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Immunomodulatory Activities of *Ganoderma lucidum* Polysaccharide and *Pleurotus tuberregium* Polysaccharide Extracts on Lead-Intoxicated Wistar Rats

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Abstract

Exposure to lead (Pb) impairs the immune function of the spleen, making it difficult for the body to fight infection. *Ganoderma lucidum* polysaccharide (GLP) and *Pleurotus tuberregium* polysaccharide (PTP) have biological activities that boost host immunity by enhancing immune cell function and promoting the release of immune factors. This study aimed to evaluate the immunomodulatory activities of *Ganoderma lucidum* and *Pleurotus tuberregium* polysaccharide extracts in Pb-intoxicated Wistar rats. Fifty-six (56) male Wistar rats were randomly divided into seven groups of eight animals each. Group 1 was administered rat pellets and water (*ad libitum*). The other six groups were treated with Pb (25 mg/kg) for 14 days to determine the pathological effects of Lead (Pb). Fourteen (14) days after Pb administration, the animals were treated as follows: group 2 served as the Pb only group, group 3 received PTP (100 mg/kg), group 4 received GLP (100 mg/kg), group 5 received 2,3-Dimecarptosuccinic acid (DMSA) (50 mg/kg), group 6 received penicillamine (PENN) (30 mg/kg), and group 7 received calcium disodium ethylenediaminetetracetic acid ($CaNa_2EDTA$) (50 mg/kg). Haematological parameters, oxidative stress, and histopathological analyses were performed. The results of this investigation showed that GLP and PTP attenuated Pb-induced haematological derangement with a significant increase in the activities of catalase, superoxide dismutase (SOD), and non-enzymatic glutathione (GSH). Histology of the spleen of rats administered with Pb only showed splenic tissue with areas of inflammation, while the rats treated with GLP and PTP exhibited splenic tissue with normal red pulp and normal white pulp follicles. This study suggests that GLP and PTP have strong immunomodulatory properties by increasing anti-oxidant activities and alleviating histological alteration

Keywords: *Ganoderma lucidum*, *Pleurotus tuberregium***,** lead, spleen, hematology

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Introduction

Although the spleen is not as well-known as other organs, it performs multiple important functions. The spleen participates in the synthesis of blood cells and helps filter blood, removing old blood cells and fighting infection (Muselin *et al*., 2010). The spleen also helps control the amount of blood circulating through the body by creating a reserve pool of blood that can be released during severe bleeding to help improve circulation, oxygenation, and blood pressure in dire circumstances. Exposure to lead (Pb) impairs the immune function of the spleen, making it difficult for the body to fight off infections. Pb reduced phagocytic activity and disrupted cytokine production. Lead (Pb) also has a direct effect on the blood content of red blood cells, as it affects their size and shape (Kim *et al*., 2020). Lead affects the percentage of hemoglobin in the blood, as it leads to a decrease in the level of hemoglobin and many other blood variables (Ekanem *et al*., 2015). The effect of Pb-poisoning on the level of anti-oxidant production and other changes in the body in general, extends from the smallest cell to the largest organ (La-Llave-León *et al*., 2016). Lead poisoning can be treated with chelating agents such as 2,3-dimecaptosuccinic acid (DMSA), pennicillamine (PENN), and calcium disodium ethylenediaminetetracetic acid ($CaNa_2EDTA$); however, these agents have secondary toxic effects. Therefore, it is necessary to address lead poisoning using a healthy natural substance. Polysaccharides obtained from medicinal mushrooms can be used as immunomodulators and antioxidant agents to boost the immune system (*Zheng et al.*, 2010; Li *et al*., 2022). Examples of such medicinal mushrooms include *Ganoderma lucidum* and *Pleurotus tuberregium. Ganoderma lucidum* polysaccharide (GLP), a natural polysaccharide, has been widely used in the field of immune enhancement (*Ahmad et al*., 2021). *Ganoderma lucidum*, from which GLP is derived, is a traditional Chinese medicine that has been used for thousands of years in Asia because of its immunomodulatory effects (Wei *et al*., 2018; Li *et al.,*2022). Previous studies have shown that *Ganoderma lucidum* polysaccharide (GLP) has different bioactivities, such as antioxidant properties, sleep-protecting effects, and amelioration of oxidative stress, and its most essential biological activity is to enhance immune cell function and promote the release of immune factors (Zeng *et al*., 2018). The enhanced immune function of polysaccharides obtained from *Ganoderma lucidum* has been reported to reduce the side effects associated with Pb toxicity (Lin *et al.*, 2019; Seweryn *et al*., 2021). Another important medicinal mushroom is *Pleurotus tuberregium*. It is found in both tropical and subtropical regions of the world (Okhuoya and Okogbo, 1991; Ogbo *et al.*, 2011), especially in the southern part of Nigeria. *Pleurotus tuberregium* polysaccharide (PTP) obtained from *Pleurotus tuberregium* is used in traditional practice to boost the immune system and treat headache, colds, fever, asthma, smallpox, and high blood pressure (Okhuoya and Okogbo, 1991; Fasidi and Olorunmaiye, 1994; El Enshasy *et al*. 2013). PTP is sometimes used to induce weight gain in malnourished babies (Alobo, 2003; Isikhuemhen *et al*., 2003; El Fakharany *et al*., 2010). *Ganoderma lucidum* polysaccharide (GLP) and *Pleurotus tuberregium* polysaccharide (PTP) not only play a role in resisting Pb toxicity but also avoid the adverse effects of artificial metal-chelating agents (Li *et al*. 2021). Therefore, this study was designed to investigate the potential roles of GLP and PTP in ameliorating the effects of lead poisoning in the spleen and blood of Wistar rats.

Materials and Methods

Collection of Mushrooms and Reagents

Ganoderma lucidum and Pleurotus tuberregium mushrooms were collected from the forest in Iwaro Oka Akoko, Ondo State, Nigeria, and identified by Dr. Ademola Olatokunbo from the Department of Plant Science and Biotechnology, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo State. Penicillamine (PENN), 2,3-Dimecarptosuccinic Acid (DMSA), and calcium disodium ethylenediaminetetraacetic acid ($CaNa_2EDTA$) were obtained from Sigma-Aldrich (St. Louis, MO, USA), and other reagents were of analytical grade or higher.

Mushrooms Preparation

The mushrooms were cleaned, rinsed thoroughly with tap water, and cut into smaller pieces. *Ganoderma lucidum* was spread under the shade and allowed to dry for 15 days, while *Pleurotus tuberregium* was oven-dried at 50^0C for seven days. After drying, the mushroom was pulverized into a powdered form with the aid of a mechanical engine.

Extraction of polysaccharides from the fruiting body of Ganoderma. lucidum and Pleurotus tuberregium

Dried powdered G*anoderma lucidum* and *Pleurotus tuberregium* **(**500 g**)** were separately added to 4000 ml of water and boiled for 60 min at 50 °C. The mixtures were then filtered using a filtered funnel with Whatman paper grade 1 circles (diameter 45 mm, pack of 100) to obtain the filtrate and residue. The filtrates were centrifuged separately at 4000 rpm for 10 min at room temperature to obtain the supernatant. The supernatants obtained were concentrated in a water bath set at 55 °C. The concentrates were dissolved in four volumes of absolute ethanol (1:4) and centrifuged to separate the precipitate. The precipitates were dissolved in a small volume of water and concentrated in a water bath to obtain a polysaccharide-rich extract (Xiao et al., 2017: Xu *et al.,* 2017). GLP and PTP

extracts were stored in a refrigerator and then dissolved in distilled water before oral administration for 28 days.

Acute Toxicity Test

The Organization for Economic Co-operation and Development (Citarish, 2022) limit test guideline (2022) was used to determine the acute toxicity of the extract. Eighteen (18) adult male rats (184 – 222 g) were used in this study. The rats were randomly divided into two groups of three animals. The animals were fasted overnight (without food but were given water) and then sacrificed. A Single oral dose of each extract of *Ganoderma lucidum* Polysaccharide (GLP) and *Pleurotous tuberregium* Polysaccharide (PTP) at 300 mg/kg and 2000 mg/kg were administered to groups 1 and 2 and the control were fed with rat pellet and water *ad libitum.* The animals were observed for 2 h for any behavioral and neurological features, then intermittently over the next 72 h and daily for 14 days with special attention to any moribund state or death.

Experimental Design

Fifty-six (56) male Wistar rats were randomly divided into seven groups of eight animals each. Group 1 was administered rat pellets and water. The other six groups were treated with Pb (25 mg/kg) for 14 days Fourteen (14) days after Pb administration, the animals were treated as follows, group 2 served as Pb only, group 3 received PTP (100 mg/kg), group 4 received GLP (100 mg/kg), group 5 received 2,3- Dimecarptosuccinic acid (50 mg/kg), group 6 received Penicillamine (PENN) (30 mg/kg), and group 7 received Calcium disodium Ethylenediaminetetracetic acid ($CaNa_2EDTA$) (50 mg/kg). All animals in each group were weighed twice a week.

Ethical approval

Animal handling procedures followed the guidelines published by the National Institute of Health (Clark *et al*., 1997) and the University of Ilorin Ethical Review Committee (UIERC). Approval number: UERC/ASN/2023/2572.

Sacrifice and Collection of organs

At the end of the 14 days treatment, the rats were anesthetized with ketamine. Blood was collected intracardially into plain tubes to obtain serum and into EDTA bottles for haematological analysis. The spleen was carefully removed, rinsed, and weighed. The splenic index was determined using relative organ weights.

Evaluation of Haematological Indices

Haemoglobin (Hb), red blood cell (RBC), hematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were measured using an automated hematology analyzer. White blood cell counts were also determined**.**

Assay of Lead Concentration in Tissues Homogenate (Principle of Flame Atomic Absorption Spectroscopy

A volume (10 ml) of nitric acid and hydrogen perchloride in a ratio 5 of2 was introduced into a conical flask with 0.5 ml of the tissue homogenate. The flask was heated in the fume chamber for 30–45 min, until the colored fumes gradually disappeared and the volume of the sample with the mixed acid was reduced to approximately half of the original volume with the flask becoming clear. The digested sample was filtered using a Whatman filter paper No. 1 into a 100 ml standard flask. The Pb concentration in the sample was determined using a flame atomic absorption spectrophotometer, and the data obtained were recorded. (Haswell 1991; Sobowale and Saliu, 2025).

Determination of Anti-oxidant Parameters

Catalase activity in the supernatants of the spleen was determined using a colorimetric assay based on the yellow complex with molybdate, as described by Goth et al. (1991). Catalase enzyme activity was expressed as U/mg protein. Absorbance at 405 nm was measured using an LT-4500 microplate reader (Labtech, UK). Reduced glutathione (GSH), a non-enzymatic anti-oxidant marker, was measured in the supernatant. The glutathione concentration was extrapolated from the standard curve of glutathione (0-200 µM) and expressed as µM GSH/mg protein, according to Nagadabu et al. (2010). The absorbance was measured within 5 min at 405 nm using an LT-4500 microplate reader (Labtech, UK). Superoxide dismutase (SOD) was assayed in the supernatant using the method described by Misra and Fridovich, which depends on the auto-oxidation of adrenaline (epinephrine) in aqueous solution to adrenochrome. The concentration of adrenochrome formed was measured using an LT-4500 microplate reader at 420 nm. Malondialdehyde (MDA) level in the supernatant was also measured with microplate reader. This method is based on the reaction between MDA and thiobarbituric acid to form a pink-colored MDA-TBA adduct that can be measured at 535 nm.

Histology of the Spleen

Individual spleen specimens were submerged in Bouin's fixative, dehydrated in alcohol, and embedded in paraffin. Five-metre sections were cut, deparaffinized, and stained with hematoxylin and eosin. The stained slides were captured using a light microscope at 400 × magnification, and the resulting histomicrograph was evaluated for Pb-induced histoarchitectural alterations (Asiwe et al., 2021).

Statistical Analysis

Data are presented as mean ± standard error of mean (SEM), and n = 8 rats per group. Comparisons were made between the treated and control groups using one-way analysis of variance (ANOVA) followed by *Tukey's post-hoc test*. All data were analyzed using GraphPad Prism (UK) version 5. Statistical significance was set at 0.05 ($P < 0.05$) were considered significant.

Results

Effect of Ganoderma lucidum and Pleurotus tuberregium Polysaccharide extract on Organ weights of Pb-intoxicated Wistar Rats.

Lead (Pb) intoxication significantly ($P < 0.05$) reduced spleen weight compared to the control group. In the lungs, the group treated with ($CaNa_2EDTA$) significantly ($P < 0.05$) reduced compared with the Pb-only group (Table 1). The weights of the other organs treated with Pb only (kidney, liver, testes, and brain) were not statistically significant ($P > 0.05$) when compared with the control group.

Effect of GLP and PTP on Pb-Induced Alteration in Anti-oxidant Parameters

Exposure of the kidney to Pb for 14 days significantly ($P < 0.05$) reduced the levels of non-enzymatic glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) compared to the control group. However, treatment with PTP and GLP significantly (***$P < 0.001$) increased the levels of catalase and superoxide dismutase but did not increase the activity of non-enzymatic reduced glutathione (GSH). Moreover, the groups treated with DMSA and PENN significantly (***$P < 0.001$, **$P < 0.001$) increased the level of non-enzymatic reduced glutathione, catalase and superoxide dismutase activity compared with the Pb-only group.

Effect of GLP and PTP on Lipid Peroxidation

The administration of Pb resulted in a significant (#$P < 0.05$) elevation of MDA levels in the spleen. In groups treated with GLP and PTP, the levels of MDA were reduced significantly (**$P < 0.001$, ***$P < 0.001$ (Figure 4)

Effect of the polysaccharides on haematological parameters

Lead exposure for 14 days significantly (#$P < 0.05$**)** reduced the level of red blood cells (RBC) when compared with the control. However, treatment with GLP, DMSA, PENN, and $CaNa_2EDTA$ significantly (***$P < 0.001$**)** increased the level of red blood cells. Moreover, treatment with PTP significantly (***$P < 0.001$**)** increased the levels of MCH and MCV compared with the Pb-only group.

Exposure to Pb significantly (#$P < 0.05$) reduced the level of neutrophils (NEU) compared with the control group. There was a non-significant ($P > 0.05$) increase in the levels of these parameters by GLP and PTP treatments. Moreover, 14 days post-treatment with PTP significantly (***$P < 0.001$) increased the levels of EOS and LYMP. Also, GLP and PENN significantly (***$P < 0.001$) increased the level of LYMP and DMSA significantly (***$P < 0.001$) increased the activities of EOS and BAS

Effects of polysaccharides on histoarchitectural alterations in the spleen of lead-induced rats.

Pb exposure caused histoarchitectural alterations in the splenic tissue with areas of inflammation (white arrow), and there was vascular congestion (white arrow) compared to the control group. Administration of PTP and GLP ameliorated alteration caused by Pb in the splenic tissue with normal red pulp (blue arrow) (Figure 5)

Table 1: Effect of *Ganoderma. lucidum* and *Pleurotus tuberregium* Polysaccharide extract on Organ weights of Pb-induced Wistar Rats.

Groups	Doses	Kidney(g)	Liver(g)	Spleen(g)	Testes(g)	Lungs(g)	Brain(g)	Heart(g)
Control	-	1.4±0.1	6.9±03	1.4 ±0.2	2.8±0.9	1.5±0.7	1.7±0.6	0.8±0.1
Pb	25	1.4 ± 0.1	7 ± 0.5	0.8± 0.1#	2.4 ± 0.3	1.9 ±0.2	1.5±0.9	0.8± 0.1
Pb - PTP	25- 100	1.4 ± 0.1	7.2±04	1.1± 0.1	2.7 ± 0.1	1.5± 0.1	1.6±0.9	0.9± 0.1
Pb - GLP	25- 100	1.4 ± 0.1	7.9±06	1.3 ± 0.2	2.6 ± 0.2	1.9 ± 0.2	1.5±0.7	0.9± 0.1
Pb -DMSA	25 - 50	1.5 ± 0.1	7 ± 0.3	0.9 ± 0.1	2.8 ± 0.1	1.7 ± 0.2	1.6±0.7	0.9± 0.1
Pb -PENN	25 -30	1.4 ± 0.1	7.1±06	0.6± 0.1	2.7 ± 0.2	1.8± 0.1	1.5±0.4	0.8± 0.1
Pb-Ca Na_2EDTA	25 -s50	1.5 ± 0.1	7 ± 0.4	0.7± 0.1	2.6± 0.1	1.3± 0.1*	1.5±0.4	0.9± 0.1

#P < 0.05 compared with the control group and *P < 0.05 compared with the Pb-only group. Values are expressed as Mean ± SEM (n=5). PTP- *Pleurotus tuberregium* polysaccharide, GLP *Ganoderma lucidum* polysaccharide, DMSA- 2,3- dimecarptosuccinic acid, PENN – Penicillamine, Pb-Lead, $CaNa_2EDTA$- Calcium Disodium Ethylenediaminetetraacetic Acid

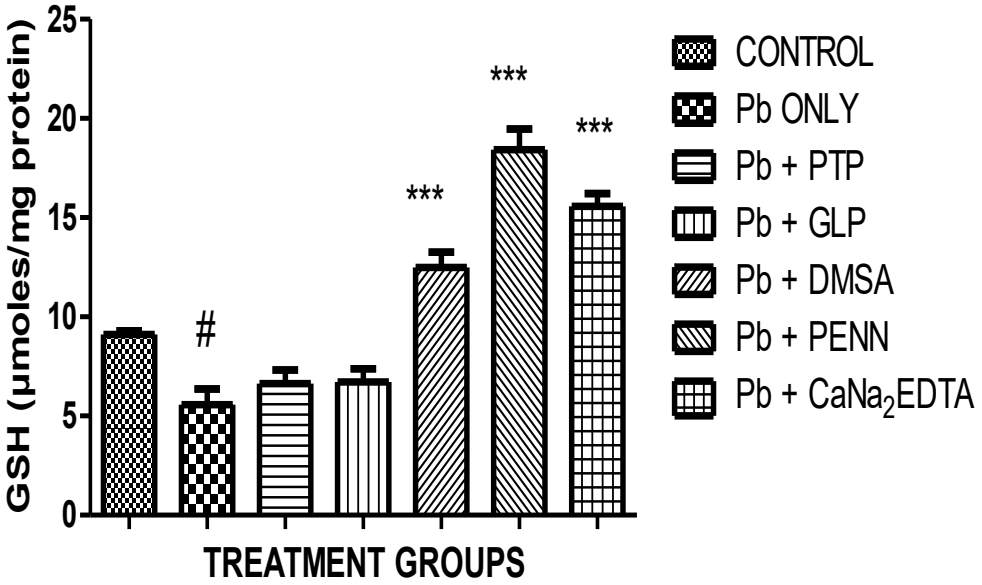

Figure 1: Effect of GLP and PTP on non-enzymatic reduced glutathione (GSH) levels in the spleen. Values are represented as mean ± SEM (n=5). #P < 0.05 compared with control and ***P < 0.001 compared with the Pb-only group. PTP- *Pleurotus tuberregium* polysaccharide, GLP- *Ganoderma lucidum* polysaccharide, DMSA- 2,3- dimecarptosuccinic acid, PENN – Penicillamine, Pb-Lead acetate, $CaNa_2EDTA$- Calcium Disodium Ethylenediaminetetraacetic acid

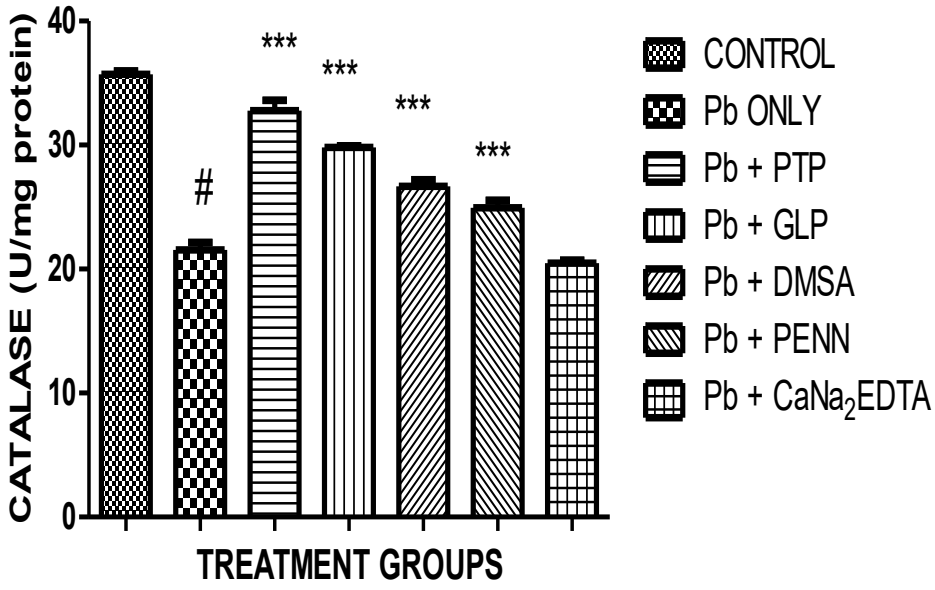

Figure 2: Effect of GLP and PTP on catalase levels in the spleen.
Values are represented as mean ± SEM (n=5). #P < 0.05 compared with control and ***P < 0.001 compared with the Pb-only group. PTP- *Pleurotus tuberregium* polysaccharide, GLP- *Ganoderma lucidum* polysaccharide, DMSA- 2,3- dimecarptosuccinic acid, PENN –Penicillamine, Pb-Lead acetate, $CaNa_2EDTA$- Calcium Disodium Ethylenediaminetetraacetic acid

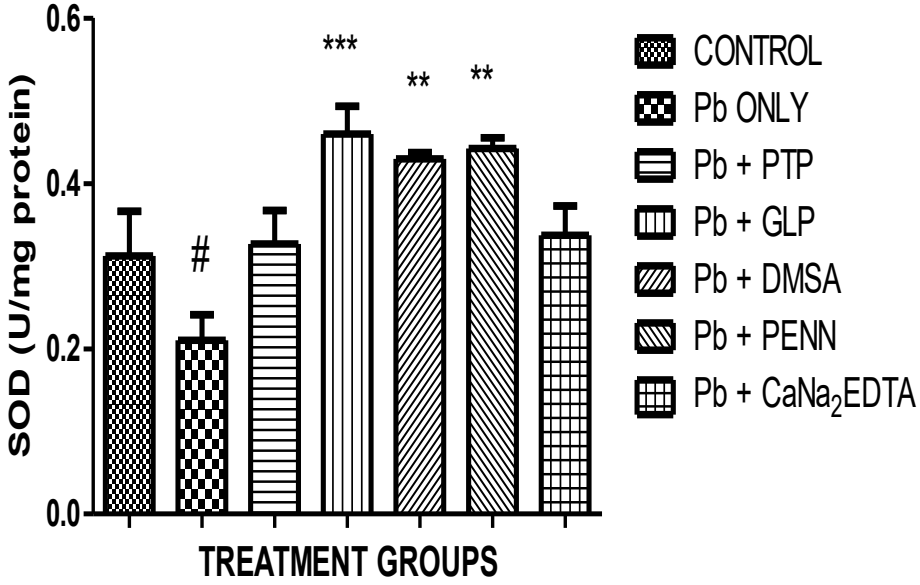

Figure 3: Effects of GLP and PTP on superoxide dismutase levels in the spleen.
Values are represented as mean ± SEM (n=5). #P < 0.05 compared with control and **P < 0.001, ***P < 0.001 compared with the Pb-only group. PTP- *Pleurotus tuberregium* polysaccharide, GLP- *Ganoderma lucidum* polysaccharide, DMSA- 2,3- dimecarptosuccinic acid, PENN – Penicillamine, Pb-Lead acetate, $CaNa_2EDTA$- Calcium Disodium Ethylenediaminetetraacetic acid

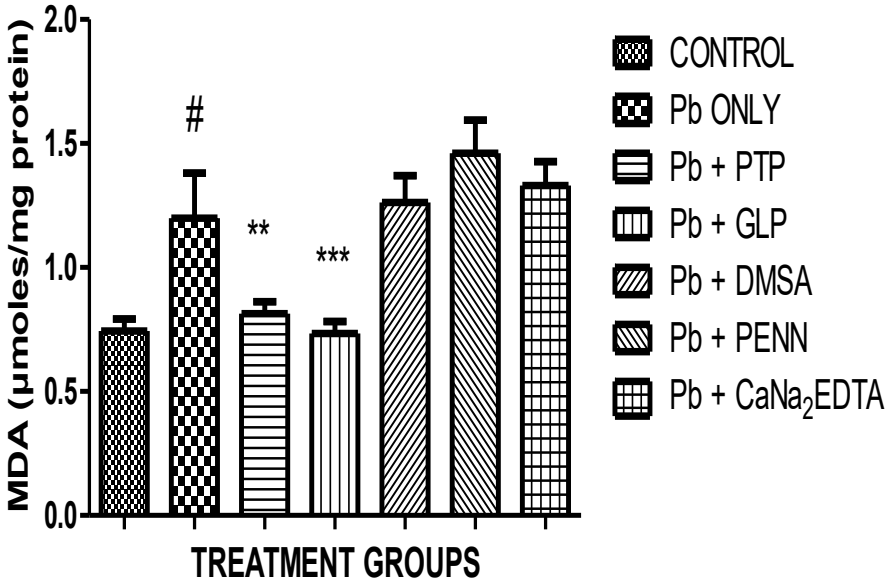

Figure 4: Effect of GLP and PTP on MDA levels in the spleen.
Values are represented as mean ± SEM (n=5). #P < 0.05 compared with control and **P < 0.001, ***P < 0.001 compared with the Pb-only group. PTP- *Pleurotus tuberregium* polysaccharide, GLP- *Ganoderma lucidum* polysaccharide,MSA- 2,3- dimecarptosuccinic acid, PENN – Penicillamine, Pb-Lead acetate, $CaNa_2EDTA$- Calcium Disodium Ethylenediamine

Table 2. Effects of treatment with GLP and PTP extract on red blood cell parameter

Groups	Doses	PCV (%)	Hb g/dl)	RBC (106/ul)	MCH (pg/RBC)	MCHC(g/dl)	MCV(g/dl)
Control	-	60.0±0.3	17.4±0.1	7.5± 0.1	24.5± .1	33.4±0.2	71.6±0.1
Pb Only	25	48.4±0.3	16.5±0.1	6.5± 0.1#	25.4±0.3	33.5±0.2	75.6±0.1
Pb – PTP	25- 100	45.4±0.1	15.4±0.1	4.6± 0.1	33.7±0.1***	33.7±0.1	100.5±0.1***
Pb –GLP	25- 100	44.9±0.1	13.4±0.1	7.7± 0.1***	20.9±0,1	33.1±0.1	63.5±0.1
Pb – DMSA	25- 50	48.1±0.1	16.3±0.1	7.5± 0.1***	24.6±0.2	33.5±0.3	72.5±0.1
Pb- PENN	25-30	48.4±0.1	16.5±0.1	7.2± 0.1***	23.6±0.2	33.8±0.6	70.5±0.1
Pb-$CaNa_2$ EDTA	25-50	46.4±0.1	15.1±0.1	7.2± 0.1***	22.5±0.1	33.4±0.2	66.2±0.1

#P < 0.05 compared with the control and ***P < 0.001 compared with the Pb-only group. PCV-Packed cell volume; Hb: Hemoglobin; RBC: Red blood cell; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; MCV: Mean corpuscular volume. PTP- *Pleurotus tuberregium* polysaccharide, GLP- *Ganoderma lucidum* polysaccharide, DMSA- 2,3- dimecarptosuccinic acid, PENN – Penicillamine, Pb-Lead acetate, $CaNa_2EDTA$- Calcium Disodium Ethylenediamine

Table 3. Effects of treatment with *G. lucidum* polysaccharide and *P. tuberregium* polysaccharide on blood indices.

Groups	Doses	WBC	EOS	BAS	LYMP	MONO	NEU
Control	-	7376±0.9	6 ± 0.4	2± 0.0	23 ± 0.8	8 ± 0.4	61± 0.4
Pb Only	25	8906±2.0	8 ± 0.4	3± 0.8	25 ± 0.8	10 ± 0.8	52± 0.8#
Pb – PTP	25 - 100	6477±0.9	14± 0.4***	2± 0.4	31.75±0.5***	12 ± 0.5	41± 0.8
Pb - GLP	25 - 100	6029±1.7	7 ± 0.8	2± 0.4	29 ± 0.8***	11 ± 0.4	53± 0.8
Pb - DMSA	25 - 50	5954±2.0	13± 0.4***	6± 0.8**	19 ± 0.8	11 ± 0.4	53± 0.8
Pb - PENN	25 - 30	6777±1.2	5 ± 0.0	1± 0.0	29 ± 0.8***	9 ± 0.3	55 ±0.4
Pb-$CaNa_2EDTA$	25 - 50	5637±1.3	5 ± 0.4	3± 0.4	25 ± 0.8	12 ± 0.8	55.75±0.8*

#P < 0.05 compared with the control and ***P < 0.001 compared with the Pb-only group. WBC- White blood cell; EOS- Eosinophil; BAS- Basophill; LYM- Lymphocytes; MONO- Monocytes; NEU- Neutrophil. PTP- *Pleurotus tuberregium* polysaccharide, GLP- *Ganoderma lucidum* polysaccharide, DMSA- 2,3- dimecarptosuccinic acid, PENN – Penicillamine, Pb- Lead acetate, $CaNa_2EDTA$- Calcium Disodium Ethylenediaminet

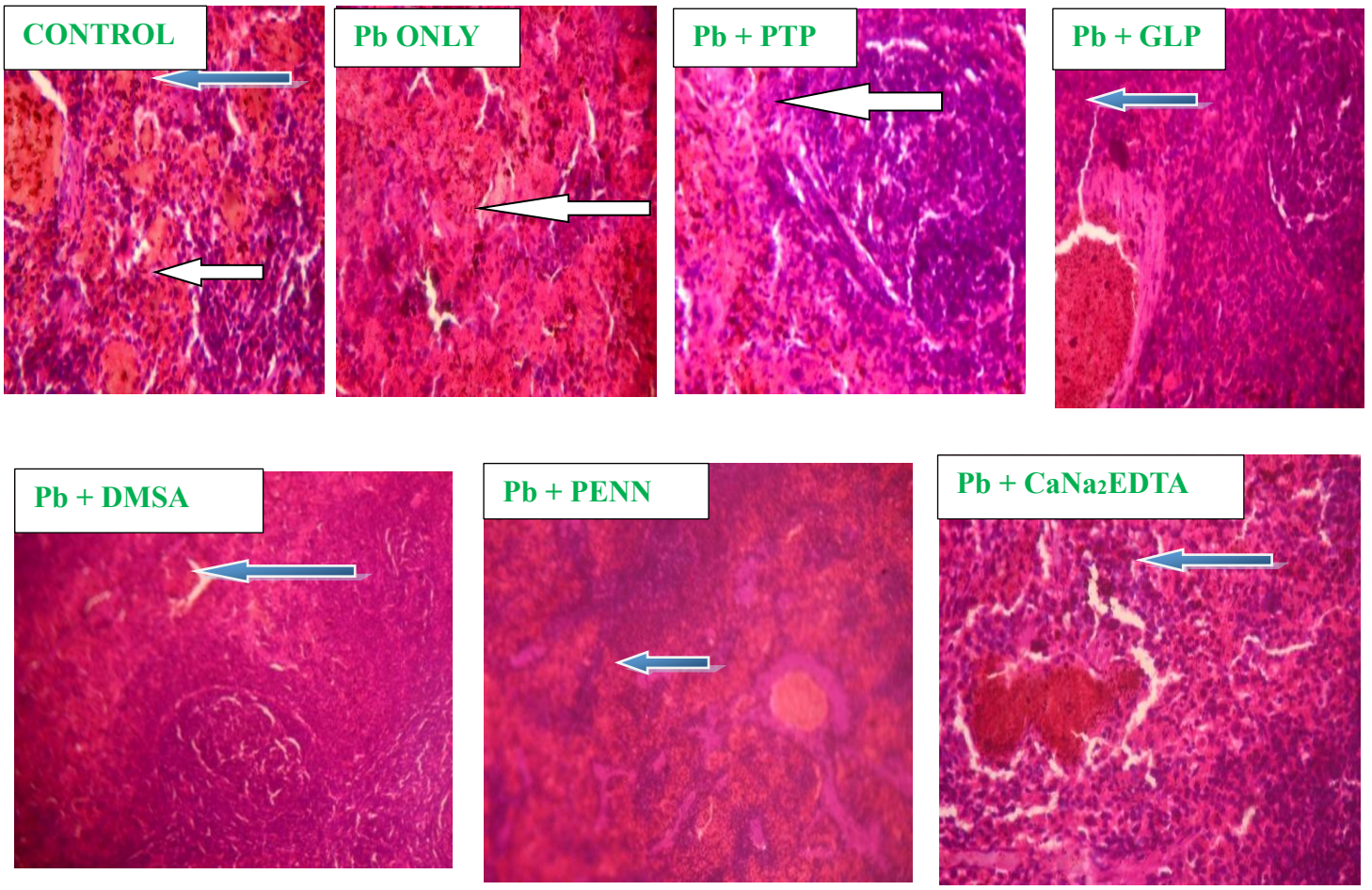

FIGURE 5: Effect of PTP and GLP on lead-induced histoarchitectural alterations in the spleen. Hematoxylin and eosin staining, original magnification × 400. Arrows indicates significant alteration in splenic tissues

Discussion

Ganoderma lucidum polysaccharide (GLP) and *Pleurotus tuberregium* polysaccharide (PTP) exhibit a wide range of pharmacological activities. However, the ability of GLP and PTP to reverse Pb – induced immunosuppression has not been studied. Hence, this study explored the roles of GLP and PTP in attenuating Pb-induced immunosuppression. Administration of GLP and PTP after 14 days of pre-treatment with Pb increased the levels of GSH, catalase, and SOD and downregulated the levels of MDA. The red and white blood cell parameters were also elevated. The study also showed that GLP and PTP reversed the histoarchitectural alterations in the spleen.

The spleen plays an important role in filtering blood and storing immune cells. A reduction in spleen weight may impair the immune function. Administration of Pb led to a reduction in spleen weight. However, PTP and GLP enhanced spleen weight, thereby ameliorating the toxic effect of Pb on the spleen. The findings of this study are consistent with those of previous reports (Fasidi and Olorunmaiye 1994; Jin *et al*. 2016; Ahmad *et al*. 2021).

The role of oxidative stress is to show the level of free radicals that are generated in organisms, which are known to be some of the important mechanisms of lead poisoning (Patra *et al.* 2011). GLP and PTP have significant anti-oxidant properties, which may be related to their polysaccharide content of up to 87.17%, containing 16 amino acids with a total amino acid content of 5.04% (Lin *et al*., 2019). This polysaccharide content is believed to be associated with their major pharmacological effects, including improved memory and learning, increased anti-oxidant effects, and promotion of longevity (Du *et al.* 2013). This study showed that the integrity of endogenous antioxidants (SOD and GSH) was improved by PTP and GLP because GLP and PTP significantly elevated the activity of SOD and GSH, which were inhibited by Pb after 14 days of oral exposure. The data suggest that GLP and PTP possess potent anti-oxidant activities by scavenging reactive oxygen species (ROS), thus providing a splenic protective effect against oxidative damage induced by oral exposure to Pb. (Jun *et al*., 2003)

The common enzyme found in nearly all living organisms exposed to oxygen, such as bacteria, plants, and animals, is catalase (CAT), which catalyzes the decomposition of hydrogen peroxide to water and oxygen (Misra and Fridovich, 2012). It is an essential enzyme that protects cells from oxidative damage by reactive Oxygen Species. Since GLP and PTP significantly increased the activity of CAT in rats exposed to Pb and effectively alleviated lead-induced toxicity in Wistar rats, it suggests that GLP and PTP fruiting bodies, as reported by Li *et al*. (2008), can perform the role of antioxidants by inhibiting oxidative stress.

Reactive oxygen radicals can affect signal transduction by damaging DNA and regulating gene expression pathways mediated by oxidative stress (He *et al*., 2010). Malondialdehyde (MDA) is the final product of lipid peroxidation. The accumulation of free radicals in the body can accelerate lipid peroxidation and produce large amounts of MDA, which causes damage to cells and tissues. Determination of the MDA content is frequently used to reflect the degree of lipid peroxidation (Yao *et al*., 2003) and, hence, the degree of accumulation of free radicals. In this study, 14 days post-treatment with GLP and PTP protected against lipid peroxidation, as indicated by the reduction in MDA levels, which was initially increased by Pb. Treatment with GLP and PTP diminished oxidative stress as it retarded splenic MDA and elevated splenic GSH levels in the GLP and PTP-treated groups in comparison with the Pb group (El sanshansy *et al.*, 2012: Wei *et al*.,2018). The MDA reduction and oxidative stress protection by GLP and PTP may be due to the scavenging of superoxide anion (O_2), hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), and peroxyl radical (ROO) (Isikhuenhen *et al*., 2003: Jin *et al*., 2012). This study suggests that PTP and GLP fruiting body can inhibit lipid peroxidation caused by Pb. The main components of the water-soluble extracts of *Ganoderma lucidum* and *Pleurotus tuberregium* mushrooms are polysaccharides (Zhang *et al*., 2024). Modern pharmacological studies on *Pleurotus tuberregium* polysaccharides (PTP) and *Ganoderma lucidum* polysaccharides (GLP) have clearly shown their immunomodulatory and anti-diabetic activities (Ogbo *et al.* 2011; Susilowati *et al*., 2021). The immunomodulatory effects of *Ganoderma lucidum* polysaccharides (GLP) and *Pleurotus tuberregium* polysaccharides (PTP) include cellular immunity, humoral immunity, and the mononuclear phagocyte system (Wu *et al*., 2022). *Ganoderma lucidum* polysaccharides (GLP) and *Pleurotus tuberregium* polysaccharides (PTP) can reduce the rate of lymphocyte apoptosis induced by lead acetate (Pb) (El Fakharany *et al*., 2010: Xiao *et al*., 2017). Lead (Pb) accumulation following oral exposure results in weight loss, inhibition of haem synthesis, decrease in lymphocytes, and reduction of white blood cells (Okediran *et al.* 2016). However, treatment with PTP and GLP reduced the increase in Pb accumulation caused by oral exposure to Pb after 14 days, with increased red blood cells, elevated lymphocyte levels, and upregulation of eosinophils, monocytes, and neutrophils. The findings of this study are in agreement with those of earlier reports (Murata *et al*. 2003; Babalola *et al.* 2010; Okediran *et al.* 2016).

In toxicological studies, histopathological examination provides supportive evidence for biochemical and haematological observations (Eroschencho and Lippincott (2000). In this study, the spleens of rats after 14 days of Pb pre-treatment showed an invasion of the red pulp into the white pulp with vascular congestion and splenic tissue with an area of inflammation. Exposure to Pb resulted in the invasion of the red pulp into the white pulp, which is a common phenomenon. The main reason for this is that Pb affects the immune system. When Pb is absorbed into the body, it is distributed to various tissues, including bone marrow. The harmful effect of Pb on bone marrow cells weakens the immune system, causing white pulp cells to lose their ability to prevent the growth of red pulp cells.

Consequently, red pulp cells invade the white pulp, causing structural changes in the spleen tissue of animals exposed to Pb (Department of Health US, Human Services AfTSaDR, 2020). However, administration of PTP and GLP reversed the histopathology alteration of the spleen showing splenic tissue with normal red pulp (Blue arrow) and normal white pulp follicle (blue arrow).

Conclusion

This study indicates that *Ganoderma lucidum* polysaccharide ((GLP) and *Pleurotus tuberregium* polysaccharide (PTP) extracts have immunomodulatory properties, specifically boosting anti-oxidant activities, elevating blood parameters, and ameliorating histological alterations that occurred as a result of Pb toxicity. These results suggest that GLP and PTP fruiting bodies play positive roles in facilitating the recovery of suppressed immune function. This medicinal mushroom may be administered for prophylaxis or treatment of diseases in immunocompromised patients.

Conflict of Interest

All authors declare no conflicts of interest.

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References

Ahmad R, Riaz M, Khan A, Aljamea A. et al (2021). Ganoderma lucidum (Reishi) an edible mushroom; a comprehensive and critical review of its nutritional, cosmeceutical, mycochemical, pharmacological, clinical, and toxicological properties. *Phytotherapy Research 35*(11): 6030-6062.

Asiwe JN, Asiwe N, Onuh JE (2021). The effect of high dietary salt consumption on renal function in Streptozotocin-induced diabetic male Wistar rats. *International Journal of Nutrition Sciences 6*(4): 201-207.

Babalola OO, Okonji RE, Atoyebi J. O, Sennuga TF et al (2010). Distribution of lead in selected organs and tissues of albino rats exposed to acute lead toxicity. *Scientific Research and Essay 5*(9): 845-848.

Canton H (2021). Organisation for economic co-operation and development—OECD. In *The Europa Directory of International Organizations 2021* (pp. 677-687). Routledge.

Clark JD, Gebhart GF, Gonder JC, Keeling ME Kohn DF (1997). The 1996 guide for the care and use of laboratory animals. *ILAR Journal 38*(1): 41-48.

Du QH, Peng C, Zhang H (2013). Polydatin: a review of pharmacology and pharmacokinetics. *Pharmaceutical Biology 51*(11): 1347-1354.

Ekanem AU, Kwari HD, Garba SH, Salami HA (2015). Effect of lead acetate on spleen and blood parameters in albino rats. *IOSR Journal of. Dental and Medical Sciences 14*(3): 43-49.

EL-Fakharany EM, Haroun B, Ng T. Redwan ER (2010). Oyster mushroom laccase inhibits hepatitis C virus entrintoperipheral blood cells and hepatoma cells. *Protein and Peptide Letters 17*(8): 1031-1039.

El Enshasy H, Maftoun P, Abd Malek R (2012). Pleuran: Immunomodulator polysaccharide from Pleurotus ostreatus, structure, production and application. *Mushrooms: Types, Properties and Nutrition* 153-172.

Eroschencho VP, Lippincott W (2000). Atlas of histology with functional correlations. *Williams and Wilkins Lippincott*, *12*.

Fasidi, IO, Olorunmaiye KS (1994). Studies on the requirements for vegetative growth of Pleurotus tuber-regium (Fr.) Singer, a Nigerian mushroom. *Food Chemistry 50*(4): 397-401.

Goth L (1991). A simple method for determination of serum catalase activity and revision of reference range. *Clinica Chimica Acta 196*(2-3): 143-151.

He XM, Mo XM, Gu Q, Chen F et al (2010). Effect of diazoxide on oxygen free radicals and cell apoptosis in brain tissue after deep hypothermia cerebral ischemia reperfusion injury in young rats. *Zhonghua wai ke za zhi [Chinese Journal of Surgery 48*(2): 142-145.

Isikhuemhen OS, Anoliefo GO, Oghale OI (2003). Bioremediation of crude oil polluted soil by the white rot fungus, Pleurotus tuberregium (Fr.) Sing. *Environmental Science and Pollution Research 10:* 108-112.

Jun Y, Guoqing L, Yuefei Z, Dewei C, Lirong Y (2003). Determination and significance of the level of super oxide dismutase and lipid per oxidation and tumor necrosis factor-α in serum of patients with nasopharyngeal carcinoma. *Hebei Medicine 9*(3): 200-203.

Kim HJ, Mahboob S, Viayaraghavan P, Al-Ghanim KA et al (2020). Determination of toxic effects of lead acetate on

different sizes of zebra fish (Danio rerio) in soft and hard water. *Journal of King Saud University-Science 32*(2): 1390-1394.

La-Llave-León O, Salas Pacheco JM, Estrada Martínez S, Esquivel Rodríguez E et al (2016). The relationship between blood lead levels and occupational exposure in a pregnant population. BMC Public Health 16: 1231. https://doi.org/10.1186/s12889-016-3902-3 1-9.

Li Y, Liang W, Han Y, Zhao W et al (2022). Triterpenoids and polysaccharides from Ganoderma lucidum improve the histomorphology and function of testes in middle-aged male mice by alleviating oxidative stress and cellular apoptosis. Nutrients 14(22): 4733. doi: 10.3390/nu14224733

Lin DM (2019). Structure and application of Ganoderma polysaccharide peptide GL-PPSQ2. *Zhongcaoyao*, 336-343.

Misra HP, Fridovich I (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry 247*(10): 3170-3175.

Muselin F, Trif A, Brezovan D, Dumitrescu, E (2010). Lead in liver, kidney, spleen and lungs consecutive chronic exposure in rats. Lucrari Stiintifci Medicina Verterinara 43 (2)

Murata K, Sakai T, Morita Y, Iwata T, Dakeishi M (2003). Critical dose of lead affecting δ-aminolevulinic acid levels. *Journal of Occupational Health 45*(4): 209-214.

Nagababu E, Rifkind JM, Boindala S, Nakka L (2010). Assessment of antioxidant activity of eugenol in vitro and in vivo. In *Free radicals and antioxidant protocols*, pp. 165-180. Totowa, NJ: Humana Press.

Okediran BS, Kasali OB, Omotainse SO, Akinloye QA (2016). Haemato-Biochemical alterations as biomarkers of Lead-induced toxicity in male wistar rats. *Bangladesh Journal of Veterinary Medicine 14*(2): 227-232.

Okjuoya JA (1991). Cultivation of *Pleurotus tuber-regium* (Fr) Sing on various farm wastes. In *Proceedings of the Oklahoma Academy of Science* 1-3

Patra RC, Rautray AK, Swarup D. (2011). Oxidative stress in lead and cadmium toxicity and its amelioration. *Veterinary Medicine International*, 457327.

Seweryn E, Ziała A, Gamian A. (2021). Health-promoting of polysaccharides extracted from Ganoderma lucidum. *Nutrients 13*(8): 2725.

Sobowale MT, Saliu JA (2025). Ameliorative Effects of Ganoderma lucidum on Pb-induced Toxicity in Wistar Rats. *Pharmacology and Toxicology of Natural Medicines 5*(1-2): 31-40.

Tyagi B, Chandrasekaran B, Tyagi A, Shukla V et al (2023). Exposure of environmental trace elements in prostate cancer patients: A multiple metal analysis. *Toxicology and Applied Pharmacology 479:* 116728.

Wang CL, Lu CY, Pi CC, Zhuang YJ et al (2012). Extracellular polysaccharides produced by Ganoderma formosanum stimulate macrophage activation via multiple pattern-recognition receptors. *BMC Complementary and Alternative Medicine 12:* 1-10.

Wei B, Zhang R, Zhai J, Zhu J et al (2018). Suppression of Th17 cell response in the alleviation of dextran sulfate sodium-induced colitis by Ganoderma lucidum polysaccharides. Journal of Immunology Research 2018(1): 2906494.

Wu KJ, Wang SQ, Shi RP, Qin LH et al (2022). Neuroprotective Effect of Ganoderma lucidum Polysaccharides in an Epileptic Rat Model. *Current Topics in Nutraceutical Research 20*(2).

Xiao C, Wu Q, Zhang J, Xie Y et al (2017). Antidiabetic activity of Ganoderma lucidum polysaccharides F31 down-regulated hepatic glucose regulatory enzymes in diabetic mice. *Journal of Ethnopharmacology 196:* 47-57.

Xu S, Dou Y, Ye B, Wu Q et al (2017). *Ganoderma lucidum* polysaccharides improve insulin sensitivity by regulating inflammatory cytokines and gut microbiota composition in mice. *Journal of Functional Foods 38:* 545-552.

Zeng P, Guo Z, Zeng X, Hao C et al (2018). Chemical, biochemical, preclinical and clinical studies of *Ganoderma lucidum* polysaccharide as an approved drug for treating myopathy and other diseases in China. *Journal of Cellular and Molecular Medicine 22*(7), 3278-3297.

Zhang H, Li N, Zhang Y, Xu Y et al (2024). *Ganoderma lucidum* polysaccharide peptide alleviates cyclophosphamide-induced male reproductive injury by reducing oxidative stress and apoptosis. Biomedicines. 12(8): 1632. doi: 10.3390/biomedicines12081632.

Zheng S, Liu Q, Zhang G, Wang HT (2010). Purification and characterization of an antibacterial protein from dried fruiting bodies of the wild mushroom *Clitocybe sinopica. ACTA Biochimica Polonica 57*(1): 43-48.

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