How to Cite:
Alkenany, M. R., & Khalil, L. W. (2022). The effect of Shiitake mushroom derived Lentinan (β-glucan) administration on some hematological and biochemical parameters in intact rabbits. *International Journal of Health Sciences*, *6*(S3), 5845–5853.
https://doi.org/10.53730/ijhs.v6nS3.7276

The effect of Shiitake mushroom derived Lentinan (β-glucan) administration on some hematological and biochemical parameters in intact rabbits

Muhammad Riyadh Alkenany
PhD candidate, MSc, BVMS, Dept. of Physiology &Pharmacology/College of Veterinary Medicine/University of Baghdad-Iraq | Al-Zahrawi University college,
Scopus affiliation ID: 122473929
*Corresponding author email: Drmr1.alkenany@gmail.com

Luma Waleed Khalil
PhD, MSc, BVMS, Dept. of Physiology &Pharmacology/College of Veterinary Medicine/University of Baghdad-Iraq
Email: Waleedluma@covm.uobaghdad.edu.iq

***Abstract*---**This study was conducted to investigate the nutritional effect of shitake mushroom extract (Lentinan) on some hematological and biochemical parameters in healthy female rabbits. The study included ourteen rabbits at age of (7-8) weeks. After acclimatization, animals were divided into two equal groups and as follows: first group (G1) included seven intact rabbits fed with food and distilled water (IDW) ad libitum,. The second group (G2) or (IL) included seven rabbits that were supplemented with Lentinan (10mg/kg/BW) daily and orally. The results of this study showed that the effect of supplementation the animals with mushroom extract (Lentinan) at a dose 10mg/kg/BW on G2 (IL) cause a significant increase (P≤0.05) in platelets counts but there were no significant (P≤0.05) change appeared on red blood cells. The results of experiment also confirmed the protective role of mushroom extract (Lentinan) against oxidative stress through the significant increase (P≤0.05) in the activity of glutathione peroxidase (GSH-PX) and decrease in concentration of peroxynitrite radical (ONOO-) in treatment groups (IL) as compared with control group(IDW). On the other hand, the results showed that there were no significant change in the activity levels of liver enzymes that included in this study which are Alanine transaminase (ALT) and Aspartate transaminase (AST) between the both groups (IDW) and (IL). Nevertheless, the supplementation with Lentanin had no clear

Manuscript submitted: 9 March 2022, Manuscript revised: 18 April 2022, Accepted for publication: 1 May 2022

damaging effect at the genetic level, which was monitored using DNA fragmentation test.

***Keywords*---**Lentinan, β-glucan, liver, enzyme, DNA.

Introduction

Lentinan is a polysaccharide isolated from the fruit body of shiitake mushroom (Lentinula edodes mycelium). Lentinan is a glucohexose-based fungal β-glucan derived from Lentinus edodes, a common edible mushroom, which was shown to be active against several different allogeneic and syngeneic tumors[1]. Lentinula edodes has been used in Asia for thousands of years to improve general health. It is the world's second most cultivated and popular edible mushroom, known in China as "Xianggu" and in Japan as "Shiitake." Lentinan is a polysaccharide found in the Lentinula edodes Lentinan's main bioactive component, -glucan, has an immunostimulatory effect[2]. It improves the host body balance, restores, and improves the reactivity of host cells to lymph, hormones, and other physical activity factors by stimulating immune cell maturation, differentiation, and proliferation[3]. Immune function is reduced in late high-intensity exercise training athletes (especially endurance athletes), making them susceptible to infectious diseases[4]. Lentinan, prepared from *Lentinus edodes* (Shiitake mushroom), has been reported to exhibit anti-coagulant, anti-viral, anti-cancer, antitumor, and anti-coagulant effects. [5].

Reactive oxygen species (ROS) include radical and non-radical oxygen species formed by partial reduction of oxygen, such as superoxide anion (O2), hydrogen peroxide (H2O2), and hydroxyl radical (HO•). Cellular ROS are produced either endogenously, such as during mitochondrial oxidative phosphorylation, or exogenously, such as through interactions with xenobiotic compounds. Oxidative stress occurs when ROS overwhelm the cellular antioxidant defense system, whether due to an increase in ROS levels or a decrease in cellular antioxidant capacity. ROS-mediated damage to nucleic acids, proteins, and lipids occurs as a result of oxidative stress, which has been linked to carcinogenesis. [6], neurodegeneration [7,8], atherosclerosis, diabetes [9], and aging [10] and metabolic disorders[11]. The role of ROS in the pathogenesis of disease states, however, is not limited to macromolecular damage. There is mounting evidence that ROS signaling plays a role in disease. [12]. Some studies used vitamins or minerals to ameliorating the deleterious effect of oxidative stress like vitamin c and zinc[13]. In the current study, Lentinan which is macrofungus extracted from *shitake* mushroom was used to know its effect on some oxidative and antioxidant parameters, but there are some studies that confirm the relationship of Lentinan to the oxidative state in the body. In human immortalized keratinocytes, Lentinan (LNT) has been shown to have significant protective and restorative effects against H2O2-induced oxidative damage in some studies (HaCaT cells). In HaCaT keratinocytes, Benzo(a)pyrene(BaP) caused the formation of reactive oxygen species (ROS). The presence of LNT in addition to BaP significantly reduced the production of ROS caused by the latter. The presence of LNT in addition to BaP, on the other hand, strongly inhibited ROS production due to the latter[14].

Design of experiment

Design of experiment to achieve the aim which is the role of *Shiitake* mushroom derived **Lentinan** on some biochemical and hematological parameters of intact female rabbits. Fourteen intact female rabbits aged 7-8 weeks and weighing 850-1100 gram (g) were divided into two groups(figure 1:graphical abstract) and treated as follows :

- Group one (G1)-control: seven intact rabbits were received distilled water daily and orally.
- Group two (G2)- Lentinan: seven intact rabbits were received (10 mg/Kg B.W) *Shiitake* mushroom derived Lentinan component daily and orally

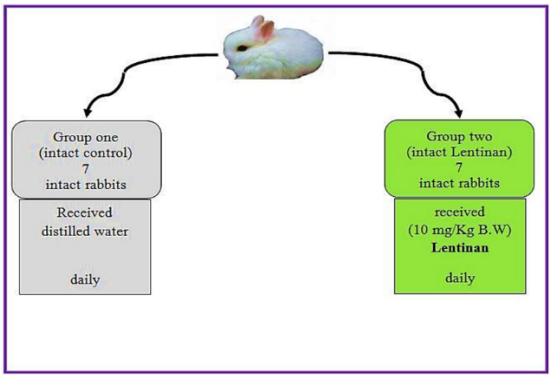

Figure 1. Graphical abstract of experimental design.

Preparation of Lentinan dose

Lentinan (β-glucan) could be found as a powder and administered orally with the dose of 10 mg/kg/B.w for each rabbit according to (McCormack *et al* ,. 2013) by dissolving 10 mg of Lentinan powder in one milliliter of distilled water, and well mixed then was given as 1 ml/kg B.W for each rabbit of G2 and G4 daily by gastric intubation for sixty days.

Figure 2. Powder of *Shiitake* mushroom derived Lentinan (β-glucan)

Blood samples collection

At the end of period (60 day) of the experiment, animals were prepared for blood samples collection. Blood was obtained from the heart directly from each rabbit using disposable syringes. Samples were divided into two divisions, first part is collected by vacuum anticoagulant tube for blood parameters, and the second part placed into vacuum tubes (gel tube) and allowed to clot. Serum was isolated after centrifugation at a speed of 5000 revolution/minute (rpm) for 5 minutes, and then serum samples were stored in freezer at -18 C° until use.

Parameters determination

Red blood cells count and Platelets count was measured manually[15]by using hemocytometer slide (neubauer slide). Glutathione peroxidase activity (GPX) was measured using a special kit (Bio Assay Systems) by the quantitative colorimetric glutathione peroxidase determination[16]. The method described by Beckman *et al* (1992) was used to Determination of serum Peroxynitrite radicals (ONOO-) concentration (MM/L) [17]. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) activity, these two enzymes were measured by using the kit manufactured by SYRBIO/Switzerland. Lastly, alkaline gel electrophoresis assay method as described by Zirkle and Krieg (1996), was used to detect DNA strand breaks[18].

Statistical analysis

The statistical analysis of the data of the experiment was measured by using the SAS (Statistical Analysis System - version 9.1), Using of T- test and p-value were performed to assess significant differences among means of the groups.The results were expressed as mean ± stander errors and $P < 0.05$ was considered statistically significant[19].

Results

The results represented in table (1) reveals the effect of oral supplementation of *Shiitake* mushroom derived Lentinan (β-GLUCAN) (10 mg/kg B.W) on rabbits. There were significant ($P \leq 0.05$) increase in platelet counts in group two (G2) that supplemented with Lentinan in comparison with control group (G1) that received distilled water. From the other hand, this table shows also that there is non-significant ($P \leq 0.05$) change in red blood cell counts between both groups of this experiment. Table (2) concerning the mean values of serum Glutathione peroxidase (GSH-PX) activity and peroxynitrite concentration(mmol/L) in intact rabbits in response to supplementation with Lentinan (β-glucan) (10 mg/kg B.W). There were a significant ($P \leq 0.05$) increase in (GSH-PX) activity in treated group with Lentinan (G2) in comparison with group that received distilled water (G1). At the same time, this table shows that the intact group(G2) reveals a significant ($P \leq 0.05$) decrease in the Peroxynitrite concentration as compared to rabbits of (G1) which received distilled water.

The effect of oral administration of *Shiitake* mushroom derived Lentinan on both alanine transaminase (ALT) and aspartate transaminase(AST) activity in intact

rabbits is illustrated in table (3). The table shows non-significant (P≤0.05) change in ALT value between control group (G1) as compared to Lentinan group (G2). Moreover, the supplementation with Lentinan also caused no significant (P≥0.05) change in AST between both groups (G1 and G2). The figure-3 represents the results which revealed the effect of oral Lentinan on DNA strand breaks in intact rabbits. The results of this marker showed no significant differences appeared among both group of this study (G1, G2) after 60 days of supplementation with distilled water and Lentinan respectively.

As shown in present study there is significant increase in platelet count in group that administered with Lentinan as compared to control group. These findings were consistent with several experimental studies in vivo have shown that β-glucans administered orally, intravenously or enterally enhance hematopoietic regeneration without side effects[20][21][22]. In current study there is a significant increase in (GSH-PX) activity while decrease in the Peroxynitrite concentration in treated group with Lentinan in comparison with group that received distilled water The current study agree with Yu et al., (2009) which found that L. edodes promote the production of antioxidant enzymes, especially GSH-Px.[23] This study found that edible and medicinal mushrooms can stimulate the activities of antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase, and catalase, to prevent oxidative stress and fatigue. SOD is a natural enzyme that can neutralize specifically superoxide radicals by utilizing different positively charged metal ions like copper or zinc. GSH-Px is also a natural antioxidant enzyme which can scavenge and inactivate hydroxyl radicals. Glutathione reductase and GSH-Px make up the glutathione system of antioxidant enzymes to reduce lipid hydroperoxides and free hydrogen peroxides. Catalase is an antioxidant enzyme working with SOD to prevent free radical damage. SOD converts superoxide radicals to hydrogen peroxides and catalase converts them into harmless water and oxygen .[23]

The antioxidant and anti- inflammatory properties of shiitake are related to its ability to trap reactive oxygen/nitrogen species and inflammatory mediators such as hydroxyl radicals, hypochlorous acid and peroxynitrite (ONOO–) which is formed endogenously by reaction of nitric oxide (NO) with superoxide (O2–). Another antioxidative attribute of shiitake is the intermediate value of the standard redox potential of the thiol/disulfide couple (–0.06V) compared with other naturally occurring thiols (from –0.2 to –0.32V). This property confers greater stability under physiological conditions, hence shiitake does not readily undergo auto-oxidation as rapidly as other antioxidant thiols such as glutathione or lipoic acid which can generate hydrogen peroxide (H2O2) in the process.[24]

The production of cytokines is not directly induced by H2O2 and NO but by thiol compounds, mainly glutathione. The ratio of reduced (GSH) to oxidized glutathione (GSSG) plays an essential role in regulating thiol-dependant signalling pathways. Exercise-induced changes in thiol redox status lead to conformational changes in transcriptional factors, release of inhibitory subunits, or promotion of protein complex formations which are necessary for signal transduction or transcription to proceed.[25] However, there have been some reports of shiitake protective effects in erythrocytes and peripheral blood mononuclear cells.[26] Its known that the ROX affect the genomic material and can cause significant DNA

damage[27]. Therefore, it is logical that the use of antioxidants gives an efficient protective effect to the genetic material against the deleterious and destroying effects of oxidative stress (ROS) on the DNA. Some research has proven the effect of antioxidants as food additives that protect cells from the risk of genetic damage due to oxidative stress.[13,28]

Table 1
The effect of *Shiitake* mushroom derived Lentinan(β-GLUCAN) on Red blood cell counts (RBC nx10^6 cells/mm^3) in intact rabbits

Groups / Parameter	G1 Intact rabbits Received distilled water (IW)	G2 Intact rabbits Received Lentinan (IL)	p-value
Red blood cell counts (N×10^6cells/mm^3).	5.35 ± 0.26 A	5.48 ± 0.27 A	0.38
Platelet counts (N×10^3 cells/mm^3).	180.42 ± 1.26 B	316.42 ± 6.43 A	<0.0001

Values represent mean(N=7) ± SE. Different capital letters in the same row denote a significantly difference between groups (p≤0.05).

Table 2
The effect of *Shiitake* mushroom derived Lentinan (β-GLUCAN) on serum Glutathione peroxidase (GSH-PX) activity (IU/L) and Peroxynitrite concentration (mmol/L) in intact rabbits

Groups / Parameter	G1 Intact rabbits Received distilled water (IW)	G2 Intact rabbits Received Lentinan (IL)	p-value
Alanine transaminase (ALT) (IU/L)	15.48 ± 0.14 A	15.24 ± 0.15 A	0.27
Aspartate transaminase (AST) (IU/L)	13.03 ± 0.33 A	12.56 ± 0.89 A	0.63

Values represent mean(N=7) ± SE. Different capital letters in the same row denote a significantly difference between groups (p≤0.05).

Table 3
The effect of Shiitake mushroom derived Lentinan (β-glucan) on serum(ALT) activity (IU/L) and serum (AST) activity in intact rabbits

Groups / Parameter	G1 Intact rabbits Received distilled water (IW)	G2 Intact rabbits Received Lentinan (IL)	p-value
Glutathione peroxidase (GSH-PX) (IU/L)	71.42 ± 0.48 B	77.43 ± 0.89 A	<0.0001
Peroxynitrite (mmol/L)	1.42 ± 0.007 A	0.93 ± 0.01 B	<0.0001

Values represent mean (N=7)± SE. Different capital letters in the same row denote a significantly difference between groups (p≤0.05).

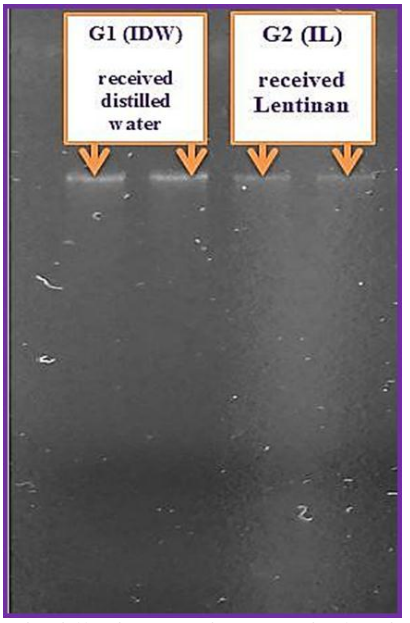

Figure 3. The effect of *Shiitake* mushroom derived Lentinan (β-glucan) supplementation on DNA fragmentation (DNA damage) in intact female rabbits.

Conclusion

From the results of this study, we mention the following conclusions based on the findings and discussion in this study. The supplementation with Lentinan (β-glucan) at the dose (10 mg/Kg/B.w)in intact rabbits significantly increase the platelets. On the other hand, Lentinan affect significantly some biochemical markers, include increasing the glutathione peroxidase activity and decrease peroxynitrite radical concentration. While there were no deleterious effect on DNA appeared or on alanine aminotransferase activity (ALT) and aspartate aminotransferase activity (AST).

Acknowledgments

Authors would like to express our sincere thanks and Praise to almighty Allah, the most gracious, the most merciful. Glory and praise be to Allah and his blessing upon Prophet Muhammad (peace be upon him and his family) and his household especially imam Hussain (peace be upon him) for giving us the strength for accomplish this work.

References

1. Hasan, Mohammad Nazmul, SM Shatil Shahriar, Jagannath Mondal, Md Nurunnabi, and Yong-kyu Lee. "Bioinspired and biomimetic materials for oral drug delivery." In *Bioinspired and Biomimetic Materials for Drug Delivery*, pp. 89-104. Woodhead Publishing, 2021.
2. Zhang, Meng, Yiran Zhang, Lijuan Zhang, and Qingwu Tian. "Mushroom polysaccharide lentinan for treating different types of cancers: A review of 12 years clinical studies in China." *Progress in molecular biology and translational science* 163 (2019): 297-328.
3. Liu D, Qiang J and Bu M. Studies on the anti-tumor effects of lentinan. *China Edible Fungus* 22: 6–7. 2002.
4. Huang, Jian, Yali Shi, and Minjun Sun. "Effects of lentinan on the function of phagocyte in long-term heavy-duty exercising mice." *European Journal of Inflammation* 16 (2018): 1721727X17752239.
5. Zi, Yusha, Biao Jiang, Congfen He, and Lei Liu. "Lentinan inhibits oxidative stress and inflammatory cytokine production induced by benzo (a) pyrene in human keratinocytes." *Journal of Cosmetic Dermatology* 19, no. 2 (2020): 502-507.
6. Trachootham, Dunyaporn, Jerome Alexandre, and Peng Huang. "Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach?." *Nature reviews Drug discovery* 8, no. 7 (2009): 579-591.
7. Andersen, Julie K. "Oxidative stress in neurodegeneration: cause or consequence?." *Nature medicine* 10, no. 7 (2004): S18-S25.
8. Shukla, Varsha, Santosh K. Mishra, and Harish C. Pant. "Oxidative stress in neurodegeneration." *Advances in pharmacological sciences* 2011 (2011).
9. Paravicini, Tamara M., and Rhian M. Touyz. "Redox signaling in hypertension." *Cardiovascular research* 71, no. 2 (2006): 247-258.
10. Haigis, Marcia C., and Bruce A. Yankner. "The aging stress response." *Molecular cell* 40, no. 2 (2010): 333-344.
11. Sabreen Momammed, Muhammed R. Al-Kenany and Tahani S. Al-Azawi. The antioxidant role of vitamin Cand zinc in ovariectomized rabbits with metabolic disorder. *Indian Journal of Natural Sciences.* Vol.(8): 48 (2018). 0976 – 0997.
12. Ishikawa, Kaori, Keizo Takenaga, Miho Akimoto, Nobuko Koshikawa, Aya Yamaguchi, Hirotake Imanishi, Kazuto Nakada, Yoshio Honma, and Jun-Ichi Hayashi. "ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis." *Science* 320, no. 5876 (2008): 661-664.
13. Al-Azawi, Tahani SS, and Muhammad Riyadh Alkenany. "Complementary effect of vitamin C and Zinc on some blood cells and serum proteins related to immunity in intact and ovariectomized rabbits. 5(5): 1296-1303. 2320-7078 (2017).

14. Zi, Yusha, Bo Zhang, Biao Jiang, Xingyao Yang, Zilu Liang, Weiyi Liu, Congfen He, and Lei Liu. "Antioxidant action and protective and reparative effects of lentinan on oxidative damage in HaCaT cells." *Journal of Cosmetic Dermatology* 17, no. 6 (2018): 1108-1114.
15. Johnstone A and Robin T. Immunochemistry in Practice. Black well Scientific Publications.Oxford.1982; P-92.
16. Pascual, p.; et al. (1992). Direct assay of glutathione peroxidase activity using high-performance capillary electrophoresis. J chromatogr. 581:49-56.
17. Beckman, J.S.; Ischiroponlos, H.; Zhu, I.; Van der Woerd, M.; Smith, C.; Chen, J.; et al. (1992). Kinetics superoxide dismutase and iron catasyzed nitration of phenolics by peroxynitrite. Arch BiochemBiophys. 298:438-445.
18. Zirkle, R. E. and Krieg, N.R. (1996). Development of a method based on alkaline gel electrophoresis for estimation of oxidative damage to DNA in *Escherichia coli. J. Appl. Bacteriol.*, 81, 133-138
19. SAS®. SAS/STAT Users Guide for Personal Computer. Release 9.13.SAS Institute, Inc., Cary, N.C., USA.2010.
20. Vetvicka V, dvorak B, Vetvickova J, et al: Orally administered marine (1→3)-beta-d-glucan phycarine stimulates both humoral and cellular immunity. int J Biol macromol 40: 291-298, 2007.
21. Baran J, allendorf dJ, hong F and Gordon rd: Oral β-glucan adjuvant therapy converts nonprotective th2 response to protective th1 cell mediated immune response in mammary tumor-bearing mice. Folia histochem cytobiol 45: 107-114, 2007.
22. liu J, Gunn l, hanses r and Yan J: combined yeast-derived β-glucan with anti-tumor monoclonal antibody for cancer immu- notherapy. Exp mol pathol 86: 208-214, 2009.
23. Yu, Z., LiHua, Y., Qian, Y., & Yan, L. (2009). Effect of Lentinus edodes polysaccharide on oxidative stress, immunity activity and oral ulceration of rats stimulated by phenol. Carbohydrate Polymers, 75(1), 115-118.
24. Cheah IK, Halliwell B. Ergothioneine; antioxidant potential, physiological function and role in disease. Biochim Biophys Acta 2012; 1822: 784-793.
25. Gambelunghe C, Rossi R, Micheletti A, Mariucci G, Rufini S. Physical exercise intensity can be related to plasma glutathione levels. J Physiol Biochem 2001; 57: 9-14.
26. Kuppusamy UR, Chong YL, Mahmood AA, Indran M, Abdullah N, Vikineswary S. Lentinula edodes (Shiitake) mushroom extract protects against hydrogen peroxide induced cytotoxicity in peripheral blood mononuclear cells. Indian J Biochem Biophys 2009; 46: 161-165.
27. Chen, H., Wang, P., Du, Z., Wang, G., & Gao, S. (2018). Oxidative stress, cell cycle arrest, DNA damage and apoptosis in adult zebrafish (Danio rerio) induced by tris (1, 3-dichloro-2-propyl) phosphate. *Aquatic Toxicology*, *194*, 37-45.
28. Ali, Z. S., & Khudair, K. K. (2019). Synthesis, Characterization of Silver Nanoparticles Using Nigella sativa Seeds and Study Their Effects on the Serum Lipid Profile and DNA Damage on the Rats' Blood Treated with Hydrogen Peroxide. *The Iraqi Journal of Veterinary Medicine*, *43*(2), 23-37.