Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Sep 05; 10(A):1450-1455.
https://doi.org/10.3889/oamjms.2022.10421
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Pharmacolgy*

In Vitro Antioxidant Activity and *In Vivo* Hepatoprotective Effects of Ethanolic Extracts from Wall-Broken *Ganoderma Lucidum* Spores

Nguyen Huu Lac Thuy[1], Vo Thi Diem[1], Trinh Thi Dieu Thuong[2], Tran Tuyet Anh[1], Truong Minh Nhut[1], Truong Van Dat[1], Huynh Ngoc Trinh[1]*

[1]Faculty of Pharmacy, University of Medicine and Pharmacy, Ho Chi Minh City, Viet Nam; [2]Faculty of Traditional Medicine, University of Medicine and Pharmacy, Ho Chi Minh City, Viet Nam

Edited by: Sinisa Stojanoski
Citation: Thuy NHC, Diem VT, Thuong TTD, Anh TT, Nhut TM, Dat TV, Trinh HN. *In Vitro* Antioxidant Activity and *In Vivo* Hepatoprotective Effects of Ethanolic Extracts from Wall-Broken *Ganoderma Lucidum* Spores. Open Access Maced J Med Sci. 2022 Sep 05; 10(A):1450-1455. https://doi.org/10.3889/oamjms.2022.10421
Key words: Antioxidant; Cyclophosphamide; Ethanolic extracts; Total triterpenoid; Wall-broken *Ganoderma lucidum* spores
***Correspondence:** Huynh Ngoc Trinh, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, Viet Nam. Email: hntrinh@ump.edu.vn
Received: 15-Jun-2022
Revised: 07-Aug-2022
Accepted: 26-Aug-2022

Funding: This work was supported by The Department of Science and Technology at Ho Chi Minh City, Vietnam (Number 888/QĐ-SKHCN)
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: The wall-broken Ganoderma lucidum spores are widely used in recent years in the belief that active components inside the spores are better released and well absorbed when taken orally.

AIM: In this study, the sporoderm of G. lucidum was broken by autoclaving at a high temperature.

METHODS: The powder of wall-broken spores was then extracted by soaking with ethanol at different concentrations (50%, 70% and 96%).

RESULTS: The 70% and 50% ethanol extracts had the highest total triterpenoid content, in which ganoderic acid A was predominant. In the 2,2-Diphenyl-1-picrylhydrazyl free radical scavenging test, 70% ethanol extract exhibited the highest in vitro antioxidant activity. This 70% ethanol extract was also safe in mice at the dose of 2000 mg/kg body weight. Moreover, this extract protected the liver from acute injury induced by cyclophosphamide.

CONCLUSION: The pretreatment by oral administration of 70% ethanol extract prevented serum alanine aminotransferase and aspartate aminotransferase activities elevation and attenuated hepatic MDA formation and GSH depletion following administration of cyclophosphamide in mice.

Introduction

Ganoderma lucidum (Leyss. ex Fr.) Karst, a wood fungus, is found throughout Vietnam, from North to South. The fruiting body of *G. lucidum* is rich in active components, such as triterpenoids, steroids, polysaccharides, and phenol compounds, which have been studied and used for a long time in traditional medicine to treat and improve health, especially to protect and support hepatic function [1]. On the other hand, in recent decades, *G. lucidum* spores have the similar active components as found in the fruiting body, even at higher content. However, due to the complicated spore collection, the yield is low and the cost of *G. lucidum* spore powder is much higher than that of the fruiting body. On the contrary, some studies have shown that *G. lucidum* spores contain fewer triterpenoid compounds than in the fruiting body [2]. This is likely due to two thick and hard chitin shells of the spores of *G. lucidum* making it difficult for the active components inside the spores to be absorbed when taken orally [3]. In addition, this hard sporoderm protected also the release of bioactive ingredients from *G. lucidum* spores under extracting process, resulting in the lower concentrations of active ingredients in extracts from the spores as compared to the fruiting bodies [2].

Thus, various methods in breaking the sporoderm have been used in recent years such as using low-temperature ultrasound, soaking [4], physical smashing [3], supercritical carbon dioxide [5], and enzyme hydrolysis methods [6]. After breaking the wall, the dissolution rate of the active components inside the spore is significantly increased, which can be applied to develop quality products that improve human health.

In the present study, the sporoderm was broken by autoclaving at a high temperature. Some of the advantages of this approach include: Saving time and effort; using available equipment in laboratories, and performing on a large amount of *G. lucidum* spores in a short time.

The aim of this study was to screen the *in vitro* antioxidant effects of the total ethanol extracts from

wall-broken *G. lucidum* spore powder, thereby selecting the optimal extract to investigate the acute toxicity as well as the *in vivo* hepatoprotective effects on the model of cyclophosphamide-induced acute hepatic injury.

Materials and Methods

Medicinal materials

Spores of *G. lucidum* were provided by Linhchi Vina Joint Stock Company. The wall of these spores was then broken by autoclaving twice at a high temperature of 120°C for 30 min.

Chemical materials

The solvents, methanol (HPLC-grade), water (HPLC-water), acetonitrile (HPLC-grade) and ethanol were purchased from Merck, Germany. Ascorbic acid (The Institute of Drug Quality Control - Ho Chi Minh City, Viet Nam) and silymarin 70 mg (Legalon® 70 Protect, Madaus, Germany) were used as *in vitro* and *in vivo* reference drugs, respectively. The quantitative reagents of ALT (alanine aminotransferase) and AST (aspartate aminotransferase) were purchased from EliTech Clinical System, France. Cyclophosphamide (Endoxan 500) was purchased from Baxter Oncology GmbH, Germany; DPPH (2,2-Diphenyl-1-picrylhydrazyl), 5,5'-Dithio-bis-(2-nitrobenzoic acid), reduced L-glutathione (GSH), and thiobarbituric acid, 1,1,3,3-Tetramethoxypropane (MDA) were from Sigma-Aldrich, Germany.

Animals

Healthy Swiss albino mice of both sexes, weighing 25–30 g, were obtained from Institute of Vaccines and Biological Products – IVAC, Nha Trang and kept in the animals' house of the Separtment of Pharmacology, faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh city. The animals were acclimatized for at least 5 days before the study and were given standard pellet food (from IVAC, Nha Trang) and *ad libitum* water. The experiment was conducted according to the ethical norms approved by the Animal Ethics Committee of Nong Lam University, Ho Chi Minh city, Viet Nam (Number NLU-2112101).

Extraction procedure

100 g of wall-broken powder of *G. lucidum* spores was soaked by ethanol at different concentrations (50%, 70% and 96%) for a period of 24 h at room temperature. The mixture was then placed in an ultrasonic bath (Branson 3800, Danbury, USA) at 40 kHz for 2 h before filtering. The process was repeated 3 times; the solvent of pooled filtrate was then evaporated to obtain the various ethanolic extracts (Table 1).

Table 1: Extraction efficacy and humidity of ethanolic extracts from the wall-broken *G. lucidum* spores

Samples	Extraction efficacy (%)	Humidity (%)
96% ethanol extract	6.0	11.5
70% ethanol extract	7.5	12.0
i50% ethanol extract	4.2	16.0

G. lucidum: Ganoderma Lucidum.

Determination of triterpene composition of ethanolic extracts

The determination of triterpene composition was performed with the Agilent 1290 UHPLC system according to USP43–NF38 with slight modifications [7]. The separation was achieved by a HPLC-C_{18} column (150 × 2 mm; 3 μm) at 25°C. The mobile phase consisted of Solution A (aqueous phosphoric acid solution, 0.075%) and Solution B (acetonitrile) with a gradient elution program were presented in Table 2.

Table 2: Gradient elution program

Time (min)	Solution A (%)	Solution B (%)
0	80.0	20.0
3	73.5	26.5
34	73.5	26.5
52	61.5	38.5
53	80.0	20.0
58	80.0	20.0

A total of 0.15 g each extract was dissolved in a 10-mL volumetric flask with methanol, using an ultrasonic bath at room temperature, dilute with methanol to volume, and mixed well. The sample solutions were filtrated through a 0.45 μm membrane filter before being subjected to HPLC analysis. The UV absorption of the standards and samples was recorded in 271 nm. The relative retention time and relative response factor of each compound were presented in Table 3; thereby, the content of triterpenoid compounds in each extract was then calculated.

Table 3: The relative retention time and relative response factor of 10 triterpenoid compounds

Analyte	Relative retention time	Relative response factor
Ganoderenic acid C	0.36	0.51
Ganoderic acid C_2	0.42	1.05
Ganoderic acid G	0.56	1.18
Ganoderenic acid B	0.60	0.45
Ganoderic acid B	0.66	1.10
Ganoderic acid A	1.00	1.00
Ganoderic acid H	1.05	1.54
Ganoderenic acid D	1.25	0.51
Ganoderic acid D	1.33	1.08
Ganoderic acid H	1.54	1.45

Evaluation of in vitro antioxidant activity

A solution of 0.05 mg/ml DPPH was prepared in absolute ethanol immediately before using and stored in a dark bottle. Different ethanolic extracts of wall-broken *G. lucidum* spores and ascorbic acid were prepared in absolute ethanol at different concentrations by dilution method. The equivalent amounts of the *G. lucidum* extracts or ascorbic acid

and DPPH solution were then added to the test-tube. The mixture was shaken well and incubated for 45 min in dark at room temperature. Absorbance was determined at 515 nm using a spectrophotometer (UV-VIS Shimadzu). The free radical scavenging effect or inhibition percentage (I %) was calculated using the following formula:

$$I\ (\%) = \frac{A_c - A_t}{A_c} \times 100$$

Where: A_c: Absorbance of control sample with DPPH

A_t: Absorbance of test sample with DPPH

Each sample was repeated 3 times. From the curve showing the relationship between sample concentration and the inhibition percentage, the linear equations were extrapolated to determine the IC_{50} (effective concentration that scavenges 50% DPPH free radical). The extract having the highest antioxidant activity was further evaluated for the *in vivo* oral acute toxicity and hepatoprotective effect.

Oral acute toxicity testing

In this experiment, mice were deprived from food for the 12 h before oral administration of the studied extract. The promising extract of wall-broken *G. lucidum* spores was suspended in distilled water to the maximum concentration that could be aspired through a gavage needle. The obtained preparation was then orally administered to 10 mice (five female and five male mice) with the gavage volume of 10 ml/kg body weight [8]. The control group (five female and five male mice) was given an equal volume of water. The animals were closely monitored during the first 72 h, and occasionally thereafter, for 14 days for the toxic signs and symptoms (asthenia, hypoactivity, anorexia, diarrhea, and syncope) or the change in general behaviors of the mice. The number of dead mice was recorded, a concentration range of tested extract would be conducted to determine the lethal dose of 50% of the animals (LD_{50}).

Evaluation of hepatoprotective effects

Cyclophosphamide was dissolved in water for injection (Vidipha, Viet Nam) at the concentration of 17.5 mg/mL immediately before the injection in mice and was injected in mice at the volume of 10 mL/kg body weight; thus, the injected dose of cyclophosphamide in mice was 175 mg/kg body weight.

In physiological control group, mice were orally administered distilled water (10 mL/kg body weight) by gavage for 7 days, then they received through an intraperitoneal (i.p.) injection of the water for injection (10 mL/kg body weight) on 7th day. In untreated-group, mice received a single i. p. toxic dose of cyclophosphamide at the dose of 175 mg/kg. In pretreated groups, mice were daily pretreated by oral administration of the promising extract of wall-broken *G. lucidum* spores (20 mg/kg) and the reference drug Silymarin (100 mg/kg), respectively, for 7 consecutive days. On 7th day, 1 h after the last gavage of *G. lucidum* extract and Silymarin, mice were given a single i. p. injection of cyclophosphamide at the dose of 175 mg/kg. On the 8th day (24 h after cyclophosphamide administration), mice were sacrificed by CO_2 dry ice. Blood was then removed by cardiac puncture into the non-heparinized bottles for determination of serum enzyme activity (ALT and AST) by enzymatic kinetic method at 340 nm. The liver of mice was rapidly excised, separately washed 3 times in ice-cold 0.09% NaCl solution, blotted, and weighed. Each organ was then homogenized in ice-cold 1.15% KCl solution at 0–4°C using a warring blender. The resulting homogenate was used for qualification of different hepatic oxidative stress markers (MDA and GSH)

Statistical analysis

All data were presented as the mean ± standard error of mean. Statistical analysis was processed using Minitab 16.0, version 3.5. Mean values were assessed for significance by Student's t-test at $p < 0.05$. One-way ANOVA and Dunnett's method were used for multiple comparisons of data.

Results

Triterpenoid composition of ethanolic extracts

Ganoderic acid A and ganoderic acid D reached the highest proportion of content in all three ethanol extracts (Table 4). The content of ganoderic acid A was much higher in the 70% and 50% ethanol extracts while

Table 4: The triterpenoid compounds and total triterpenoid contents of the ethanolic extracts

Samples	Content in triterpenoid compounds (µg/g)										Total triterpenoid content (µg/g)
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
90% ethanol extract	0.55	0.78	-	1.84	6.69	11.03	-	0.45	32.68	1.62	55.64
70% ethanol extract	4.93	6.52	-	4.27	17.29	45.77	-	2.14	47.33	1.47	129.72
50% ethanol extract	9.21	9.81	-	4.71	16.70	45.31	-	1.96	43.93	2.19	133.82

(1): ganoderenic acid C; (2): Ganoderic acid C_2; (3): Ganoderic acid G; (4): Ganoderenic acid B; (5): Ganoderic acid B; (6): Ganoderic acid A; (7): Ganoderic acid H; (8): Ganoderenic acid D; (9): Ganoderic acid D; (10): Ganoderic acid F.

Table 5: Linear equations and IC_{50} values of *G. lucidum* extracts and ascorbic acid

Samples	Equations	IC_{50} (ppm)
90% ethanol extract	y = 0.014x + 0.064 R^2 = 0.996	3,493.8
70% ethanol extract	y = 0.048x + 14.878 R^2 = 0.993	737.8
50% ethanol extract	y = 0.029x + 2.144 R^2 = 0.993	1,728.2
Ascorbic acid	y = 2.805x + 2.438 R^2 = 0.998	16.96

G. lucidum: Ganoderma lucidum.

there was a slight difference of the content of ganoderic acid D between 3 extracts (Table 5). Ganoderic acid A is a marker compound when controlling the quality of *G. lucidum* according to USP43-NF38 [7].

Oral acute toxicity of 70% ethanol extract

The maximum concentration of the total 70% ethanol extract was of 200 mg/mL in distilled water. Therefore, the maximum dose of this total extract in the acute toxicity testing was 2000 mg/kg. No significant toxic signs and no death was reported during the 14-day observation period. The general behavior of mice was normal with no diarrhea and no urine discoloration. Mice gained weight steadily during 14 days of follow-up. Thus, the 70% ethanol extract at the dose of 2,000 mg/kg body weight is regarded as practically non-toxic.

Hepatoprotective effects of 70% ethanol extract

Activities of Hepatic Marker Enzymes

The effects of pretreatment with the total 70% ethanol extract and silymarin on elevation of the serum ALT and AST activities following cyclophosphamide injection are shown in Table 6. Serum ALT and AST activities were significantly increased in untreated mice relative to the physiological control animals ($p < 0.05$). Pre-treatment with the total extract and Silymarin for 7 consecutive days mitigated cyclophosphamide toxicity and was associated with decreasing serum ALT and AST activities. The serum ALT and AST activities of the cyclophosphamide group were 71.87 ± 2.40 and 245.76 ± 24.82 U/L, respectively, while the pretreated groups had significantly lower serum ALT and AST activities. Indeed, the serum ALT and AST activities in the total extract group declined to 51.74 ± 4.75 and 148.30 ± 46.75 IU/L, respectively, and comparable to those of silymarin pretreated group. The results indicate that pre-treatment of mice with the 70% ethanol extract of wall-broken *G. lucidum* spores prevented serum ALT and AST activities elevation following administration of cyclophosphamide.

Table 6: ALT and AST activities

Group (n = 7)	ALT (IU/L)	AST (IU/L)
Control	43.76 ± 1.83	76.73 ± 3.46
Untreated	71.87 ± 2.40*	245.76 ± 24.82*
Total extract	51.74 ± 4.75#	148.30 ± 46.75#
Silymarin	59.66 ± 3.03#	104.57 ± 9.01#

*p<0.05 relative to Control group, #p<0.05 relative to Untreated group. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase.

MDA and GSH levels

The *in vivo* antioxidant effect was assessed through the production of MDA and the total amount of GSH in the liver. MDA is a product of cell membrane peroxidation caused by free radicals; the higher the hepatic MDA level, the more severe the liver damage is. GSH is a free glutathione in a reduced form with a -SH group that protects the body against the oxidation of free radicals; the higher the hepatic GSH level, the more antioxidant, and liver-protective effects the sample has.

As shown in Table 7, the single intraperitoneal injection of cyclophosphamide caused serious oxidative stress by significantly increasing MDA and reducing GSH levels in the untreated group as compared to the physiological control group. The pre-treatment with the 70% ethanol extract as well as silymarin attenuated cyclophosphamide-induced MDA elevation ($p < 0.05$) and GSH depletion with respect to untreated group (Table 7). These findings support the *in vivo* antioxidant of 70% ethanol extract of wall-broken *G. lucidum* spores.

Table 7: Hepatic levels of MDA and GSH (nmol/g)

Groups	MDA (nmol/g)	GSH (nmol/g)
Control	73.85 ± 7.05	7.177.55 ± 196.69
Untreated	84.83 ± 7.94*	6.024.98 ± 371.45*
Silymarin	51.62 ± 5.32**#	10.385.88 ± 1453.62**#
Total Extract	58.69 ± 3.58**#	10.370.81 ± 2.267.96**#

*p<0.05 and ** P<0.01 relative to Control group, #p<0.05 relative to Untreated group.

Discussion

G. lucidum spores were proved to contain genes inherited from the fungus and contain active components with biological effects similar to those of the fruiting body, the most important of which are triterpenoid compounds and polysaccharides [9], [10], [11]. Various biological activity of *G. lucidum* spores was also elucidated, such as antioxidant activity [12], [13], anticancer activity [14], [15], and liver protection from damage caused by CCl_4 [16], [17] or cyclophosphamide [18]. Moreover, many triterpenoid compounds from the spores of *G. lucidum* have been isolated and determined [9], [19].

In this study, eight triterpenoid compounds in three ethanolic extracts from wall-broken *G. lucidum* spores were identified by HPLC analysis. Ganoderic A and D and the total triterpenoid content in 70% and 50% ethanol extracts were higher. The 50% ethanol extract was an effective solvent for the extraction of triterpenoid compounds. However, there were many other ingredients not having biological effect in this extraction such as polysaccharides. There were not only difficulties in the granulation process but also affecting the peak separation ability in HPLC analysis.

Moreover, in DPPH test, the 70% ethanol extracts showed the highest *in vitro* antioxidant activity. Therefore, the 70% ethanol extract was selected to investigate the safety and hepatoprotective efficacy in mice. The results of the acute toxicity test showed that this extract did not cause death in mice at the maximum dose of 2000 mg of extract/kg body weight (corresponding to 26.7 g of *G. lucidum* spore powder/kg body weight). This proves that the 70% ethanol extract from *G. lucidum* spore is safe in animals. Moreover, this extract also protects the liver from acute damage caused by cyclophosphamide. Indeed, liver injury was the most common regimen – related organ toxicity of cyclophosphamide in human and experimental animal [20]. Cyclophosphamide requires hepatic activation by microsomal cytochrome P450 system for its therapeutic action. However, the resulting metabolites themselves caused damage to liver tissues indicated by the increased liver enzymes (AST and ALT) in serum. These marker enzymes were significantly restored in animals intoxicated with cyclophosphamide and pretreated with the 70% ethanol extract suggest the hepatoprotective activity of *G. lucidum* spores. In addition, this extract also exhibited antioxidant effects *in vivo* through a decrease in MDA content and an increase in GSH content in hepatocytes. Recent studies have reported the formation of reactive oxygen species such as superoxide anion, hydroxyl radical, and hydrogen peroxide which cause lipid peroxidation of cellular membrane in mice intoxicated by cyclophosphamide [21]. During their oxidative metabolism, they depress the antioxidant defense mechanisms by the enhanced formation of MDA and the increased depletion of endogenous hepatic GSH [22].

These *in vitro* and *in vivo* results demonstrated that 70% ethanol extract from wall-broken spores of *G. lucidum* could be a promising candidate to use concomitantly as a supplement agent against hepatotoxicity of cyclophosphamide. Further study focused on the formulation of potential extract from wall-broken *G. lucidum* spores should be carried out, thereby performing clinical trials to evaluate the safety and effectiveness of the product in the treatment of acute liver injuries, especially in patients undergoing chemotherapy for cancer.

Conclusion

The results demonstrated the highest content of total triterpenoid in 50 % and 70% ethanol extracts, of which 70% ethanol extract exhibited the strongest *in vitro* antioxidant activity. This extract was also safe and protected the liver from acute damage caused by cyclophosphamide in experimental mice.

Acknowledgement

This work was financially supported by The Department of Science and Technology at Ho Chi Minh City, Vietnam. We greatly thank our colleagues from laboratories of the Pharmacology department and the Analytical Chemistry department, faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam for their helpful technical assistance.

Ethics approval

None to declare.

References

1. Li-Li Y, Zhi-Qin D, Wei-Yuan H, Na L, Li-Xin C, Lin-Yan Z. Protective effect of *Ganoderma lucidum* against sub-acute alcoholic hepatic injury in mice. Lishizhen Med Mater Med Res. 2013;24(3):513-4.
2. Liu MT, Chen LX, Zhao J. *Ganoderma* spore powder contains little triterpenoids. Chin Med. 2020;15(1):111. https://doi.org/10.1186/s13020-020-00391-1
   PMid:33062047
3. Fu YJ, Liu W, Zu YG, Shi XG, Liu ZG, Schwarz G, *et al.* Breaking the spores of the fungus *Ganoderma lucidum* by supercritical CO_2. Food Chem. 2009;112(1):71-6. https://doi.10.1016/j.foodchem.2008.05.044
4. Zhao D, Chang MW, Li JS, Suen W, Huang J. Investigation of ice assisted sonication on the microstructure and chemical quality of *Ganoderma lucidum* spores. J Food Sci. 2014;79(11):E2253-65. https://doi.org/10.1111/1750-3841.12681
   PMid:25319018
5. Ma J, Fu Z, Ma P, Su Y, Zhang Q. Breaking and characteristics of *Ganoderma Lucidum* spores by high speed entrifugal shearing pulverizer. J Wuhan Univ Technol Mater Sci Edit. 2007;22(4):617-21. https://doi.org/https://doi.org/10.1007/s11595-006-4617-6
6. Chaiyasut C, Kruatama C, Sirilun S. Breaking the spores of *Ganoderma lucidum* by fermentation with *Lactobacillus plantarum*. Afr J Biotechnol. 2010;9(43):7379-82.
7. United States Pharmacopoeia-National Formulation. *Ganoderma Lucidum* Fruiting Body Powder, USP43-NF38, Electronic Version; 2020.
8. OECD. Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure. OECD Guidelines for the Testing of Chemicals, Section 4. 2022; 1-27.
9. Ma B, Ren W, Zhou Y, Ma J, Ruan Y, Wen CN. Triterpenoids from the spores of *Ganoderma lucidum*. N Am J Med Sci. 2011;3(11):495-8. https://doi.org/10.4297/najms.2011.3495
   PMid:22361494
10. Xu J, Li P. Researches and application of *Ganoderma* spores powder. Adv Exp Med Biol. 2019;1181:157-86. https://doi.org/10.1007/978-981-13-9867-4_6
    PMid:31677143
11. Soares AA, de Sá-Nakanishi AB, Bracht A, da Costa SM, Koehnlein EA, de Souza CG, *et al.* Hepatoprotective effects of mushrooms. Molecules. 2013;18(7):7609-30. https://doi.

org/10.3390/molecules18077609
PMid:23884116

12. Mau JL, Lin HC, Chen CC. Antioxidant properties of several medicinal mushrooms. J Agric Food Chem. 2002;50(21):6072-7. https://doi.org/10.1021/jf0201273
PMid:12358482
13. Wachtel-Galor S, Choi SW, Benzie IF. Effect of *Ganoderma lucidum* on human DNA is dose dependent and mediated by hydrogen peroxide. Redox Rep. 2005;10(3):145-9. https://doi.org/10.1179/135100005X57355
PMid:16156953
14. Yuen JW, Gohel MD. Anticancer effects of *Ganoderma lucidum*: A review of scientific evidence. Nutr Cancer. 2005;53(1):11-7. https://doi.org/10.1207/s15327914nc5301_2
PMid:16351502
15. Zaidman BZ, Yassin M, Mahajna J, Wasser SP. Medicinal mushroom modulators of molecular targets as cancer therapeutics. Appl Microbiol Biotechnol. 2005;67(4):453-68. https://doi.org/10.1007/s00253-004-1787-z
PMid:15726350
16. Kim DH, Shim SB, Kim NJ, Jang IS. β-Glucuronidase-inhibitory activity and hepatoprotective effect of *Ganoderma lucidum*. Biol Pharm Bull. 1999;22(2):162-4. https://doi.org/10.1248/bpb.22.162
PMid:10077435
17. Lin JM, Lin CC, Chen MF, Ujiie T, Takada A. Radical scavenger and antihepatotoxic activity of *Ganoderma formosanum*, *Ganoderma lucidum* and *Ganoderma neo-japonicum*. J Ethnopharmacol. 1995;47(1):33-41. https://doi.org/10.1016/0378-8741(95)01251-8
PMid:7564419
18. Pham HN, Hoang LS, Phung VT, Shu TC. Hepatoprotective activity of *Ganoderma lucidum* (Curtis) P. Karst against cyclophosphamide-induced liver injury in mice. Cogent Biol. 2016;2(1):1267421. https://doi.org/10.1080/23312025.2016.1267421
19. Chen RY, Yu DQ. Studies on the triterpenoid constituents of the spores from *Ganoderma lucidum* Karst. J Chin Pharm Sci. 1993;2(2):91-6.
20. McDonald G, Frieze D. A problem-oriented approach to liver disease in oncology patients. Gut. 2008;57(7):987-1003. https://doi.org/10.1136/gut.2007.131136
PMid:18559388
21. Shokrzadeh M, Ahmadi A, Naghshvar F, Chabra A, Jafarinejhad M. Prophylactic efficacy of melatonin on cyclophosphamide-induced liver toxicity in mice. BioMed Res Int. 2014;2014:470425. https://doi.org/10.1155/2014/470425
PMid:25101283
22. Zhu H, Long MH, Wu J, Wang MM, Li XY, Shen H, *et al*. Ginseng alleviates cyclophosphamide-induced hepatotoxicity via reversing disordered homeostasis of glutathione and bile acid. Sci Rep. 2015;5:17536. https://doi.org/10.1038/srep17536
PMid:26625948