Anti-proliferative Effects of *Ganoderma lucidum* in Cell Culture on Vero Cells

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***Abstract*---**There are studies showing anticancer effects of Ganoderma extract in herbal medicine literature. The aim of this study was to determine the effects of *Ganoderma lucidum* on vero cancer cell line viability in cell culture**.** In this laboratory experimental study, we used MTT assay to determine cell viability following administration of different doses of *Ganoderma lucidum* in vero cell culture. The data were statically analyzed using ANOVA. The results showed that the administration of 10 µg/ml and 100 µg/ml of *Ganoderma lucidum* resulted in decreased viability of vero cells ($P<0.05$), however, 1 mg/ml of *Ganoderma lucidum* extract resulted in increased viability of vero cells ($P<0.05$). Our findings indicate that viability of vero cells in cell culture is influenced by administration of *Ganoderma lucidum* and based on the dose of extract the viability may be reduced or increased**.**

***Keywords*---** Ganoderma lucidum, Vero Cell Line, Viability.

I. Introduction

GANODERMA lucidum (figure I) has been used in traditional Chinese medicine for more than 4,000 years to treat liver disorders, high blood pressure, arthritis, and other ailments. Other promising uses for which there is still inconclusive evidence include diabetes, heart disease and pain. *Ganoderma lucidum* is also believed to reduce cholesterol levels and has an anticoagulant effect, which may make it useful in coronary heart disease prevention [1], [2].

Some experts believe that *Ganoderma lucidum* promotes longevity and maintains vitality of the human body. *Ganoderma lucidum* major benefit appears to be its immunomodulating action [3], improvement of liver function, and improvement and restoration of the normal functions of the respiratory system. Antioxidant effects, which contribute to the overall well-being of patients, have been proposed. It is believed that *Ganoderma lucidum* increases intellectual capacity, and banish forgetfulness [1] –[3].

Mushrooms such as *Ganoderma lucidum* are considered one of the richest sources of natural antibiotics, and various species of them inhibit the growth of a wide diversity of microorganisms. *Ganoderma lucidum*, a well-known mushroom has many pharmacological and biological activities including an antimicrobial effect, although few studies have investigated the antibacterial and antifungal effects of its purified compounds [3]. *Ganoderma lucidum* is one of the most extensively studied mushrooms as a functional food and as a chemopreventive agent due to its recognized medicinal properties. Some *G. lucidum* extracts have shown promising antitumor potential [4]. The results of studies provide evidence that *Ganoderma lucidum* suppresses protein synthesis and tumor growth by affecting survival and proliferative signaling pathways that act on translation, suggesting that *Ganoderma lucidum* is a potential natural therapeutic for breast and other cancers [5]. *Ganoderma lucidum* polysaccharide (GLP) is a biologically active substance reported to possess anti-tumor ability. Nonetheless, the mechanisms of GLP-stimulated apoptosis are still unclear [6]. Degrading multi-enzymes from *Ganoderma lucidum* have been also characterized [7].

In modern times, the available data from human trials together with evidence from animal studies suggest that *Ganoderma lucidum* may have some positive benefits for cancer patients. However, the number and quality of trials is very limited. The main aim of this study was to determine the effects of *Ganoderma lucidum* on vero (kidney cancer) cell line viability in cell culture.

Fig. 1 *Ganoderma lucidum.*

II. Material and Methods

A. Extract preparation

Ganoderma lucidum was prepared and different concentrations of extract (10 µg/ml, 100 µg/ml and 1mg/ml) were used in our study.

B. Protocol of Study

We used MTT assay in this work to determine the effects of ganoderma extract on vero cells viability in cell culture. Briefly, the procedure was carried out in the following steps:

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DAY ONE: 100 µl of cells (15000 cells) was added into each well (96 well plate) and incubate at 37 with 5% co2 overnight.
DAY TWO: The media was removed and extract was added and incubated at 37 with 5%co2 overnight. For control 10%FBS was added to media.
DAY THREE: extract was removed from media. 20 µl of 5 mg/ml MTT was added to each well and incubated for 4 hours at 37oC. 150 µ isopropanol was added and covered with tinfoil and agitate cells on orbital shaker for 15 min. Absorbance was read at 570 nm with a reference filter of 630 nm and recorded.

C. Statistical Analysis

Statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS 19. Significance was measured using Tukey's test. Differences with $P<0.05$ were considered significant

III. Results

Figure 1 represents viability of vero cells related to different doses of ganoderma extract.

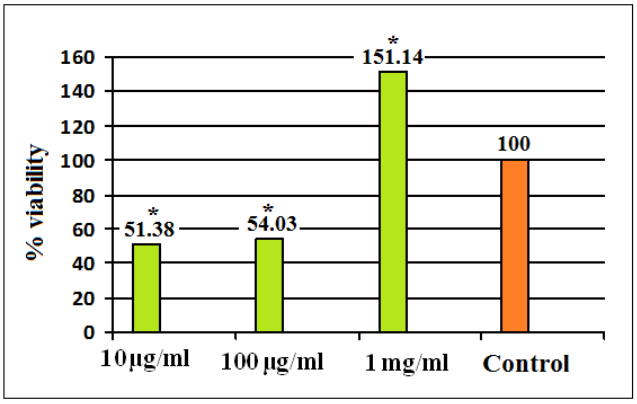

Figure1. Viability of vero cells in response to different doses of *Ganoderma lucidum.*

Our results show that administration of lower doses (10 and 100 µg/ml) of ganoderma extract have anti-proliferative effects on vero cell line, however, higher dose (1mg/ml) of ganoderma extract resulted in increased vero cell viability.

IV. Discussion

In our study, we reported the appropriate dose of *Ganoderma lucidum* extract has antiproliferative effects on kidney cancer cells in cell culture. *Ganoderma lucidum* is among the worldwide well-known medicinal basidiomycetes. A variety of commercial *G. lucidum* products are available in various forms, such as powders, dietary supplements, and tea. These are produced from different parts of the mushroom, including mycelia, spores, and fruit body. The specific applications and attributed health benefits of lingzhi include controling of blood glucose levels, modulation of the immune system, hepatoprotection, bacteriostasis, and more. The various beliefs regarding the health benefits of *G. lucidum* are based largely on anecdotal evidence, traditional use, and cultural mores. Studies in recent years have been concerned with *G. lucidum* structure and components [7]-[10]. A bioactive fraction was extracted and purified from the mycelia of *Ganoderma lucidum* indicating antiviral activities against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) [11]. It has also been shown that *Ganoderma lucidum* suppresses breast-to-cancer metastasis through the inhibition of pro-invasive genes [12]. However, it is said that *G. lucidum* is used mostly as an immune enhancer and a health supplement, not therapeutically [13]. And in contrast to our finding some authors believe that there is not sufficient evidence to justify the use of *G. lucidum* as a first-line treatment for cancer [14].

V. Conclusion

We have shown that appropriate dose of *Ganoderma lucidum* extract has anti-proliferative effects on vero cancer line cells in cell culture, indicating the potential power of *Ganoderma lucidum* extract in treatment of cancer cells.

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References

[1] Shafiee-Nick R, Parizadeh SM, Zokaei N, *et al*. Effect of Ganoderma lucidum hydroalcoholic extract on insulin release in rat-isolaeyted pancreatic islets, Avicenna J Phytomed. 2012 Fall;2(4):206-11.
[2] Bhardwaj N, Batra P, Sharma AK1. Suppression of Inflammatory and Allergic Responses by Pharmacologically Potent Fungus Ganoderma lucidum, Recent Pat Inflamm Allergy Drug Discov. 2014 Jun 19.
[3] Vazirian M1, Faramarzi MA2, Ebrahimi SE3 *et al*. Antimicrobial Effect of the Lingzhi or Reishi Medicinal Mushroom, Ganoderma lucidum (Higher Basidiomycetes) and Its Main Compounds, Int J Med Mushrooms. 2014;16(1):77-84. http://dx.doi.org/10.1615/IntJMedMushr.v16.i1.70
[4] Oliveira M1, Reis FS, Sousa D, *et al.* A methanolic extract of Ganoderma lucidum fruiting body inhibits the growth of a gastric cancer cell line and affects cellular autophagy and cell cycle, Food Funct. 2014 Jun 25;5(7):1389-94. http://dx.doi.org/10.1039/c4fo00258j
[5] Suarez-Arroyo IJ, Rosario-Acevedo R, Aguilar-Perez A, et al, Anti-tumor effects of Ganoderma lucidum (reishi) in inflammatory breast cancer in in vivo and in vitro models. PLoS One. 2013;8(2):e57431. http://dx.doi.org/10.1371/journal.pone.0057431
[6] Liang Z1, Yi Y2, Guo Y3 *et al*. Chemical characterization and antitumor activities of polysaccharide extracted from Ganoderma lucidum, Int J Mol Sci. 2014 May 22;15(5):9103-16. http://dx.doi.org/10.3390/ijms15059103
[7] Li YL1, Xin XM, Miao ZM,et al, Study on effective constituents extracted from fibrous roots of Salvia miltiorrhiza with degrading multi-enzymes from taishan Ganoderma lucidum, Zhongguo Zhong Yao Za Zhi. 2013 Mar;38(5):678-82.
[8] Wachtel-Galor S, Yuen J, Buswell JA, et al, Ganoderma lucidum (Lingzhi or Reishi): A Medicinal Mushroom. Wachtel-Galor S, Yuen J, Buswell JA, Benzie IFF. In: Benzie IFF, Wachtel-Galor S, editors. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. Boca Raton (FL): CRC Press; 2011. Chapter 9. http://dx.doi.org/10.1201/b10787
[9] Joo SS1, Ryu IW, Park JK,et al, Molecular cloning and expression of a laccase from Ganoderma lucidum, and its antioxidative properties. Mol Cells. 2008 Feb 29;25(1):112-8.
[10] Wang HX1, Ng TB, A laccase from the medicinal mushroom Ganoderma lucidum, Appl Microbiol Biotechnol. 2006 Sep;72(3):508-13. Epub 2006 Apr 25. http://dx.doi.org/10.1007/s00253-006-0314-9
[11] Liu J1, Yang F, Ye LB,et al, Possible mode of action of antiherpetic activities of a proteoglycan isolated from the mycelia of Ganoderma lucidum in vitro. J Ethnopharmacol. 2004 Dec;95(2-3):265-72. http://dx.doi.org/10.1016/j.jep.2004.07.010

[12] Loganathan J, Jiang J, Smith A, et al,The mushroom Ganoderma lucidum suppresses breast-to-lung cancer metastasis through the inhibition of pro-invasive genes. Int J Oncol. 2014 Jun;44(6):2009-15.

[13] Batra P, Sharma AK, Khajuria R. Probing Lingzhi or Reishi medicinal mushroom Ganoderma lucidum (higher Basidiomycetes): a bitter mushroom with amazing health benefits. Int J Med Mushrooms. 2013;15(2):127-43. Jin X, [14]. Ruiz Beguerie J, Sze DM, et al, Ganoderma lucidum (Reishi mushroom) for cancer treatment. Cochrane Database Syst Rev. 2012 Jun 13;6:CD00773.