

## Immunostimulatory and anti-inflammatory effects of Pleuran purified from the edible mushroom *Pleurotus ostreatus* var. *ostreatus* in the hydrogen peroxide-induced inflammation in white rats

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<https://doi.org/10.54153/sjpas.2024.v6i2.689>

### Article Information

Received: 23/8/2023

Revised: 26/9/2023

Accepted: 2/10/2023

Published: 30/6/2024

### Keywords:

*Pleurotus ostreatus* var. *ostreatus*, Pleuran Anti-inflammatory, Interleukin-6, Immunomodulatory, Tumor Necrosis Factor-Alpha (TNF- $\alpha$ ).

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### Abstract

This study was conducted at the laboratories of the College of Agriculture and Animal House in the college of Veterinary Medicine, Tikrit University, during 2022/2023, with the aim of evaluating the effect of Pleuran purified from the edible mushroom *Pleurotus ostreatus* var. *ostreatus* as immunomodulatory and antioxidant agent in the Inflammatory rats with Hydrogen peroxide. The results of the Pleuran effect In vivo (white rats) showed that all Pleuran concentrations did not have a negative impact on the hematological parameters in healthy animals. However, these treatments led to improvements in the white blood cell counts, restoring them to normal levels in animals induced with inflammation by hydrogen peroxide, the highest improvements were observed at 15 mg/kg body weight of Pleuran, with white blood cell and platelet counts reaching 7500 and 188000 cells/ $\mu$ L, respectively. Neutrophil/lymphocyte index decreased from 2.58 in the Inflammatory animals to 1.12 in the presence of Pleuran at 15 mg/kg/day, compared to 1.3 in the levamisole group. The lowest immunoglobulin G (IgG) and M (IgM) was 406.3 and 45.3 mg/dL in the hydrogen peroxide treatment alone, then increased in all pleuran concentration, the highest values were 746.3 and 118.3 mg/dL in pleuran at 15 mg/kg/day, compared to 585.3 and 90.3 mg/dL in the levamisole group, while the results showed increase in Tumor Necrosis Factor-Alpha (TNF- $\alpha$ ) and Interleukin-6 (IL-6) to 103.8 pg/ml and 37.8 ng/L in the hydrogen peroxide treatment alone, and increased in all pleuran concentration, with highest values 68.4 pg/ml and 9.66 ng/L in present of pleuran at 15 mg/kg/day, compared to 89.8 pg/ml and 9.66 ng/L in the levamisole group, respectively.

### Introduction:

Pleuran, (related to  $\beta$ -glucan) a non-soluble polysaccharide, is sourced from *Pleurotus* spp. It consists of glucose molecules linked via  $\beta$ -(1,3/1,6)-glucan bonds, forming branched chains interconnected by glycosidic linkages. While  $\beta$ -glucan is obtained from barley, oats, algae, seaweeds, various microfungi and bacteria, pleuran, particularly related to *Pleurotus*

spp., like other type of  $\beta$ -glucan derived from other Mushrooms such as lentinan (from *Lentinus edodus*) and schizophyllan (from *Schizophyllum commune*), and all these stand out as a predominant medicinal reservoir of  $\beta$ -glucans (Kozarskia *et al.*, 2023).

In Iraq, the initial cultivation of this mushroom was undertaken by Hassan in 1996, with studies on its many medicinal properties (Hassan and Mahmoud, 2003; Hassan, 2005; Hassan and Awad, 2023). Extensive animal experimentation and selected human clinical trials have revealed the immunomodulatory attributes of Pleuran. Furthermore, Pleuran showcases a multifaceted profile encompassing antioxidant, anti-inflammatory, anti-cancer, anti-diabetic, antimicrobial, lipid-lowering, hypoglycemic, and immune-modulatory activities (Bai *et al.*, 2019). This wide-ranging functionality has conferred substantial significance upon Pleuran across realms including nutrition, healthcare, pharmaceuticals, cosmetics, chemical industries, and agro-food production (Mirończuk-Chodakowska *et al.*, 2021). These effects are notably attributed to its interactions with immune cells situated in Peyer's patches within the gastrointestinal tract, subsequent to oral ingestion. Pleuran engages with immune cells bearing receptors such as Dectin-1, Complement receptor-3, and Scavenger receptor, which possess the capability to recognize diverse forms of  $\beta$ -glucan (Kozarskia *et al.*, 2023). The binding of Pleuran to these receptors elicits both innate and adaptive immune responses, leading to the release of anti-inflammatory cytokines and complement components ( $\beta/\alpha$  1 Interleukins, Interleukin-6, Interleukin-8, Interleukin-12, and tumor necrosis factor TNF) that exhibit resistance against pathogens Volman, (2008). Pleuran was extracted and purified for the first time in Iraq from three *Pleurotus* spp. namely *P. pulmonarius* strain Has.AA-1(ON834486.1), *P. ostreatus* var. *ostreatus* strain Has.AA-2(ON834487.1), and *P. eryngii* var. *eryngii* strain Has.AA-3 (ON834484.1) by Hassan and Muhammad (2023) , According to this study, *Pleurotus ostreatus* var. *ostreatus* was the highest producer of pleuran. In another study, Pleuran purified from *Pleurotus ostreatus* var. *ostreatus* has powerful antioxidant activity (Muhammad *et al.*, 2023).

Recognizing the pivotal status of Pleuran as a natural compound coupled with its diverse therapeutic attributes, this investigation is aimed at the evaluation of Immunostimulatory and anti-inflammatory effects of Pleuran purified from the edible mushroom *Pleurotus ostreatus* var. *ostreatus* in the hydrogen peroxide-induced inflammation in white rats

## **Materials and Methods**

### **Source of Pleuran**

Pleuran purified from *Pleurotus ostreatus* var. *ostreatus* (ON834487.1) was obtained from the laboratories of the Plant Protection Department, University of Tikrit (Hassan and Muhammad , 2023)

### **Extraction and purification of pleuran**

The pleuran was purified according to what was mentioned by Byron (1993), by mixing 200 grams of mushroom powder with (1000 ml) of NaOH (5 M1), and the mixture was sterilized in an autoclave for 60 minutes and centrifuged in a centrifuge at 5000 rpm for 15 minutes. It was discarded. The filtrate and the remaining fraction were washed 3 times, each time with 1 liter of distilled water and centrifuged (5000 rpm/15 minutes). The product was

mixed with acetic acid (3%) at 85 °C and left to mix continuously for 3 hours, then centrifuged and the fraction was suspended. The acid was dissolved in 600 ml of absolute ethanol and centrifuged. The ethanol washed portion was suspended in 600 ml of acetone and centrifuged. The precipitate was mixed with the filtrate. The acetone was withdrawn from the vacuum filter funnel and the resulting product was placed in a petri dish and dried under pressure in a desiccator. The clumps were crushed and stored for tests.

### ***In vivo* Experiments using white rats**

In vivo experiments were executed within the premises of the laboratory and animal housing facilities situated at the College of Veterinary Medicine, University of Tikrit. These experiments were carried out over a duration spanning from July 2022 to October 2022. 45 white rats, with an age of 2 months and weighing within the range of 200 to 250 g, were employed as subjects for this investigation. Rats were maintained under standardized laboratory conditions, encompassing proper ventilation, and a controlled temperature spanning from 20 to 25°C. Additionally, the rats were subjected to a light: dark photoperiod of 12:12 hours. Throughout the course of the experiment, the rats had unrestricted access to both water and a standard diet, as per the approach outlined by (Balducci *et al.* 2001).

### **Experimental Design**

The animals were partitioned into nine distinct groups, with each group comprising five individual subjects. This division aimed to investigate the inflammatory and immunomodulatory responses elicited by Hydrogen peroxide, coupled with the administration of varied concentrations of Pleuran. The experimental design encompassed the following treatment regimens::

1. Control Group (without treatment).
2. Hydrogen peroxide Group
3. Pleuran 5 mg/kg/day + Hydrogen peroxide Group
4. Pleuran 10 mg/kg/day + Hydrogen peroxide Group
5. Pleuran 15 mg/kg/day + Hydrogen peroxide
6. levamisole + Hydrogen peroxide Group
7. Three Pleuran groups: 5 , 10 and 15 mg/kg/day.

Animal groups treated with formalin received an injection of 0.1 ml of 2% formalin solution into the footpad. Conversely, animal groups treated with Pleuran were subjected to oral administration of Pleuran, following the indicated dosages. In the positive control group treated with Piroxicam, animals were orally administered a dose of 10 mg/kg/day of levamisole.

## **Hematological Analysis**

Following a 14-day period of the aforementioned treatments, blood specimens were procured using capillary tubes from the ocular conjunctiva and deposited into plastic test tubes containing the anticoagulant Ethyl Diamine Tetra Acetic Acid (EDTA). Another set of blood samples was obtained without anticoagulant for the assessment of blood parameters.

The total leukocyte count (TLC) and differential percentages of white blood cells (WBCs) were determined following the methodology outlined by Johnstone and Robin (1982). In addition, a serum blood sample was acquired for the quantification of immunoglobulin IgG and IgM levels through the utilization of the enzyme-linked immunosorbent assay (ELISA), as delineated by (Newkirk *et al.* 2003).

## **Statistical Analysis**

A Completely Randomized Design (CRD) was used for all the experiments and the collected data were subsequently analyzed utilizing the Gene Stat software. Comparative analysis of means was executed employing both the Least Significant Difference (L.S.D.) test and Duncan's Multiple Range Test, both conducted at a significance level of 0.01, following the methodology stipulated by Al-Rawi and Khalafallah (1980).

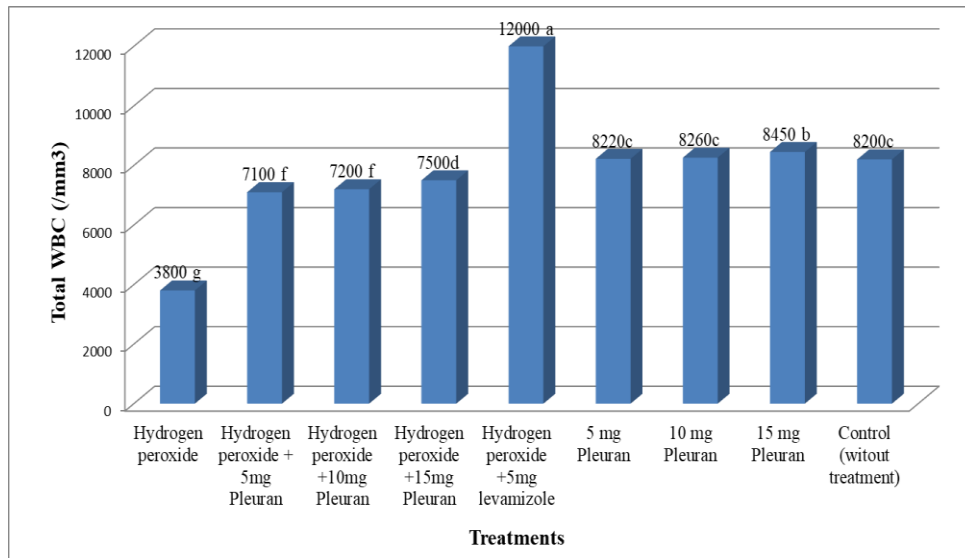
## **Results**

### ***In vivo* Experiments**

Effect of Pleuran on White Blood Cell Count, Platelets, and Differential White Blood Cell Ratios in Rats Exposed to an Induced Inflammation Model using Hydrogen Peroxide.

### **White Blood Cell Count**

The results presented in Figure (1) demonstrate that Pleuran had no effect on healthy animals. However, it exhibited a proportional increase in white blood cell count in animals treated with hydrogen peroxide-induced inflammation. Specifically, Pleuran treatment at a concentration of 15 mg in animals afflicted with hydrogen peroxide-induced inflammation yielded a white blood cell count of 7500 cells/mm<sup>3</sup>, in comparison to the hydrogen peroxide treatment which exhibited a lower white blood cell count of 3800 cells/mm<sup>3</sup>, followed by the Pleuran treatment at a concentration of 10 mg, which showed a white blood cell count of 7200 cells/mm<sup>3</sup>. Conversely, the anti-inflammatory drug, Levamisole, registered an elevation in white blood cell count, reaching 12000 cells/mm<sup>3</sup>.

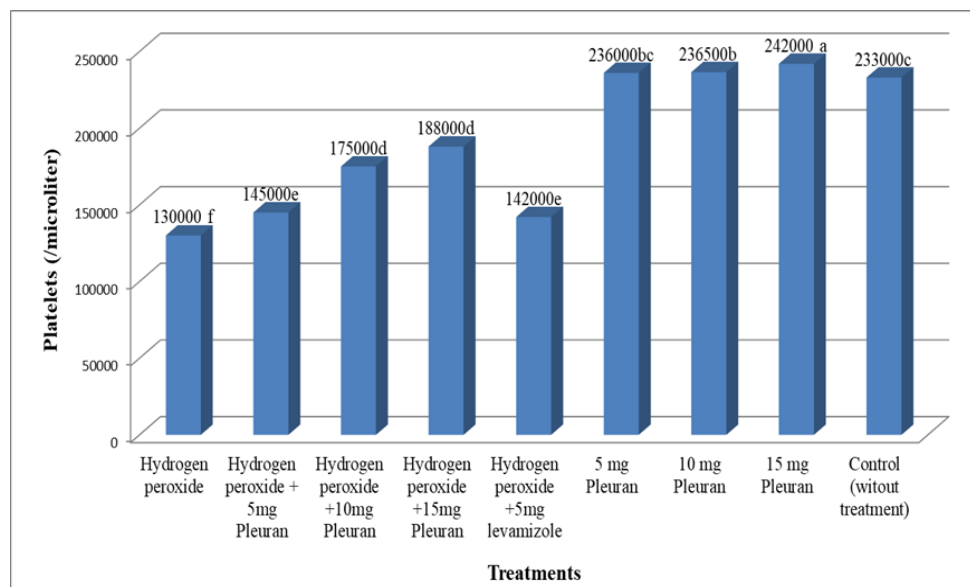


**Fig. 1** Impact of Pleuran on white blood cell count resulting from the hydrogen peroxide-induced inflammation model in rats.

Ref. range; WBC=4000-10000, Hydrogen peroxide was administered orally with drinking water at 0.5% , Pleuran was administered orally /kg of body weight/day.

### Platelet Count

The results depicted in Figure (2) reveal that Pleuran had no impact on healthy animals. However, it displayed an elevation in platelet count for animals affected by hydrogen peroxide-induced inflammation. This elevation reached its peak at a concentration of 15 mg of Pleuran, reaching 188,000 platelets/ $\mu$ L, as opposed to the hydrogen peroxide treatment alone which recorded 130,000 platelets/ $\mu$ L.



**Fig. 2** demonstrates the influence of Pleuran on platelet count resulting from the hydrogen peroxide-induced inflammation model in rats.

Ref. range; Platelet= 140000-400000, Hydrogen peroxide was administered orally with drinking water at 0.5%, Pleuran was administered orally /kg of body weight/day.

## Differential White Blood Cell Ratios

The results presented in Table (1) indicate the effect of Pleuran on differential white blood cell ratios resulting from the hydrogen peroxide-induced inflammation model in rats. The results reveal that there were no statistically significant differences observed in various types of white blood cell populations when treating healthy animals with various concentrations of Pleuran, compared to the control animals (untreated healthy animals). However, when subjecting animals to hydrogen peroxide treatment to induce inflammation, there was a significant reduction in the lymphocyte cell population, decreasing to 24% compared to the 40% observed in the control treatment (untreated healthy animals). Moreover, these ratios increased to 38%, 40%, and 42% in the presence of Pleuran concentrations of 5, 10, and 15 mg/kg/day, respectively, when subjected to hydrogen peroxide treatment, in contrast to levamisole treatment which reached 40%. Additionally, the proportions of Neutrophil, Monocyte, Basophil, and Eosinophil cells rose in response to hydrogen peroxide treatment, reaching 62%, 8.5%, 2%, and 3.5%, respectively, compared to the control treatment (untreated healthy animals) which recorded proportions of 49%, 7%, 1%, and 3%, respectively. However, these proportions declined when treated with Pleuran, reaching their lowest values of 47%, 6%, 1%, and 2.5% in the presence of Pleuran concentrations of 15 mg/kg/day, 10 mg/kg/day, and (all concentrations), respectively, compared to levamisole treatment which recorded proportions of 52%, 5%, 1%, and 2%, respectively. Furthermore, the results also indicated that the lowest ratio (index) of neutrophil-to-lymphocyte cells was 1.17 in treatment with Pleuran concentrations of 10 and 15 mg/kg/day in healthy laboratory animals, compared to 1.23 in the control treatment (untreated healthy animals). However, when inducing inflammation in animals through hydrogen peroxide treatment, this indicator significantly increased to 2.58. This indicator subsequently decreased to 1.31, 1.27, and 1.12 in hydrogen peroxide treatment with the presence of Pleuran concentrations of 5, 10, and 15 mg/kg/day, respectively, in comparison to levamisole treatment which registered an indicator value of 1.3.

**Table 1:** Effect of Pleuran on Differential Leukocytes (%) in the hydrogen peroxide-induced Inflammation Model in Albino Rats

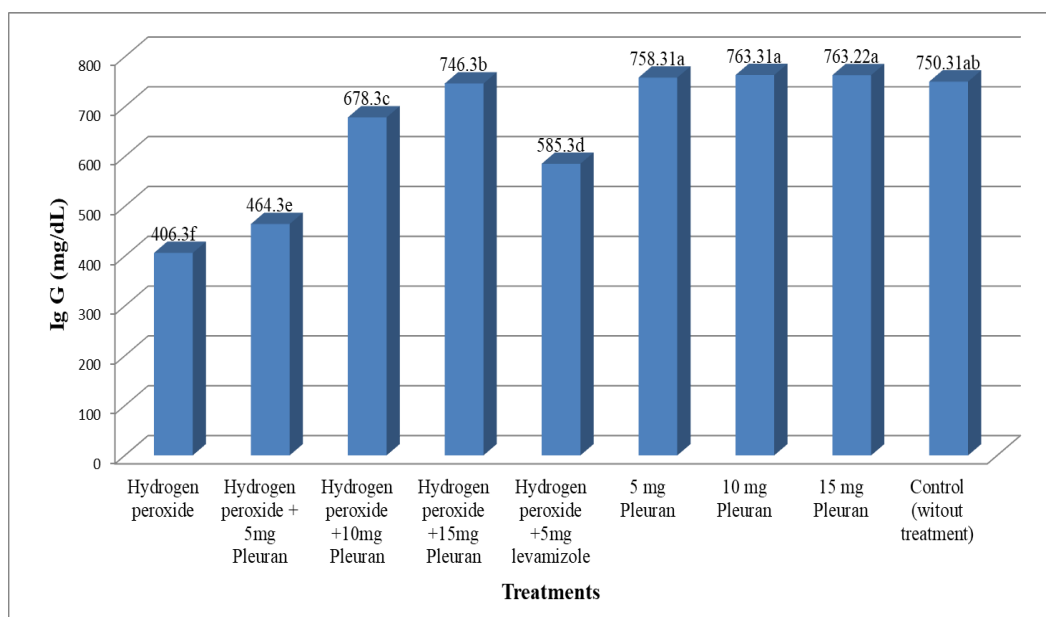
Animals groups	Differential Leukocytes count (%)					Neutrophil/ Lymphocytes Index
	Lymphocyte	Neutrophil	Monocyte	Basophil	Eosinophil	
Control (untreated)	40	49	7	1	3	1.23
Pleuran (5 mg/kg)	40	49	7	1	3	1.23
Pleuran (10 mg/kg)	41	48	7	1	3	1.17
Pleuran (15 mg/kg)	41	48	7	1	3	1.17
H <sub>2</sub> O <sub>2</sub> + Pleuran (5 mg/kg)	38	50	7	1	4	1.31
H <sub>2</sub> O <sub>2</sub> + Pleuran (10 mg/kg)	40	51	6	1	2	1.27
H <sub>2</sub> O <sub>2</sub> + Pleuran (15 mg/kg)	42	47			2.5	1.12
H <sub>2</sub> O <sub>2</sub> + levamisole (10 mg/kg)	40	52	7.5	1	2	1.30
H <sub>2</sub> O <sub>2</sub> alone	24	62	5	1	3.5	2.58
LSD <sub>0.01</sub>	1.08	1.31	8.5	2	0.042	0.061

Ref. range (%), lymphocyte; 20-40, Neutrophil; 40-60, Monocyte; 2-8, Basophil; 0.5-1, Eosinophil; 1-4.

The Influence of Pleuran on Some Immune Indices Resulting from the Hydrogen Peroxide-Induced Inflammation Model in Rats.

## Immunoglobulin G

The results presented in Figure (3) indicate that Pleuran demonstrated an elevation in the concentration of immune globulin G (IgG) in animals afflicted with hydrogen peroxide-induced inflammation, particularly at a concentration of 15 mg. The IgG concentration reached 746.3 mg/dL compared to 585.3 mg/dL in the levamisole treatment at a concentration of 5 mg. Subsequently, Pleuran treatment at a concentration of 10 mg resulted in a concentration of 678.3 mg/dL. Conversely, the lowest concentration of immune globulin G (IgG) was recorded in the hydrogen peroxide treatment alone, with a value of 406.3 mg/dL. The results also illustrate the lack of effect of Pleuran on healthy animals.

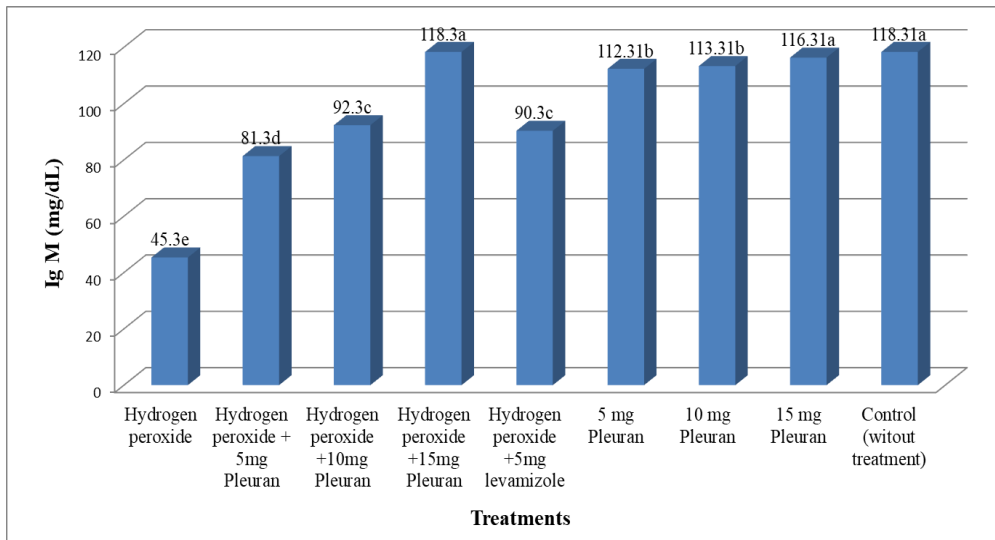


**Fig. 3** Effect of pleuran on the concentration of immunoglobulin G (mg/dL) resulting from the hydrogen peroxide-induced inflammation model in albino rats.

Ref. range; Ig G = 650-1600 mg/Dl. Hydrogen peroxide was administered orally with drinking water at 0.5%. Pleuran was administered orally /kg of body weight/day.

## Immunoglobulin M (IgM)

The results illustrated in Figure (4) reveal that Pleuran had no impact on healthy animals. However, it exhibited a noticeable effect in the inflammation-induced treatments with hydrogen peroxide. This effect became more pronounced as the concentration of Pleuran increased. The highest concentration of immune globulin M (IgM) was recorded at 118.3 mg/dL for Pleuran treatment with a concentration of 15 mg, compared to 90.3 mg/dL in the hydrogen peroxide treatment with levamisole. Conversely, the lowest concentration of immune globulin M (IgM) was observed in the hydrogen peroxide treatment alone, reaching 45.3 mg/dL.

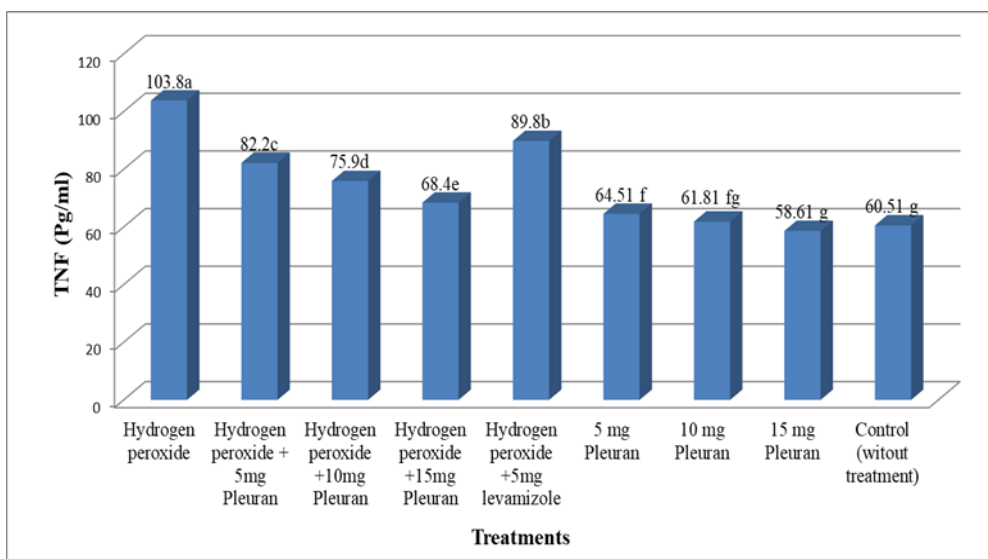


**Fig. 4** Effect of pleuran on the concentration of immunoglobulin M (mg/dL) resulting from the hydrogen peroxide-induced inflammation model in albino rats

Ref. range; Ig M = 54-300 mg/dL, Hydrogen peroxide was administered orally with drinking water at 0.5%, Pleuran was administered orally /kg of body weight/day.

### Interleukin-6 (IL-6)

The results depicted in Figure (5) indicate that Pleuran had no impact on healthy animals. However, it exhibited a reduction in the concentration of IL-6, reaching 9.66 nanograms/liter at a concentration of 15 mg in animals afflicted with hydrogen peroxide-induced inflammation, compared to 8.81 nanograms/liter in the control treatment. Notably, the concentration of IL-6 was notably higher in the hydrogen peroxide treatment alone, registering at 37.8 nanograms/liter. Subsequently, the Pleuran treatment at a concentration of 10 mg recorded a concentration of 12.04 nanograms/L.



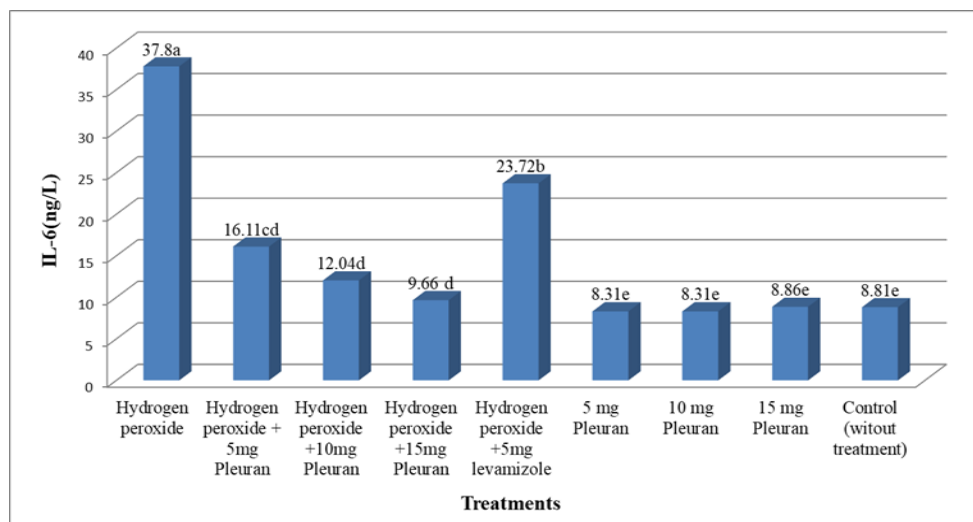
**Fig. 5** Effect of pleuran on the concentration of Interleukin-6 (IL-6) resulting from the hydrogen peroxide-induced inflammation model in albino rats.

Ref. range; IL-6 = 10 ng/L, Hydrogen peroxide was administered orally with drinking water at 0.5% , Pleuran was administered orally /kg of body weight/day



## Tumor Necrosis Factor-Alpha (TNF- $\alpha$ )

The results presented in Figure (6) reveal that Pleuran treatment did not exhibit statistically significant differences in healthy animals. However, it demonstrated a reduction in the concentration of Tumor Necrosis Factor-Alpha (TNF- $\alpha$ ) at a concentration of 15 mg. The concentration of TNF- $\alpha$  reached 68.4 picograms/mL in animals afflicted with hydrogen peroxide-induced inflammation, compared to 103.8 picograms/mL in the hydrogen peroxide treatment alone.



**Fig. 6** Effect of pleuran on the concentration of Tumor Necrosis Factor-Alpha (TNF- $\alpha$ ) resulting from the hydrogen peroxide-induced inflammation model in albino rats.

Ref. range ; TNF =  $75 \pm 15$  Pg/ml, Hydrogen peroxide was administered orally with drinking water at 0.5% , Pleuran was administered orally /kg of body weight/day

## Discussion

Hydrogen peroxide ( $H_2O_2$ ) exhibited an inflammatory effect in the treated animals, as evidenced by the reduction in white blood cell count. This phenomenon can likely be attributed to the functional role of  $H_2O_2$  during inflammation, as it is responsible for activating NF $\kappa$ B, which serves as a pivotal regulatory molecule in the transcription of numerous genes involved in inflammation. NF $\kappa$ B constitutes a well-known family of transcription factors that are sensitive to oxidation and reduction. Its activation involves tyrosine phosphorylation and the triggering of IKK (I $\kappa$ B kinase) through the influence of  $H_2O_2$  (Schoonbroodt *et al.*, 2000; Yin *et al.*, 2000). In a study conducted by Khudair (2008), the effects of 0.5%  $H_2O_2$  and levamisole on cellular and humoral immunity in rabbits male were elucidated. The results revealed that exposing animals to  $H_2O_2$  for four weeks led to a significant decrease ( $P < 0.05$ ) in the percentage of lymphocyte cells compared to the control treatment. A notable increase in the lymphocyte cell percentage was observed after levamisole administration in the group treated with  $H_2O_2$ , in contrast to the treatment period. Oral administration of 0.5%  $H_2O_2$  to male rabbits resulted in immunosuppressive effects manifested by reduced white blood cell count, decreased platelets, a significant decrease in the total and active lymphocyte cell percentage, reduced phagocytic activity, and decreased IgG concentration in the blood. The results demonstrated that hydrogen peroxide ( $H_2O_2$ ) led to a reduction in the concentration of immunoglobulins G (IgG) and IgM. This decrease can be

attributed to oxidative stress induced by hydrogen peroxide, which negatively affects the animal's health. This effect might be connected to the immune-inhibitory impact of H<sub>2</sub>O<sub>2</sub> due to the oxidative stress resulting from increased reactive oxygen species (ROS) production, particularly hydroxyl radicals (Fariss *et al.*, 2005; Schumaker, 2006; Newsholme *et al.*, 2007; Robertson, 2007; Tang, 2007). It has been noted that exposure to high concentrations of H<sub>2</sub>O<sub>2</sub> externally causes oxidative stress in animals (Tritto *et al.*, 1998; Khudair, 2008). When compared to other cell types, immune cells are highly susceptible to oxidative damage, with increased sensitivity to programmed cell death (apoptosis) and cell membrane damage resulting in impaired immune response (Hana *et al.*, 2006; Maies *et al.*, 2007). On the other hand, high-risk oxidative damage may arise from the additional generation of ROS through elevated NADPH oxidase activity (Babior, 2002; Ganong, 2005). Moreover, the stress condition resulting from exposure to H<sub>2</sub>O<sub>2</sub> might lead to excessive cortisol secretion, which is an established immune modulator (Ganong, 2005). These findings also highlight the role of levamisole in enhancing the immune inhibitory effect of H<sub>2</sub>O<sub>2</sub>. Levamisole is utilized in immunotherapy for both experimental animals and humans, often employed in cases involving immune suppressants (Szeto *et al.*, 2000; Khalel, 2007). It has been noted that levamisole increases the levels of guanosine monophosphate (GMP) in both monocyte and neutrophil cells, enhancing hexose monophosphate activity and thereby stimulating phagocytosis and enhancing the chemical response (Marlton & Kurzrock, 1993; Demicrt, 2005). The results also demonstrated the role of Pleuran in increasing the concentration of immunoglobulins G (IgG) and IgM. This finding aligns with the results of a study by Yalu *et al.* (2020), which found that a high dose of *P. ostreatus* Glucan (POG) at 0.9 g/kg of body weight can stimulate the immune response by increasing the concentration of cytokines and immunoglobulins in the serum after inducing inflammation with cyclophosphamide. In another study by Suhad *et al.* (2018), the efficacy of crude *P. ostreatus* extract as an immune stimulant was evaluated by studying the contribution of certain immune variables to the response to the crude extract in white rats. The effective dose of the *P. ostreatus* extract was determined by treating rats with different doses of the extracts and then evaluating them through complete blood count (CBC). The results indicated that a dose of 100 mg/kg was the most effective in stimulating the immune response. The results also showed a significant increase in IgM and IgG levels at the sixth dose after 14 days, with recorded values of 34.11 and 71.35 mg/dL, respectively.

Furthermore, the results revealed an elevation in the tumor necrosis factor (TNF) in the hydrogen peroxide treatment, indicating the induction of inflammation by hydrogen peroxide. This can be attributed primarily to its superior oxidative reactivity when compared to other reactive oxygen species (ROS), along with its longer half-life, enabling better oxidizing activity and enhanced cell membrane penetration. These findings are in accordance with the study by Liao and Huang (2019), where hydrogen peroxide was used to stimulate gastric mucosal cells in humans as a model for oxidative damage, demonstrating its effectiveness in inducing oxidative damage. In a study by Min and Kang (2021), the anti-inflammatory properties of *Pleurotus ostreatus* were elucidated. *In vitro* and *In vivo* testing of *Pleurotus ostreatus* powder revealed that this mushroom significantly reduced the secretion of IL-6, IL-12, and TNF-. These findings are consistent with Jedinak *et al.* (2011), who demonstrated that oyster mushrooms can be used as a dietary supplement with anti-inflammatory properties. The results of this study indicate the absence of statistically significant differences in the

proportions of all white blood cell types. Furthermore, the ratio of neutrophils to lymphocytes in the control animals remained consistent across various concentrations of Pleuran, suggesting that there is no detrimental effect of Pleuran in these animals. The results of this study indicate a reduction in the inflammatory response due to all pleuran concentrations, as evidenced by the normalization of white blood cell proportions and the decrease in the neutrophil/lymphocyte ratio. This reduction in inflammation could be attributed to the high molecular weight of  $\beta$ -glucan produced by *P. ostreatus*.  $\beta$ -glucans are known for their anti-tumor, anti-inflammatory, and immune-stimulating activities. Immune cells express pattern recognition receptors (PRRs) like dectin-1 that recognize pathogen-associated molecular patterns, such as  $\beta$ -glucans and fungal cell wall components. When  $\beta$ -glucans bind to immune cells, particularly macrophages expressing dectin-1, it activates immune responses. This activation leads to the secretion of various cytokines, such as interleukins (IL-6 and IL-4) and tumor necrosis factor-alpha (TNF- $\alpha$ ), as well as the activation of lymphocytes like B and T cells and natural killer (NK) cells.  $\beta$ -glucans that are water-insoluble tend to have stronger immune-modulating effects compared to their water-soluble counterparts. These findings align with another study that highlight the immune-enhancing properties of *P. ostreatus* polysaccharide by Bulam *et al.* (2019) who demonstrated that the polysaccharides extracted from *P. ostreatus* can improve the effectiveness of peripheral white blood cells and stimulate the secretion of interleukin-6 (IL-6) along with the enhanced function of specialized white blood cells.

## Conclusion

The present study concludes that the pleuran (purified from the fungus *P. ostreatus* var. *ostreatus*) has immunomodulatory anti-inflammatory properties. All pleuran concentrations in healthy animals did not negatively affect the Hematological properties, while these treatments led to an improvement in white blood and platelet account, interleukin-6 and TNF- $\alpha$  factor, as well as a reduction in the lymphocyte/neutrophil index to its normal state in the H<sub>2</sub>O<sub>2</sub>-induced inflammation model in albino rats.

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## استخلاص البليوران من انواع الفطر الغذائي Pleurotus spp وتقييم كفاءتها كمضادات للالتهابات والاكسدة والتعزيز المناعي

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### الخلاصة:

أجريت هذه الدراسة في مختبرات كلية الزراعة وبيت الحيوان بكلية الطب البيطري، جامعة تكريت، خلال عام 2023/2022، بهدف تقييم تأثير بلوران المنقى من الفطر الصالح للأكل *Pleurotus ostreatus* var. *ostreatus* كعامل مناعي ومضاد للأكسدة في الفئران الالتهابية مع بيروكسيد الهيدروجين. أظهرت نتائج تأثير Pleuran في الجسم الحي (الجرذان البيضاء) أن جميع تركيزات Pleuran لم يكن لها تأثير سلبي على المعلمات الدموية في الحيوانات السليمة. ومع ذلك، أدت هذه العلاجات إلى تحسين تعداد خلايا الدم البيضاء، وإعادتها إلى المستويات الطبيعية في الحيوانات التي يسببها الالتهاب بفعل بيروكسيد الهيدروجين، ولوحظت أعلى التحسينات عند 15 مجم / كجم من وزن الجسم من بلوران، مع تعداد خلايا الدم البيضاء والصفائح الدموية. تصل إلى 7500 و188000 خلية / ميكرو لتر، على التوالي. انخفض مؤشر العدلة / اللمفاويات من 2.58 في الحيوانات الالتهابية إلى 1.12 في وجود Pleuran عند 15 مجم / كجم / يوم، مقارنة بـ 1.3 في مجموعة الليفاميزول. كان أدنى غلوبولين مناعي (IgG)(G) و 406.3 M (IgM) و 45.3 مجم / ديسيلتر في علاج بيروكسيد الهيدروجين وحده، ثم زاد في جميع تركيز الجنب الجنب، وكانت أعلى القيم 746.3 و 118.3 مجم / ديسيلتر في الجنب عند 15 مجم / كجم / يوم، مقارنة بـ 585.3 و 90.3 مجم / ديسيلتر في مجموعة الليفاميزول، بينما أظهرت النتائج زيادة في عامل نخر الورم ألفا (TNF-α) وإنترلوكين 6 (IL-6) إلى 103.8 بيكوغرام / مل و 37.8 نانوغرام / ل. في المعالجة ببيروكسيد الهيدروجين وحده، وتزداد في جميع تركيزات الجنب، بأعلى قيم 68.4 بيكوغرام / مل و 9.66 نانوغرام / لتر في الوقت الحاضر من بلوران عند 15 مجم / كجم / يوم، مقارنة بـ 89.8 بيكوغرام / مل و 9.66 نانوغرام / لتر في مجموعة الليفاميزول، على التوالي.

### معلومات البحث:

تاريخ الاستلام:

تاريخ التعديل:

تاريخ القبول:

تاريخ النشر:

### الكلمات المفتاحية:

مضاد لالتهابات، المناعي، عامل نخر

الورم ألفا (TNF-α)

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