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IC_{50} OF GANODERMA LUCIDUM EXTRACT ON ORAL CANCER CELLS, ORL-48T

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ABSTRACT

Oral cancer is the sixth most common cancer worldwide and Malaysia ranked 14 in the world for oral cancer incidence. Despite recent advancement in treatment modalities, the prognosis and survival rates for oral cancer patients have not improved significantly over the past decades. Ganodermalucidum has long been used in Chinese traditional and conventional medicine for prevention and treatment of various diseases. The cytotoxic effect of G. lucidum towards oral cancer cell line was determined in this study using in vitro model study. After 24 h treatment with G. lucidum, the IC_{50} of G. lucidum extract obtained was 310 ± 0.1 µg/mL. Thus, this study shows a preliminary evidence of G. lucidum cytotoxic activity towards oral cancer cell line, ORL-48T.

Keywords: oral cancer; ganodermalucidum; MTT assay; cytotoxic effect; IC_{50}.

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1. INTRODUCTION

Oral cancer is the sixth most common cancer worldwide and Malaysia is ranked as number 14 in the world with annual oral cancer deaths of 1,587 [1-2]. In 2011, the World Health Organization (WHO) reported that the rate of oral cancer deaths reached to 1.55% of the total deaths [2]. Significant numbers of oral cancer cases were reported in the Asia region including India, Sri Lanka, Pakistan and Taiwan [1]. Even with recent advancement in treatment modalities, the prognosis and survival rates of oral cancer patients have not improved notably over 50% in the past decades [3].

Ganodermalucidum (G. lucidum) has long been used in Chinese traditional and conventional medicine for prevention and treatment of various human diseases [4]. The primary reason of using this type of plant-based medicine is because there are no reported side effects that could lead to serious complication as compared to current treatment such as radiotherapy and chemotherapy. G. lucidum extract exerts a wide variety of pharmacological properties such as antioxidant, anti-tumour, anti-inflammatory, antinociceptive, anti-hypertension, anti-bronchitis, anti-arthritis, anti-chronic hepatitis, anti-gastric ulcer, anti-hypercholesterolemia, immunological disorders and scleroderma [5]. There have been reports on the cytotoxic effects of G. lucidumin vivo and in vitro studies [6]. Extracts of G. lucidum have shown to assert cytotoxic effects in various cancer cell lines, including breast, pancreas, lung, colon, skin and prostate [6].

IC_{50} is used to indicate the concentration of inhibitor that require to inhibit a given biological or biochemical function by half. A larger IC_{50} values signify that the inhibitor tested interact less effectively with the tested biological sample than inhibitor that has small IC_{50} values [7].To my knowledge, the cytotoxic effect and IC_{50} of G. lucidum towards oral cancer cells is yet to be known. Thus, in this study, the cytotoxic effect of G. lucidum towards oral cancer cell line was determined using in vitro model. MTT assay for cytotoxicity are widely used in testing the sensitivity of inhibitor towards cultured cell, as it is an economical and rapid test that does not requires the use of animal model.

2. METHODOLOGY

2.1. G. lucidum Extract

G. lucidum capsules were purchased from Shanghai Green Valley Pharmaceuticals, China. The capsule content was dissolved in dimethyl sulfoxide (DMSO) to make a stock solution of 10 mg/mL (less than 1 % DMSO). The mixture was sonicated at room temperature for 30 minutes. The suspension was then centrifuged and the supernatant was filtered to remove any remaining particles.

2.2. Cell Line and Culture Condition

The human oral squamous cell carcinoma cell line, ORL-48T was used in this study. The cell were cultured in DMEM (Dulbecco's Modified Eagle Medium) F-12 medium (Gibco, USA) supplemented with 10 % fetal bovine serum and maintained at 37 ˚C in a 5 % CO_2 humidified atmosphere.

2.3. Cell Viability Assay

Cell viability was determined using MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay (Thermofisher Scientific, USA). ORL-48T was seeded at 1.4×10^4 cells/mL on a 96-well plate. It was treated for 24 h with 10 different fifty-fold concentrations (50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 µg/mL) of the extracts in triplicate wells. Test control was applied by not adding any treatment of G. lucidum (0 µg/mL). After 24 h, 20 µL of MTT reagent was added into each well. After 4 h incubation at 37 °C in 5 % CO_2 in humidified atmosphere, 50 µL SDS-0.1 M HCL was added and the mixture was incubated overnight. The absorbance of cell viability was read using microplate reader (TECAN) at a wavelength of 540 nm. The percentage of cell viability at every concentration was calculated using the formula below.

$$\text{Percentage of cell viability} = \frac{\text{OD sample}}{\text{OD control}} \text{ x } 100\%$$

The percentage of cell viability was plotted against concentration and the IC_{50} was then determined using the graph of percentage of cell viability versus concentrations of the G. lucidum. The experiment was repeated three times (n=9).

3. RESULTS AND DISCUSSION

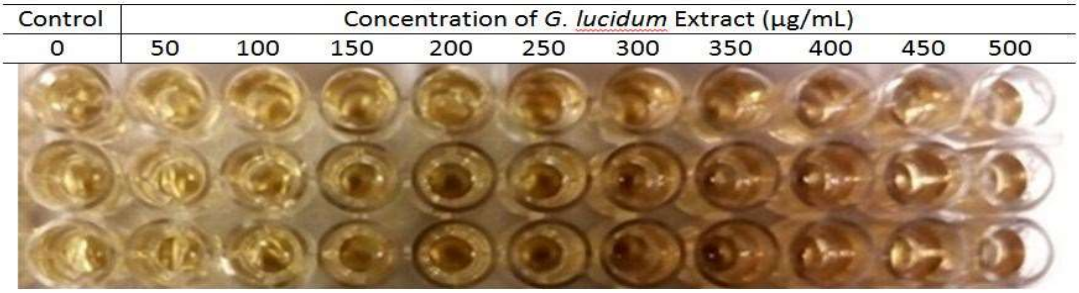
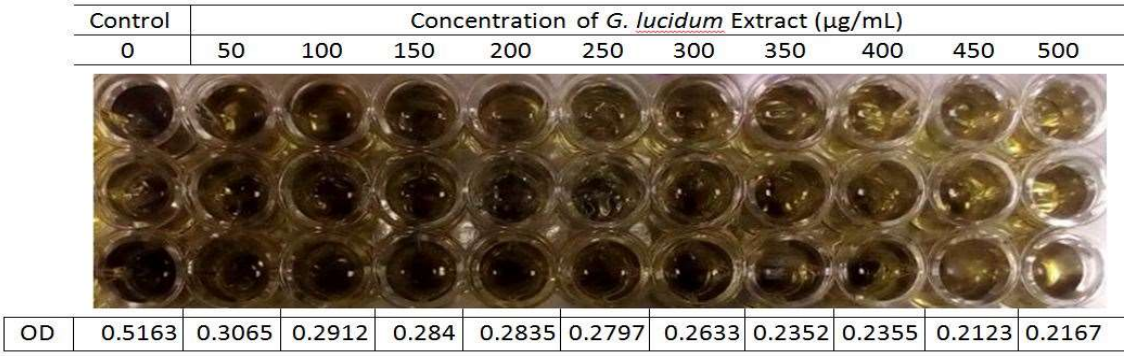


Fig.1. Each well in the 96-well plate shows colour intensity of cell viability before MTT reagent was added



OD	0.5163	0.3065	0.2912	0.284	0.2835	0.2797	0.2633	0.2352	0.2355	0.2123	0.2167

Fig.2. Each well in the 96-well plate shows colour intensity of cell viability after MTT reagent was added and OD measurement

As shown in Fig. 1 and 2, the colour changes of media before and after the addition of MTT reagent were very noticeable in the cell viability assay. The difference in media colour is due to the resulted formation of purple colourformazan derivatives after 24 h of incubation with MTT reagent.

From Fig. 2, it can be observed that the intensity of purple colourformazan decreases from 0 µg/mL to 500 µg/mL. This is supported by the optical density (OD) measurement of the cultured cell line where it was observed that the OD measurement decreased from 0 µg/mL to 350 µg/mL and increased slightly at concentration of 400 µg/mL. At concentration of 450 µg/mL, the OD measurement increased slightly and decreased slightly again at concentration of 500 µg/mL. It can also be observed that the colour intensity of purple colourformazan was the darkest and the OD measurement obtained was the highest at concentration of 0 µg/mL. The obtained result suggest that higher intensity of purple colourformazan signifies the presence of actively proliferating viable cells to transform the tetrazolium salt into

colouredformazan derivatives [8], hence, higher OD measurement. While, lower intensity of purple colourformazan indicates lower presence of actively proliferating viable cells, hence, lower OD measurement. Thus, from the obtained OD measurement, this suggests that the cells treated with lower concentration of G. lucidum extract contained higher number of viable cells whereas cells treated with higher concentration of G. lucidum extract contained lower concentration of viable cells.

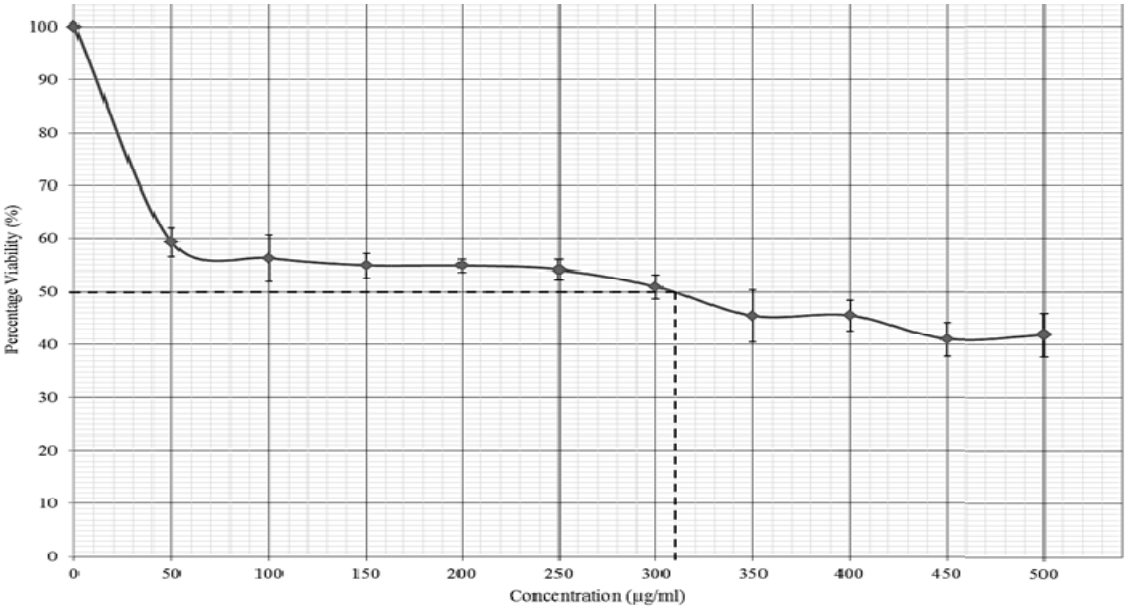


Fig.3. Graph shows percentage of cell viability in each concentration and IC_{50} value of G. lucidum in oral cancer cell ORL-48T (p = 0.00)

Table 1. IC_{50} value of G. lucidum towards ORL-48T

Treatment	IC_{50} (µg/mL)
Ganodermalucidum	310 ± 0.1

From Fig. 3, it was observed that the percentage of viable cell significantly decreased from concentration of 0 to 50 µg/mL and gradually decreased from concentration of 50 to 350 µg/mL of G. lucidum extract. At concentration of 400 µg/mL, the percentage of viable cell increases slightly and decreases again at concentration of 450 µg/mL. The precentage of cell viability increases slightly again at concentration of 500 µg/mL.From the plotted graph of percentage of cell viability versus concentration of G. lucidum extract, the IC_{50} of G. lucidumextract obtained was 310 ± 0.1 µg/mL (Fig. 3).

This study was done to determine the IC_{50} of G. lucidum extracts using human oral cancer cell lines, ORL-48T. From the result, it was observed that G. lucidum extract decreased cell proliferation significantly in a concentration related manner. The data also reflects the cytotoxic effect of G. lucidum towards ORL-48T with an IC_{50} determined at 310 ± 0.1 µg/mL. This suggests that G. lucidum have active compounds that contributed to the inhibition of ORL-48T cell proliferation. Hence, this may imply that G. lucidum may have potential anticancer properties towards oral cancer cells.

Previous studies determining the cytotoxicity of G. lucidum in other cancer cell lines, e.g. mouse myeloma cancer, inflammatory breast cancer, prostate cancer and colorectal cancer [9–12], reported a lower concentration of IC_{50} with valuesbelow 300 µg/mL. Previous study done by [9] used two type of extraction solvent to acquired G. lucidum powder crude extract. The extracts were then treated to mouse myeloma cancer cell line and the IC_{50} data obtained differ significantly from our present study. It was observed that the concentration of IC_{50} was considerably low at 44 ± 3.8 µg/mL for G. lucidum methanol extract and 82 ± 6.1 µg/mL for G. lucidum hot water extract.

Besides that, previous report about the cytotoxic effect of different extraction solvent of G. lucidum toward prostate cancer cell line (PC3) showed that G. lucidum whiskey extract has the strongest inhibition of proliferation with IC_{50} of 4.52 µL, followed by G. lucidum whiskey only extract (5.7 µL) and G. lucidum ethanol only extract (5.38 µL) [11]. Most of active compounds in G. lucidum can be extracted using water or ethanol [13,6]. It is possible that G. lucidum whiskey extract resulted in lower IC_{50} concentration because it may contain active ingredients found in both G. lucidum water- and ethanol-based extracts as this type of liquors are a mixture of ethanol and water solvents.

In the present study, the solvent used for diluting G. lucidum is DMSO. DMSO is known to be an universal solvent due to its amphipathic nature [14]. It is applied as a solvent for many drug types and used as drug vehicle controlin both in vitro and in vivo studies [15,10]. However, the result of our present study may differ from previous reports due to the difference in the extraction method done for the crude G. lucidum and/or the type of cancer cell lines used. In addition, different extraction method yielded different amount and type of the desired anti-tumour compound [13]. Thus, future study needs to be done in order to

specifically determine the active biological compound presence in the extract of G. lucidum using different type of solvent.

Besides that, the possibility of non-specific cytotoxicity of G. lucidum should also be considered. In the research for potent anti-tumour agents, it is essential to distinguish between non-specific cytotoxicity and specific anti-neoplastic activity. As stated in [16,9]suggested that non-specific cytotoxic drugs are unable to differentiate between normal and tumour cells, whereas specific anti-neoplastic activity drugs are more active against tumour cells than normal cells. Hence, a comparison with normal cells should be done to distinguish the efficiency of G. lucidum inhibition activity towards oral cancer cells.

4. CONCLUSION

In conclusion, this present study demonstrates that G. lucidum may have cytotoxic effect towards oral cancer cell line, ORL 48T. Moreover, this study serves as a preliminary evidence of G. lucidum inhibition activity towards oral cancer cells.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

[1]Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer.Oral Oncology, 2009,45(4):309–316

[2] World Health Organisation (WHO). Oral health fact sheet no. 318.Geneva: WHO, 2012

[3] Razak A A, Saddki N, Naing N N, Abdullah N. Oral cancer presentation among Malay patients in hospital Universiti Sains Malaysia, Kelantan.Asian Pacific Journal of Cancer Prevention, 2009, 10:1131–1136

[4] Wu Q P, Xie Y Z, Li S Z, La Pierre D P, Deng Z, Chen Q, Li C, Zhang Z, Guo J, Wong C KA. Tumour cell adhesion and integrin expression affected by Ganoderma lucidum.Enzyme and Microbial Technology, 2006, 40(1):32–41

[5] Liu X, Yuan J P, Chung C K, Chen X J.Antitumour activity of the sporoderm-broken germinating spores of Ganoderma lucidum.Cancer Letters, 2002, 182(2):155–161

[6] Wachtel-Galor S, Tomlinson B, Benzie I F. Ganoderma lucidum ('Lingzhi'), a Chinese medicinal mushroom: Biomarker responses in a controlled human supplementation study. British Journal of Nutrition, 2004, 91(2):263-269

[7] CaldwellG W, Yan Z, Lang W, Masucci J A.The IC_{50}concept revisited.Current Topics in Medicinal Chemistry, 2012, 12(11):1282–1290

[8] Stoddart M J.Cell viability assays: Introduction.In M. Stoddart (Ed.), Mammalian cell viability: Methods and protocols.New York: Humana Press, 2011, pp. 1–6

[9] Tong C C, Choong Y K, Aini N B U, Noordin M M, Mohamed S. Cytotoxic activity induced by crude extracts of Ganoderma lucidum (W. Curt.: Fr.) P. Karst. on mouse myeloma cancer cell-line. World Journal of Microbiology and Biotechnology, 2009, 25(4):687-695

[10] Martínez-Montemayor M M, Otero-Franqui E. Ganoderma lucidum (Reishi) Inhibits Cancer Cell Growth and Expression of Key Molecules in Inflammatory Breast Cancer.Nutrition and Cancer, 2011, 63(7):1085–1094

[11] Kao C H J, BishopK S, Xu Y, Han D Y, Murray P M, Marlow G J, Ferguson L R.Identification of potential anticancer activities of novel Ganoderma lucidum extracts using gene expression and pathway network analysis. Genomics Insights, 2016, 9:1–16

[12] Xie J T, Wang C Z, Wicks S, Yin J J, Kong J, LiJ, LiY C, Yuan C S, Ganoderma Lucidum extract inhibits proliferation of SW 480 human colorectal cancer cell.Experimental Oncology, 2006, 28(1):25–29

[13] Kao C H J, Jesuthasan A C, Bishop K S, Glucina M P. Anti-cancer activities of Ganoderma lucidum: Active ingredients and pathways. Functional Foods in Health and Disease, 3(2):48–65

[14]GalvaoJ, DavisB, TilleyM, Normando E, Duchen M R, Cordeiro M F.Unexpected low-dose toxicity of the universal solvent DMSO. The FASEB Journal, 2017, 28(3):1317–1330

[15] Li SY, Lo A C Y. Lutein protects RGC-5 cells against hypoxia and oxidative stress.International Journal of Molecular Sciences,2010, 11(5):2109–2117

[16] Wilson A. P. Cytotoxicity and viability assays. England: Oxford University Press, 2000

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