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Potential Genotoxicity Effect of *Ganodermalucidum* Extracts on DNA Damage in Bone Marrow Cells of Albino Mice

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Abstract: The present study investigated the genotoxicity effects of three types of crude extracts, which include two aqueous extracts (hot aqueous extract, cold aqueous extract) and ethanolic extract of *Ganodermalucidum*(*G. lucidum*) powder from DXN Company (Malaysia) on albino mice bone marrow cells *in vivo* using comet assay. The extracts were subjected to analysis using GC-MS technology. The analysis revealed that *G. lucidum*predominantly contains organic compounds like alcohols, aldehydes, esters, and ketones. Each type of extract (alcoholic, cold aqueous, and hot aqueous) was administered to three mice at low, moderate, and high concentrations (1000, 2000, and 4000) mg/kg (three replicate for each dose) at this study and injected with 0.3 ml in each mouse intraperitoneally. The control group received an injection of 0.3 ml of normal saline using the same method for 24 hours. The results showed that the extracts' genotoxic effect on the genetic material of bone marrow cells depends on the type of extract and dose. Furthermore, DNA damage increased significantly ($P \leq 0.05$) and proportionally to higher extracts doses as demonstrated by the increased values of comet length (hot aqueous extract=62.50, cold aqueous extract= 81.00, ethanolic extract=89.50), head diameter (hot aqueous extract= 88.30, cold aqueous extract= 78.53, ethanolic extract= 72.17), and tail length (hot aqueous extract= 19.86, cold aqueous extract= 20.32, ethanolic extract= 23.80) at the concentration 4000 mg/kg. The identification and assessment of bioactive compounds in the three *G. lucidum*extracts suggest these extracts have slight DNA damage effect in bone marrow cells.

Keywords: Genotoxicity, *Ganodermalucidum*, Bone marrow cells, Comet assay.

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Introduction:

Ganodermalucidum, commonly known as "Lingzhi or Reishi," is a mushroom believed to have longevity and health-promoting properties. It has been used for over 2000 years in Traditional Chinese medicine due to its various therapeutic activities, including its ability to combat tumors, allergies, viruses, liver damage, oxidative stress, high blood pressure, and inflammation. It also acts as an immunomodulator, lowers blood sugar levels, prevents blood clot formation, and exhibits antibacterial properties, among other health benefits. In addition, the therapeutic mechanism of *G. lucidum* involves preventing and treating various diseases, including chronic bronchitis, hypertension, hyperlipidemia, diabetes, hepatitis, tumor, and aging, through its antioxidative and free radical scavenging effects (1).

The *Ganoderma* species also contains a diverse range of 400 important bioactive chemical

constituents, such as polysaccharides, triterpenoids, polysaccharide-peptide complex, β-glucans, lectins, natural germanium, adenosine, phenols, steroids, amino acids, lignin, vitamins, nucleotides, and nucleosides. These constituents possess distinct healing properties and are utilized to create various useful products derived from *Ganoderma* fruiting bodies, mycelia, and spores(2).

Figure (1): *Ganodermalucidum*(3)

This study aimed to compare the genotoxic effects of three different types of extracts from *G. lucidum* (hot aqueous, cold aqueous, and ethanolic) on bone marrow cells of albino mice, to provide a genotoxic evaluation of *G. lucidum* extracts, especially the activity of bioactive compounds and their relevance to damage the genetic material. This may enable the development of more efficient and less toxic alternatives to synthetic therapeutic agents.

Materials and method

Mushroom collection

Ganodermalucidum (Reishi mushroom powder) were purchased from DXN Company (Malaysia).

Figure (2): Reishi mushroom powder

Preparation of ethanolic extract

Ethanolic extract was prepared by dissolving 25 gm of *G. lucidum* fine powder in 250 ml of 80% ethanol at a ratio of 1:10 w/v between the powder and the solvent. The maceration process was conducted on a magnetic stirrer at room temperature for 48 hours. The flask was securely sealed using aluminum foil and parafilm during extraction. The extraction solution underwent centrifugation at 3000 rpm and filtration through the Whatman No. 1 filter paper. The resulting supernatant was then dispensed into glass Petri dishes and dried in an incubator at 40°C until complete desiccation. The dried extract was dissolved in a small quantity of DMSO solvent, and then the

volume was completed to the mark with D.W. before utilization (4, 5).

Preparation of aqueous extracts

In the case of hot aqueous extract, 25 gm of powdered sample was dissolved in 250 ml of sterilized distilled water at a ratio of 1:10 w/v on a hot plate at 80°C for the first 6 hours; then, the extraction continued for 48 hours without heating. Meanwhile, the cold aqueous extract was subjected to continuous agitated for period of 48 hours at room temperature. To prevent contamination and ensure airtight conditions, the flask was securelysealed using aluminum foil and parafilm during the maceration process. The aqueous phase underwent centrifugation at 3000 rpm,and the resulting supernatant was filtered, dried, dissolved in a proper solvent, and subsequently preserved at 4°C in a refrigerator (6, 7).

Gas chromatography-mass spectrum analysis

The bioactive compounds in *G. lucidum* extracts (ethanolic, hot aqueous, and cold aqueous) were analyzed using an Agilent 6890 GC-mass system (Agilent, USA) connected to an Agilent 5973N MSD. The ion source temperature was set to be 200°C, and the injection volume was 1.0 µl with a split ratio of 50:1. The capillary column used was HP-5MS (30 m x 0.25 mm ID x 0.25 µm film) from Agilent J and W, USA. The oven temperature was initially set at 100°C and then increased at a rate of 10°C/min until it reached a final temperature of 275°C after 20 minutes. The transfer line temperature was set at 220°C. Helium gas was used as the carrier at a constant 1 ml/min of flow rate. Data was acquired using the Agilent GC/MSD Chem-Station Version D.02.00. This test was assessed inMinistry of Science and Technology (8).

Genotoxic effects of *Ganodermalucidum* extracts on DNA damage in bone marrow cells

The comet assay (single-cell gel electrophoresis) is highly sensitive and cost-effective for measuring DNA damage and repair. The standard procedure involves performing the alkaline comet assay with slight modifications (9).

Samples and slides preparation

The complete bone marrow was drained from the femur bone with warm PBS (37 °C) after the injection and sacrifice of albino mice. The cell suspension in the final concentration was 1×10^5 cells/ml.LM Agarose was prepared then mixed with the cell suspension at 37°C in a ratio of 1:10 (v/v). The resulting mixture was immobilized onto a Comet slide in a volume of 50 µl.The slide was submerged in a dark container with the lysis buffer (25 ml/slide), at 4^{o}C for 30 to 60 minutes. To increase sensitivity, it could be incubated overnight. Gently remove slides from the lysis buffer and immerse in alkaline solution (pH>13) for 20 minutes inside the dark container at 4^{o}C to allow DNA unwinding.The slide was then held horizontally electrophoresis chamber filled with alkaline electrophoresis buffer while a 21 volts was applied for 30 min.Subsequently, the slides were transferred from the electrophoresis chamber into a neutralization buffer (25 ml/ slide) for 5 min, which was repeated twice. The slides were stained with ethidium bromide for 5 min then washed with distilled water and examined immediately using 40X objective lense in a fluorescent microscope (10).The method of quantification using the Comet score image analysis program. For each

comet, the analysis software will determine a fresh set of parameters. Three parameters, comet length, head diameter, and tail lengthwere estimated (11, 12, 13).

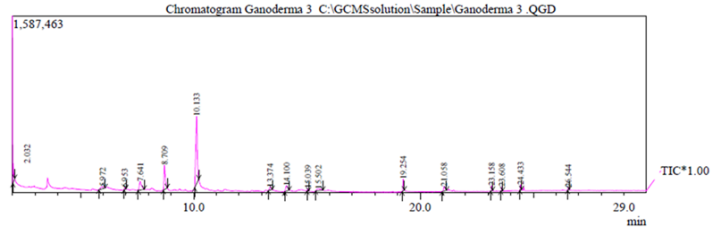
Results

GC- MS analysis

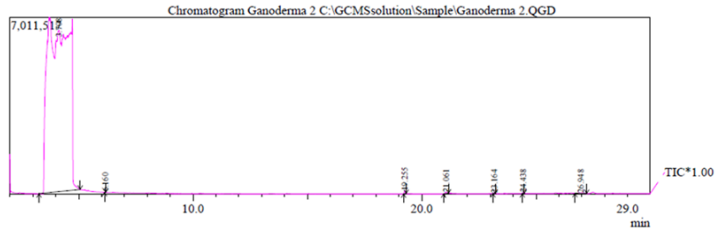
GC-MS chromatograms of the Reishi mushroom crude extracts exhibited different active compounds. *G. lucidum* is composed mainly of organic compounds such as alcohols, aldehydes, esters, and ketones. Some ketones are cyclohexanone, 3-hydroxy, cyclohexanone, 4h-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, and ethanone, 1-(2-methylcyclopropyl)-. Alcohols are abundant in edible mushrooms and found in different forms, such as 2-furan methanol as furfural alcohol, 3-hexanol, cyclopentanol as a primary alcohol, and 2-methyl-2-nonanol as a fatty alcohol. The main volatile aroma compounds identified were aldehydes, such as 2-ethyl-trans-2-butenal.

The total sum of fatty acids was relatively high in *G. lucidum*. The primary fatty acid identified in *G. lucidum* extracts was palmitic acid, including forms like hexadecanoic acid, 2,3-dihydroxypropyl ester, and n-hexadecanoic acid. Other significant fatty acids included oleic acid and fatty acid esters such as 3-methylpentanoic acid and 2-hydroxy-, methyl ester. Additionally, organic compounds like pyridines were present, including 2-picoline and 6-nitro-.

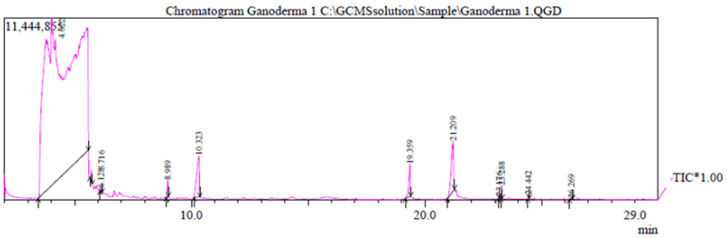
Other organic compounds found in the form of Epoxides cyclic ether such as Oxirane, 3-butenyl-, in the form of Aldoxime such as Pentanal, oxime, in the form of polyether such as oxirane, ethoxymethyl-, in the form of nitrile such as Pentanenitrile, 4-methyl-, and flavonoid organic compound such as 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (Figure 3 a, b, c).



(a)



(b)



(c)

Figure (3): Gas chromatogram of *Ganodermalucidum* extracts. (a): Cold aqueous extract, (b): Hot aqueous extract, (c): Ethanolic extract

Genotoxicity effect on DNA damage

Comet Length

The results in Figure 4 showed significant differences at (P≤0.05) where the values of the effect of *G. lucidum* extracts on the Comet length were recorded (65.00 ±2.31, 63.00 ±4.35, 62.50 ±2.93) of hot aqueous extract, (65.60 ±7.79, 69.50 ±0.86, 81.00 ±1.73) of cold aqueous extract, (64.00 ±2.31, 75.50 ±3.75, 89.50 ±0.86) of erhanolic extract at the doses (1000, 2000 and 4000) mg/kg when compared with the control group (65.67 ±2.18). At the same time, there are no significant differences (P>0.05) when comparing the control value with the values of hot aqueous extract at the doses 1000, 2000, and 4000 mg/kg and with the values of cold aqueous extract and ethanolic extract at the concentration 1000 mg/kg. There are significant differences between the three extracts at the three doses.

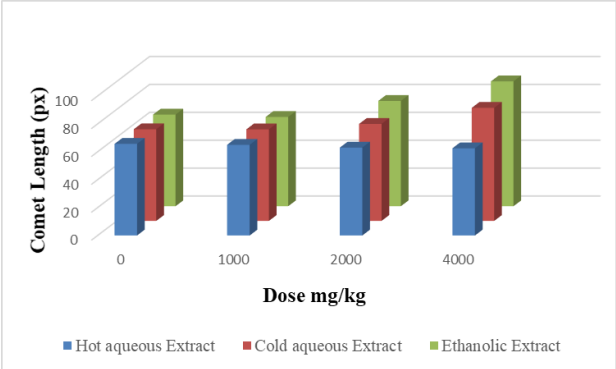


Figure (4): Effect of *Ganodermalucidum* extracts on DNA damage (comet length) in mice bone marrow cells

Head Diameter

The results of the statistical analysis of the effect of *G. lucidum* three extracts show a significant decrease at (P≤0.05) in the head diameter values from the control value (94.40 ±0.95) reaching 88.30 ±1.56, 78.53 ±0.28, and 72.17 ±0.55 of hot aqueous, cold aqueous, and ethanolic extract with the doses (1000, 2000, and 4000) mg/kg. Also, there is a significant difference (P≤0.05) between the values of the three extracts at the three concentrations (horizontal comparison), as shown in Figure 5.

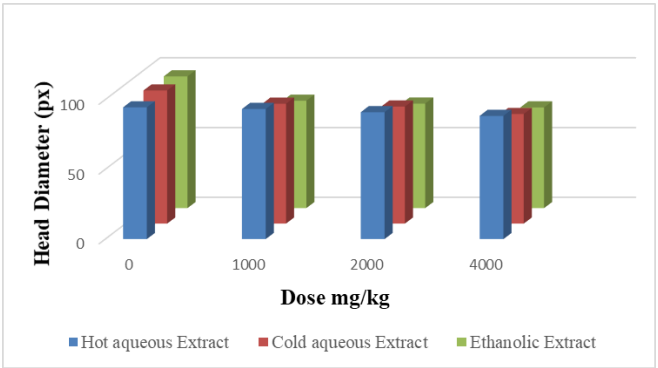


Figure (5): Effect of *Ganodermalucidum* extracts on DNA damage (head diameter) in mice bone marrow cells

Tail Length

In Figure 6, the results revealed that there are no significant differences (P ≥0.05) from comparing the tail length values of (16.76 ±0.83, 17.00 ±0.44) of hot aqueous extract at the doses (1000, 2000) mg/kg and a significant difference (P≤0.05) in the value (19.86 ±0.54) at the dose 4000 mg/kg comparing with the control value (16.00 ±1.15). Also, the comparison between all doses of tail length value of the three extracts showed significant differences with the control value.

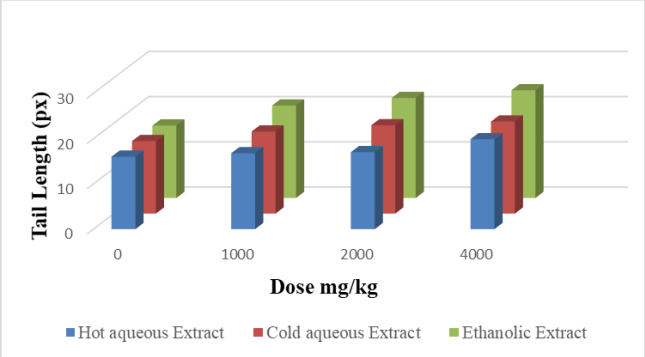


Figure (6): Effect of *Ganodermalucidum* extracts on DNA damage (Tail length) in mice bone marrow cells

Discussion:

In this study, a genotoxic evaluation using non-tumorous cells (bone marrow cells) to determine the potential toxic effects of *G. lucidum* on immune cells and its impact on genetic material. It was found that *G. lucidum* caused slight changes in the three parameters in a dose-dependent manner (Figure 7).

A studydemonstrated that GLE had genotoxic effects on the two tumour cell lines by inducing oxidative stress inside these cells. High concentrations of GLE were the only ones that had a harmful effect on lymphocytes, decreasing their viability. Conversely, low concentrations of GLE increased the vitality of lymphocytes. Furthermore, GLE only caused primary DNA damage at the highest concentration by the formation of H_2O_2(14).

Ganodermalucidum has been found to possess a genoprotective function at low concentrations while causing DNA damage at high ones. It can reverse the harmful effects on genetic material by repairing DNA damage in tissues (15).

In this study, the cold aqueous extract contains organic compounds like aldehydes, which exhibit cytotoxic effects by inducing gene expression changes and causing cell damage at certain concentrations (16). Another organic compound, epoxide, has potential cytotoxic effects by alkylating DNA and proteins, leading to cellular damage or death, making it a candidate for development as a chemotherapeutic agent (17). On the other hand, the hot aqueous extract contains fatty acids such as palmitic acid, which acts as an antioxidant (18). Additionally, the ethanolic extract contains fatty acids like oleic acid and palmitic acid, as well as organic compounds like ketones and alcohols, all of which function as antioxidants (19, 20, 21).

A study, using *in vitro* and *in vivo* experiments, shows that *G. lucidum* is highly effective in protecting against DNA damage. This protection is achieved through radical scavenging, interaction with apurinic/ apyrimidinic endonucleases, and restoration of enzymatic antioxidant activity. The effectiveness is attributed to its abundant triterpenes, polyphenols, and

other active ingredients, similar to its free radical scavenging activity (22).

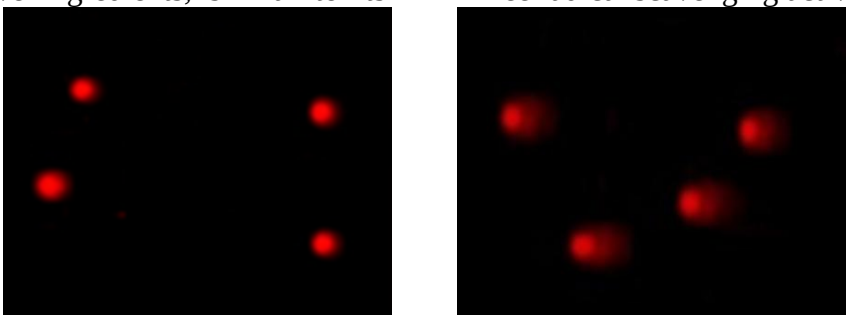


Figure (7): DNA damage in bone marrow cells in albino mice, A: Normal cells, B: Cells treated with *Ganodermalucidum* extracts appear DNA damage (comet shape).

Conclusion

Three experimental results have revealed that the three extracts (cold aqueous, hot aqueous, ethanolic) of *G. lucidum* cause slight damage to DNA in high concentrations, so there is no cause for concern over the safety of *Ganoderma* extract. Some bioactive components may show antioxidant properties, but we cannot rule out the potential benefits of larger doses and longer durations of supplementation.

Recommendation

Reducing oxidation-induced damage to nuclear DNA suggests that *G. lucidum* extracts could be recommended as a nutraceutical. It is recommended to detect the cytotoxic activity of *G. lucidum* extracts on different cancer cell lines (23, 24, 25). Finally, it could be detected in potential effect on bacteriocins (26).

Experimental animals and ethics statement

A total of 48 mice included male Albino mice aged 6-8 weeks and weighing 20-25 grams. The animals were treated following the "Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines" guidelines. The mice were housed in plastic cages with unrestricted access to water and a pellet diet, and the temperature was maintained at 23 ± 2◦C. The mice were given anaesthesia and rested overnight for a 12-hour light cycle, ensuring minimal stress effects. They were provided with water and a pellet diet (27). Approval number: (Ref. CSEC/1221/0082).

Conflict of interest

The authors have no conflicts of interest to declare.

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Author's contribution

Study conception and design, Acquisition of data analysis, drafting of manuscript: (Hala M. Mahmud). Interpretation of data, critical revision: (Hind H. Obaid).

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