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The effect of Lentinan administration on some immunological 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

*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 fourteen rabbits at age of (7-8) weeks. After acclimatization, animals were divided into two equal groups and as follows: first group (G1) or (DW) included seven intact rabbits fed with food and distilled water ad libitum,. The second group (G2) or (L) 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 (L) cause a significant increase ($P \leq 0.05$) in total white blood cell. The results also showed that the administration of Lentinan caused a significant increase ($P \leq 0.05$) in lymphocytes and monocytes percentage in G2. Also, the effect of Lentinan showed a significant decrease ($P \leq 0.05$) in neutrophil/lymphocytes (N/L) ratio and the Arneth's index (neutrophil nucleus segmentation test) for neutrophil maturation stages, showed a significant ($P \leq 0.05$) shift to right in both groups above that administered with Lentinan, while there were no significant ($P \leq 0.05$) change in Lymphocytes/monocytes (L/M) ratio. At the meantime, the concentration of total protein, globulin and gammaglobulin (Ab) increased significantly ($P \leq 0.05$) in group that treated with Lentinan as compared to control group, while there were no significant effect of Lentinan on albumin and albumin/globulin (AL/GL) ratio. Finally, The effect of mushroom extract (Lentanine) was clearly shown on the levels of interleukin 2 (IL-2) and interleukin 10 (IL-10), where there was a significant ($P \leq 0.05$) increase in the level of IL-2 accompanied by significant decrease

($P \leq 0.05$) in the level of IL-10 in G2 (L) that supplemented with Lentanin as compared with the control groups (DW).

Keywords---Lentinan, β -glucan, immunity, lymphocyte, interleukin.

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].

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 [6]. Some studies used vitamins or minerals to ameliorating the deleterious effect of oxidative stress like vitamin c and zinc and proven the effect of them as food additives that protect the immune system and cells from the risk of genetic damage due to oxidative stress[7,63]. In the current study, Lentinan which is macrofungus extracted from *shiitake* 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.

β -glucans were reported to improve antigen presenting function of DCs [8], As a result, tumor-specific cytotoxic T cells are produced. Furthermore, when the constant region (Fc) of an immunoglobulin interacts with receptors on leukocytes for the Fc domain of IgG (Fc gamma R), phagocytosis, enhanced antigen presentation, release of inflammatory mediators, and antibody-dependent cellular cytotoxicity (ADCC) are all triggered [9]. The Fc gamma R (FcR) protein is a crucial connection between humoral and cellular immunity. FcR expression has been demonstrated to be boosted by β -glucans [10], as well as complement activation [11]. As a result, β -glucans act in conjunction with anti-tumor monoclonal antibodies (mAbs), which are routinely employed to treat cancer [12,13].

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.

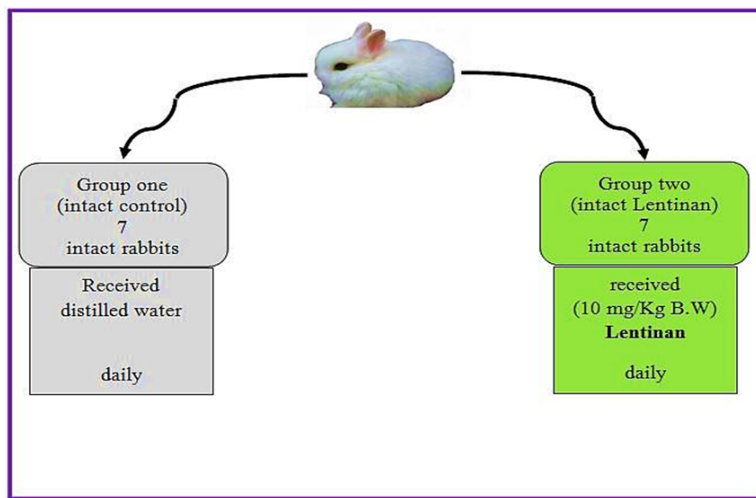


Figure 1. Graphical abstract of experimental design

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 [14] 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 $-18\text{ }^{\circ}\text{C}$ until use.

Parameters determination

Total Leukocytes count was measured manually[15] by using hemocytometer slide (neubauer slide). Differential Leukocytes count percentage (%) was calculated using blood smears from each animal and stained with Giemsa stain [15]. Neutrophil / Lymphocytes ratio (N/L) and Lymphocytes/monocytes (L/M) ratio was measured as described by Campbell (1988)[16]. Arneth's index was measured according to Ghai(2013) and Khurana(2009)[17, 18]The procedure as follow:

- Prepare and stain a fresh blood film in the usual manner
- Examine 100 (or 200) neutrophils, noting the number of lobes in each cell and calculate the percentages of each stage.

Proteins profiles

The estimation Total serum proteins concentration (g/L) was carried on at the laboratory of physiological department/collage of veterinary medicine/University of Bagdad. Serum total protein was measured using a special kit (LiNEAR, Spain) by the biuret method[19]. Serum albumin was measured using a special kit

(Biosystem, Spain) by the BROMOCRESOL GREEN method[20]. Globulin is the second part of the contents of protein and contains several compounds (alpha 1, alpha 2, beta 1, beta 2 and gamma). Total globulin concentration measured as described by Pulmmer (1971)[21]. Albumin/Globulin ratio (Alb/Glob), It was measured by dividing the amount of albumin on the amount of globulin to estimate the ratio [22].

Electrophoresis of Gamma globulin (immunoglobulin)

Protein fraction percent (%) were measured by electrophoresis, by which (electric deportation of all types of serum proteins with explanation to the amount of proteins, albumin and globulin components, namely, (alpha 1, alpha 2, beta and gamma). It is measured by using protein electrophoresis apparatus (Helena Bioscience /Europe).it is very modern special and sophisticated device, consist of two parts (SAS 1and SAS 2) as described in (figure 3). The result was calculated by control software "analysis-platinum 4V" as described and shown.



Figure 3. Protein fraction electrophoresis "analysis-platinum 4V".

Enzyme linked immunoassay

Interleukin-2 (IL-2) and Interleukin-10 (IL-10) estimation done by using ELISA kit which use the principle of Sandwich-ELISA method as descried in the procedure of this kit that provided by SUNLONG CO/china.

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 $\pm$ stander errors and $P < 0.05$ was considered statistically significant[23].

Results

The results represented in table (1) reveal the effect of oral supplementation of Shiitake mushroom derived Lentinan (β -GLUCAN) (10 mg/kg B.W) on rabbits. There is a significant ($P \leq 0.05$) increase in Leukocytes counts in group two (G2) that supplemented with Lentinan in comparison with the control group (G1) that received distilled water. From the results illustrated in the table (2), it's obvious that there is a significant ($P \leq 0.05$) decrease only in neutrophils in the intact Lentinan group (G2) that administered with Lentinan at a dose of (10 mg/kg B.W) as compared with the control group (G1). In the meantime, the table shows that the administration of Lentinan to groups of rabbits caused a non-significant ($P \geq 0.05$) effect on other types of granulocytes (eosinophils, basophils). The values represented in table (3) reveal the effect of oral supplementation of Lentinan at a dose of (10 mg/kg B.W) on Agranulocytes (lymphocytes, monocytes) in intact rabbits groups (G1 and G2). This table shows that the intact Lentinan group (G2) has significantly ($P \leq 0.05$) higher values of both types of cells (lymphocytes, monocytes) than the group of rabbits that received distilled water (G1).

Table (4) reveals the effect of oral administration of (10 mg/kg B.W) of the mushroom derived Lentinan(β -GLUCAN) on the ratio between neutrophil and lymphocyte. On the other hand, the table also reveals the ratio between lymphocyte and monocyte in intact rabbits. There is a significant ($P \leq 0.05$) decrease in the value of both ratio neutrophil/ Lymphocyte ratio in the group supplemented with Lentinan (G2) in comparison with the intact group that received distilled water (G1). In contrast, there was no significant ($P \leq 0.05$) change in the Lymphocyte/monocyte ratio value between the groups. Table (5) and figure (4) represent the stages of neutrophil's segmentation and lobulation of the nucleus in the blood (Arneth's index) that subjected to the Lentinan (β -GLUCAN) supplementation at a dose of 10 mg/kg B.W. However, the intact rabbits that received Lentinan (G2) showed a significant ($P \leq 0.05$) percentage in the mature (hypersegmented) stages (25.08%) more than the immature neutrophils which have one and two lobes (23.57%) of the same group compared to the same stages of the control group (G1) that received distilled water, revealed a shift to the right pattern in G2 compared to the control.

The results obtained in the table (6) represent the effect of (10 mg/kg B.W) Shiitake mushroom derived Lentinan (β -GLUCAN) on total serum protein (g/L), serum Albumin(g/L) and globulin(g/L) in intact rabbits. The supplementation with Lentinan (β -GLUCAN) showed a significant ($P \leq 0.05$) increase in values of total serum protein(T.S.P) and serum globulin(S.G) in the Lentinan group (G2) compared to the control group(G1). On the other hand, there was no significant ($P \leq 0.05$) change in serum Albumin(g/L) between groups (G1 and G2). The results obtained in the table (7) represent the effect of (10 mg/kg B.W) Shiitake mushroom derived Lentinan (β -GLUCAN) on serum gamma globulin which is immunogammaglobulin(Ig) and Albumin/Globulin ratio in intact rabbits. The supplementation with Lentinan (β -GLUCAN) induces a significant ($P \leq 0.05$) increase in the serum gamma globulin(Ig) in the Lentinan group (G2) compared to the control group (G1). On the other hand, the supplementation with Lentinan (β -GLUCAN) also induces no significant ($P \leq 0.05$) change in Albumin/Globulin ratio in the Lentinan group (G2) compared with those received distilled water (G1).

Table (8) reveals the effect of oral administration of (10 mg/kg B.W) of the mushroom derived Lentinan (β -GLUCAN) on interleukin 2 (IL-2) and interleukin 10 (IL-10) in intact rabbits. The supplementation with Lentinan (β -GLUCAN) induced a higher significant ($P \leq 0.05$) value of interleukin 2 (IL-2) in the Lentinan group (G2) in comparison with the group that received distilled water (G1). In contrast, the G2 which received Lentinan (β -GLUCAN) represents the lower significant ($P \leq 0.05$) value of interleukin 10 in comparison with the group that received distilled water (G1).

Discussion

As shown in the present study, there is a significant increase in leucocyte and platelet count in the group administered with Lentinan compared to the control group. These findings were consistent with several experimental studies in vivo that have shown β -glucans administered either orally, intravenously or enterally enhance hematopoietic regeneration without side effects [24] [25] [26]. Brown and Gordon (2005) showed that Beta-glucans appear to be able to activate leukocytes directly, stimulating their phagocytic, cytotoxic, and antimicrobial activities and stimulating the production of pro-inflammatory mediators and cytokines and chemokines, including IL-8, IL-1b, IL-6, and TNF- α . Further, another study was consistent with our results and found that many β -glucans have demonstrated in vivo the significant bioactivity, including antitumor efficacy and stimulation of hematopoiesis [27][28]. For example, a good manufacturing process (GMP)-produced β -glucan, poly-(1,6)- β -D-glucopyranosyl- (1,3)- β -D-glucopyranose (β -glucan PGG), was shown in vivo and ex vivo models to enhance murine and primate myelopoiesis [29]. Although, mushrooms Shiitake (*Lentinula edodes*) produces high quantities of branched β (1-3)(1-6)-D-glucan (Lentinan) [30][31]. Interaction of β -glucans with different cellular receptors inducing intracellular signaling may activate different profiles of the immune response [32][33].

The result in the current study demonstrated a significant decrease only in neutrophils in the intact Lentinan group compared with the control group. Previous studies have shown that the binding site of low-molecular-weight soluble β -glucans to neutrophil CR3 was mapped to the lectin-like domain (LLD) of CD11b-located C terminal with respect to the iC3b- and ICAM-1-binding I domain [29]. Liu *et al.* (2016) found that non-cytotoxic levels of LNT strongly increased blood myeloperoxidase (MPO) activity; improved BM structural injuries; increased the numbers of leukocytes and neutrophils in the blood and BM; elevated the blood levels of granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF). Since, M-CSF (or CSF-1) is a hematopoietic growth factor involved in the proliferation, differentiation, and survival of monocytes, macrophages, and bone marrow progenitor cells [34]. M-CSF affects macrophages and monocytes in several ways, including stimulating increased phagocytic and chemotactic activity and increased tumour cell cytotoxicity. [35]. G-CSF affects the bone marrow and stimulates neutrophilic granulocytes production and their release into the bloodstream [36]. Granulocyte macrophage-colony stimulating factor (GM-CSF) was firstly identified as being able to induce in vitro the proliferation and differentiation of bone marrow progenitors into granulocytes and macrophages.

The present study shows that the intact Lentinan group has significantly higher lymphocyte values than the control group of rabbits that received distilled water. Lentinan was found to be safe, and the rats demonstrated a significant increase in B-cells, monocytes, and interferon-gamma. No sign of toxicity was observed. [37]. L. edodes are composed of polysaccharides, such as β -glucan, which enhance the immune function of T-cells and B-cells through activation of macrophages and are regarded as a potent antioxidant and anticarcinogen [38]. The effects seem to occur by stimulating the maturation, differentiation, or proliferation of immune cells involved in host defence mechanisms [39]. Beta-glucans also activate NK-cells and stimulate T helper cells[40][41].

The supplementation with Lentinan (β -glucan) showed a significant increase in values of total serum protein and serum globulin in the Lentinan group compared to the control group. Current results agree with Abo Ghanima *et al.* (2020), which found that administering rabbits with β -glucan at a dose of 0.5 ml per one-litre of drinking water increased blood total protein and globulin values[42]. Concerning the biochemical findings, El-Sawy *et al.* (2015) reported that oral administration of yeast β -glucan did not alter serum protein, albumin, and globulin of chicks compared to control chicks.[43] Belhassen *et al.* (2016) reported that dietary administration of *S. cerevisiae* did not alter the blood parameters of growing rabbits. The changes in mouse serum protein distribution after administration of Lentinan, an antitumor polysaccharide, were examined using micro two-dimensional electrophoresis without denaturing agents[44]. Three protein spots were markedly increased in level and two of them were identified as haptoglobin and haptoglobin partially bound with hemoglobin. After complex formation with hemoglobin and ceruloplasmin, activity staining of haptoglobin was demonstrated [45].

In the current study, the supplementation with Lentinan (β -glucan) induced a higher significant value of interleukin 2 (IL-2) in the Lentinan group compared to the group that received distilled water. There are many kinds of research on antitumor activity and mechanism of action for polysaccharides, including Lentinan [46][47]. For example, Chihara *et al.* (1970) reported that Lentinan caused complete regression of Sarcoma 180 (S-180) cells transplanted into mice at a dose of 1 mg/kg for 10 days[48], while a large dose of 80 mg/kg for 5 days showed no antitumor activity in comparison with the untreated control mice[49]. Therefore, The anti-tumor mechanism was summarized to improve immune function [46], induce apoptosis of tumor cells [50][51], increase NK and T helper cells and stimulate the synthesis of interleukins [52]. the lentinan effect was attributed to the regulation of the innate immune responses and specific immunity and the down-regulation of the inflammatory IL-2 [53]. The carriers of specific cellular immunity are T lymphocytes. We can discriminate between T helpers (TH), T cytotoxic and T regulatory lymphocytes. To date, several subpopulations of TH lymphocytes have been detected based on the characteristic spectrum of produced cytokines. The two best-known and important subpopulations are TH1 and TH2 lymphocytes. TH1 characteristically produces and secretes interleukin 2 (IL-2), interferon-gamma (IFN- γ) and tumour necrosis factor-beta (TNF- β), which induce immune tolerance and the reactions of cellular immunity. The other important subpopulation is TH2 lymphocytes, which produce interleukins 4, 5, 6, 10 and 13 and activate humoral immunity and

typically contribute to the development and persistence of allergic inflammation. TH0 lymphocytes simultaneously produce IL-4 and also IFN- γ . [54]. β -glucan reduced the TH2 response with concomitant stimulation of an anti-tumour TH1 reaction. [41]. In animal experiments, intraperitoneal application of lentinan improved the capacity of peritoneal macrophages to produce IL-12, which directed the immune response towards TH1 and stimulated the T lymphocytes to produce IFN- γ [55].

Whereas in the present study, the group received Lentinan (β -glucan), representing the lower significant value of interleukin 10 compared to the control group that received distilled water. McCormack *et al.* (2010) investigated the chemo-immunostimulatory properties of Lentinan, extracted from *Lentula edodes*, in male BN/RijHsd rats[37]. There is a significant increase in weight gains, monocytes, blood cells, circulatory cytotoxic T-cells, and a reduction in anti-inflammatory cytokines such as IL-10. Other work showed that after dietary intake of β -glucans from *Lentinula edodes* macrophages clearly downregulate IL-10 expression, indicative of immune stimulation, and IL-4 and IFN- γ induced expression, clearly suggesting activation of pro-inflammatory but also anti-inflammatory pathways. This can be achieved by the presence of M1 and M2 differentiated macrophages in the periphery or by inducing these cytokines in the same cell. In any case, the response to β -glucans involves activation of both subpopulations, likely leading to an overall activation state useful in the fight against a broad spectrum of pathogens and avoiding the exacerbation of the inflammatory response [56]. By depleting potentially involved cytokines from the supernatant, we could confirm the pivotal role of IL-10 in the activation of porcine NK cells upon β -glucan priming of monocytes. Although traditionally classified as an immunosuppressive cytokine, IL-10 has been described as an enhancer of NK cell cytotoxicity in humans and mice [57][58].

Furthermore, IL-10-mediated increased NK cell cytotoxicity has been suggested to facilitate antigen uptake from dead cells by antigen-presenting cells, thereby enhancing the cross-talk between the innate and adaptive immune systems during infection [59]. Interestingly, Holder and Grant (2019) described that cmvIL-10, a virokin homologous to human IL-10 encoded by human cytomegalovirus (HCMV), increased NK cell cytotoxicity in vitro as well. Taken together, these results show that β -glucans can significantly enhance NK cell effector functions by triggering NK cell-stimulating IL-10 secretion by monocytes[60]. These findings again highlight the broad influence that β -glucans can exert on the immune response and, therefore, the potential of these molecules in the development of novel therapeutic strategies, both in human and veterinary medicine [61]. β -glucans are a group of biologically active polysaccharides of natural origin with a proven pleiotropic immunomodulation effect [54]. The observed increase of serum IL-10 after subcutaneous administration of β -glucans could modulate allergic sensitization with the restoration of the TH1/TH2 equilibrium, given the fact that low serum levels of IL-10 are associated with the dysregulation of T lymphocytes [62].

Tables

Table 1
The effect of *Shiitake* mushroom derived Lentinan(β -GLUCAN) on Total Leukocyte counts ($N \times 10^3$ cells/ mm^3) in intact rabbits

Groups Parameter	G1 Intact rabbits Received distilled water (IW)	G2 Intact rabbits Received Lentinan (IL)	p-value
Lymphocytes (%)	54.77 $\pm$ 1.42 B	62.36 $\pm$ 2.94 A	<0.0001
Monocytes (%)	3.04 $\pm$ 0.21 B	3.81 $\pm$ 0.14 A	<0.0001

Values represent mean($N=7$) $\pm$ SE.

Different capital letters in the same row denote a significant difference between groups ($p \leq 0.05$).

Table 2
The effect of *Shiitake* mushroom derived Lentinan(β -GLUCAN) on granulocytes (neutrophils, eosinophils, basophils) in intact rabbits

Groups Parameter	G1 Intact rabbits Received distilled water (IW)	G2 Intact rabbits Received Lentinan (IL)	p-value
Total Leukocyte counts ($N \times 10^3$ cells/ mm^3).	8.10 $\pm$ 0.07 B	13.98 $\pm$ 0.04 A	<0.0001

Values represent mean($N=7$) $\pm$ SE

Different capital letters in the same row denote a significant difference between groups ($p \leq 0.05$).

Table 3
The effect of *Shiitake* mushroom derived Lentinan(β -glucan) on Agranulocytes
(Lymphocytes, Monocytes) in intact rabbits

Groups Parameter	G1 Intact rabbits Received distilled water (IW)	G2 Intact rabbits Received Lentinan (IL)	p-value
Neutrophil/ Lymphocyte ratio (N/L)	0.73 $\pm$ 0.04 A	0.52 $\pm$ 0.08 B	<0.0001
Lymphocyte/ Monocyte ratio (L/M)	18.64 $\pm$ 1.61 A	16.57 $\pm$ 1.11 A	0.33

Values represent mean(N=7) $\pm$ SE

Different capital letters in the same row denote a significant difference between groups ($p \leq 0.05$).

Table 4
The effect of *Shiitake* mushroom derived Lentinan(β -GLUCAN) on
neutrophil/Lymphocyte ratio and Lymphocyte /Monocyte ratio in intact rabbits

Groups Neutrophil segmentation	G1 Intact rabbits Received distilled water (IW)	G2 Intact rabbits Received Lentinan (IL)	p-value
One lobe(%)	4.6 $\pm$ 0.621 Db	5.32 $\pm$ 0.451 Ca	0.014
Two lobe(%)	28.22 $\pm$ 1.169 Ba	18.25 $\pm$ 1.055 Bb	
Three lobe(%)	49.13 $\pm$ 1.747 Ab	51.34 $\pm$ 1.651 Aa	
Four lobe(%)	14.8 $\pm$ 0.530 Cb	17.76 $\pm$ 0.863 Ba	
Five or more lobe(%)	3.5 $\pm$	7.36 $\pm$	

	0.312 Db		0.382 Ca		
LSD	2.50				

Values represent mean(N=7) $\pm$ SE. Different capital letters in the same row denote a significant difference between groups ($p \leq 0.05$).

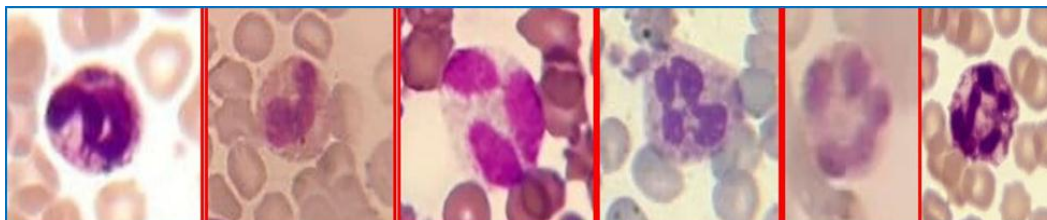
Table 5
The effect of *Shiitake* mushroom derived Lentinan (β -GLUCAN) supplementation on stages of neutrophils maturation (Arneth's index %) in intact rabbits

Groups Parameter	G1 Intact rabbits Received distilled water (IW)	G2 Intact rabbits Received Lentinan (IL)	p-value
Neutrophils (%)	39.48 $\pm$ 1.23 A	30.96 $\pm$ 2.84 B	<0.0001
Eosinophils (%)	1.36 $\pm$ 0.16 A	1.47 $\pm$ 0.2 A	0.31
Basophils (%)	1.26 $\pm$ 0.15 A	1.33 $\pm$ 0.21 A	0.07

Values represent mean(N=7) $\pm$ SE.

Means with different small letters in the same row are significantly different ($P < 0.05$).

Means with different capital letters in the same column are significantly different ($P < 0.05$).



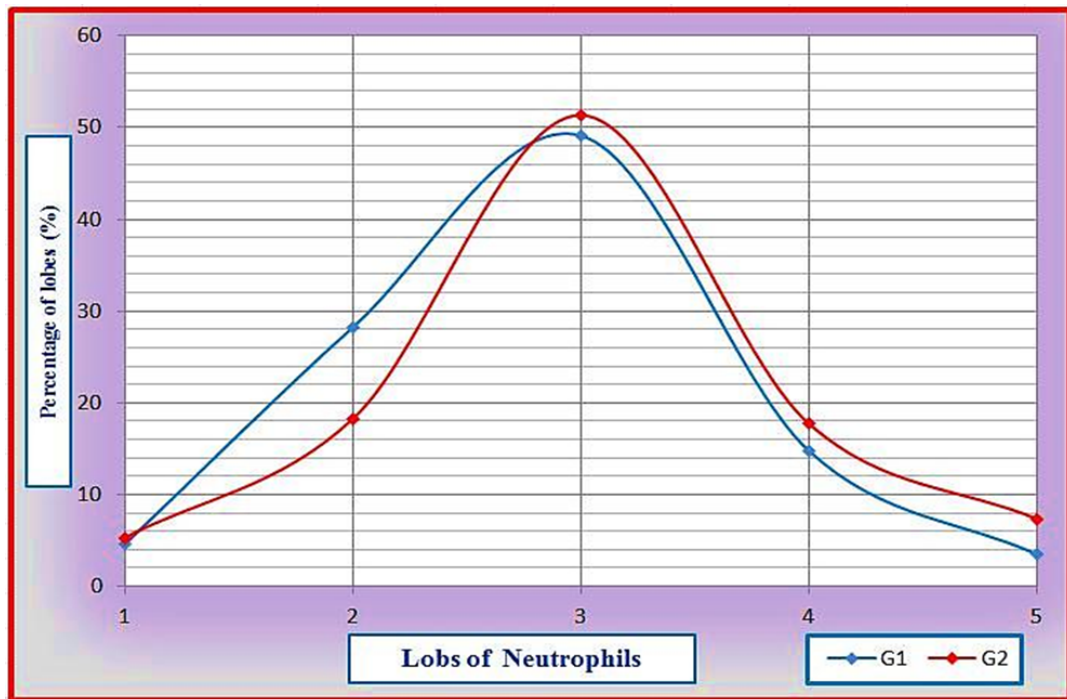


Figure 4. The effect of *Shiitake* mushroom derived Lentinan (β -GLUCAN) supplementation on stages of neutrophils maturation (Arneth's index %) in intact rabbits.

Group one: Blue curve

Group two: Red curve

Table 6

The effect of *Shiitake* mushroom derived Lentinan (β -GLUCAN) on total serum protein(g/L), serum albumin(g/L) and serum globulin(g/L) in intact rabbits

Groups Parameter	G1 Intact rabbits Received distilled water (IW)	G2 Intact rabbits Received Lentinan (IL)	p-value
Total serum protein(T.S.P) (g/L)	65.7 ± 1.41 B	70.01 ± 0.97 A	0.02
Serum Albumin(S.A) (g/L)	35.01 ± 0.64 A	35.03 ± 0.91 A	0.99
Serum Globulin(S.G) (g/L)	30.69 ± 1.00 B	34.98 ± 0.42 A	0.02

Values represent mean(N=7) ± SE.

Different capital letters in the same row denote a significant difference between groups ($p \leq 0.05$).

Table 7

The effect of *Shiitake* mushroom derived Lentinan (β -GLUCAN) on gamma globulins (g/L) and Albumin/Globulin ratio (Alb/Glob) in intact rabbits

Groups Parameter	G1 Intact rabbits Received distilled water (IW)	G2 Intact rabbits Received <u>Lentinan</u> (IL)	p-value
Interleukin 2 (<u>pg</u> /ml)	22.35 ± 1.54 B	44.94 ± 1.27 A	<0.0001
Interleukin 10 (<u>ng</u> /L)	23.45 ± 0.82 A	14.06 ± 0.81 B	<0.0001

Values represent mean(N=7) ± SE.

Different capital letters in the same row denote a significant difference between groups ($p \leq 0.05$).

Table 8

The effect of *Shiitake* mushroom derived Lentinan (β -GLUCAN) on Interleukin-2 (IL-2)(pg/ml) and Interleukin-10 (IL-10) (ng/L) in intact rabbits

Groups Parameter	G1 Intact rabbits Received distilled water (IW)	G2 Intact rabbits Received <u>Lentinan</u> (IL)	p-value
gamma globulins (g/L)	8.30 ± 0.12 B	13.62 ± 0.15 A	<0.0001
Albumin/Globulin ratio (<u>Alb/Glo</u>)	1.14 ± 0.3 A	1.0 ± 0.3 B	0.01

Values represent mean(N=7) ± SE.

Different capital letters in the same row denote a significant difference between groups (p≤0.05).

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 leukocytes count. On the other hand, Lentinan affect significantly on white blood cells type, include increasing the lymphocytes and monocytes percentage, decrease neutrophil percentage and neutrophil/ lymphocytes ratio. The neutrophil segmentation (Arneth's index) test showed a shift to the right after supplementation with Lentinan (β -GLUCAN). While there were no significantly effect on eosinophil, basophil percentages and L/M ratio appeared. Protein profile showed a significant increasing in total serum protein, globulin, immunogammaglobulin and a significant decrease in albumin/ globulin ratio in group that supplemented with Lentinan at the dose mentioned above as compared with control group. The supplementation with Lentinan (β -GLUCAN) at the dose (10 mg/Kg/B.w)in intact rabbits significantly modulates and regulates the immune function, and that include a significant increase in interleukin 2 (IL_2) and significant decrease in interleukin 10 (IL_10) as compared with control. from all results mentioned, it obviously clear the effect of *Shiitake* mushroom derived Lentinan(β _glucan) on the immune system and its important role in protecting and treating it against invading microbes.

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