RESEARCH PAPER

Oxidative Stress Modulation by Oyster Mushroom (Pleurotussajorcaju) in Cyclophosphamide-Induced Female Wistar Rats

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ABSTRACT

Oxidative stress from excess ROS impairs immunity and health. Post-COVID-19, interest in natural antioxidants has grown. Pleurotus sajor-caju shows antioxidant and immunomodulatory properties, but limited evidence exists. This study evaluates its effects in a cyclophosphamide-induced rat model. In vitro antioxidant activity of the extract was evaluated using DPPH, ABTS, hydrogen peroxide, and superoxide scavenging assays (IC_{50} values calculated). In vivo, female Wistar rats (n=6 per group) were assigned to six groups: control, cyclophosphamide (10 mg/kg), cyclophosphamide plus mushroom extract (800 mg/kg), cyclophosphamide plus levamisole (50 mg/kg), mushroom extract alone, and levamisole alone. Treatments were administered orally for 28 days. The liver and spleen tissues were examined for reduced glutathione (GSH) and malondialdehyde (MDA). Tukey's post hoc test ($p < 0.05$) was used in conjunction with a one-way ANOVA to examine the data. Cyclophosphamide notably increased MDA (~75%) and decreased GSH (~60%) levels ($p<0.001$). P. sajor-caju treatment reduced MDA by ~40% and restored GSH ($p<0.05$). In vitro, IC_{50} values were 5.33 mg/mL (DPPH) and 0.94 mg/mL (ABTS). Pleurotus sajor-caju extract effectively mitigates cyclophosphamide-induced oxidative stress and supports immune function. It shows promise as a natural antioxidant therapeutic. Further studies are required to clarify its mechanism and clinical potential.

Keywords: Antioxidant, Cyclophosphamide, Immune modulation, Oxidative stress, *Pleurotus sajor-caju*, Wistar rats.

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INTRODUCTION

Oxidative stress has gained considerable attention in biomedical research due to its established role in the pathogenesis of numerous diseases, including cardiovascular, neurodegenerative, hepatic, renal, metabolic, and inflammatory disorders[1]. It happens when the quantity of reactive oxygen species (ROS) generated cannot be counteracted by endogenous antioxidant systems. These ROS compromise cellular structure and function by causing oxidative damage to proteins, lipids, and DNA. They include hydrogen peroxide ($H2O_2$), superoxide anion (O_2^-), and hydroxyl radical (•OH)[2,3].

This imbalance becomes particularly critical in immunocompromised states. Following the COVID-19 pandemic, increased focus has been directed toward understanding the oxidative stress–immune interaction. It has been shown that SARS-CoV-2 infection causes cytokine storms and increased ROS generation, which impairs host defenses and exacerbates inflammatory reactions[4]. As a result, researchers are exploring natural interventions that could modulate oxidative stress and simultaneously enhance immune function.

Mushrooms of the genus *Pleurotus*, especially *Pleurotus sajor-caju* (commonly known as oyster mushroom), have become viable options because of their diverse range of bioactive substances. These include β-glucans, phenolics, flavonoids, terpenoids, polysaccharides, & essential vitamins such as A, C, and E[5]. Studies by Mridha, (2017) reported that *Pleurotus* species contain high levels of flavonoids and phenols, which contribute to antioxidant

activity via the processes of single electron transfer (SET) and hydrogen atom transfer (HAT)[6]. Similarly, Anguiano et al., (2007) demonstrated potent scavenging of ABTS and hydroxyl radicals, linking this activity to the phenolic content in the mushroom extract[7].

The immunomodulatory effects of *Pleurotus sajor-caju* are also well-documented. These mushrooms' β-glucans promote phagocytosis and cytokine release via binding to pattern recognition receptors (PRRs) on immune cells, including Dectin-1 and TLR-2. These properties make *Pleurotus* not only an antioxidant but also an effective immune stimulant[8]. However, while in vitro studies confirm its antioxidative and immunological potential, in vivo research- particularly in immunosuppressed models- remains sparse[9,10,11].

Cyclophosphamide (CYP), an alkylating agent used in chemotherapy, serves as a well-established model for inducing oxidative stress and immunosuppression (11). Metabolized in the liver to form acrolein and phosphoramide mustard, CYP disrupts antioxidant defense mechanisms, generates ROS, and induces cellular damage. According to Hussain et al., (2023) and Otitoloju & Olagoke, (2011), CYP decreases glutathione (GSH), an essential intracellular antioxidant, while increasing malondialdehyde (MDA), a sign of lipid peroxidation. Both MDA and GSH are critical biomarkers for evaluating oxidative injury and antioxidant therapy efficacy[12,13,14].

This study assesses the antioxidant and immune-protective effects of *Pleurotus sajor-caju* extract in female Wistar rats with cyclophosphamide-induced oxidative stress, measuring MDA and GSH levels in liver and spleen, along with histopathological analysis of key organs. The aim is to explore its potential as a nutraceutical against oxidative and immune dysfunction.

Materials and Methods

Study Design

This study investigated the in vitro antioxidant capacity and in vivo immunomodulatory effects of an aqueous extract of *P. sajor-caju* (oyster mushroom) using a cyclophosphamide-induced oxidative stress paradigm in Wistar rats. The research was conducted in two integrated phases, beginning with in vitro antioxidant assays to assess the extract's free radical scavenging activity, followed by in vivo investigations involving six treatment groups to examine a range of biochemical, histopathological, and immunological parameters.

Animals

The Central Animal Facility at the All-India Institute of Medical Sciences (AIIMS), New Delhi, provided healthy adult female Wistar rats weighing 140–160 g (6–8 weeks old) (Reg. No. 10/GO/ReBiBt/S/99/CPCSEA). The mice were acclimated in the Division of Animal Biotechnology, College of Biotechnology, SVPUAT, Meerut, under conventional laboratory conditions (temperature: 22–25 °C; relative humidity: 50–65%; 12-hour light/dark cycle). They were given a pelleted feed that was nutrient-dense and unlimited access to potable water. The CPCSEA requirements were strictly followed when handling and caring for the animals.

Extract Preparation

Fresh fruiting bodies of *Pleurotus sajor-caju* were procured from AICRP–Mushroom, MPUAT, Udaipur. After being pulverized into a fine powder (particle size <1 mm) and shade-dried at 25 °C, the mushrooms were kept in airtight containers. A hot aqueous extract was prepared as described by Devi and Krishnakumari, (2015) with some modifications[15]. A mixture of mushroom powder and distilled water, 1:10 (w/v), was decocted for two hours at 100°C in a water bath. Whatman No. 1 filter paper was used to filter the extract, and a vacuum evaporator was used to evaporate the filtrate at a lower pressure. The weight difference was used to compute the yield. The stock extract was newly diluted with distilled water for oral administration, resulting in a working dosage of 800 mg/kg body weight, or 1/16th of the documented LD_{50}.

Phytochemical Screening of *P. sajor-caju*

To identify the main bioactive ingredients, a preliminary phytochemical screening of the *P. sajor-caju* aqueous extract was carried out using accepted techniques. The tests listed below were carried out: Salkowski's test for triterpenoids, Borntrager's test for anthraquinones, Braymer's test for tannins, the foam test, the Effervescence test for carboxylic acids, Mayer's test for alkaloids, Benedict's test for reducing sugars, Legal's test for glycosides, Keller-Killani's test for cardiac glycosides, Biuret's test for proteins and amino acids, Shinoda's test for flavonoids, Ferric chloride's test for phenolics, Benedict's test for reducing sugars, Legal's test for glycosides, and Molisch's test for carbohydrates. Positive reactions were indicated by color changes or the formation of precipitates specific to each ingredient[16].

In Vitro Antioxidant Assays

The antioxidant properties of the mushroom extract were assessed using DPPH, ABTS, hydrogen peroxide (H_2O_2), and superoxide scavenging assays.

DPPH assay

The method used to evaluate the samples' capacity to scavenge free radicals included only minor adjustments to Kumari et al. (2016)'s procedure[17]. The 0.1 mM DPPH in methanol solution was treated with the same quantity of test extract. It was incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm. Standard ascorbic acid was used.

ABTS assay

Barbosa et al., (2018) used their approach to analyse the ABTS Assay[18]. Following 16 hours of dark incubation, a combination of 2.45 mM ammonium persulfate and 7 mM ABTS produced the ABTS+. The absorbance of 0.70 at 734 nm from the working solution was used to normalize it. The absorbance was determined after 190 µL of ABTS+ solution and 10 µL of test extract were combined and incubated for 6 minutes. The outcome was represented using Trolox equivalents.

Hydrogen peroxide scavenging assay

Hydrogen Peroxide Scavenging Activity was performed using the method of Hakkani et al., (2022), which included preparing a solution of 40 mM H_2O_2 in phosphate buffer

(pH 7.4). The absorbance at 230 nm was measured after 0.6 mL of extract and 0.6 mL of H_2O_2 were combined and incubated for 10 minutes[19].

Superoxide scavenging assay

Using Li's, (2012) approach, the Superoxide Scavenging Assay was conducted using 50 µL of 60 mM pyrogallol in 100 µL of extract and 2.85 mL of Tris-HCl buffer (0.05 M, pH 7.4). Absorbance at 325 nm was measured after 5 minutes. Trolox or ascorbic acid were used as benchmarks[20]. Standard curves of all the assays were drawn, and the scavenging activity was expressed in terms of percentage inhibition and equivalent antioxidant concentration.

In Vivo Experimental Protocol

Thirty-six Wistar rats were randomly assigned to six groups, each consisting of six rats (Table 1):

Table 1. Treatment groups and dosage regimen used in Wistar rats.

Group	Treatment	Dose / Route
I	Control (Distilled water)	0.1 mL/100 g b.wt / oral
II	Cyclophosphamide only	10 mg/kg b.wt / oral
III	Cyclophosphamide + Mushroom Extract	10 mg/kg + 800 mg/kg b.wt / oral
IV	Cyclophosphamide + Levamisole	10 mg/kg + 50 mg/kg b.wt / oral
V	Mushroom Extract only	800 mg/kg b.wt / oral
VI	Levamisole only	50 mg/kg b.wt / oral

Cyclophosphamide was administered in normal saline, levamisole in DMSO[21]. For 28 days, all therapies were administered orally once daily. After the experimental period, animals were euthanized humanely as per CPCSEA guidelines. Liver, spleen, kidney, and lungs were harvested for biochemical and histological assessments[22,23].

Biochemical Estimations

For lipid peroxidation (LPO) and total protein estimates, tissue homogenates (10%) were produced in ice-cold 0.9% saline. For reduced glutathione (GSH) analysis, they were prepared in 0.02 M EDTA buffer[24].

Total Protein

The total quantity of protein in the liver and spleen was ascertained using the biuret method, which was first published by Parvin et al., (1956)[25]. After TCA precipitation and centrifugation (5000 rpm, 10 min), the pellet was dissolved in NaOH and reacted with Biuret reagent. At 540 nm, absorbance was measured.

GSH

GSH was estimated using the methodology outlined by Sedlak and Lindsay, (1968)[26]. In which the material was deproteinized using sulphosalicylic acid. Tris buffer and DTNB were used to incubate the resultant supernatant. At 412 nm, absorbance was measured.

Lipid Peroxidation (LPO)

The Jordan and Schenkman (1982) approach, which included reacting homogenates with TBA after TCA precipitation, was used to estimate LPO. After 20 minutes of boiling, the samples were cooled, and the absorbance at 532 nm was measured[27].

Histopathological Examination

A small piece of liver, kidneys and lungs of rats of each group was collected and processed as per the method of Bancroft & Gamble, (2008). After being stored in 10% neutral buffered formalin, the liver, kidney, and lung tissues were dried and paraffin embedded. The 5 µm slices were stained with hematoxylin and eosin and examined under a microscope to look for structural alterations and pathological abnormalities[28].

Ethical Considerations

All animal experiments were conducted in accordance with the regulations set out by the Government of India's Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The Institutional Animal Ethics Committee (IAEC) at SVPUA&T, Meerut, authorized the research on November 1, 2022, with permission number IAEC/SVPUAT/2022/75. Without sacrificing the experiment's scientific validity, every attempt was made to reduce the number of animals and their suffering.

Statistical Analysis

GraphPad Prism version 8 and IBM SPSS version 26 were used for statistical analysis. The results are shown using the mean ± standard error of mean (SEM) format. The Shapiro-Wilk test was used to confirm that the data was normal. Tukey's post hoc analysis was used with a one-way ANOVA to compare groups. $P < 0.05$ is the cutoff point for statistical significance.

Results

Phytochemical screening of *P. sajor-caju*

The hot aqueous extract of *Pleurotus sajor-caju* yielded 16.5%. Qualitative analysis showed high levels (+++) of carbohydrates, reducing sugars, glycosides, cardiac glycosides, proteins, saponins, and alkaloids. Moderate amounts (++) of flavonoids, phenols, tannins, and carboxylic acids were present, while triterpenoids and anthraquinones were detected at low levels (+) (Table 2).

Table 2. Qualitative phytochemical screening of *P. sajor-caju* extract (+ to +++ indicating increasing presence).

Test	Presence
Carbohydrates	+++
Reducing sugars	+++
Glycosides	+++
Cardiac glycosides	+++
Protein & amino acids	+++
Flavonoids	++
Phenols	++
Tannins	++
Triterpenoids	+
Anthraquinones	+
Carboxylic acids	++
Saponins	+++
Alkaloids	+++

In Vitro Antioxidant Activity of *P. sajor-caju*

The aqueous extract of *Pleurotus sajor-caju* exhibited significant antioxidant potential, as demonstrated by strong free radical scavenging activities: DPPH (49.73 ± 6.03 mg AAE/g), superoxide (155.33 ± 12.37 mg AAE/g), hydrogen peroxide (195.76 ± 4.60 mg AAE/g), and ABTS (9.4 ± 0.00 mg TE/g) (Table 3). Bioactive substances including tannins, flavonoids, phenols, alkaloids, proteins, and carbohydrates were found by phytochemical analysis; these substances most likely support these antioxidant actions (Table 4). These findings support the extract's ability to neutralize various reactive oxygen species through multiple mechanisms.

Table 3: In Vitro Antioxidant Activity of *P. sajor-caju* Aqueous Extract

Assay	Activity (mg AAE/g or mg TE/g) Mean ± SEM
DPPH Radical Scavenging	49.73 ± 6.03 mg AAE/g
Superoxide Scavenging	155.33 ± 12.37 mg AAE/g
Hydrogen Peroxide Scavenging	195.76 ± 4.60 mg AAE/g
ABTS Radical Scavenging	9.4 ± 0.00 mg TE/g

Table 4. Quantitative Estimation of Key Phytochemicals in *Pleurotus sajor-caju* Aqueous Extract

Metabolite	Quantity (mg/g) Mean ± SEM
Protein	8.11 ± 0.08
Tannins	6.86 ± 0.003
Flavonoids	5.34 ± 0.003
Phenols	3.21 ± 0.006
Amino Acids	2.90 ± 0.009
Carbohydrates	2.67 ± 0.02
Alkaloids	2.79 ± 0.003

Effect on Tissue Protein Concentration

Total protein content in liver and spleen homogenates was significantly influenced by treatment (liver: $F_{5,30} = 31.959$, $p < 0.0001$; spleen: $F_{5,30} = 20.921$, $p < 0.0001$). Group II (cyclophosphamide alone) demonstrated a marked reduction in protein concentration compared to the normal control (Group I), indicating tissue degradation and catabolism due to oxidative stress. Co-administration of *Pleurotus sajor-caju* extract (Group III) or levamisole (Group IV) restored protein levels significantly ($p < 0.05$) relative to Group II. Groups V and VI, which received extract and levamisole respectively without cyclophosphamide, did not differ significantly from the control, indicating the absence of adverse effects on protein metabolism (Figure 1).

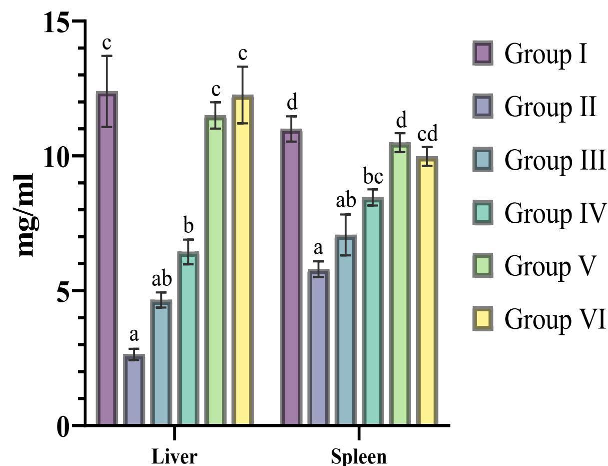


Figure 1. **Total protein levels in liver and spleen tissues of Wistar rats following treatment with cyclophosphamide alone or in combination with *Pleurotus sajor-caju* extract or levamisole.**

Lipid Peroxidation (LPO) Levels

Rats treated with cyclophosphamide alone (Group II) had considerably higher levels of malondialdehyde (MDA), an end-product of lipid peroxidation, in their liver tissues than controls ($F_{5,30} = 78.646$, $p < 0.0001$), indicating increased oxidative stress. Similar trends were observed in spleen tissue ($F_{5,30} = 13.972$, $p < 0.0001$). Treatment with mushroom extract (Group III) or levamisole (Group IV) significantly reduced MDA levels ($p < 0.05$), demonstrating their antioxidative efficacy. Groups V and VI showed MDA values comparable to Group I, indicating that neither treatment induced oxidative damage (Figure 2).

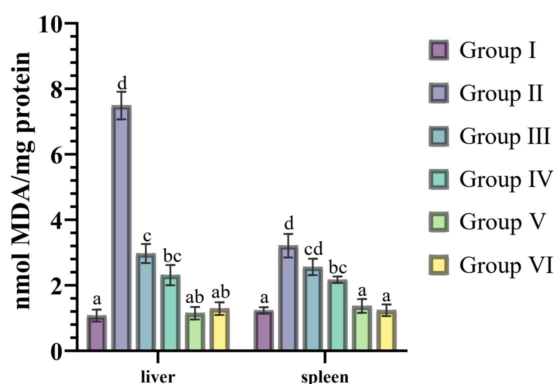


Figure **2. Lipid peroxidation (LPO) levels in liver and spleen tissues of Wistar rats following treatment with cyclophosphamide alone or in combination with *Pleurotus sajor-caju* extract or levamisole.**

Reduced Glutathione (GSH) Levels

Cyclophosphamide significantly decreased GSH levels in liver homogenates ($F_{5,30} = 8.312$, $p < 0.0001$) relative to control group. Groups III & IV revealed a significant restoration of GSH concentration following administration of extract or levamisole, respectively ($p < 0.05$). Similar protective trends were observed in spleen GSH content

($F_{5,30} = 25.118$, $p < 0.0001$). Extract or levamisole alone did not cause any significant deviation from control levels (Groups V and VI), supporting their safety and adaptogenic nature (Figure 3).

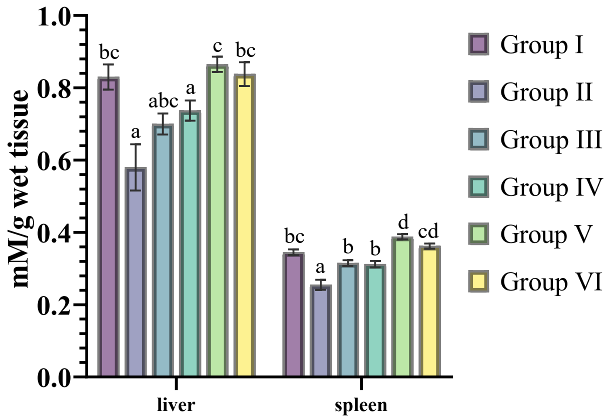
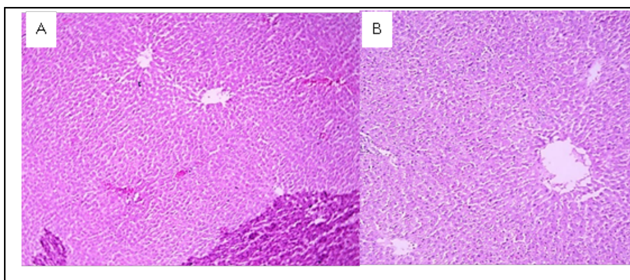


Figure 3. **Decreased levels of glutathione (GSH) in Wistar rat liver and spleen tissues following administration of cyclophosphamide alone or combined with *Pleurotus sajor-caju* extract or levamisole.**

Histopathological Findings

Cyclophosphamide-treated rats (Group 1) exhibited severe tissue damage, including hepatocellular degeneration and sinusoidal congestion in the liver (Plate 4A), white pulp depletion in the spleen (Plate 5A), glomerular atrophy and tubular necrosis in the kidneys (Plate 6A), and alveolar hemorrhage with bronchopneumonia in the lungs (Plate 7A). Co-treatment with *Pleurotus sajor-caju* (Group 2) showed marked histological improvement, with only mild liver congestion (Plate 4B), reduced splenic depletion (Plate 5B), mild renal congestion (Plate 6B), and minimal alveolar changes (Plate 7B). Levamisole (Group 3) offered similar protection, though mild bronchopneumonia and hemosiderosis persisted (Plates 4C–7C). No pathological alterations were seen in Groups 4, 5, and 6 (Plates 4D–7F). These results confirm the tissue-protective effects of *P. sajor-caju* against cyclophosphamide-induced organ damage.



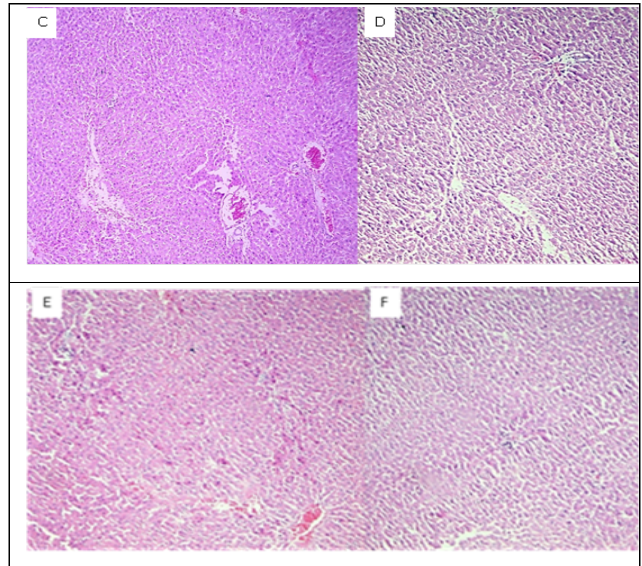


Figure 4. Liver histology (H&E, 10×)

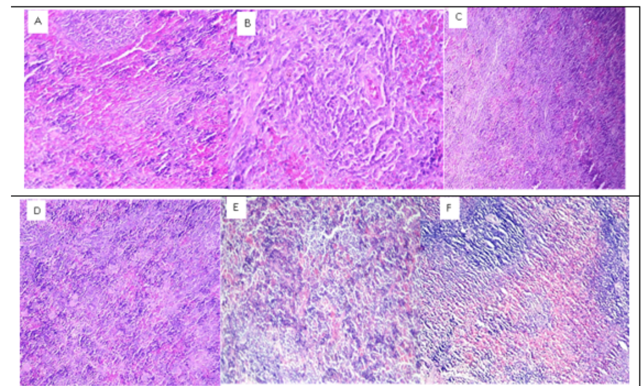


Figure 5. Spleen histology (H&E, 10×)

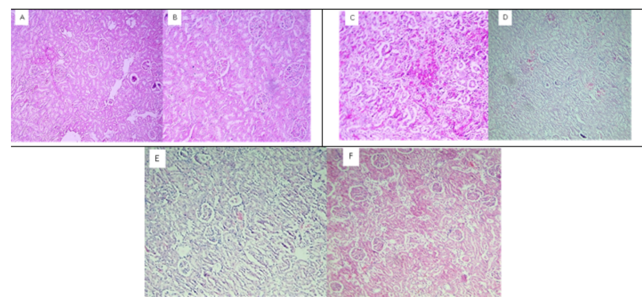


Figure 6: Kidney histology (H&E, 10×)

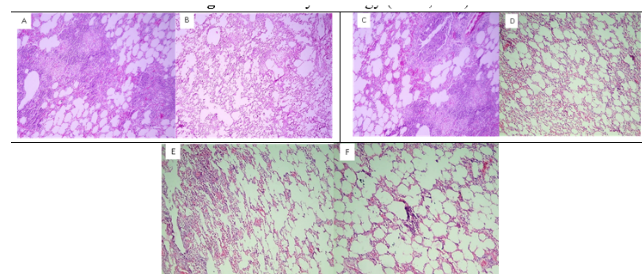


Figure 7. Lung histology (H&E, 10×)

DISCUSSION

Pleurotus sajor-caju's rich composition of bioactive chemicals has attracted a lot of research because of its immunomodulatory and antioxidant qualities. Significant radical scavenging activity was shown in our in vitro tests for superoxide, DPPH, ABTS, and $H2O_2$. According to earlier research by Devi, (2024) and Saini et al., (2024), these benefits are mostly attributable to the extract's high quantities of flavonoids, polyphenols, tannins, and antioxidant vitamins like C, E, and A[29,30]. These phytochemicals efficiently neutralize free radicals and stop cellular damage by primarily acting via the processes of hydrogen atom transfer (HAT) and single electron transfer (SET). The ABTS and hydrogen peroxide scavenging potentials observed in our study align with findings by Pinto et al., (2023)[31], who demonstrated that phenolic-rich mushroom extracts possess substantial reactive oxygen species (ROS)-scavenging capabilities.

Cyclophosphamide (CYP), a well-established chemotherapeutic agent, induces oxidative stress by generating reactive metabolites such as acrolein and phosphoramide mustard. Acrolein interacts with cellular macromolecules—especially thiol-containing antioxidants like glutathione (GSH)—and promotes lipid peroxidation. These oxidative insults are fundamental to CYP's immunosuppressive effects. Consistent with reports by Sebaey et al., (2019) and Mohamed and Houseiny, (2020) CYP administration in our study significantly elevated malondialdehyde (MDA) levels and depleted GSH in hepatic and splenic tissues, confirming its role in oxidative damage[32,33]. These observations are further corroborated by Awadallah et al. (2020), who reported dose-dependent oxidative impairment in rat liver following CYP exposure[34].

Treatment with *P. sajor-caju* extract significantly mitigated these biochemical alterations. The marked reduction in MDA and restoration of GSH levels suggest that the mushroom extract reinstated redox homeostasis, likely by scavenging ROS and/or enhancing endogenous antioxidant defenses. This is consistent with the findings of Abdelkader et al., (2024) and Adesida & Alimba, (2025), who demonstrated that mushroom-derived phenolics promote GSH synthesis while inhibiting lipid peroxidation[35,36]. According to Zhou et al., (2018), antioxidant substances including ergothioneine and β-glucans, which are present in mushrooms, are also known to enhance mitochondrial activity and maintain membrane integrity under oxidative stress[37].

Histopathological analysis further supported our biochemical results. CYP exposure resulted in significant hepatocellular degeneration, sinusoidal congestion, glomerular shrinkage, and pulmonary hemorrhages, all of which were substantially ameliorated by treatment with the mushroom extract. These findings agree with Dubey et al., (2023), who observed improved hepatic and renal histoarchitecture in rodents treated with *Pleurotus* species following toxic insult[38]. The preservation of hepatic and renal structures may be attributable to the membrane-stabilizing properties of mushroom polysaccharides, as detailed by Adil et al., (2016)[39].

Interestingly, CYP-induced pulmonary histopathological alterations, including alveolar congestion and bronchopneumonia, were also partially reversed by P. sajor-caju extract. This observation aligns with Haider et al., (2024), who noted that oxidative stress from CYP compromises pulmonary tissue integrity and that antioxidant therapy can attenuate alveolar inflammation[40]. The antioxidant potential of P. sajor-caju may also indirectly enhance lung health by reducing circulating pro-inflammatory cytokines and systemic inflammation.

Within the spleen, a crucial immune organ, oxidative damage manifests as diminished protein content and white pulp degeneration. Administration of the mushroom extract preserved splenic architecture and total protein levels, likely due to the immunomodulatory role of β-glucans, as discussed by Hobbs, (2017)[41]. These polysaccharides stimulate innate immunity by activating macrophages and dendritic cells, enhancing phagocytosis, and promoting cytokine secretion. The observed enlargement of white pulp in treated groups further suggests lymphoid follicle regeneration, consistent with the findings of Murad, (2023)[42].

Collectively, these findings emphasize the dual antioxidant and immunomodulatory roles of Pleurotus sajor-caju. The flavonoids and polysaccharides within the extract not only neutralize ROS but also facilitate immune cell recovery, providing a comprehensive protective mechanism. Singh and Bhardwaj, (2023) highlighted that mushroom-derived β-glucans engage Toll-like receptors and dectin-1 receptors, potentiating immune responses without triggering excessive inflammation[43].

Despite these promising results, the precise molecular mechanisms underlying the dose-dependent effects of P. sajor-caju on oxidative pathways remain to be fully elucidated. Furthermore, translating these findings into clinical applications will require detailed studies on pharmacokinetics, bioavailability, and potential drug interactions.

In conclusion, research highlights *P. sajor-caju's* medicinal potential in reducing oxidative stress and immunological dysfunction brought on by CYP. The mushroom's rich phytochemical profile supports its further development as a nutraceutical intervention for illnesses linked to oxidative stress by providing strong ROS-scavenging action and tissue protection.

CONCLUSION

This study demonstrates that Pleurotus sajor-caju mushroom extract effectively counteracts cyclophosphamide-induced oxidative stress and immune suppression in Wistar rats. Cyclophosphamide significantly increased lipid peroxidation, reduced glutathione levels, and caused tissue damage in the liver, spleen, kidney, and lungs. Treatment with the mushroom extract reversed these effects, indicating strong antioxidants and immunomodulatory properties, likely due to its rich content of flavonoids, phenolics, and vitamins C and E. These findings suggest its potential as a natural therapeutic or nutraceutical candidate. However, limitations such as the lack of molecular mechanism studies, long-term safety

evaluation, and clinical validation must be addressed. Future research should explore active compounds, dose optimization, and translational relevance in human health applications.

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AUTHOR CONTRIBUTIONS

Dr. Pallav Kumar Trar was responsible for the study concept and design, conducted the experiments, analysed the data, plotted the figures and drafted the manuscript.
Dr. Rachna Varma, Dr. Rajesh Mandiland, Dr. Shweta Anand supervised, guided in conducting the experiment and helped in correcting the manuscript.
Dr. Vikas Jaiswal guided in performing histopathology.
Dr. Amit Kumar helped in animal handling and housing during the experiment period.

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REFERENCES

1. Ukwubile CA, Mathias SN, Pisagih PS. Acute and subchronic toxicity evaluation and GC-MS profiling of Ajumbaise: a traditional Nigerian polyherbal formulation for labor enhancement and pain relief. J Pharm Sci Comput Chem. 2025; 1(2):154-173.
2. Ozougwu JC. The role of reactive oxygen species and antioxidants in oxidative stress. Int J Res. 2016; 1(8):1-8.
3. Juan CA, Pérez de la Lastra JM, Plou FJ, Pérez-Lebeña E. The chemistry of reactive oxygen species (ROS) revisited: outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. Int J Mol Sci. 2021; 22(9):4642.
4. Wieczfinska J, Kleniewska P, Pawliczak R. Oxidative stress-related mechanisms in SARS-CoV-2 infections. Oxid Med Cell Longev. 2022; 2022:5589089.
5. Oloke JK, Adebayo EA. Effectiveness of immunotherapies from oyster mushroom (Pleurotus species) in the management of immunocompromised patients. Int J Immunol. 2015; 3(2-1):8-20.
6. Mridha MH. In-vitro assessment of phenolic, flavonoid, flavonolic contents and antioxidant activities of plant Pleurotus ostreatus for neurodegenerative disorders [dissertation]. Dhaka: East West University; year not stated.
7. Ramírez-Anguiano AC, Santoyo S, Reglero G, Soler-Rivas C. Radical scavenging activities, endogenous oxidative enzymes and total phenols in edible mushrooms commonly consumed in Europe. J Sci Food Agric. 2007; 87(12):2272-2278.
8. Bhagarathi LK, Subramanian G, DaSilva PN. A review of mushroom cultivation and production, benefits and therapeutic potentials. World J Biol Pharm Health Sci. 2023; 15:1-56.
9. Magrone T, Magrone M, Russo MA, Jirillo E. Recent advances on the anti-inflammatory and antioxidant properties of red grape polyphenols: in vitro and in vivo studies. Antioxidants. 2019; 9(1):35.
10. Shaikh SS, Deshmukh SA, Satpute RB, Pawar VV, Gangurde HH. Formulation and evaluation of green tea-based herbal anti-aging cream for effective skin care. J Pharm Sci Comput Chem. 2025; 1(2):69-82.
11. Jadhav SS, Dighe PR, Kumbhare MR. Synthesis, in vitro evaluation, and molecular docking studies of novel pyrazoline derivatives as promising bioactive molecules. J Pharm Sci Comput Chem. 2025; 1(3).
12. Srirangan P, Sabina EP. Protective effects of herbal compounds against cyclophosphamide-induced organ toxicity: a pathway-centered approach. Drug Chem Toxicol. 2025; 1-43.
13. Hussain S, Jali AM, Alshahrani S, Khairat KH, Siddiqui R, Alam MI, et al. Hepatoprotective and antioxidant effects of nanopiperine against cypermethrin via mitigation of oxidative stress, inflammation and gene expression using qRT-PCR. Int J Mol Sci. 2023; 24(20):15361.
14. Otitoloju A, Olagoke O. Lipid peroxidation and antioxidant defense enzymes in Clarias gariepinus as useful biomarkers for monitoring exposure to polycyclic aromatic hydrocarbons. Environ Monit Assess. 2011; 182(1):205-213.
15. Devi MR, Krishnakumari S. Quantitative estimation of primary and secondary metabolites in hot aqueous extract of Pleurotus sajor-caju. J Pharmacogn Phytochem. 2015; 4(3):198-202.
16. Chinala KM, Chaithanya A, Sawrov M, Sahithi A, Achyuth C. Phytochemical and antimicrobial evaluation of Laurus nobilis leaves against acne- and dandruff-causing microorganisms. J Pharm Sci Comput Chem. 2025; 1(1):50-57.
17. Kumari S, Elancheran R, Kotoky J, Devi R. Rapid screening and identification of phenolic antioxidants in Hydrocotyle sibthorpioides Lam. by UPLC-ESI-MS/MS. Food Chem. 2016;203:521-529.
18. Barbosa EA, Oliveira A, Plácido A, Socodato R, Portugal CC, Mafud AC, et al. Structure and function of a novel antioxidant peptide from the skin of tropical frogs. Free Radic Biol Med. 2018; 115:68-79.

19. Al-Hakkani MF, Gouda GA, Hassan SH, Saddik MS, El-Mokhtar MA, Ibrahim MA, et al. Cefotaxime removal enhancement via bio-nanophotocatalyst α-Fe_2O_3 using photocatalytic degradation technique and its eco-biomedical applications. Sci Rep. 2022; 12(1):1.
20. Li X. Improved pyrogallol autoxidation method: a reliable and cheap superoxide-scavenging assay suitable for all antioxidants. J Agric Food Chem. 2012; 60(25):6418-6424.
21. Hou FX, Yang HF, Yu T, Chen W. The immunosuppressive effects of 10 mg/kg cyclophosphamide in Wistar rats. Environ Toxicol Pharmacol. 2007; 24(1):30-36.
22. Savant C, Kulkarni AR, Mannasaheb BA, Gajare R. Immunostimulant phytoconstituents from Mangifera indica L. bark oil. J Phytopharmacol. 2014; 3(2):139-148.
23. Shruthi S, Vijayalaxmi KK, Shenoy KB. Immunomodulatory effects of gallic acid against cyclophosphamide- and cisplatin-induced immunosuppression in Swiss albino mice. Indian J Pharm Sci. 2018; 80(1).
24. Ng SH, Mohd Zain MS, Zakaria F, Wan Ishak WR, Wan Ahmad WA. Hypoglycemic and antidiabetic effect of Pleurotus sajor-caju aqueous extract in normal and streptozotocin-induced diabetic rats. Biomed Res Int. 2015; 2015:1.
25. Parvin R, Pande SV, Venkitasubramanian TA. On the colorimetric biuret method of protein determination. Anal Biochem. 1965; 12(2):219-229.
26. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968; 25:192-205.
27. Jordan RA, Schenkman JB. Relationship between malondialdehyde production and arachidonate consumption during NADPH-supported microsomal lipid peroxidation. Biochem Pharmacol. 1982; 31(7):1393-1400.
28. Bancroft JD, Gamble M, editors. Theory and practice of histological techniques. 6th ed. London: Elsevier Health Sciences; 2008.
29. Devi A. Estimation of phytochemical constituents and antioxidant potential of underutilized ethnobotanically important wild edible fruits of Manipur. Indian J Tradit Knowl. 2024; 23(5):478-488.
30. Saini R, Kumar V, Upadhyay N, Sharma S, Sourirajan A, Kumar D, et al. Comparative analysis of medicinal and nutritional properties of three varieties of Phyllanthus emblica fruits of North-West Himalayas. Chem Afr. 2024; 7(2):705-723.
31. Pinto D, Moreira MM, Vieira EF, Švarc-Gajić J, Vallverdú-Queralt A, Brezo-Borjan T, et al. Development and characterization of functional cookies enriched with chestnut shells extract as source of bioactive phenolic compounds. Foods. 2023; 12(3):640.
32. El-Sebaey AM, Abdelhamid FM, Abdalla OA. Protective effects of garlic extract against hematological alterations, immunosuppression, hepatic oxidative stress, and renal damage induced by cyclophosphamide in rats. Environ Sci Pollut Res. 2019; 26(15):15559-15572.
33. Mohamed AA, El-Houseiny W, Abd Elhakeem EM, Ebraheim LL, Ahmed AI, Abd El-Hakim YM. Effect of hexavalent chromium exposure on liver and kidney tissues related to the expression of CYP450 and GST genes of Oreochromis niloticus: role of curcumin-supplemented diet. Ecotoxicol Environ Saf. 2020; 188:109890.
34. Awadallah N, Proctor K, Joseph KB, Delay ER, Delay RJ. Cyclophosphamide has long-term effects on proliferation in olfactory epithelia. Chem Senses. 2020; 45(2):97-109.
35. Abdelkader MA, Mediatrice H, Lin D, Lin Z, Aggag SA. Mitigating oxidative stress and promoting cellular longevity with mushroom extracts. Foods. 2024; 13(24):4028.
36. Adesida SO, Alimba CG. Anticholesterol, antihepatotoxic, and immunomodulatory activities of bioactive compounds of edible mushrooms. In: Bioactive compounds in edible mushrooms: sustainability and health applications. Cham: Springer Nature Switzerland; 2025. p. 707-738.
37. Zhou L. Bioactive compounds and health benefits of common edible mushrooms. [Details not provided].
38. Dubey SK, Rai SN, Singh VK, Bajpeyee AK, Singh MP. Evaluation of Pleurotus mushroom effects on histopathological changes in organs of diabetic rats. Dis Markers. 2023; 2023:1520132. doi:10.1155/2023/1520132.
39. Adil M, Kandhare AD, Ghosh P, Venkata S, Raygude KS, Bodhankar SL. Ameliorative effect of naringin in acetaminophen-induced hepatic and renal toxicity in laboratory rats: role of FXR and KIM-1. Ren Fail. 2016; 38(6):1007-1020. doi:10.3109/0886022X.2016.1163998.
40. Haider R, Mehdi A, Das GK. Pulmonary and respiratory health antioxidants and nutraceuticals. i-manager's J Life Sci. 2024;3(1):25-37. doi:10.26634/jls.3.1.20867.
41. Hobbs C. The health and clinical benefits of medicinal fungi. Adv Biochem Eng Biotechnol. 2023; 184:285-356. doi:10.1007/10_2023_230.
42. Murad AH. Immunomodulatory biomaterials in dental pulp regeneration: towards enhancing endodontic treatment outcomes. Future Dent Res. 2023; 1:1-11.

doi:10.3390/fdr1010001.

43. Singh RP, Bhardwaj A. β-Glucans: a potential source for maintaining gut microbiota and the immune system. Front Nutr. 2023; 10:1143682. doi:10.3389/fnut.2023.1143682
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