorcctong@fsas.upm.edu.my

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Introduction

Ganoderma lucidum, a mushroom of biomedical importance, contains a number of bioactive components biological response modifiers, which activate our immune systems for a multitude of defensive functions. These identified compounds (polysaccharides and triterpenes) of known molecular structures account for a range of reported beneficial biomedical effects, most notably in the prevention of physiological disorders and diseases.

Bioactive mycelial polysaccharides differ from the ones isolated from the fruiting body in *Ganoderma* species (Kim *et al.*, 1993; Mizuno, 1992; Sohn, 1995). These two growth stages produce some similar types but different forms of bioactive components derived form different stages of primary (polyglycans) or secondary (triterpenes) fungal metabolites.

In Malaysia, There is an appalling paucity of information in Malaysia on the efficacy of our own *Ganoderma* products. Thus, it is only obvious that there is notable discrepancy with the public's frequent impression that *Ganoderma* is a wonderful health supplement which may be taken as a cure for so many life-threatening illnesses and the lack of experimental and clinical trials be conducted locally in order to evaluate and ascertain the quality of our local products. Tong *et al.* (1994a; 1994b) recommended the use of *Ganoderma* mycelium in addition to the fruiting bodies for the manufacture of the *Ganoderma* products. "Ganocelium" with the product number of FM0002, is the recent product in Malaysia using mycelium of *G. lucidum* as its source.

Hence, this work attempts to investigate the efficacy of the water soluble extract from *G. lucidium* fruiting body and mycelium grown in soy waste on (a) the levels of plasma lipids, lipoproteins and related enzymes in rats. (b) cholesterol induced cardio-atherosclerosis, hepatic and renal functions in rats, (c) the cytotoxic activity on mouse myeloma, leukaemia and human breast cancer cell-line, (d) changes of lipid peroxidation and antioxidant enzymes in rats fed with a cholesterolemic diet and (e) skin and lung tumor induced by Bap in rats.

Materials and Methods

Source of microorganism: *Ganoderma lucidum* strain 021.

Cultivation techniques: Fungal mycelium either grown in solid soy waste as substrate for 2½ months in sterile plastic bags

at 28°C or soy bean extract in medical flat bottles at 25°C.

Experimental Animals : Twenty-four Male Sprague-Dawley rats weighing 300-350 g (six months old) were divided into

four groups comprising of 6 rats per group. The formula diet for each group was planned (not shown). At regular interval,

blood samples were collected via transecting the tail or intra-cardiac puncture and analyzed for various enzymatic and

vitamin parameters. Histology of the liver and kidney were also examined.

Cell cultures : Balb/C mouse myeloma, J558 (ATCC); Human breast ductal carcinoma, MDA-MB-435 (ATCC); Leukemia

cell-line, PN6/CEM-SS (National Cancer Institute, USA); and Mouse fibroblast normal cell-line, 3T3 (RIKEN Cell Bank,

Japan).

Enzymatic analysis : Serum cholesterol and related enzymes levels were quantified using an Cobas Intrega Multipurpose

Auto-analyser machine.

Fruiting body and mycelial extraction : Hot water and methanol extraction.

Determination of cellular growth: Microculture Tetrazolium Assay (MTT), Scanning Electron Microscopy (SEM),

Transmission Electron Microscopy (TEM), Acridine Orange and Propidium Iodide Staining, DNA Fragmentation Assay.

Results and Discussion

The *in vivo* and *in vitro* effect of a locally grown *G. lucidum* fruiting body powder (GF) and *G. lucidum* mycelium (GM) grown in soy waste on tumor and hyper-cholesterolaemic rats were studied.

Administration of 1% cholesterol diet in the cholesterol (Chol) group caused a significant ($p<0.05$) increase in the serum total cholesterol (TC), triglyceride (TG) and low density lipoprotein cholesterol (LDL-C) levels while reducing serum high density lipoprotein cholesterol (HDL-C) level. In the case of the Chol+GF and Chol+GM groups, the initial serum TC, TG and LDL-C levels showed a much higher levels ($p<0.05$) compared to the Chol group. However, the levels gradually decreased towards the end of the experiment. There was no significant difference in the lipid profiles amongst the Control, GF and GM groups. Serum alanine transferase (ALT), gamma glutamyltransferase (GGT) and creatine kinase (CK) in the Chol+GF group as well as the ALT, GGT level in the Chol+GM group were found to be significantly ($p<0.05$) lower than those in the Chol group for both the experiments using GF and GM. Despite the fact that all groups showed levels of serum urea and creatinine within the normal range, mild degenerative changes in the glomeruli were seen in the Chol group. In addition, higher level of serum uric acid was observed in the Chol group compared to the Chol+GF and Chol+GM groups. In term of the lipid peroxidation and antioxidant enzymes analyses, the Chol+GF and Chol+GM groups showed a significantly ($p<0.05$) lower level of malondialdehyde (MDA), catalase and glutathione peroxidase (GSH-Px) activities but higher vitamin C level compared to the Chol group.

The anti-carcinogenesis test indicated that hypercholesterolaemic rats (the Chol group) demonstrated a significantly ($p<0.05$) higher serum MDA level as compared to the GF and Chol+GF groups. Furthermore, *Ganoderma* supplemented groups had significantly ($p<0.05$) lower levels of serum MDA, catalase, GSH-Px and GGT activities but higher in the ascorbic acid level as compared to the Chol group. Histological, the Chol+GF group showed a much reduced thickened coronary vessel wall as well as normal hair growth in the anti-carcinogenesis test. The Chol+GM group registered 61.9% normal hepatocytes as compared to only 5.6% in the Chol group. The presence of epithelization of lung epithelium in the Chol group which were not severe in the Chol+GM group indicated the anti-tumor effect of 10% GM.

The cytotoxicity effect of the various *Ganoderma* crude extracts on MDA-MB-435 cell lines was determined by measuring the cytotoxic dose that killed 50% of the cell population in 72 hours as compared to the untreated control. The mycelial results obtained from *G. lucidum* was the most effective towards MDA-MB-435 with an IC_{50} value of 148 ± 9.07 µg/mL.

The percentage viability of MDA-MB-435 cells exposed to *Ganoderma* crude extracts was dose-dependent and there was significant reduction when the dose was increased to 400µg/ml. Under the phase contrast microscopy, a mixture of cells in necrosis and apoptosis was apparent. Apoptotic bodies and cells with condensed nuclei were conspicuous as representative of typical morphology of apoptotic death mode. Necrotic cells were seen as rupture cells with an irregular shape due to the loss of membrane integrity. In this case, these cells were detached from basement and remained floating in the medium. The fluorescence microscopic examination of MDA-MB-435 cells treated with commercial *G. lucidum* powder crude extract for 24, 48 and 72 hours revealed that the percentage of apoptotic cells and necrotic cell increased significantly within 72 hours of treatment, whereas the viable cells decreased by as much as 55%. It is apparent that the mode of cell death via apoptosis is 18.4% more than necrotic. Both the scanning electron micrograph and the transmission electron micrographs further confirmed the *Ganoderma* powder crude extract induced MDA-MB-435 cell death by apoptosis and necrosis.

When the cells were treated at IC_{50} with commercial *G. lucidum* crude extract for 72 hours, internucleosomal DNA cleavage was obvious. Thus, the biochemical hallmark observed in *G. lucidum* treated MDA-MB-435 cells as demonstrated by internucleosomal DNA cleavage confirmed the apoptotic mode of cell death.

Conclusions

The effectiveness of GF and GM in inhibiting cholesterol deposition resulting in plaque and cholesterol synthesis was indicated. The results also showed a redistribution of lipoprotein cholesterol leading to the formation of pronounced anti-atherogenic lipoprotein profile and demonstration of antioxidant properties. *G. lucidum* crude extract exhibited the highest cytotoxic activity (lower IC_{50}) towards J558 Balb/C mouse myeloma, MDA-MB-435 human breast ductal carcinoma, PN6 leukemia T-cell but not against 3T3 mouse fibroblast normal cell-line as compared to crude extract from *G. tsugae* and *G. tropicum*. Among these cancer cell-lines, J558 cells were most sensitive to the cytotoxic effects of all the three *Ganoderma* spp. Furthermore, the suppressive effect of *G. lucidum* crude extract on LOOH formation would partly contribute to the anti-tumor promoting activity of *G. lucidum* crude extract in rat skin. This pilot study suggests the potential use of commercial *G. lucidum* powder and *G. lucidum* mycelium in the prevention of diseases related to hypercholesterolaemia and lipid peroxidation.

Benefits from the study

Malaysia currently imports millions of Ringgit worth of health products processed from *Ganoderma* annually. It is imperative that experimental and clinical trials be conducted locally in order to evaluate and ascertain the quality of our own local *Ganoderma* products. Research findings of this nature will provide sound scientific backing to the establishment of local industries in the manufacture of high quality *Ganoderma* health products well supported by scientific evaluation data . Through direct linkages and providing consultation services, the research findings will be channeled to local potential producers/industries with the hope of establishing collaborative venture with UPM. New information networks between local and overseas scientists laid the foundation for infrastructural development through active participation in international conferences/seminars. This project has trained postgraduates capable of contributing further advancement to this area of research. A total of 4 journal papers intended for international publication are in preparation

Patent(s), if applicable:

Nil

Stage of Commercialization, if applicable:

Nil

Project Publications in Refereed Journals:

Nil

Project Publications in Conference Proceedings

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Graduate Research

Name of Graduate	Research Topic	Field of Expertise	Degree Awarded	Graduation Year
Law Buon Jong	Production and Properties of Polysaccharides from Mycelia of Three *Ganoderma* sp.	Mushroom Biotechnology	M.Sc	2000
Choong Yew Keong	In Vivo and In Vitro Studies of Anti-Cholesterol and Anti-Carcinogenic Effects of *Ganoderma* Crude Extracts	Mushroom Biotechnology	Ph.D	2003

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