

In Vitro and in vivo Studies of *Ganoderma lucidum* in Cancer

Khor Goot Heah^{1,2,3*}, Syairah Nabila Bt Suhaimi⁴, Nur Rawaidah Bt Mohd Shobri⁴,
Hong-Jian Zhu³, GRA Froemming⁵

1. Centre of Preclinical Science Studies, Faculty of Dentistry, Universiti Teknologi MARA, Sungai Buloh Campus, Selangor, Malaysia.
2. Oral and Maxillofacial Cancer Research Group, Universiti Teknologi MARA, Selangor, Malaysia.
3. Department of Surgery, University of Melbourne, Royal Melbourne Hospital, Parkville, Victoria, Australia.
4. Faculty of Dentistry, Universiti Teknologi MARA Sungai Buloh Campus, Selangor, Malaysia.
5. Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, Sarawak, Malaysia.

Abstract

Current cancer therapy such as chemotherapy and radiography are known to possess many side effects that could lead to serious complications. The application of natural product as complementary and alternative treatment provides significant advantages. It could increase the sensitivity of chemotherapy and radiography while at the same time reducing their associated side effects and complications. With regards to anticancer drugs, more than 50% of the modern oncological drugs are derived from natural products. An edible mushroom, *Ganoderma lucidum* has long been used in traditional and conventional medicine in China, for the prevention and treatment of various human diseases.

The fungus possesses a wide variety of bioactive compounds present in its fruiting bodies, mycelium and spores, including anticancer property that has been proven in in vitro and in vivo studies. The carcinostatic effects of *G. lucidum* have been shown in a variety of cancer cell lines, including breast, pancreas, lung, colon, skin and prostate. This paper presents in vitro and in vivo studies of the anticancer properties of *G. lucidum* in cancers.

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Introduction

From thousands of years ago, medicinal herbs have long been used as a natural remedy by the ancient people. Even in this 21st century, medicinal plants still continue to play a central role in the healthcare system of the world's population¹. Currently, western medicine has started accepting natural products from Traditional Chinese Medicine. There is also an increasing popularity of herbal therapies for the treatment of cancer in the United States.² Most of the commercially available mushrooms contain free radical scavenging, reducing power, chelating effects on metal ions, and antioxidant properties.³ Among these mushrooms, *G. lucidum* has long been used in traditional and

conventional Chinese medicine for the prevention and treatment of various human diseases.^{4, 5} Contemporary research indicates that *G. lucidum* and some other species of the *Ganoderma* genus contain bioactive constituents, including polysaccharides like beta-glucans and triterpenes like ganderic acids.⁶ Hence, scientific justification based on the elucidation of mechanisms responsible for the biological effects of these natural products could help validate their use in alternative or adjuvant cancer therapies.⁷

The primary reason for using this type of plant-based medicine is because it could reduce the side effects and complications associated with chemotherapy and radiotherapy. Currently, the major standard cancer therapies widely used are still surgery, chemotherapy, and radiotherapy.⁸ Although chemotherapy and radiotherapy are effective against cancer, they also possessed serious side effects and complications such as fatigue, pain, diarrhea, nausea, vomiting, and hair loss.⁹ The clinical use of the chemotherapy drug is limited due to cellular resistance and dose-dependent toxicity in normal tissue.¹⁰ Hence, the use of natural

*Corresponding author:

Khor Goot Heah
Centre of Preclinical Science Studies,
Faculty of Dentistry, Universiti Teknologi MARA,
Sungai Buloh Campus, Selangor, Malaysia.
E-mail: gootheah@uitm.edu.my; gootheah@gmail.com

products as complementary and alternative medicine could be applied to treat cancer, as it has significant advantages in terms of increasing the sensitivity of chemotherapy and radiotherapy, as well as reducing their associated side effects and complications.¹¹

***Ganoderma lucidum* and Its Major Bioactive Compound**

Ganoderma lucidum (*G. lucidum*) belongs to the family of Ganodermataceae (Basidiomycetes), which is a type of an edible mushroom used in complementary and alternative medicine particularly in Asian countries.¹² This mushroom is used traditionally for treating many kinds of diseases, including cancer.¹³ *Ganoderma lucidum* exerts a wide variety of pharmacological properties, such as antioxidant, antitumor, anti-inflammatory, antinociceptive, hypertension, bronchitis, arthritis, chronic hepatitis, gastric ulcer, hypercholesterolemia, immunological disorders, and scleroderma.¹⁴

There are over 300 bioactive compounds from the fruiting body, spores and mycelia of *G. lucidum* that have been isolated or detected.¹⁵ The main known bioactive molecules from *G. lucidum* are triterpenoids, polysaccharides, nucleotides, fatty acids, glycoproteins, sterols, steroids, proteins or peptides and trace elements.¹⁶ Among these bioactive molecules, the two main groups which have been identified to exhibit anti-cancer effects are triterpenoids and polysaccharides.¹⁷

In general, the basic chemical structure of triterpenoids in *G. lucidum* is that of an oxygenated lanostane.¹⁸ Lanostane-type triterpenoids in *G. lucidum* can be divided into about ten groups depending on structural similarities and functional groups.¹⁵ These includes ganoderic acid (GA), ganosporeric acid, methyl ganoderate, ganosporelactone, ganoderenic acid, ganolucidic acid, methyl ganolucidate, ganoderiol, ganoderal, epoxyganoderiol, methyl lucidenate, lucidone, ganolactone, lucidumol, lucialde- hyde and so on.¹⁵ At least 50 triterpenoids are unique to *G. lucidum*. The first triterpenoids isolated from *G. lucidum* are GA-A and GA-B in 1982.¹⁹ Structure-activity relationship assays of 43 triterpenoids separated from *G. lucidum* revealed that the type of side-chain, C-3 carbonyl group, double bonds (D7,8, D9,11), and number of hydroxyl groups

are important in the cytotoxicity of *G. lucidum* triterpenoids.¹⁵

The major component of polysaccharides in *G. lucidum* is glucose, where it was indicated from the structural analyses of *G. lucidum* polysaccharides (GL-PS).²⁰ However, GL-PS can also contain xylose, mannose, galactose, and fucose in different conformations as it is heteropolymers.²¹ Besides that, the anti-tumorigenic properties of these polysaccharides are affected by branching conformation and solubility characteristics.²²

The function of polysaccharides of *G. lucidum* mainly acts as immunomodulators or antioxidants in vivo to resist cancer, while triterpenoids, mainly function to inhibit cancer cell proliferation and metastasis.^{23,24} The proportions of triterpenoids and polysaccharides in *G. lucidum* are not fixed in *G. lucidum*.²⁵ This may be due to variations in species, cultivation, growing area and extraction method of *G. lucidum*.¹⁵ Even in the same mushroom, the composition of triterpenoids and polysaccharides varies greatly from the fruiting body, spore and mycelia of *G. lucidum*.¹⁵

***Ganoderma lucidum* Treatment in Cancer**

Progression of cancer involves a series of multiple processes of abnormal genetic and epigenetic events which leads to malignant transformation.^{26,27} *G. lucidum* demonstrates an anti-cancer activity in an in vivo and in vitro studies.²⁸ Various types of solvent have been used to extract the bioactive compound of *G. lucidum* and these extracts have shown to have carcinostatic effects in a wide variety of cancer cell lines, including breast, pancreas, lung, colon, skin, and prostate.²⁸

G. lucidum also contributes to anticancer activity by inhibiting the signalling pathways involved with cell adhesion, proliferation, survival, invasion, and degradation of the extracellular matrix.¹³ Several studies had conducted on the effects of *G. lucidum* towards cancer cell are tabulated in Table 1.

A previous study done by Dai et al. (2014) reported that both CX43 and VEGF play an important role in the genesis and development of ovarian cancer.²⁹ It was demonstrated that *G. lucidum* inhibits ovarian cancer by down-regulating the expression of VEGF and up-regulating the downstream Cx43 expression.²⁹

using the human ovarian cancer cell line (HO 8910).

No	Author	G. lucidum sample (Region of origin)	Type of cancer/ cell line/ animal model	In vivo / In vitro Study
1	29	<i>G. lucidum</i> (Reishimax): 6% triterpenes and 13.5% polysaccharides (China - cultivated)	1) Human ovarian cancer cells HO 8910 (HOCC)	In vitro
2	34	fruiting bodies: polysaccharide (India - wild)	1) DMBA-induced mammary carcinogenesis in Sprague Dawley rats	In vivo
3	31	1. fruiting bodies 2. fruiting bodies: Polysaccharides (Slovenia - cultivated)	1) Human umbilical vein endothelial (HUVEC) 2) Human colon adenocarcinoma (CaCo-2)	In vitro
4	32	whole <i>G. lucidum</i> (Himalayan region - cultivated)	1) Prostate cancer cell line (PC3 and DU145)	In vitro
5	30	Spore: triterpenoids (China - cultivated)	1) Human cholangiocarcinoma cells (TFK-1)	In vitro
6	33	6% triterpenes and 13.5% polysaccharides	1) Human breast cancer cells (MDA-MB-231)	orthotopic xenograft model
7	37	Ganoderic acid DM (triterpenoid)	1) Estrogen receptor-positive human breast cancer MCF-7 cells 2) Estrogen receptor-negative MDA-MB-231 cells	In vitro
8	38	Ganoderic acid T	1) Lung cancer cell line (95-D) 2) Liver cancer cell line, (HepG2) 3) Cervical cancer cell line (HeLa)	In vitro
9	39	Total triterpenes	1) Human breast adenocarcinoma (MCF-7) 2) Dimethylbenz [a] anthracene (DMBA) induced skin papilloma 3) Mammary adenocarcinoma in Swiss albino mice and Wistar rats	In vitro and in vivo
10	40	Ganoderic acid A	1) Lung cancer cells (H460)	In vitro

Table 1. Summary of the in vitro and in vivo studies using *G. lucidum* in anti-cancer treatment.

In human bile duct cancer, *G. lucidum* supercritical carbon dioxide extract was reported to suppress cholangiocarcinoma migration in human cholangiocarcinoma cell line (TFK-1) through the inhibition of TGF- β 1 induced epithelial-mesenchymal transition (EMT).³⁰ Besides that, Gregori and Pohleven (2014) reported that *G. lucidum* supercritical carbon dioxide extract shows a targeted cytotoxic activity solely towards the human colon adenocarcinoma cell line (CaCo-2) and not the human umbilical vein endothelial cell line (HUVEC).³¹

Kao et al. (2016) have done a study using gene and pathway analyses associated with anticancer activities of *Ganoderma lucidum* Whisky Extract (GWh) in two types of prostate

cancer cell lines which are PC3 and DU145.³² The study suggested that several biologically active pathways, including the apoptotic pathway were identified to be associated with GWh anticancer activities.³² Thus, this shows the potential anticancer effects of *G. lucidum*, including the inhibition of cell cycle, induction of apoptosis, and the reduction of tumour progression.³²

Loganathan et al. (2015) reported that *G. lucidum* extract suppressed the expression of genes involved in the invasive behaviour of human breast cancer cells (MDA-MB-231) cell line.³³ In addition, it was also reported that *G. lucidum* extracts inhibit breast-to-lung cancer metastasis of the highly invasive human breast cancer cells implanted in mouse mammary tissue.³³

An in vivo study done by Deepalakshmi et al. (2013) using Sprague Dawley rats reported that *G. lucidum* ethanolic extract exhibited potent anti-oxidant effects and could enhance enzymatic antioxidants like superoxide dismutase, catalase, and glutathione peroxidase in rat mammary carcinogenesis.³⁴

A preliminary study was done by Syairah et al. (2017) to determine the effects of *G. lucidum* extract towards oral cancer cell (ORL-48T). The study reported that *G. lucidum* extract decreased cell viability significantly in a concentration related manner, which reflects the cytotoxic effect of *G. lucidum* towards ORL-48T.

Hence, there are many mechanisms by which the *G. lucidum* extracts exhibit anti-cancer activities, including the direct inhibition of cell viability, inhibition of cell proliferation through cancer-specific cell cycle arrest and apoptosis.^{13,35} Besides, several studies have shown that the treatment of cancer cells with *G. lucidum* extracts can lead to down-regulation of cell cycle-associated proteins resulting in cell cycle arrest in cancer cells.³⁶

Conclusions

In conclusion, the application of an alternative treatment in cancer treatment using natural products is crucial as it could increase the sensitivity of chemotherapy and radiotherapy, as well as reduce their associated side effects and complications. The use of traditional Chinese medicinal mushroom, *G. lucidum* as an alternative cancer treatment could be employed.

This is because *G. lucidum* has been shown to have anti-cancer activity in the in vivo studies and cytotoxic effects in the in vitro studies, including breast, prostate, ovary, colon, and liver cancers. However, a thorough research regarding the effects of *G. lucidum* on human cancer is essential before the development of *G. lucidum* as an alternative medicine could be established.

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Declaration of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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