ISSN: 1813-1638

The Medical Journal of Tikrit University

Available online at: www.mjotu.com

Evaluation of the Effect of Reishi Mushroom `*Ganoderma lucidum*` on Antioxidant in Rats

Buthina A. Abdullah [(1)]
Maad A. Alfahad [(2)]
Husamuldeen S. M. S. Alnajar [(3)]
Takea S. A. [(4)]
Noor A. Abdullah [(5)]

(1) Dept. of Pharmacology, College of Veterinary Medicine, University of Tikrit.
Iraq
(2) Plants Protection, College of Agriculture, University of Tikrit.
Iraq
(3) Dept. of Pharmacology, College of Medicine, University of Tikrit.
Iraq
(4) Dept. of Basic Medical Sciences, Dentistry college, University of Tikrit.
Iraq
(5) Dept.of Zoonotic Dis, College of Veterinary Medicine, University of Baghdad.
Iraq

***Keywords*:**

Ganoderma lucidum, antioxidant, liver, cholesterol, tricholesterol, HDL, VLDL, LDL, glutathione, rat

ARTICLE INFO

Article history:

Received	03 Jul 2022
Accepted	25 Jul 2022
Available online	01 Sep 2022

ABSTRACT

There are many incidents that lead to oxidative stress resulting from reactive oxygen species. Many plants including fruits, vegetables and fungi are recognized as sources of natural antioxidants that can protect against oxidative stress such as mushrooms like, ***Ganoderma lucidum*** including many compounds can protect against oxidative stress. In this study we aimed to investigate the antioxidant effects of Ganoderma lucidum (Red Reishi Mushroom) on liver histologically by light microscopy, measurement of some biochemical parameters (**cholesterol, tricholesterol , HDL,VLDL, LDL and glutathione)**. We use 20 male rats weighing around 200-250g , which were divided into 4 groups. The first group as a control group 1 didn't receive any medication and gained free access to food and water. The second group received hydrogen peroxide H_2O_2. The third group received vitamin C + H_2O_2. The fourth group received Ganoderma lucidum + H_2O_2 for 30 days. After experiment, we analyze the results (histopathological sections of liver and detection of biochemical parameters such as **cholesterol, tricholesterol ,HDL,VLDL.LDL and glutathione**) to reveal the effects of Ganoderma lucidum and antioxidant compared with the other groups with duration of exposure.

DOI: http://dx.doi.org/10.25130/mjotu.28.2022.15

**Corresponding author E mail :* Sbc.s4@tu.edu.iq

Introduction:

Oxidative stress is an imbalance in the production of free radicals. Free radicals can chemically interact with cell components such as lipids, proteins and DNA. Therefore, oxidative stress also has a useful part in physiologic acclimation and in the regulation of intracellular signal transduction. Oxidative stress leads to many pathophysiological conditions in the body. It is not only causes harmful events such as lipid peroxidation and oxidative DNA damage, also physiologic acclimation phenomena and regulation of intracellular signal transduction(Toshikazu, Yuji 2002,271).

Antioxidants control oxidative homeostasis, therefore it concede protection mechanisms . Numerous synthetic antioxidants can effectively progress protection mechanisms, like dietary intake (Maja, *et. al* 2015, 1420). The reports of the *National Health and Medical Research 2009* have said (National Health and Medical Research Council ,2009) "There are many types of antioxidants, like vitamins (eg. vitamins C, E), minerals (eg. selenium, copper) and a range of non-nutrient compounds (eg. carotenoids, bioflavonoids, phenolic). The body produces its reign antioxidant compounds, such as superoxide dismutase, catalase and glutathione peroxidase, while benefiting from the further antioxidants provided in the diet, but because of their harmful toxic effects under certain conditions, preference is given to natural compounds. Alternative sources of antioxidant foods are mushrooms. Also the mechanism of action involved in their antioxidant properties, have increased rapidly.

Antioxidant factors, including CAT, SOD, GPx, GSH, and TAC, comprise the first line of defense against oxidative damage. SOD is a sensitive marker of liver damage, The most important detoxifying systems for peroxides include GSH and CAT in hepatic cells. Therefore, reduction of

these factors may produce some negative effects, given the accumulation of H_2O_2 and superoxide radicals. (Roghani et al., 2020,1933)

Several researchers have pointed out the long history of traditional medicinal uses of mushrooms, especially G. lucidum, mostly in Far East countries, dating back more than 4000 years . For a long time, G. lucidum has been used as a traditional medicine for treating neurasthenia, debility of prolonged illness, insomnia, anorexia, dizziness, chronic hepatitis, hypercholesterolemia, mushroom poisoning, coronary heart disease, hypertension, prevention of acute mountain sickness. These studies have continued until the present via a series of exciting discoveries related to the biological activities of Ganoderma lucidum, including antitumor and anti-inflammatory effects, as well as cytotoxicity to hepatoma cells. (Loyd, et al., 2018, 1557).

Chemical composition and antioxidant potential of mushrooms have been intensively studied. Edible mushrooms might be used directly in enhancement of antioxidant defenses through dietary supplementation to reduce the level of oxidative stress. Mushrooms also have the ability for antioxidant compounds to protect the body from ill-health. As mushrooms reside in their own biological kingdom, there is great interest in their antioxidant profile. Mushrooms have the ability to complement the benefits of antioxidants found in plant foods. Significant antioxidant activities *in vitro* have been reported in several varieties of mushrooms, with one study reporting antioxidant capacity comparable to vitamin C. Reishi mushroom has been greatly present in Japanese culture where it is investigated to be the most important of all the Japanese medicinal polypores [sarunoko shikake] (Matsumoto,1979,63). The reishi mushroom *Ganoderma lucidum* is classified within the family Ganodermaceae of Polyporales which show hard fruiting bodies (Boh,2007,265), *Ganoderma lucidum* have medicinal properties like strengthened immune response,

enhanced cancer-fighting capacities, and improved renal health (Leskosek et al.,2010,2262)(Kuo,2006,217). These researchers have confirmed "Mushrooms have attracted attention as a commercial source of antioxidants". They might be used directly in enhancement of antioxidant defenses through dietary supplementation to reduce the level of oxidative stress (Ferreira et al ,2009,1543)(Kozarski et al ,2014,305).

Material and method :

Animal Management

A total of 20 mature male mice (age 8 weeks) were used in this experiment for 30 days. The male mice were housed in an animal house at Veterinary College / Tikrit University. They were housed in polypropylene cages with paddy husk bedding under standard laboratory conditions (28±1°C temperature and 50±5% humidity). All animals feed on balanced food (Purina-Peru), water ad libitum and a photoperiod of roughly 14/10 hours (light / dark).

Drug

Preparation of Mushroom :- Buy directly from the DXN company market, prepared in Malaysia . Reishi Gano (RG) is formulated from Ganoderma lucidum. Cultivated in an organic accredited farm.

Experimental Design

The experimental trial done for 20 rats, they were divided into 4 groups (5 rats per group). The first group "Control group (I); didn't receive any medication and gained free access to food and water. The second group(II) received hydrogen peroxide H_2O_2. The third group(III) received Vitamin C 0.03gm\day + H_2O_2. The fourth group(IV) received a supplemented single dose of mushroom (0.03gm) + H_2O_2 for 30 days.

Histopathological Examination

According to Bancroft and Gamble 2002 (Bancroft,2002), the animals were sacrificed post month withdrawal of treatments, one centimeter cubes from liver were taken and fixed in 10% buffered formalin, dehydrated in ascending concentrations of ethanol and cleared in xylene followed by embedding in paraffin. Sections (5 μm) were prepared from each tissue block and stained with hematoxylin-eosin stain (H&E) for histological examination as described previously.

Results:

1-In the control group, we can see the structure of liver, the central vein, and the hepatocytes were arranged in radical form and are a normal size and shape. Also the sinusoid and the Kupffer cells appeared normal in size. As seen in figures 1 A & B.

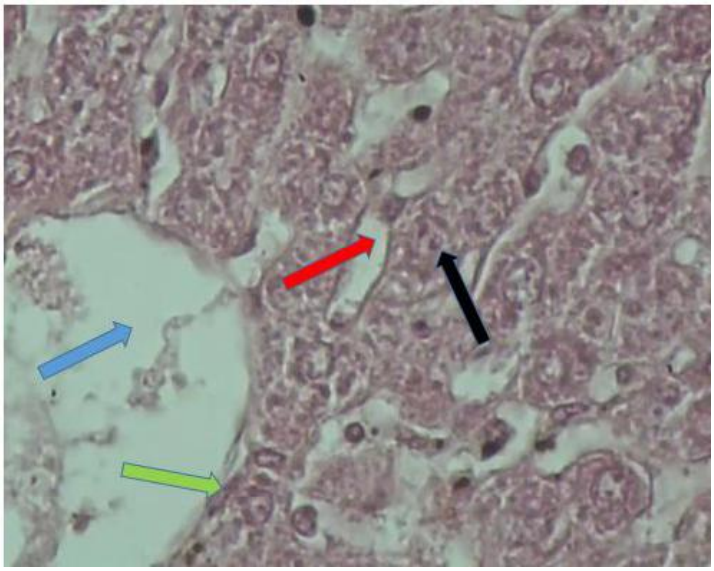

Figure (1A) : Photomicrograph of rats liver from control group 1 showing normal central vein (Blue arrow) , normal sinusoids (Red arrow) , normal hepatocyte cords (Black arrow) and kupffer cells (Green arrow) . Stain H&E . Magnification 100 X .

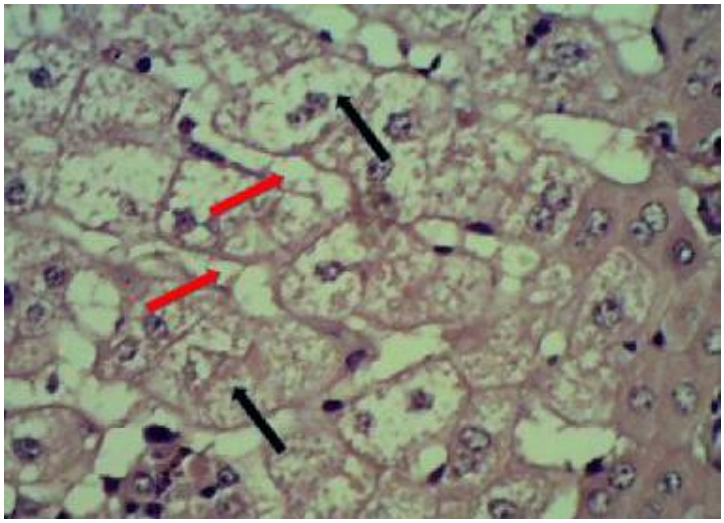

Figure (1B) : Photomicrograph of rats liver from control group 1 showing , normal sinusoids (Red arrow) , normal hepatocyte cords (Black arrow) . Stain H&E . Magnification 100 X .

2- The second group received H_2O_2. As seen in figures 2 (A & B), the parenchyma of the liver was continuing atrophied hepatocytes with degenerative process of other cells, containing Karyohexis of nuclei, the central veins were congested with blood and the sinusoid appeared without blood , kupffer cells appeared in the lumen of these sinusoid.

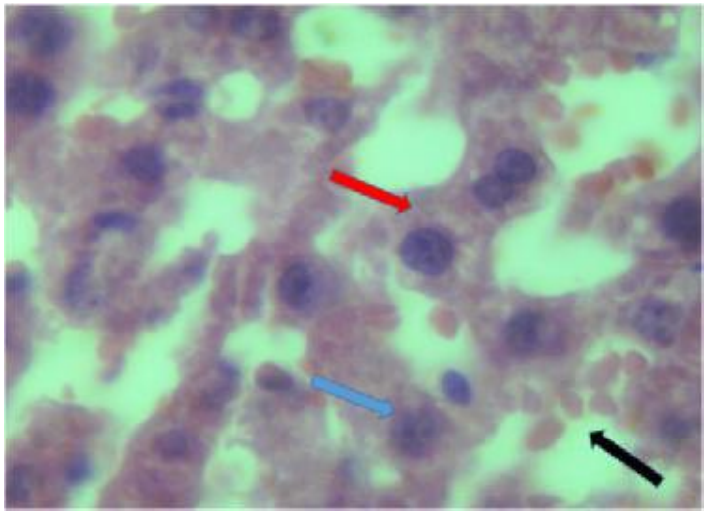

Figure 2(A) : Photomicrograph of rats liver from group II showing degenerative changes in hepatocytes (red arrow) , Distended and congested sinusoids (Black arrow) , karyorehxis in nucleus (Blue arrow) . Stain H&E . Magnification 400 X .

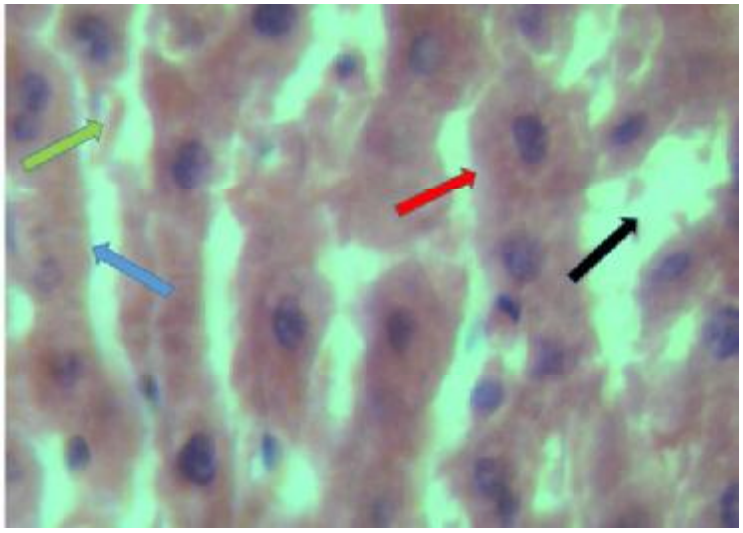

Figure 2 (B) : Photomicrograph of rats liver from group II showing degenerative changes in hepatocytes (red arrow) , Distended sinusoids (Black arrow) , atrophied hepatocyte (Blue arrow) . subsinusoidal kupffer cell (green arrow) .Stain H&E . Magnification 400 X .

3-The third group III received 0.03g of vitamin C+H_2O_2. As shown in figures 3 (A & B) the liver is in a normal condition. It's cytoplasm was acidophilic. The nucleus is a spherical-shaped in its cytoplasm . The sinusoid-like structures and Kupffer-like cells were observed between liver cell cords. (figures 3 A&B).

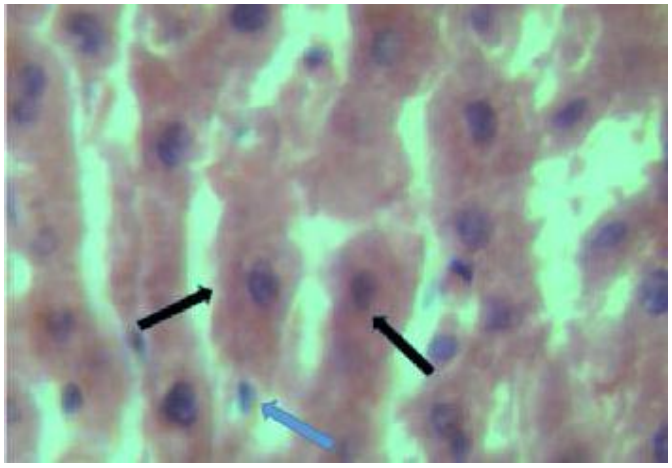

Figure 3 (A)

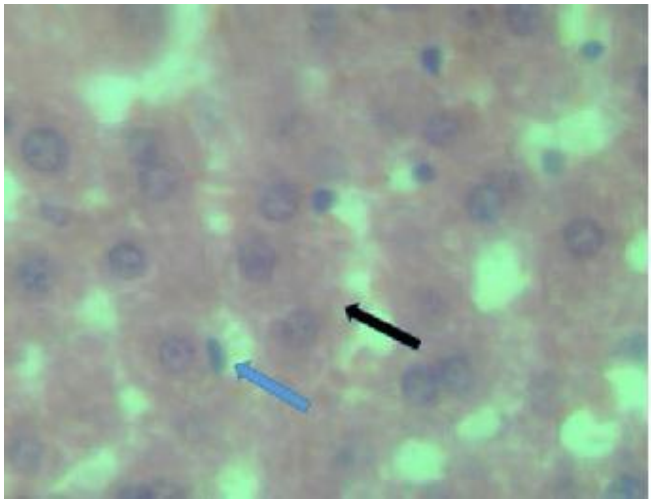

Figure 3 (B)

Figures 3(A&B):- We analyzed the liver cells in the group that received vitamin C. As seen in figures 3 (A&B) the black arrow shows normal shape and size. The blue arrow shows sinusoid-like structures with kupffer. Stain H&E . Magnification 400 X .

4- The fourth group IV received 0.03 g of mushroom Reishi +H_2O_2. The liver is in a normal condition as seen in figures 4 (A & B).

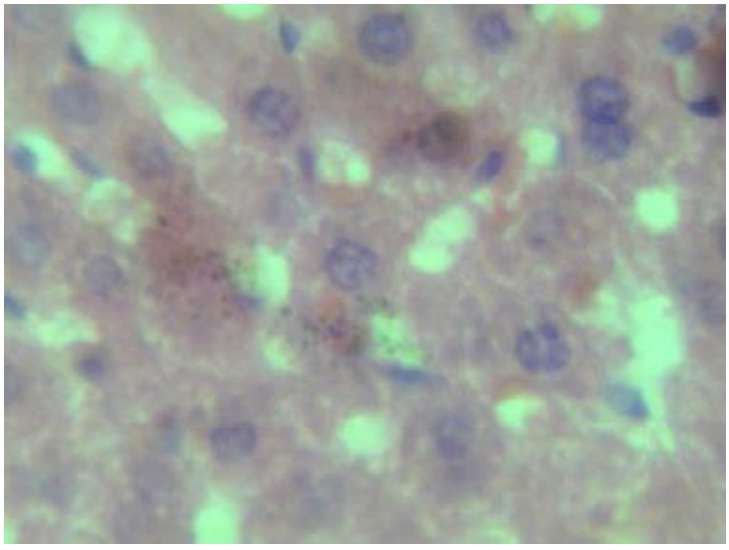

Figure (4A)

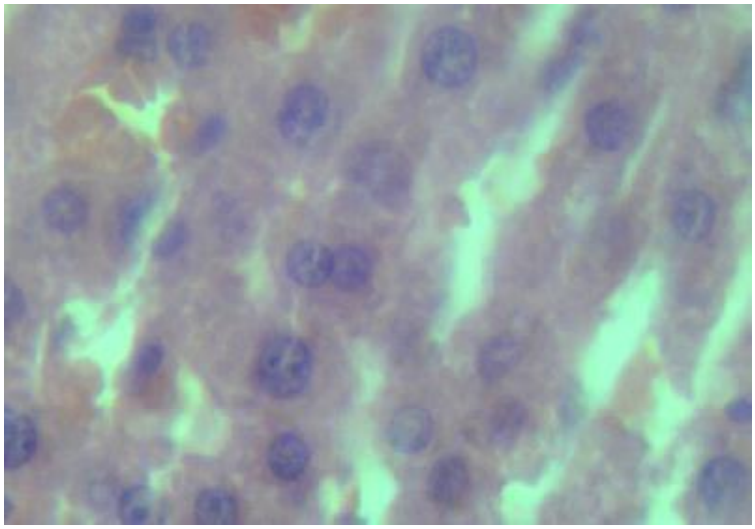

Figure (4B)

Figures 4 (A&B):- The fourth group received 0.03 g of mushroom Reishi +H_2O_2 . The liver is in a normal condition. Stain H&E . Magnification 400 X .

The table shows some biochemical parameter such as (cholesterol, tricholesterol, HDL,VLDL, LDL and glutathione). After the experiment, we observed no significant changes the control group and the vitamin C group. A statistically significant change was observed in the Ganoderma Lucidum groups after the experiment. In this study, the fourth group revealed a significant decrease in Cholesterol, Tricholosterols , VLDL, LDL and significant increase in HDL and Glutathione if compared with the second group that received H_2O_2 (($p<0.01$-Table 1).

Table 1:- Comparative values among all groups. The fourth group that used Ganoderma Lucidum, had a significant decrease in Cholesterol, Tricholosterols , VLDL, LDL and significant increase HDL, Glutathione compared with the second group that received H_2O_2.

Control Group I	**H_2O_2 Group II**	**Vit. C +H_2O_2 Group III**	**Mushroom +H_2O_2 Group IV**	**Groups / parameters**
156.21±29.26	**207.21±20.26**	**152.2±11.17**	**133.21±20.26**	**Cholesterol (mg/dl)**

119.22±21.0*	**189.21±30.17***	**119.83±29.21**	**124.01±12.0***	**Tricholosterols (mg/dl)**
59.3±12.2*	**38.02±10.06***	**49.76±8.67**	**58.02±12.06***	**HDL (mg/dl)**
28.1±2.17*	**32.3±2.37***	**22.33±1.65**	**30.1±2.17***	**VLDL (mg/dl)**
98.21±24.12	**125.21±24.12**	**99.09±12.65**	**95.21±24.12**	**LDL (mg/dl)**
***4.85±0.7**	***1.63±1.65**	**3.83±1.6**	***4.13±1.65**	**Glutathione**

***Values are statistically significant at $p \leq 0.01$, T-TEST**

Discussion:

Oxidative stress leads to an imbalanced metabolism and an increase of reactive oxygen species (ROS). This causes a range of health trouble in humans. Their compounds in the diet that show to crush the oxidation of molecules in the body that is called "Antioxidants" . Antioxidant has the ability to neutralize the effects of ROS. This helps to protect the body from damage of the free radical. As reported by the research council (National Health and Medical Research Council ,2009).

The ability for antioxidant compounds to protect the body from ill-health seems to work best when antioxidants are provided in the amounts naturally found in a healthy diet rather than through supplementation. All major health authorities promote the consumption of a range of fruit and vegetables, including mushrooms have the ability to complement the benefits of antioxidants found in plant foods. Significant antioxidant activities in vitro have been reported in several varieties of mushrooms, with one study reporting antioxidant capacity comparable to vitamin C. Many studies like Mau et al., 2001 found that mushrooms are commonly used for pharmaceutical purposes and as health foods. They contain the nutritional values (Mau et al,2001,521).

Previous studies have reported a protective effect of *Ganoderma lucidum*. For example: Boh B, et.al, 2007 reported (Boh,2007,265) they were found that homogenates of pancreas had higher lipid peroxidation

products in alloxan-treated mice than in animals treated with *Ganoderma lucidum* polysaccharides while Jia et al., 2009 (Jia,2009,32) demonstrated in their study with diabetic rats that orally administrated *Ganoderma lucidum* ameliorates hyperglycemia normalizing plasma sugar level as well as liver status that was compromised by oxidative damage. Such promising antioxidant properties and diabetes-mitigation effects may open new avenues in the treatment of diabetes and its complications. Jeng et.al., 2002 (Jeng,2002,6072), they demonstrated that the major naturally occurring antioxidant components found in methanolic extracts from medicinal mushrooms, were higher in antioxidant activity, reducing power, scavenging and chelating abilities, and total phenol content. *Ganoderma lucidum* contain active pharmaceutical compounds as the triterpenoids and polysaccharides (Boh,2007,265) .

As explained by Ruan W,et.al.2012 (Ruan,2012,203) "Triterpenoids extracted from *Ganoderma lucidum* are reported to be responsible for many of the pharmaceutical properties of the fungus. Thus far, hundreds of triterpenoids have been isolated in *Ganoderma lucidum* and many more are likely to be discovered in the future. Two major types of triterpenoids are ganoderic acids (C30) and lucidenic acids (C27), with the total triterpenoid content in *Ganoderma lucidum* ranging from 0.6 to 11 mg/g of dry powder. These triterpenoids were reported to mitigate diabetes and regulate inflammatory pathways in cell culture. Triterpenoids from *Ganoderma lucidum* also possess significant chemo-therapeutic potential and exhibit cytotoxic effects on colon carcinoma cells. Moreover, it has been reported that its activities affect tumor growth and metastasis in a triterpenoid fraction of *Ganoderma lucidum.*

References :-

- Bancroft, J.D. ; and Gamble, M. 2002 . Theory and Practice of Histological Technique. (5th edition), Churchill Livingstone,

Edinburg, London.

- Boh, B, Berovic, M, Zhang J,; Zhi-Bin, L; 2007 *Ganoderma lucidum* and its pharmaceutically active compounds. In: Biotechnology Annual Review. Elsevierp. 265-301.
- Ferreira, Isabel C.F.R.; Barros, Lillian; Abreu, Rui M.V. 2009 Antioxidants in wild mushrooms. *Curr. Med. Chem.*, *16*, 1543–1560.
- Jeng E.L. Hsiu CH.L. and Chin – CH. CH.2002, Antioxidant Properties of Several Medicinal Mushrooms, *J. Agric. Food Chem.* 2002, *50*, 6072-6077.
- Jia J, Zhang X, Hu Y, Wu Y, Wang Q, Li N, et al. Evaluation of in vivo antioxidant activities of Ganoderma lucidum polysaccharides in STZ-diabetic rats. Food Chem. 2009 7/1;115(1):32-6.
- Kozarski, M.S.; Klaus, A.S.; Niksic, M.P.; van Griensven, L.J.L.D.; Vrvic, M.M.; Jakovljevic, D.M. 2014,Polysaccharides of higher fungi: Biological role, structure and antioxidative activity. *Chem. Ind. 68*, 305–320.
- Kuo, M,; Weng, C, Ha C, Wu M. 2006 *Ganoderma lucidum* mycelia enhance innate immunity by activating NF-κB. J Ethnopharmacol. 1/16;103(2):217-22.
- Leskosek-Cukalovic I, Despotovic, S, Lakic N, Niksic M, Nedovic V, Tesevic V. 2010 *Ganoderma lucidum* — medical mushroom as a raw material for beer with enhanced functional properties. Food Res Int. 11;43(9):2262-9.
- Loyd, A.L.; Richter, B.S.; Jusino, M.A.; Truong, C.; Smith, M.E.; Blanchette, R.A.; Smith, J.A. 2018. Identifying the “mushroom of immortality”: Assessing the Ganoderma species composition in commercial Reishi products. Front. Microbiol., 9, 1557.
- Maja Kozarski, Anita Klaus, Dragica Jakovljevic, Nina

Todorovic, Jovana Vunduk, Predrag Petrović, Miomir Niksic, Miroslav M. Vrvic, and Leo van Griensven, 2015. Antioxidants of Edible Mushrooms. Molecules, ISSN 1420-3049.

- Matsumoto K. 1979. The Mysterious Reishi Mushroom. Santa Barbara: Woodbridge. p 63 .
- Mau, J.-L.; Lin, H.-C.; Chen, C.-C. 2001 Non-volatile components of several medicinal mushrooms. *Food Res. Int.*, *34*, 521-526.
- National Health and Medical Research Council. 2009 Australian Guidelines to Reduce Health Risks from Drinking Alcohol. Canberra: National Health and Medical Research Council.
- oghani M, Kalantari H, Khodayar MJ, Khorsandi L, Kalantar M, Goudarzi M, Kalantar H. 2020. Alleviation of Liver Dysfunction, Oxidative Stress and Inflammation Underlies the Protective Effect of Ferulic Acid in Methotrexate-Induced Hepatotoxicity. Drug Des Devel Ther;14:1933-1941. doi: 10.2147/DDDT.S237107. PMID: 32546960; PMCID: PMC7250701.
- Ruan W, Popovich DG. 2012 *Ganoderma lucidum* triterpenoid extract induces apoptosis in human colon carcinoma cells (caco-2). Biomedicine & Preventive Nutrition. 0;2(3):203-9.
- Toshikazu Yoshikawa and Yuji Naito, 2002. What Is Oxidative Stress? Journal of the Japan Medical Association JMAJ 45(7): 271–276.