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In Vitro Cytotoxicity of Reishi Mushroom Extract Against Two Human Cell Lines

Noor T. Hamdan [a]*, Ahmed Khayoon Abed Al-Abboodi [b]

[*a] *Biology Department, College of Science, Mustansiriyah University, Baghdad- Iraq*

[b] *Ministry of education, Iraq*

Abstract

Reishi mushroom *Ganoderma lucidum* is commonly used in conventional medicine due to its high medicinal properties. Phytochemical screening of *G. lucidum* mushroom extract found to be flavonoids, glycosides, terpenoids, phenols, Carbohydrates. The current study was applied to demonstrate the In vitro cytotoxicity of ethanolic extract of *G. lucidum* mushroom against two human cell lines breast cancer cell line MCF-7 and human embryonic liver cell line WRL-68. The measured inhibitory concentration fifty(IC_{50}) values were observed maximum dose responses (IC_{50}) of WRL68 and MCF-7 at 2.0 µg/ml ethanolic mushroom concentration reported of 210.2% and 185.1% compared to the dose-response (IC_{50}) of antidrug doxorubicin (control) at 2.0µg/ml ethanolic mushroom concentration counted to be 18.68. The highly cytotoxic activity of the extract on cell viability MCF-7 and WRL-68 were generally observed 100% and 97% at extract concentrations of 12.5 µg/ml and 25µg/ml respectively compared to antidrug was determined to be 94% cell viability at 6.25 µg/ml concentration.

Keywords: Cytotoxicity, MTT assay, Reishi mushroom, Human cell lines

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1. Introduction

Cancer seems to be the world's main reason of death. Cancer introduces a global health challenge and current data from the World Cancer Research Fund International(WCRF), indicates an approximate 18 million cases reported worldwide in 2018, and this data is predicted to rise to 24 million by 2035 (www.WCRF.org/). Breast cancer has to be the most frequent in women and the second largest source of cancer with 12.3% registered overly. In 2018, over 2 million new patients were screened [1]. Liver cancer has to be the fifth most frequent found in men and the ninth largest source of cancer found in women. More than 840,000(i.e. 5%) new patients were screened in 2018 [1].

The recent anticancer therapies present in the industry are not strict goals and present many adverse impacts and risks to treat different types of cancer, therefore, using the immediate necessity for modern efficient and low hazardous therapeutic strategies [2]. Several research experiments were performed to evaluate the influence of the usage of industrial medicinal mushroom extracts for cancer treatment which considered to supplement chemical treatment and radiation treatment through avoiding the adverse impacts of tumors, like fatigue, bone marrow destruction, anemia, and reduced tolerance [3,4].

Recently, *Ganoderma lucidum* (Reishi) considers a common easternal medicinal mushroom to encourage health and longevity [5].

In Nature, under high moisture and indefinite lighting status it can often widely cultivated on living and dead deciduous wood plants [6]. It was used to avoid and the management of numberous illness, including chronic liver disease, kidney disease, elavated blood pressure, lung disease and carcinogenesis disorders [7]. It is claimed that *G. lucidum* "the immortality's mushroom" has remarkable medical properties as well as comprises

*Corresponding author e-mail:noor.t.hamdan@uomustansiriyah.edu.iq
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more than 400 bioactive compounds [8], such as triterpenoids, polysaccharides, nucleotides, sterols, steroids, fatty acids and proteins/peptides, that posses a variety of therapeutic properties [9], like anti-tumour [10], anti-microbial [11], anti-atherosclerotic and anti-inflammatory, hypolipidemic [12], anti-diabetic, anti-oxidative and radical-scavenging, anti-aging, anti-fungal, and anti-viral (specifically against herpes and HIV) materials, and improving the immune system [13].

Our current research aims to investigate the *Ganodrema lucidum* mushroom on proliferation and tumors colony inhibition in breast cancer cell line MCF-7 and human embryonic liver cell line WRL-68.

2. Experimental

2.1. Preparation of reishi mushroom extracts

The reishi mushroom was bought from supermarket in Iraq, cleaned, dried at 40˚C, grinded and then weighed. The mushroom dried powder 1g was used for 10ml of solvents 70% ethanol, and then extracted by using soxhlet. After 24h, The solution was centrifuged for 15min at 1000 rpm/ min, and then collected liquid phase was used for further process. The liquid portion was evaporated using a rotary evaporator at 50 ˚C and then preserved at -20 ˚C for further studies.

2.2. In-vitro Anticancer activity

The anticancer efficency of ethanolic extract from reishi mushroom against breast cancer cell line MCF-7 and human embryonic liver cell line WRL-68 that we obtained from Iraqi Center For Cancer Research in Al- Mustansiriyah university. For 24 hours, cells have been grown at 37°C in a humid conditions of 5% CO2 in Minimum Essential Medium supplemented with 10% fetal bovine serum and 1% penicillin..

Cell viability MTT assay included by the standard instructions(Promega Corporation, Madison, WI, USA). At first, the 96-well tissue culture plate was filled with 100 µl/well of cells(10^6 cell/ml). Various concentrations of reishi mushroom extract test solution were prepared to evaluate cytotoxic effect against two examined cell line(400, 200, 100, 50, 25, 12.5, 6.25 µg/ml) in water. After that, 100µl of different concentrations was applied to each well in incubator at 37 ˚C with 5% CO_2 humid conditions for 24h. During the incubation, 10µl of 5mg/ml MTT solution transferred to each well and incubated at 37˚C for 2h. Each well added through the extraction buffer(20% sodium dodecyl sulphate (SDS) and 50% dimethyl formamide) and incubated overnight at 37 ˚C. A positive cytotoxic control represented as anti-tumor drug doxorubicin(1mM) in cell culture wells. The cell suspension absorbance was calculated using ELISA Microplate Reader(Bio Tek, USA) at 570nm. Cell viability was described as the ratio of the mean absorbance of the treated cells to that of control cells. The tumors cells' sensitivity to the extract was represented as IC_{50} values. This experiment was replicated three times, and the statistical data was analysed to give the final results.

2.3. Evaluation some of the active compounds in the fungal extracts

The occurrence of active compounds in the examined mushroom was calculated by standard instructions [14, 15] and summarized in table(1).

Table 1. Experiments done for the study of bioactive compounds in mushroom

S. No.	Class of compounds	Experiments	Observations
1.	Flavonoids	1 mL of mushroom extract added to 1 mL of solution of (10%) lead ethyl alcohol .	Dark bluish color
2.	Glycosides	Two ml of acetic acid and two ml of chloroform is mixed into 2 mL of mushroom extract.	Yellow discoloration
3.	Saponins	A mixture of 5 mL of distill water with a mushroom extract has been performed.	Dense foam for a long time
4.	Terpenoids	Two ml of acetic anhydride and two-three droplets of condensed H2SO4 were combined with 2 ml of mushroom extract.	turned yellow
5.	Phenols	2 mL FeCl3 solution has been combated with the crude extract (2 percent).	The appearance of a greenish-blue color
6.	Alkaloids	a solution of one milliliter of a 0.2 percent hydrochloric acid was added to the unrefined extract, it was heated slightly. Wagner and Mayer's reagents were then applied to the mixture.	The appearance of a white precipitate
7.	Carbohydrates	the contents of both Fehling B and Fehling A reagents were added to 2 mL of the resultant unrefined extract, and the mixture solution was taken to a boil.	red orange precipitate

2.4. Data analysis

For statistical research, the program SPSS 20.0 has been used. Three different values have been represented as mean + SD values. In all instances, the adopted levels was 5% ($p < 0.05$).

3. Results and Discussion

3.1. Cell line growth and cytotoxicity assay

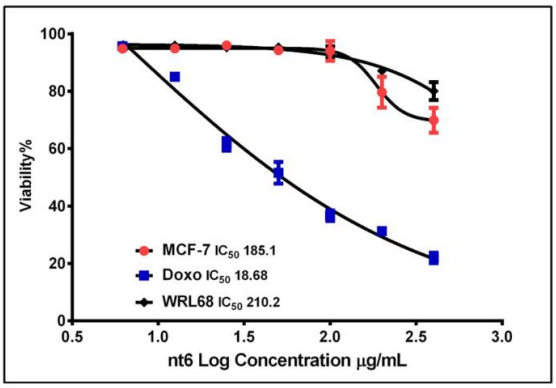

Figure1: Dose responses (IC_{50}) of anti-tumor mushroom on WRL68 and MCF-7 human cell lines.

Cells have been cultured in minimal essential medium supplemented with 10% fetal bovine serum and subjected to ethanolic extract of reishi mushroom at concentrations of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0µg/ml for 24h. In each experiment was replicated three times and two different experiments were conducted. Mean ±SD (n=6) are values shown. The cytotoxic results of ethanolic mushroom extract on WRL68 and MCF-7 human cell lines were shown to be a dose-responsive manner (Figure 1). Results of the anticancer activity WRL68 and MCF-7 found high variation compared to the antidrug doxorubicin(control).

Our data shown that the maximum dose responses (IC_{50}) of WRL68 and MCF-7 at 2.0 µg/ml ethanolic mushroom concentration reported of 210.2% and 185.1% compared to the dose-response(IC_{50}) of antidrug doxorubicin (control) at 2.0µg/ml ethanolic mushroom concentration counted to be 18.68. Findings suggest that bioactive compounds of mushroom work as a vital strategy for treating drug-resistant cancers relative to commercial anticancer drugs.

The *Christia vespertilionis* ethanolic extract was screened for cytotoxicity on WRL68 and MCF-7 and resulted the IC_{50} values reported higher in compared with antidrug cyclophosphamide(control)(Mutalib and Latip, 2019) [16]. In addition, the previous study shows that the *Lignosus rhinocerotis* mushroom extract has cytotoxicity toward solid tumor cells MCF-7 and WRL68 with IC_{50} of 20- 100mg/ml (Lau *et al*., 2013) [17]. Futhermore, and as investigated with IC_{50} cytotoxic activity ranged to be 68 to 171 µg/ml against PC-3 and MCF-7 cancers (Welti *et al*., 2010)[18]. Another study [19] represents the growth cell inhibition of MCF-7 and liver cell lines and resulted IC_{50} 93.62 and 18.37mg/l.

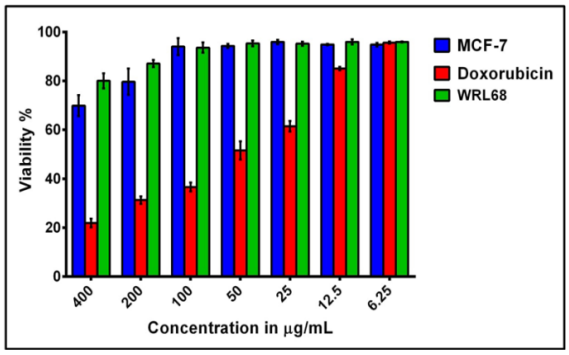

Figure 2: The effect of various concentrations of Reishi mushroom ethanolic extract on growth inhibition of two human breast cell line MCF-7 and embryonic liver cell line WRL68 after 24hr incubation time.

The cell viability 100% and 97% in WRL68 and MCF-7was determined at high rate at the concentration of 12.5 µg/ml and 25µg/ml respectively compared to antidrug was determined to be 94% cell viability at 6.25 µg/ml concentration.

In data study, Reishi mushroom extract detected that the comparision of bio-active compounds with chemotherapy improving anticancer activities. It's consider a cheap and sustainable medicine toward WRL68 and MCF-7 cell lines and can ultimately improve the life quality of the countryside and peri-urban people in developing countries.

Recent study of [19] which the inhibition cell growth against MCF-7 cell line by *Coriolus versicolor* extract. Moreover, the percentages of MCF7 cell proliferation inhibition by *Xanthium strumarium* extract have maximum compared with doxorubicin [20].

Furthermore, Cytotoxicity towards hepatoma, cervical cancer and lung carcinoma cells was demonstrated in *Ganoderma lucidum* extract [21]. Cell growth inhibition in MCF-7 at a concentration 500µg reported in study [22]. Another report of [23] showed that inhibition of tumor growth in liver and MCF-7 at 34 µM and 50-70 concentrations respectively. An approximately 70% inhibition of cell proliferation from human liver cell line reported at 500 µM/ml in *Ganoderma lucidum* extract [24].

3.2. Evaluation of Phytoactive Compounds

The primary detection (Presence or absence) for the active components shown in table(2) for ethanolic fungal extract, the results showed that the crude ethanolic extract contains many active chemical compounds such as flavonoids, glycosides, Terpenoids, phenols, Carbohydrate, the mean of pH extracts was (5.5-6).

Table 2: Presence or absence of the active compounds in Reishi mushroom ethanolic extract by standard instructions [14, 15].

Effective compounds	*Ganoderma lucidum*
Flavonoids	+
Glycosides	+
Saponins	-
Terpenoids	+
Phenols	+
Alkaloids	-
Carbohydrate	+

The bioactive compounds polyphenols and carbohydrate-flavonoid complexes derived from *G. lucidum* extract, may be usefully employed in anticancer and anti-inflammatory drugs toward breast cancer [25].Terpenoids extracted from *Ganoderma lucidum* extracts have been recognized as an anticancer drug which are structurally related to steroid hormones and have a large variety of anticancer effects [26]. In the genus *G. lucidum* extract over 150 triterpenoids compounds were known in possessing cytotoxic effects on different cancer cells including Ganoderic acids, lucidimols, ganodermanondiol, ganoderiol F and ganodermanontriol [27, 28]**.** Triterpenoids were recorded as an anti-hypertensive, hypocholesterolemic, hepatoprotective, and anti-histaminic effects, as well as anti-tumour and anti-angiogenic activity [29]**.**

G. lucidum triterpenes are able to sensitize cells to the activity of doxorubicin by elevated oxidative stress, DNA destruction, and apoptosis [30]. Cell proliferation, induced DNA destruction, the G1 phase cell cycle, as well as apoptosis in human breast cell line were inhibited by Ganoderic acid 3,7-Dioxolanosta-8,24-dien-26-oic acid(GA-DM) [31]. Two lanostane triterpenes were extracted from *G. lucidum* fruiting bodies with powerful cytotoxic effects toward A549, MCF-7 and PC-3 human cell lines [32]. Approximately more than 200 distinct polysaccharides extracted from *Ganoderma lucidum* were discovered in many reports [33]. Polysaccharides are known as a largest category of active compounds because of enormous pharmacological agents to resolve various diseases. Polysaccharides were shown about 10–50% insides fruitbodies dry matter Extracted polysaccharides improve anti-tumour immune responses by stimulating the natural killer cells activity and T-lymphocytes cytotoxicity [34, 35].

Glycoproteins, heteropolysaccharides and ganoderans A, B and C possess large molecular weights, a hydrophilic structure and major anti-tumor effects. Ganoderan refers to a biopolymer which is often used in conjunction with anticancer drugs as additional therapy [36]. The efficacy of cytotoxic drugs and immunomodulators of prostate cancer patients may be enhanced Polysaccharides β-D-glucans show that a high anticancer activity among the numberous polysaccharides [37].

4. Conclusions

Reishi mushroom *Ganoderma lucidum* are now a rising segment of medical industry due to their large variety of bioactive compounds. It's consider a cheap and sustainable medicine toward WRL68 and MCF-7 cell lines and can ultimately improve the life quality of the countryside and peri-urban people in developing countries The obtained data from the current study could offer to new insights into the potential medical properties of mushrooms and useful recommendations for the design of anti-tumor drugs from mushrooms in combating cancer owing to the existence of a broad variety of compounds that have therapeutic properties and improve the immune system.

5. Conflict of interest

The author declares no conflict of interest.

6. Acknowledgments

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