Toxicology International
DOI: 10.18311/ti/2026/v33i3/55561
INFORMATICS JOURNALS
eISSN: 0976-5131 | Vol 33(3), 2026 | pp. 649-667

Hepatoprotective Potentials of *Pleurotus pulmonarius* against Cadmium and Restraint Stress-induced Liver Injury: Integrated *in vivo* and *in silico* Evidence

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Abstract

The present investigation was carried out to evaluate the hepatoprotective activity of aqueous ethanolic extract of *Pleurotus pulmonarius* (AEEPP) against combined cadmium and restraint stress induced hepatotoxicity in rats. Preliminary mycochemical screening indicated the presence of phenolics, flavonoids and polysaccharides. AEEPP showed concentration dependent DPPH and nitric oxide radical scavenging properties with IC50 values of 16 and 55 µg/mL respectively. The disaccharide (HEX-HEX) demonstrated the most encouraging results in silico molecular docking, with a docking score of −11.109 kcal/mol and MMGBSA energy of −63.72 kcal/mol, indicating its possible interaction with the Keap1–Nrf2 pathway. Cadmium and constraint stress dramatically enhanced hepatic enzymes and oxidative stress, and depressed antioxidant defense in vivo. Treatment of AEEPP especially at 300 mg/kg dramatically restored the levels of SOD, CAT, GSH, GPx and improved the serum biochemical markers ($p<0.0001$). Liver morphology and histopathology revealed reduced inflammation, degradation of hepatocytes and tissue injury with near normal architecture at 300 mg/kg. Behavioral tests (OFT and rotarod) were considered supportive because no significant treatment-related changes were observed. To summarize, AEEPP showed hepatoprotective effect on cadmium and restraint stress produced liver injury perhaps due to its antioxidant and bioactive ingredients.

Major Findings: The phenolics, flavonoids and polysaccharides included in AEEPP demonstrated excellent radical-scavenging activity. HEX-HEX exhibited favorable Keap1–Nrf2-related docking. AEEPP at 300 mg/kg increased antioxidant and liver-function measures and greatly conserved the hepatic architecture. Behaviors: No significant treatment-related changes in behavior were observed.

Keywords: Cadmium Toxicity, Keap1-Nrf2, Hepatoprotection, *Pleurotus pulmonarius*, Molecular Docking, Oxidative Stress, Restraint Stress

1. Introduction

The liver as impressing capacity of detoxification function among multiple visceral organs, initials exposure to toxins and emotional stress leads to hepatotoxicity, which is distinguished by hepatic dysfunction and hepatic impaired function[1]. The triggering hepatotoxins, like heavy metals, may have an inducible role in hepatotoxicity. Heavy metals are high in density and atomic weight; these metallic elements can threaten the health of animals and humans. One of these toxins is cadmium (Cd), which is considered an environmental pollutant when incorporated into the food chain of humans and animals by consuming contaminated water, plants and aquatic products[2]. More than 13,000 tons of cadmium are produced annually worldwide for use in chemical stabilisers, colour pigments, metal coatings, Ni-Cd batteries and alloys[3]. The metal mixture-associated Metabolic Dysfunction-associated Steatotic Liver Disease (MASLD) showed high-risk exposure to cadmium[4].

Article Received on: 26.03.2026 **Revised on:** 15.07.2026. **Accepted on:** 16.07.2026

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Emotional stress, also known a s psychological stress, non-painful which is triggering factor and provokes mental and physical health problems and makes one susceptible to many other diseases[5]. Due to many modern lifestyle modifications and working night shifts, which can lead to the disruption of circadian rhythms and changes in neurological responses, contributing to a stress state, a previous study states that the combination of cadmium and stress can alter the physiology of the liver by hepatic dysfunction, metabolic disease, hepatotoxicity and eventually leads to hepatocellular cancer[6]. The exact mechanism by which cadmium and stress cause hepatotoxicity is not yet known; however, based on previous literature review, cadmium can be unconsciously absorbed through the food chain. It is not metabolise then accumulates mainly in the liver, where metallothionein is a metal-binding protein which helps in metal homeostasis and detoxification; cadmium as high affinity for binding to this protein, which damages the metallothionein protein[7]. Possibly by reducing the permeability of the mitochondria, which inhibits the respiratory chain's process and generates reactive oxygen species ROS further inhibiting antioxidant enzymes, inducing cell apoptosis and inhibiting DNA repair[8]. One essential cytoprotective transcription factor that may protect redox homeostasis factor is nuclear-factor erythroid 2-related factor 2 (Nrf2), as Cd exposure causes suppression of the regulation of Nrf2 signalling pathways[9]. It is still unclear exactly how stress causes hepatic damage. An increasing amount of research has shown a connection between psychological stress and hepatic injury[10]. Stress provokes activation of the HPA axis, which releases and increase level of stress hormones like glucocorticoids, cortisol, leading to liver inflammation and changes in behavioural like anxiety, locomotor activity and depression[11]. combination of cadmium-restraint stress model create oxidative stress and hepatic injury, as revealed prominent elevation of serum ALT, ALP and AST levels, increased levels of reactive oxygen species, glutathione and lipid peroxidation activity, and drain of antioxidant enzyme activities, antioxidant enzymes like Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx) and Catalase (CAT) may undergo inhibition of expressions in relation to Cd and restraint stress along with liver morphology and Histological analysis[12]. Many herbal plants and drugs have been used as hepatic therapeutic agents owing to their antioxidant properties; among them, *P. pulmonarius* is a grey oyster mushroom, and it is an edible mushroom throughout the world[13]. *P. pulmonarius* belongs to the family Pleurotaceae, which is widely used as traditional medicine in China, Vietnam, Africa and Asia, including Southeast Asia, Japan and other countries. The bioactive compounds are Carbohydrates, alkaloids, amino acids, glycosides, and triterpenoids/steroids. Polysaccharides, phenolics and other β-glucans; Pleurotus β-glucan is responsible for the antitumoral activity of *P. pulmonarius*. Further, Anti-cholesterol, Antioxidant, anti-inflammatory, Anticancer, Antiviral, Antidiabetic, Antiviral, Eye health, antibacterial, and Antiarthritic activities were reported on *P. pulmonarius*. However, there is no available data on *P. pulmonarius* extract's potential against Combined Cadmium and restraint stress-induced hepatotoxicity[14]. The potential of *P. pulmonarius* in this domain requires further investigation.

2. Materials and Methods

2.1 Collection and Preparation of Aqueous Ethanolic Extract of *P. pulmonarius*

Fresh grey oyster mushrooms (*P. pulmonarius*) were procured from Gandhi Krishi Vigyana Kendra (GKVK), Bengaluru, and taxonomically authenticated by the head of the Department of Botany, KLE Society's S. Nijalingappa College, Rajajinagar, Bengaluru. Fresh grey oyster mushrooms (*P. pulmonarius*) were shade-dried, and 100 grams of dried coarse powder were used to prepare the aqueous ethanolic extract. By using a ratio of 50:50 of ethanol: Distilled water by reflux method. The extraction was performed in three successive cycles at a constant temperature of 65°C. The first cycle was carried out for 3 h, followed by the second cycle for 2 h and the third cycle for 1 h. The combined extracts were filtered, and the filtrate was concentrated under vacuum dry and dried for 24 h. A total of 28 g of dried extract was obtained from 100g of coarse powder. The dried extract was stored in amber-colored glass bottles until further use[15].

2.2 Preliminary Phytochemical Screening

The preliminary phytochemical screening was carried out using qualitative chemical techniques. The aqueous ethanolic extract of *P. pulmonarius* was analysed for the presence of alkaloids, carbohydrates, triterpenoids, steroids, saponins, quinones and phenols, flavonoids, tannins, and protein[16].

2.3 Quantitative Phytochemical Analysis

The total phenolic content, total flavonoids, and total polysaccharides of *P. pulmonarius* were determined using a UV spectrophotometer, following a standard procedure as available in the literature[17,18].

2.4 Antioxidant Activity of *P. pulmonarius* Extract

The free radical scavenging activity of aqueous ethanolic extract of *P. pulmonarius* on 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined using the method described by Okonkwo *et al.*[18]. Nitric oxide scavenging activity of aqueous ethanolic extract of *P. pulmonarius* was carried out using a UV spectrophotometer, the method described by Ogidi *et al.*[19].

2.5 *In silico* Docking on *P. pulmonarius* Extract [20]

An *in silico* approach was employed to explore the potential molecular mechanisms of bioactive compounds of AEEPP extract for hepatoprotective activity.

Software: Molecular docking was performed using Glide (Schrödinger Suite, Maestro version 14.3, Schrödinger, LLC, New York, NY).

Protein Preparation: The crystal structure of [target protein, PDB ID: 3WN7] was prepared using the Protein Preparation Wizard in Schrödinger. This included:

Assignment of bond orders and addition of hydrogen atoms. Removal of crystallographic water molecules beyond 5 Å from the binding site. Optimisation of the hydrogen-bonding network and protonation states at pH 7.0 ± 2.0 using PROPKA Restrained energy minimisation using the OPLS4 force field (convergence to RMSD of 0.30 Å for heavy atoms).

Ligand Preparation: All ligands were prepared using LigPrep (Schrödinger) Maestro. This step generated possible ionisation states at pH 7.0 ± 2.0 using Epik, assigned correct tautomers, and performed energy minimisation using the OPLS4 force field.

Grid Generation: A receptor grid was generated using the Receptor Grid Generation module, centred on the co-crystallised ligand/active site residues (specify residues if known), with default van der Waals scaling (1.0) for non-polar receptor atoms.

Ligand Selection: The compounds, including the disaccharides, were selected for docking studies as they were identified as major constituents present in the sample through phytochemical analysis. Since these compounds are naturally abundant in the studied source, they were considered relevant candidates to evaluate their potential binding interactions with the target protein, alongside other reported bioactive compounds.

Docking Protocol: Ligands were docked using Glide in Extra Precision (XP) with flexible ligand sampling. The top-scoring pose for each ligand, ranked by Glide docking score (kcal/mol), was selected for further analysis.

2.6 *In vivo* Experimental Studies

Forty-six male Wistar rats weighing between 180-220 g (5-8 weeks old) were used for this study, has been approved by the Institutional Animal Ethics Committee (IAEC) with CPCSEA regulations (protocol No.16/KM/2025). Animals for this experiment were procured from Vaarunya Biolabs Private Limited, Bengaluru. Animals were supplied with a balanced diet and water *ad libitum* acclimatised to the environment for one week prior to experimental use. Experimental procedures adhered to CPCSEA guidelines.

Preparation of inducing agent: Here, the inducing agent, cadmium chloride monohydrate 98.0%, was

Group	Treatment dose
Group I Negative Control	Distilled water
Group II Positive Control	Cadmium chloride (2.5mg/kg b.w, i.p.) + restraint stress
Group III Standard control	Cadmium chloride (2.5mg/kg/b.w) + restraint stress + silymarin (50mg/kg/ b.w, p.o.)
Group IV Treatment group	Cadmium chloride + restraint stress and low dose oral administration of *P. pulmonarius* extract with distilled water
Group V Treatment group	Cadmium chloride + restraint stress + medium dose of *P. pulmonarius* extract
Group VI Treatment group	Cadmium chloride + restraint stress + high dose of *P. pulmonarius* extract

purchased from CDH, India. Doses were prepared daily and dissolved in sterile water.

2.6.1 Dose Determination

The selected dose of *P. pulmonarius* extract was chosen based on previously published toxicological studies on *Pleurotus* species, which have demonstrated low toxicity and good tolerability in experimental animal models. Oral administration of *P. pulmonarius* extract up to the dose of 2000mg/kg b.w showed no observable toxicity. Therefore, we have chosen 200, 300 and 400mg/kg, b.w doses as low, mid and high doses, respectively, for this study[21].

2.6.2 Experimental Design

The rats were randomly divided into six groups and each consisting of eight animals (n = 8) induction phase of cadmium chloride and restraint stress for 30 mins was given for 28 days from day 1 to 28th day followed by treatment phase for next 28 days from 29th to 56th day on 55th day, 56th day and 57th day all group of rats was performed behavioral tests and rats were euthanised in accordance with CCSEA guidelines blood and liver were harvested. Figure 1 depicts the experimental timeline[22].

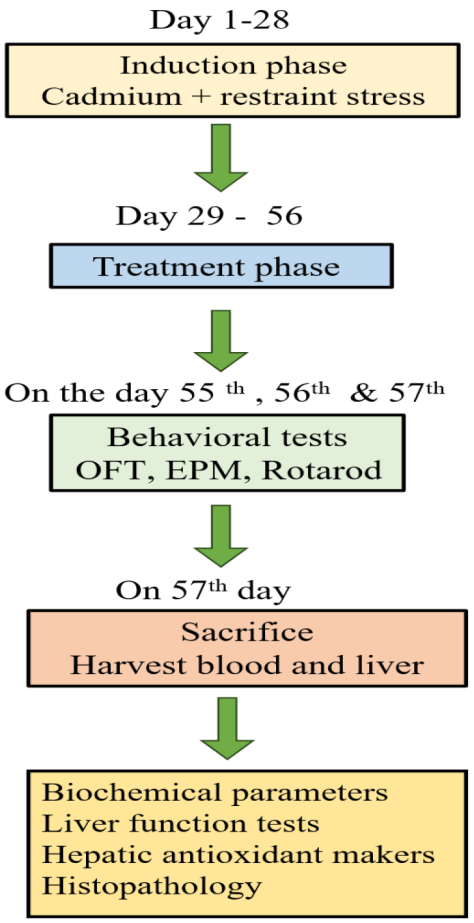


Figure 1. Schematic Experimental Timeline.

2.7 Behavioural Analysis

The Locomotor Activity Test (LAT) is widely used to measure exploratory neurobehavior in rats as well as to assess movement and anxiety-like behaviours. In this test, we recorded time and activity on the Open Field Test (OFT) and Rota rod[23].

2.8 Hepatic Antioxidant Status

Hepatic antioxidant status: the activities of superoxide dismutase (SOD), catalase (CAT), Glutathione-S-Transferase (GST), Glutathione (GSH), Malondialdehyde (MDA) and Glutathione Peroxidase (GPx) in liver tissues were measured as per standard procedure available in literature[12].

2.9 Biochemical Parameters

Liver function tests, including the enzymatic activities of serum aspartate aminotransferase, alanine aminotransferase, total protein, albumin and alkaline phosphatase, were analysed in accordance with the manufacturer's instructions of commercial kits Agappe Diagnostics, Ltd., by an automatic biochemical analyser for the liver injury in the study is listed in Table 1.

2.10 Histopathological Investigations and Morphological Images of Liver

The liver parts were fixed in formalin (10%), following the routine paraffin method procedure. Hepatic tissues were prepared into paraffin blocks. For routine histology examination, the paraffin sections (5 mm thick) were stained with haematoxylin and eosin. An experienced pathologist who was blinded through the experimental procedure and conducted the histopathological evaluation. Histopathological observations were evaluated using a semi-quantitative grading scale (0-4) based on the severity of hepatic lesions. The scores are presented descriptively as a bar graph to support the representative photomicrographs.

2.11 Statistical Analysis

The experimental data are expressed as mean ± SEM (n = 6), and statistical analysis is performed using GraphPad

Table 1. Changes in serum ALT, AST, ALP, Total protein and Albumin in extract and standard treated as well as untreated experimental animals

Parameter	Kit name	Method	Manufacture	nm	Instrument
ALT	SGPT	IFCC recommended	Agappe Diagnostics Ltd	340nm	Auto Analyser
AST	SGOT	IFCC recommended	Agappe Diagnostics Ltd	340nm	Auto Analyser
ALP	Alkaline phosphatase	DGKC-SCE recommended	Agappe Diagnostics Ltd	405nm	Auto Analyser
Total protein	Albumin and total protein	Direct biuret	Agappe Diagnostics Ltd	546nm	Auto Analyser
Albumin	Albumin and total protein	Direct biuret	Agappe Diagnostics Ltd	546nm	Auto Analyser

Table 2. Preliminary mycochemicals of aqueous ethanolic extract of *P. pulmonarius*

Mycochemicals tests	Results
Alkaloids test	Present
Carbohydrates test	Present
Quinones test	Present
Triterpenoids test	Present
Steroid test	Present
Saponins test	Present
Phenols test	Present
Flavonoids test	Present
Tannins test	Present
Protein test	Present

Quantitative detection of mycochemical compounds

Prism version 8.0.2, which involves a one-way ANOVA accompanied by Tukey's Post hoc multiple comparison test. Although the sample size was (n = 8), only 6 values were included in the statistical analysis.

Table 3. Total phenolic content of aqueous ethanolic extract of *P. pulmonarius.*

Drug/extract	Concentration (mg/ml)	Absorbance
Gallic acid	50	0.305
	100	0.462
	150	0.718
	200	0.895
	250	1.017
P. pulmonarius	0.5 ml	0.856

Table 4. Total flavonoid content of aqueous ethanolic extract of *P. pulmonarius*

Drug / extract	Concentration(mg/ml)	Absorbance
Quercetin	10	0.035
	20	0.045
	30	0.051
	40	0.052
	50	0.071

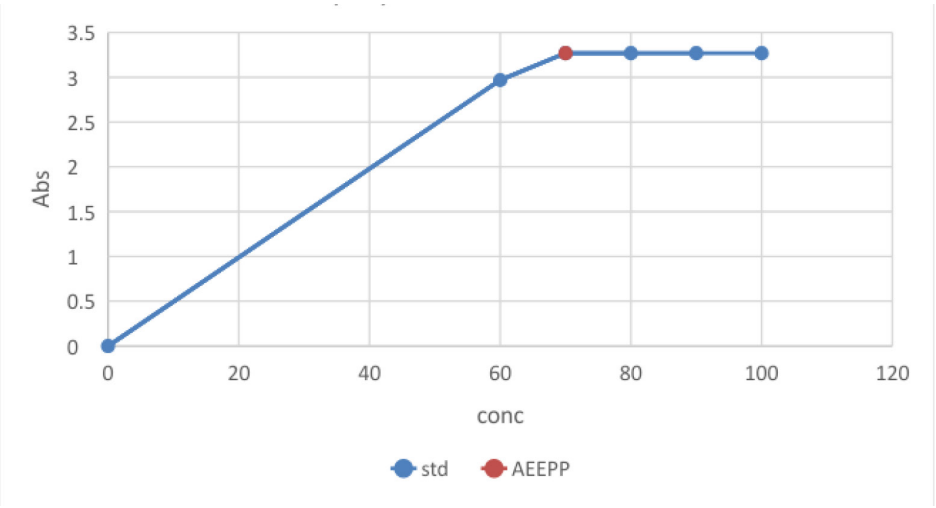


Figure 2. Total polysaccharide content.

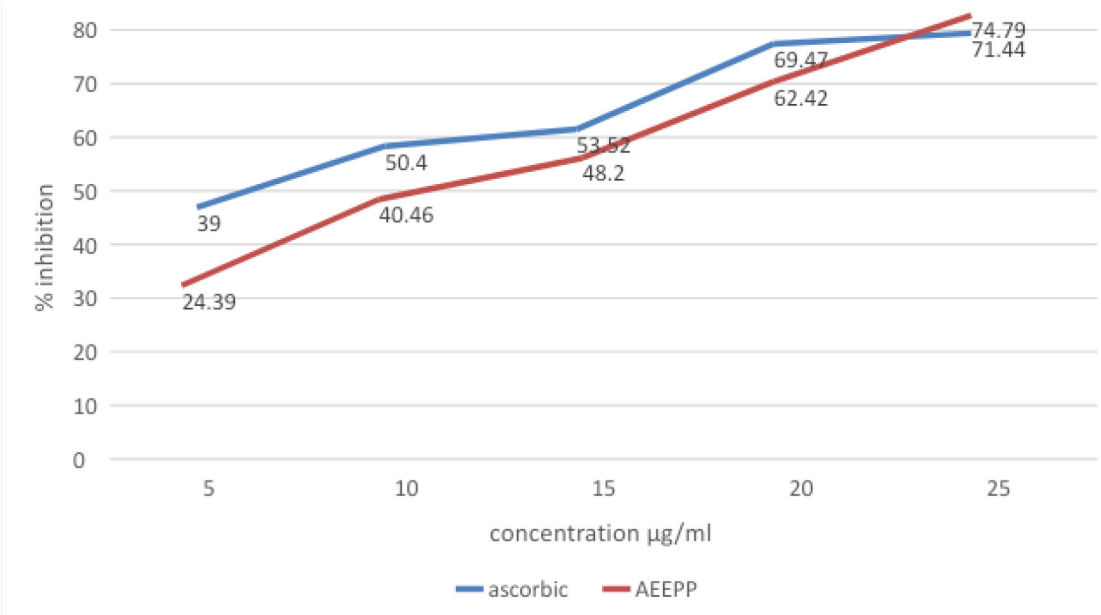


Figure 3. DPPH assay.

Table 5. Total polysaccharide content of aqueous ethanolic extract of *P. pulmonarius*

Drug / extract	Concentration(mg/ml)	Absorbance
Glucose	0	0
	60	2.968
	70	3.269
	80	3.269
	90	3.269
	100	3.269
P.pulmonarius	0.5 ml	3.269

Table 6. Percentage inhibition and IC 50 value of AEEPP and Ascorbic Acid in increasing concentration

Drug/ extract	Concentration (µg/ml)	% inhibition	IC 50 value(µg/ml)
Ascorbic acid	5	39.07	10µg/ml
	10	50.40	
	15	53.52	
	20	69.47	
	25	71.44	
AEEPP	5	24.39	16µg/ml
	10	40.46	
	15	48.20	
	20	62.42	
	25	74.79	

3. Result and Discussion

3.1 Results of Preliminary Mycochemical Screening

First, we evaluated the preliminary phytochemical test of aqueous ethanolic extract of *P. pulmonarius,* was brought out an extensive profile of phytochemicals as shown in Table 2.

Results of Total phenolic content of the extract are tabulated in Table 3. The total phenolic content in aqueous ethanolic extract of *P. pulmonarius* was found to be 190 mg/g (GAE). results of Total flavonoid content of the extract were tabulated in Table 4. The total flavonoid content in aqueous ethanolic extract of *P. pulmonarius* was found to be 40mg quercetin/g, and results of Total polysaccharide content of the extract are tabulated in Table 5 and calibration curve depicted as Figure 2. The total polysaccharide content in aqueous ethanolic extract of *P. pulmonarius* was found to be 70 mg glucose/g.

Quantitative analysis of AEEPP was carried out in triplicate, and the average value was used for concentration validation. The results were presented graphically using Microsoft Excel. No statistical analysis was conducted.

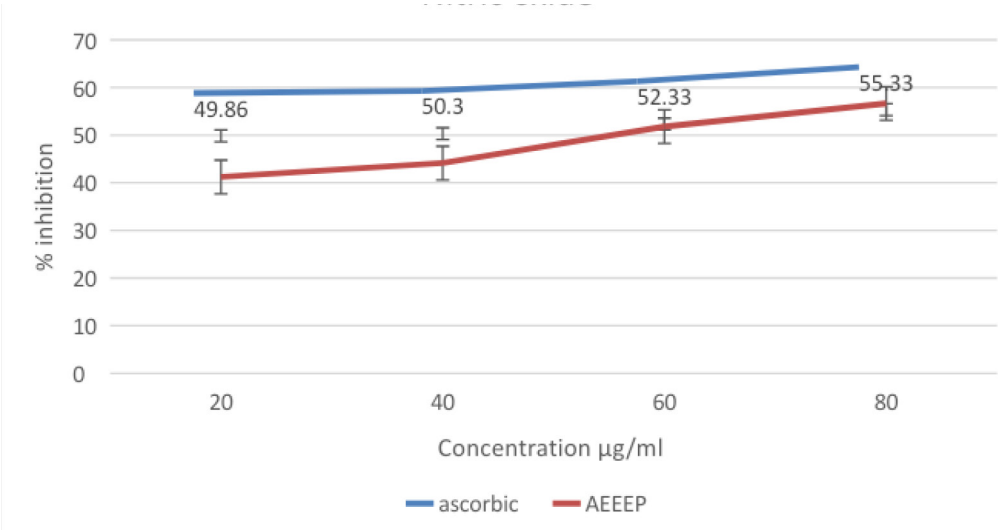


Figure 4. Nitric oxide assay.

Table 7. Nitric oxide free radical scavenging activity of Ascorbic acid, AEEPP in increasing concentration

Drug/ extract	Concentration (µg/ml)	% inhibition	IC 50 value (µg/ml)
Ascorbic acid	20	49.86	40µg/ml
	40	50.30	
	60	52.33	
	80	55.33	
AEEPP	20	41.23	55µg/ml
	40	44.14	
	60	51.80	
	80	56.65	

3.2 *In vitro* Antioxidant Activity of *P. pulmonarius* Extract

DPPH Assay: Results of the DPPH assay carried out with Ascorbic acid as standard are tabulated as shown in Table 6 and calibration curve depicted as Figure 3. IC-50 of AEEPP was found to be 16 µg/ml, and that of Ascorbic Acid was 10 µg/ml. Antioxidant assays of AEEPP were carried out in triplicate, and the average value was used for concentration validation and percentage inhibition. The results were presented graphically using Microsoft Excel. No statistical analysis was conducted.

Nitric oxide free radical scavenging activity of extract AEEPP was tabulated as shown in Table 7 and Figure 4, and IC 50 Value was found to be 55 µg/ml; the IC50 value of Ascorbic acid was found to be 40 µg/ml.

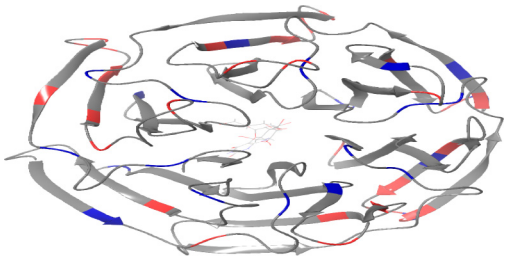


Figure 5. Crystal Structure of Keap1 in Complex with the N-terminal region of the Nrf2 transcription factor that confirms the biological Target.

3.3 *In silico* Molecular Docking Analysis of *Pleurotus pulmonarius*

Figure 5 represents the crystal structure of Keap1 in Complex with the N-terminal region of the Nrf2 transcription factor, which confirms the biological Target.

Keap1 was chosen as the hepatoprotective target protein because it is one of the most important negative regulators of the Nrf2 transcription factor, whose target genes encode antioxidant and cytoprotective proteins for cellular defence against hepatic oxidative insults and toxin-induced liver injury. Abnormal regulation of the Keap1-Nrf2 pathway is closely associated with hepatotoxicity; therefore, Keap1 is a biologically relevant molecular target in hepatoprotective studies.

For molecular docking studies, the experimentally determined crystal structure of Keap1 in complex with the N-terminal region of Nrf2 was taken directly from

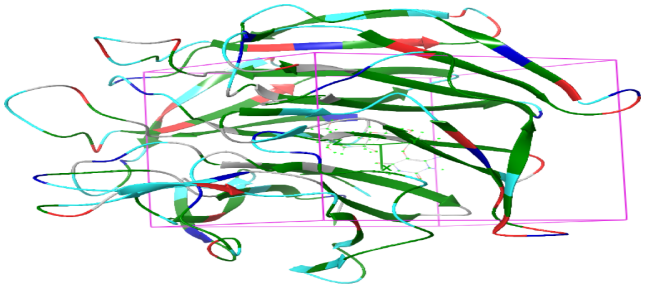


Figure 6: Pre-defined active-site region of the selected hepatoprotective target protein in the form of the docking grid.

the Protein Data Bank, PDB ID: 3WN7, without further structural prediction or modelling. This structure shows a well-resolved three-dimensional architecture of the protein with well-defined elements of secondary structure, thus representing a reliable and biologically adequate framework for investigation of ligand binding at the Keap1-Nrf2 interaction interface. The protein structure was determined from high-resolution X-ray crystallographic data. Use of a directly validated PDB structure inherently confers high confidence in the structural integrity of the target protein and supports

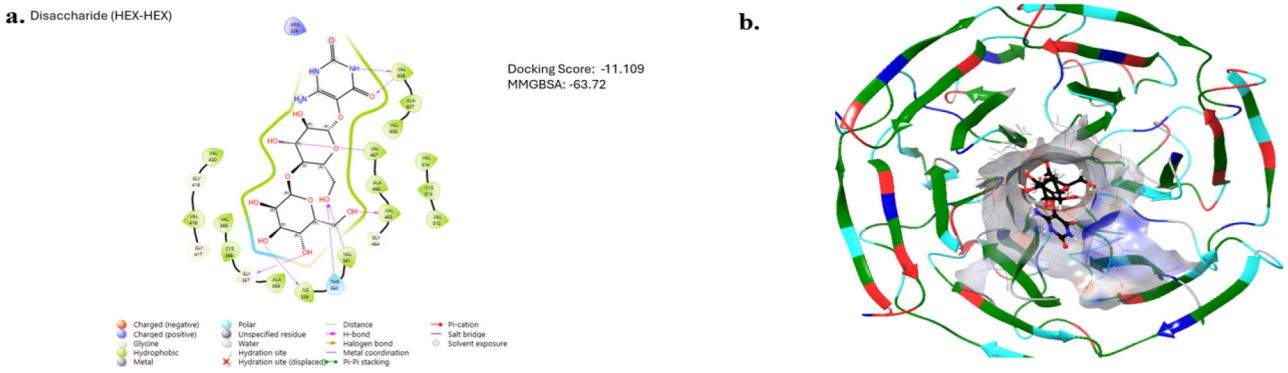


Figure 7. Molecular docking calculations of the disaccharide (HEX-HEX) into the hepatoprotective target protein

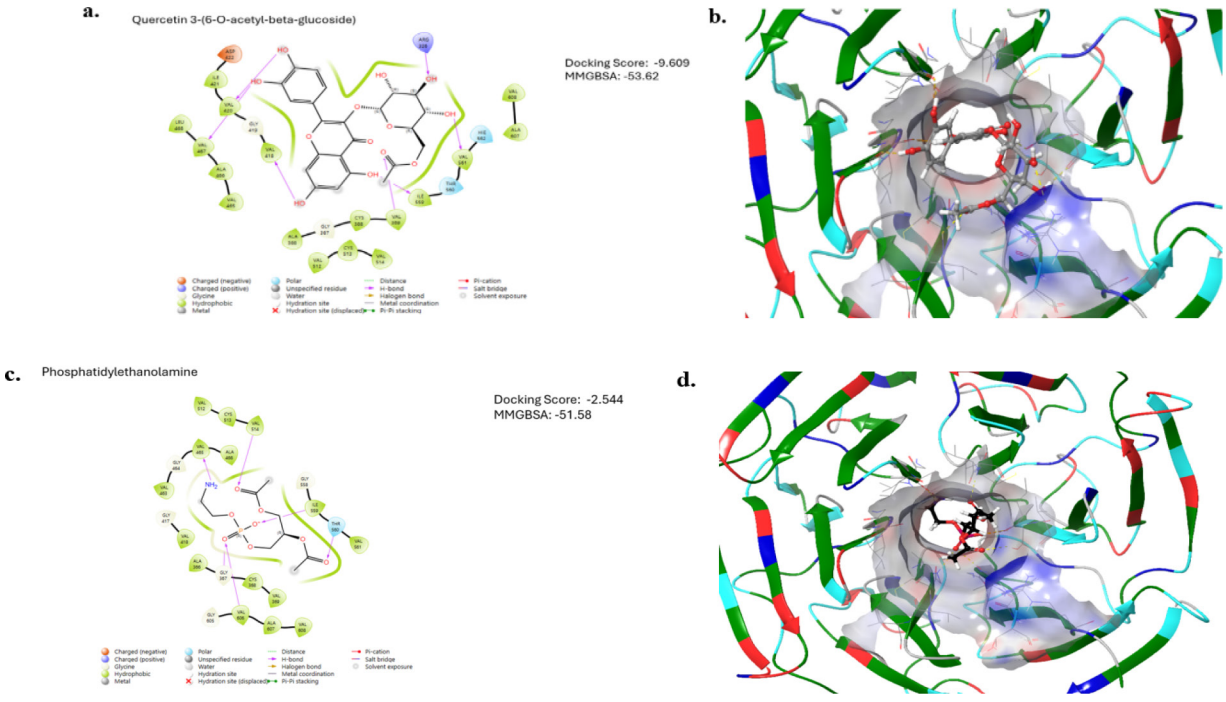


Figure 8. Total polysaccharide content. **(a, b)** Quercetin and **(c, d)** Phosphatidylethanolamine.

its suitability for docking and in silico screening of hepatoprotective phytochemicals targeting the Keap1-Nrf2 pathway.

Figure 6 shows the pre-defined active-site region of the selected hepatoprotective target protein in the form of the docking grid. The pink grid box encloses a biologically relevant binding pocket, ensuring the modelling of ligand conformation within this functionally important region rather than on the whole protein surface. The protein is shown in ribbon form with a well-organised secondary structure and connecting loops dominated by β-sheets, contributing toward pocket formation. The grid is centred around residues known to play a key role in either catalysis or regulation in mechanisms related to hepatic protection, such as antioxidant defence, modulation of inflammation, or detoxification pathways. Proper placement and size of the grid confirm that docking simulations are focused upon residues critical for ligand-protein interaction, thereby assuring higher reliability in the prediction of binding affinity and supporting the identification of a phytoconstituent or compound with potential hepatoprotective activity.

Figure 7 shows the molecular docking calculations of the disaccharide (HEX-HEX) into the hepatoprotective target protein disclosed a stable binding pose inside the active-site cavity. Accordingly, the 2D interaction map displays several hydrogen bonds via hydroxyl groups with polar amino acids such as THR466, THR467, and GLY417, while hydrophobic interactions were detected with non-polar amino acids like VAL464, VAL465, VAL606, and ALA607. This suggests that the hydroxyl substructures of the sugar constitute important hydrogen-bonding donors/acceptors, while the sugar ring backbone seems to contribute to hydrophobic stabilisation in the pocket. Indeed, the 3D binding mode poses the disaccharide nicely into the cavity in agreement with the surface topography and secondary structural elements of the protein. Overall, these interactions suggest a favourable and stable protein-ligand complex with strong binding affinity as reflected by a docking score of −11.109 k cal/mol and an MMGBSA free energy of −63.72 kcal/mol.

The docking result (Figure 8a) shows that Quercetin 3-(6-O-acetyl-β-glucoside) is bound firmly to the active site of the target protein with hydrogen bonding and hydrophobic interactions. The 2D plots revealed that the flavanoids and glycosidic part make multiple hydrogen bonds with phenolic as well as sugar hydroxyls with polar beads such as ASP422, ARG326, THR560, and HIS562, whereas the other nonpolar residues around the active site, VAL420, VAL461, VAL465, ALA466, LEU468, and PHE419, make nonpolar interactions with the ligand, which are involved in π-π interactions with the benzoids of the flavonoids. The acetylated glucose part also increases polar interactions, which increases bonding with the pocket. The 3D result (Figure 8b) shows that the ligand is nicely bound deeply inside the pocket, thereby predicting that there is a high affinity of the compound with the target protein because of the high docking score of -9.609 kcal/mol with an MMGBSA binding energy of -53.62 kcal/mol, which is a stable result.

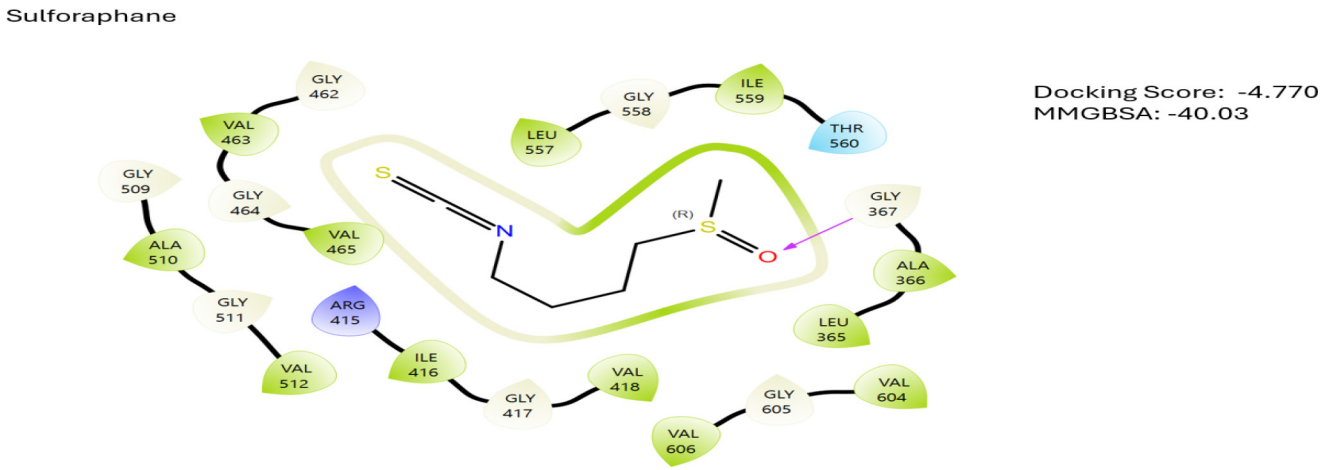


Figure 9. Docking of Sulfuraphane with disaccharide.

The structure of phosphatidylethanolamine (Figure 8 c) revealed a relatively weaker but stable binding conformation within the same active-site pocket. The soaked 2D interaction profile indicates that the polar head part of the compound is mainly involved in hydrogen bonding withThr560, Gly417, and Cys368, while the aliphatic chains are mainly involved in nonpolar interactions with VAL461, VAL465, VAL512, ALA466, and LEU468. This interaction profile indicates that the compound is mainly stabilised by nonpolar interactions, which are mostly non-directional compared to the interactions exhibited by the polyphenolic compound. Additionally, the 3D graphical analysis (Figure 8d) confirms that the compound is seated inside the pocket but with a non-directional interaction profile, which is denoted by the lack of significant interactions with the pocket residues. This is therefore, consistent with a moderate binding affinity, denoted by a binding score of -2.544 kcal/mol with a favourable MMGBSA free energy of -51.58 kcal/mol, which indicates overall stability but a lack of specificity to the pocket.

The result of the docking study revealed a hydrophobic binding mode by which the sulforaphane interacts with the active-site pocket of the target protein as depicted in Figure 9. Based on the 2D representation, several hydrophobic residues surrounding the ligand were revealed: VAL463, VAL465, VAL512, VAL418, VAL604, VAL606, LEU365, LEU557, and ILE416/559, which contribute overall to the stability of the aliphatic backbone of the sulforaphane molecule through hydrophobic interactions. However, only weak polar interactions were observed, particularly the hydrogen-bond interaction of the sulfoxide group with GLY367

Table 8. List of compounds with their docking score

Name of Compound	Docking Score	MMGBSA
Disaccharide (HEX-HEX)	−11.109	−63.72
Quercetin 3-(6-O-acetyl-β-glucoside)	−9.609	−53.62
Phosphatidylethanolamine	−2.544	−51.58
Sulfuraphene (Std)	−4.770	−40.03

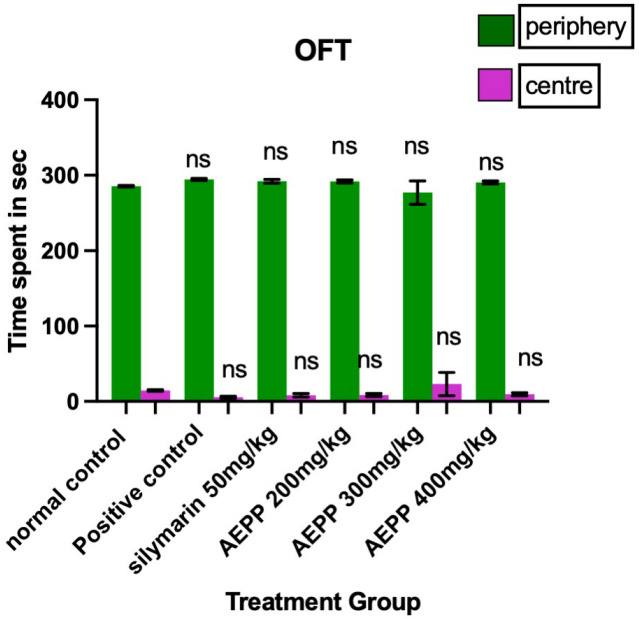


Figure 10. Changes in time spent (s) in center and periphery

Values are expressed as mean± SEM (n=6). All the values with p<0.05 were considered significant. (ns) indicates p>0.05 in comparison with normal control animals; (ns) indicates p>0.05 in comparison with positive control animals.

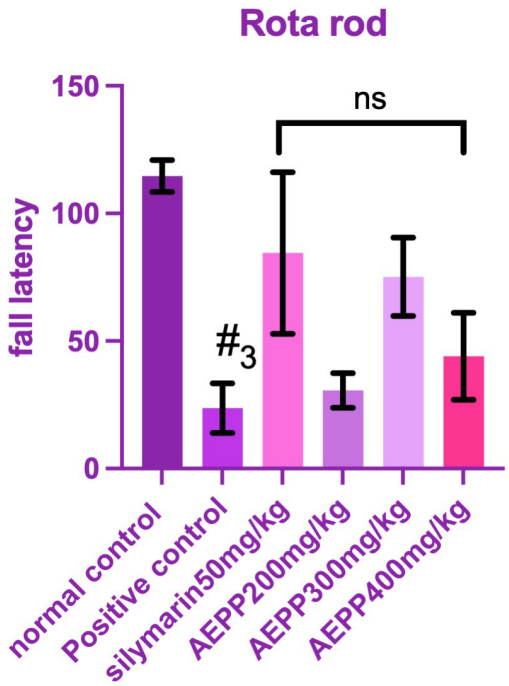


Figure 11. Changes in fall off time (s)

Values are expressed as mean± SEM (n=6). All the values with p<0.05 were considered significant. ($\#_3$) indicates p<0.05 in comparison with normal control animals, (ns) indicates p>0.05 in comparison with positive control animals.

and its approach towards THR560, while the approach of ARG415 towards the molecule was observed, indicating a possible electrostatic effect but lacking a definitive directional binding. In its 3D binding conformation (not shown), a shallow but stable binding mode of the ligand within the target pocket was indicated. The binding mode was typical of moderate affinity, as suggested by its moderate binding energy of −4.770 kcal/mol as indicated by the docking score and an MMGBSA binding free energy of −40.03 kcal/mol, tabulated in Table 8.

Out of the tested hepatoprotective compounds, the disaccharide (HEX-HEX) showed the greatest binding affinity for the target protein, as evidenced by the highest scoring docking (−11.109) and most negative MMGBSA binding free energy (−63.72 kcal/mol). This suggests that the most stable protein-ligand complex has been formed, mostly due to extensive hydrogen bonding interactions and proper binding within the active site cavity. Quercetin 3-(6-O-acetyl-β-glucoside) showed moderate levels of binding affinity with a docking score of −9.609 and an MMGBSA of −53.62 kcal/mol, with the help of a proper blend of hydrogen bonding and hydrophobic or π-π interactions. However, phosphatidylethanolamine showed the lowest affinity for docking (−2.544), although with an acceptable MMGBSA of −51.58 kcal/mol, inferring stable non-specific binding interactions mostly mediated due to hydrophobic interactions between the fatty acid moieties. However, the standard compound Sulforaphane showed moderate affinity for binding with a docking score of −4.770 and an MMGBSA of −40.03 kcal/mol. Actually, this serves as a relative parameter for comparative assessment. Hence, the relative hepatoprotective activity of the compounds as inferred from the relative docking score stands as: Disaccharide (HEX-HEX) > Quercetin Derivative > Sulforaphane (Standard) > Phosphatidylethanolamine.

3.4 Assessment of Behavioural Activity

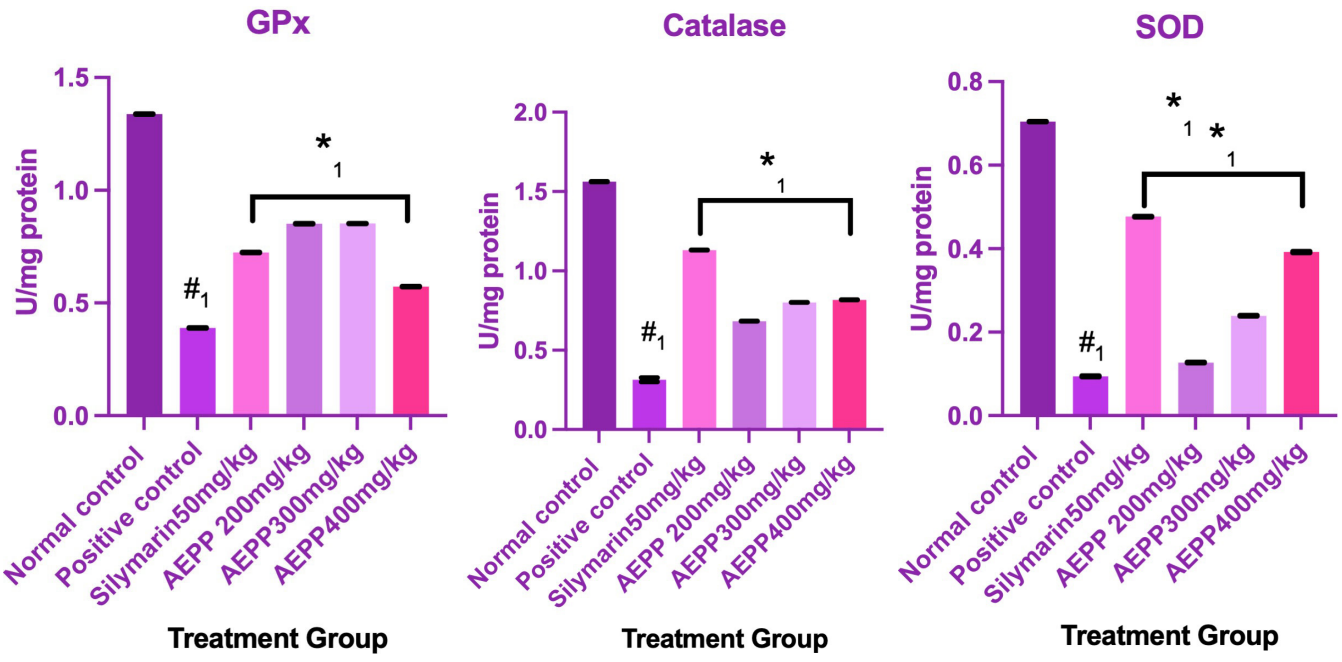


Figure 12. Changes in oxidant and anti-oxidant levels in liver homogenate.
Values are expressed as mean± SEM (n=6). All the values with $p<0.05$ were considered significant. ($\#_1$) indicates $p<0.0001$ in comparison with normal control animals, ($*_1$) indicates $p<0.0001$ in comparison with positive control animals.

3.5 Restoration of Hepatic Antioxidant Defence System

3.6 Effect of Treatment on Serum Hepatic Biochemical Parameters

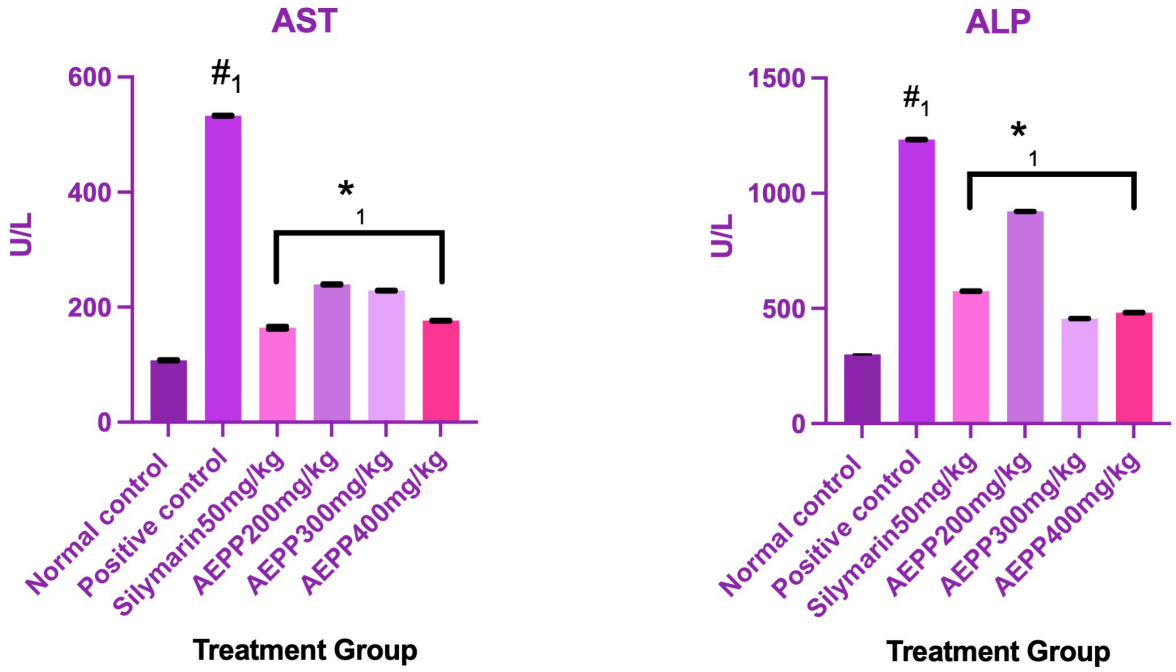


Figure 13. Values are expressed as mean± SEM (n=6). All the values with $p<0.05$ were considered significant. ($\#_1$) indicates $p<0.0001$ in comparison with normal control animals, ($*_1$) indicates $p<0.0001$ in comparison with positive control animals.

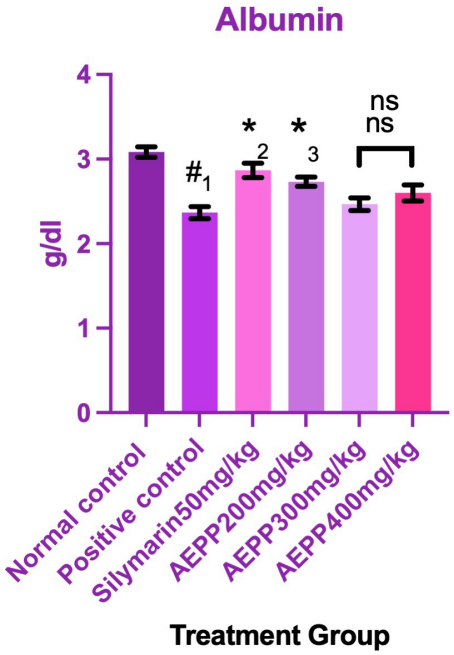


Figure 14. Changes in serum albumin.
Values are expressed as mean± SEM (n=6). All the values with $p<0.05$ were considered significant. ($\#_1$) indicates $p<0.0001$ in comparison with normal control animals, ($*_2$) indicates $p<0.001$ in comparison with positive control animals, ($*_3$) indicates $p<0.01$ in comparison with positive control animals, and ns indicates $p>0.05$ in comparison with positive control animals.

3.7 Impact of Treatment on Liver Morphological and Histopathological Observations

Macroscopic observation of liver tissue in cadmium and restraint stress-induced hepatotoxicity model. Normal control: illustrating healthy architecture, smooth texture and characteristics. Positive control: demonstrating hepatomegaly, surface irregularities, and significant lesions. Silymarin: showing significant reversal of toxic effects. AEEPP 200 mg/kg group: showed partial restoration of the liver AEEPP 300 mg/kg group exhibited improvement, with better structural integrity and reduced visible lesions than the positive control group, indicating a strong hepatoprotective effect. AEEPP 400 mg/kg group showed liver morphology that closely resembled that of the silymarin-treated groups, morphology, although mild irregularities and congestion were still visible.

Representative photomicrographs of histopathological HandE-stained liver sections (×400) from experimental groups. **(A)** Normal

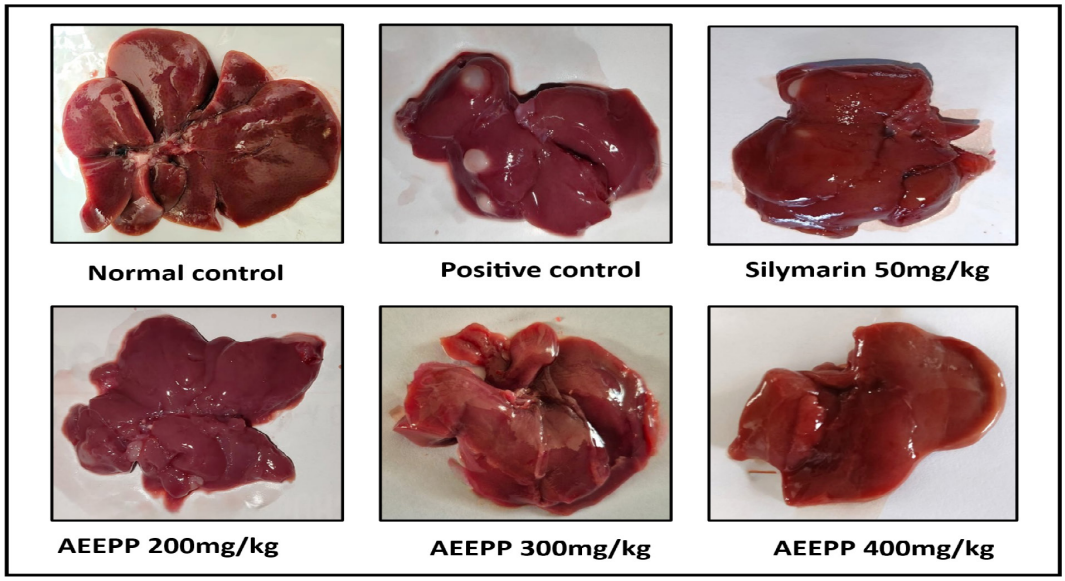


Figure 15. Impact of Treatment on Liver Morphological Observations.

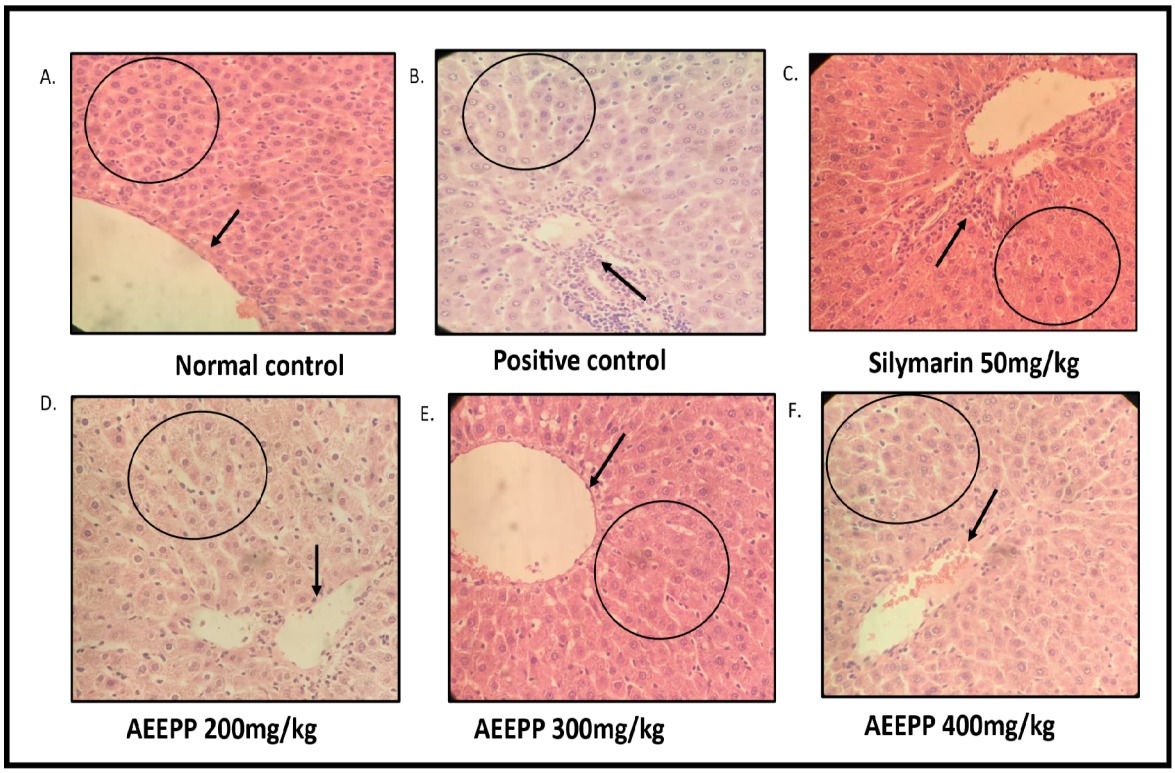


Figure 16. Impact of Treatment on Liver Histopathological Observations.

control showing intact hepatic architecture. **(B)** Positive control (cadmium + restraint stress) showing hepatocellular degeneration, sinusoidal congestion, inflammation, and necrosis. **(C)** Silymarin-treated group showing near-normal architecture. **(D)** AEEPP 200mg/kg showing moderate improvement. **(E)** AEEPP 300mg/kg showing marked restoration of hepatocytes. **(F)** AEEPP 400mg/kg showing near-normal hepatic architecture comparable to standard treatment.

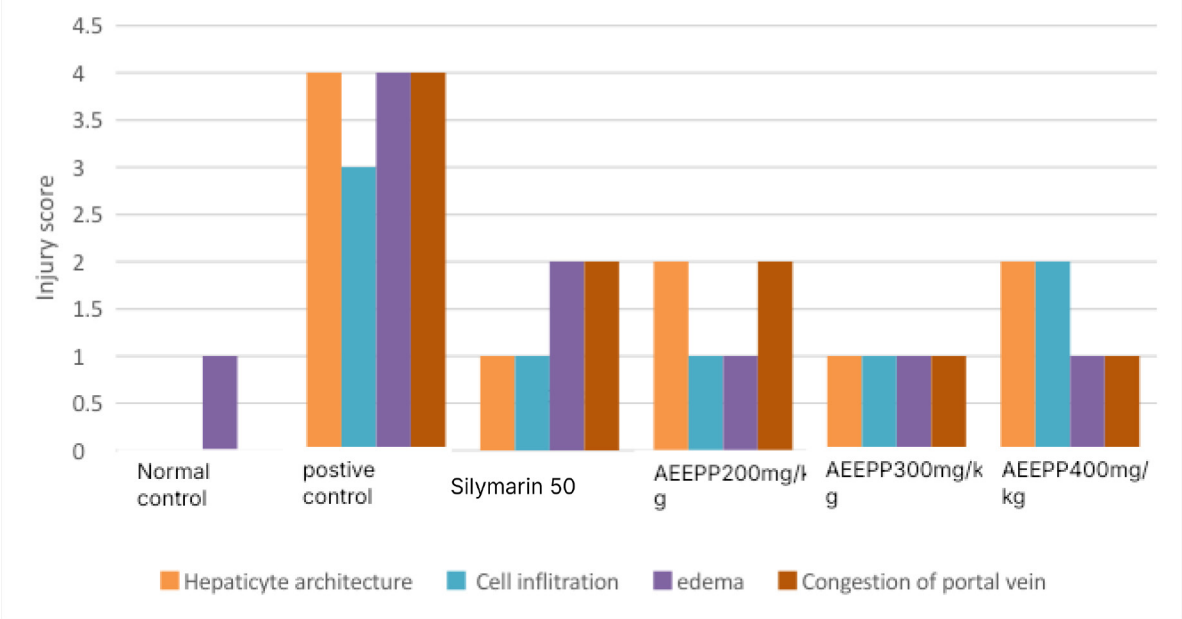


Figure 17. Histopathology injury score.

4. Discussion

Cadmium is one of the most hazardous substances which exists in the environment and work conditions. The primary ways that people are exposed to cadmium are through food, drink, and inhalation. Once absorbed, the element is stored in the body throughout life (even up to 30 years). Every year, a contaminated environment affects about 600 million individuals. When cadmium is indirectly consumed, Cd is prone to accumulate in hepatic circulation, and Cd may bind to a protein called metallothionein synthesised in the liver to form a Cd-metallothionein (Cd-MT) complex. Further exposure suppresses the binding capacity of metallothionein, thus leading to an excess of free Cd ions, leading to the release of reactive oxygen species, increasing oxidative stress markers, depleting the antioxidant enzyme system and ultimates to cell death and hepatotoxicity. Heavy metal contamination of food is more prevalent in contaminated agricultural areas and is a major global issue. To create pro-health attitudes, broadly defined preventative action should be implemented, with an emphasis on encouraging different healthy behaviours, including combating alcoholism and nicotinism, maintaining a high standard of personal cleanliness and educating workers about exposure to cadmium[24]. Restraint stress was incorporated in the present study to mimic conditions where environmental toxicant exposure is accompanied by stress-related physiological alterations, which may further intensify liver damage. Restraint stress induces hepatotoxicity through activation of the HPA axis, leading to increased glucocorticoid secretion and disruption of hepatic redox balance. This results in excessive ROS generation, suppression of the Nrf2 antioxidant pathway, inflammatory cytokine release, and activation of apoptotic and ferroptotic cell-death pathways in hepatocytes[25]. Currently, there is no specific drug approved for cadmium-induced hepatotoxicity; however, chelating agents, antioxidants, hepatoprotective drugs, and phytochemicals are widely investigated for mitigating cadmium-mediated oxidative liver injury. Shows limited effectiveness and may produce adverse effects, particularly in chronic cadmium exposure. Therefore, the search for safer and multifunctional natural therapeutic agents has gained considerable attention. Mushrooms such as *P. pulmonarius* contain bioactive compounds including β-glucans, phenolics, flavonoids and polysaccharides that exhibit strong antioxidant and hepatoprotective activities[26]. These compounds enhance endogenous antioxidant defences, reduce oxidative stress, and improve liver histopathology in cadmium- and stress-induced liver injury models. Therefore, mushroom extract was investigated as a natural therapeutic approach for cadmium- and stress-induced hepatotoxicity. Preliminary mycochemical analysis and antioxidant assays were estimated for the

conforming presence of bioactive compounds in advance of conducting an *in vivo* animal study. Multiple signalling pathways are involved in the pathogenesis of hepatic injury; the NF-κB signalling pathway plays a crucial role in regulating liver inflammation. Activation of NF-κB in hepatocytes and Kupffer cells promotes the transcription of several pro-inflammatory mediators. The increased production of these mediators contributes to oxidative stress, inflammatory responses, and hepatocellular damage, ultimately leading to liver dysfunction. Therefore, suppression of NF-κB activation and activation of the Nrf2 pathway has been considered an important therapeutic strategy for preventing or attenuating Cd and stress-induced liver injury[27]. In addition, the bioactive compounds responsible for hepatoprotective activity were further identified by in silico screening. We disclosed the potential hepatoprotective compounds in which (Table 8) disaccharide (HEX-HEX) showed the greatest binding affinity for the target protein, as evidenced by the highest scoring docking (−11.109) and most negative MMGBSA binding free energy (−63.72 kcal/mol). In accordance with the curative design of the present00 investigation may contribute to the observed therapeutic effects[20]. In the present study, the extract AEEPP demonstrated a concentration-dependent increase in DPPH radical scavenging activity (Table 6). The IC_{5_0}value of AEEPP was found to be 16 µg/ml; the extract possesses strong antioxidant activity[28]. The results show a concentration-dependent increase in nitric oxide scavenging activity of AEEPP (Table 7), showing progressive nitric oxide scavenging activity with IC_{5_0} value of 55 µg/ml is consistent with previous reports by Im *et al.*,[29]. The antioxidant activity observed in AEEPP might be due to the presence of bioactive constituents such as phenolic compounds, flavonoids, polysaccharides, and ergothioneine. Which could exert hepatoprotective effects by scavenging free radicals and enhancing endogenous antioxidant defence mechanisms. This finding supports the therapeutic potential of the extract in mitigating oxidative stress-mediated liver damage.

Further, Cadmium and restraint stress exposure is known to induce systemic oxidative stress and neurotoxicity, which can impair motor coordination, locomotor, and anxiety-related behaviour. The Open Field Test (OFT), which assesses locomotor activity and exploratory behaviour, which did not show significant alterations were observed across groups. These findings indicate that cadmium combined restraint stress exposure under the present experimental conditions produced minimal changes in anxiety-like behaviour and locomotor exploration. The rotarod test used to assess motor coordination and neuromuscular balance demonstrated (Figure 10) a significant reduction in fall latency in the positive control group compared with the normal control group, due to cadmium combined restraint stress-induced toxicity. Treatment with the standard drug silymarin (50 mg/kg) showed improvement in rotarod performance, suggesting partial restoration of motor coordination. Animals treated with AEEPP extract at different doses (200, 300, and 400 mg/kg) also showed improvement in fall latency compared with the positive control group; however, the changes were statistically Non-Significant (ns) observed in our study compared with Bai *et al.*,[30]. The behavioural assessment revealed no significant neuromotor impairment in animals exposed to the cadmium and restraint stress protocol. Similarly, treatment did not produce marked alterations in behavioural performance. These findings suggest that the experimental conditions were insufficient to induce detectable neuromotor deficits within the study duration. Therefore, the behavioural observations were interpreted as supportive assessments of animal condition rather than direct indicators of hepatoprotective activity. Oxidative stress is due to excessive generation of Reactive Oxygen Species (ROS), lipid peroxidation, and depletion of endogenous antioxidant defence systems[31]. In the present study, the positive control group (Figure 12) showed a significant elevation in Malondialdehyde (MDA) levels along with a marked reduction in antioxidant enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), reduced Glutathione (GSH) and glutathione peroxidase (GPx) when compared with the normal control group ($p<0.0001$). Elevated MDA levels indicate enhanced lipid peroxidation and oxidative damage to hepatocyte membranes caused by cadmium toxicity observed in our study compared with Asgharzadeh *et al.*,[31]. Administration of AEEPP extract at different doses (200, 300, and 400 mg/kg) also produced a significant improvement in hepatic antioxidant parameters. The treatment groups shown effect of the treatment is not strictly dose-dependent, but rather follows an optimal dose-response relationship, with a decrease in MDA levels, indicating inhibition of lipid peroxidation. Simultaneously, the activities of endogenous antioxidant enzymes such as SOD, CAT, and GPx were significantly elevated compared to the positive control group, suggesting restoration of the cellular

antioxidant defence system. The levels of reduced Glutathione (GSH) were also markedly increased following treatment. observed in our study compared with Ahmed *et al.,*[32]. Among the tested dose, AEEPP 300 mg/kg exhibited more pronounced antioxidant effects, approaching the protective efficacy observed. These results support the hepatoprotective potential of *P. pulmonarius* against cadmium and restrain stress-induced liver toxicity. Cadmium exposure combined with restraint stress produced significant hepatic injury, as evidenced by the marked alterations in serum biochemical parameters (Figure 13). The positive control group showed a significant elevation in liver enzymes such as aspartate Aminotransferase (AST), alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) when compared with the normal control group ($p<0.0001$). These enzymes are well-recognised indicators of hepatocellular damage. Similarly, administration AEEPP extract at different doses (200, 300, and 400 mg/kg) markedly AEEPP 300 mg/kg group ameliorated the elevated levels of ALT, AST, and ALP compared with the positive control group, Among the tested doses, the higher doses (300 and 400 mg/kg) exhibited reductions in enzyme levels, indicating optimal dose-response relationship hepatoprotective effect. Cadmium exposure also significantly reduced serum albumin and total protein levels (Figure 14) indicating impaired hepatic synthetic function. observed in our study compared with Sun *et al.,*[33]. Treatment with silymarin and AEEPP 300 mg/kg extract significantly restored albumin and total protein levels toward normal values, further supporting the hepatoprotective activity of the treatments. Morphological alterations in the liver, indicating substantial hepatic injury (Figure 16) The positive control group showed clear signs of hepatotoxicity characterised by irregular liver surface, discoloration, swelling, and loss of normal architecture compared with the normal control group, which exhibited a smooth surface and uniform reddish-brown appearance. Treatment with silymarin (50 mg/kg), a well-established hepatoprotective agent, markedly improved liver morphology. The liver from this group appeared relatively smooth with reduced swelling and improved colour compared with the positive control group. Administration of the AEEPP extract demonstrated protective effects against induced liver injury. The AEEPP 200 mg/kg group showed partial restoration of liver morphology, although mild irregularities and congestion were still visible, suggesting moderate protection at the lower dose. In contrast, the AEEPP 300 mg/kg group exhibited showed liver morphology that closely resembled that of the normal and silymarin-treated groups, indicating a strong hepatoprotective effect at the medium dose. Notably, the AEEPP 400 mg/kg group exhibited a more pronounced improvement, with better structural integrity and less visible lesions compared with the positive control group observed in our study comparison with Ahmed *et al.*[32] *P. pulmonarius* extract exerts significant hepatoprotective effects and role in restoring hepatic structure and function against cadmium-induced liver damage, with the medium dose (300 mg/kg) showing efficacy comparable to silymarin. Histopathological examination of liver sections (Figure 16) normal control group (A) exhibited normal hepatic architecture characterised by well-organised hepatic cords, intact hepatocytes with centrally located nuclei, and normal sinusoidal spaces surrounding the central vein. No signs of cellular degeneration, inflammation, or necrosis were observed, indicating healthy liver morphology. In contrast, the positive control group (B) exposed to cadmium with restraint stress showed disruption of normal hepatic architecture, hepatocellular degeneration, inflammatory cell infiltration, and dilation of sinusoids, indicating severe hepatic injury. Treatment with silymarin (50 mg/kg) (C) markedly improved hepatic architecture. Animals treated with AEEPP extract at 200 mg/kg (D) showed moderate improvement in liver architecture. Although some hepatocellular degeneration and mild sinusoidal dilation were still present, the degree of tissue damage was comparatively lower than that observed in the positive control group, Further improvement was observed in the AEEPP extract 300 mg/kg(E), demonstrated the most pronounced hepatoprotective effect among the treatment groups. The liver architecture appeared nearly normal with well-arranged hepatocytes and minimal inflammatory infiltration. The restoration of hepatic structure observed in this group was comparable to that of the silymarin-treated group indicating a strong protective potential of the extract, where liver sections showed better preservation of hepatic structure with reduced cellular degeneration and improved hepatocyte organisation. In AEEPP extract of (400 mg/kg) (F) where liver sections showed better preservation of hepatic structure with reduced cellular degeneration and improved hepatocyte organisation observed in our study comparison with Atia *et al.,*[34]. The histological features indicated progressive recovery of

liver tissue with medium dose of the extract. The histopathological findings support the biochemical results and suggest that *P. pulmonarius* exerts a hepatoprotective effect against cadmium-induced liver injury, however higher dose shown not significant compared to medium dose is 300 mg/kg showing the most significant restoration of hepatic architecture.

5. Conclusion

The aqueous ethanolic extract of *P. pulmonarius* has produced promising results as a hepatoprotective agent against cadmium chloride and restraint stress-induced hepatotoxicity. It was confirmed by performing various biochemical and histopathological studies. From the results, it was observed that the study extract could mitigate liver injury significantly, consistent at mid-dose (300 mg/kg) when it was compared to overall results of the parameters among tested doses. Further, the activity might be due to the presence of polysaccharides in the extract, as it was also confirmed through *in silico* docking studies via the Nrf2 pathway. This study can be further escalated to explore the molecular-level testing of these pathways and mechanisms through which the extract under study might have exhibited hepatoprotection.

6. Acknowledgement

The authors would like to acknowledge KLE College of Pharmacy for providing the platform to carry on this animal-based research work.

7. References

1. Kim HG, Lee JS, Lee JS, Han JM, Son CG. Hepatoprotective and antioxidant effects of Myelophil on restraint stress-induced liver injury in BALB/c mice. J Ethnopharmacol. 2012; 142(1):113-120. https://doi.org/10.1016/j.jep.2012.04.023 PMid:22543172
2. Souza-Arroyo V, Fabián JJ, Bucio-Ortiz L, Miranda-Labra RU, Gómez-Quiroz LE, Gutiérrez-Ruiz MC. The mechanism of cadmium-induced toxicity and cellular response in the liver. Toxicology. 2022; 480:153339. https://doi.org/10.1016/j.tox.2022.153339 PMid:36167199
3. Jomova K, Alomar SY, Nepovimova E, Kuca K, Valko M. Heavy metals: Toxicity and human health effects. Arch Toxicol. 2025; 99(1):153-209. https://doi.org/10.1007/s00204-024-03903-2 PMid:39567405
4. Li Y, Liu Z, Chang Y, Chen N, Zhang R, Liu X, *et al*. Associations of multiple toxic metal exposures with metabolic dysfunction-associated fatty liver disease: NHANES 2011-2018. Front Nutr. 2023; 10:1301319. https://doi.org/10.3389/fnut.2023.1301319 PMid:38115883 PMCid:PMC10729449
5. Rajkumar V, Lee VR, Gupta V. Heavy metal toxicity. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 [cited 2026 Mar 18]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK560920/
6. Manjunath K, Mani T. Comprehensive insights into liver diseases: Pathophysiology, experimental models, diagnostic approaches, and emerging therapeutic strategies. IJPPR Human. 2026; 32(1):93-101. https://doi.org/10.25166/IJPPR.2026.32.1.12
7. Đukić-Ćosić D, Baralić K, Javorac D, Djordjevic AB, Bulat Z. An overview of molecular mechanisms in cadmium toxicity. Curr Opin Toxicol. 2020; 19:56-62. https://doi.org/10.1016/j.cotox.2019.12.002
8. Dkhil MA, Diab MSM, Lokman MS, El-Sayed H, Aljawdah HMA, Al-Quraishy S, *et al*. Hepatoprotective effect of *Pleurotus ostreatus* extracts in cadmium-intoxicated rats. J King Saud Univ Sci. 2020; 32(8):3432-3436. https://doi.org/10.1016/j.jksus.2020.09.029
9. Vaikunthanathan T, Landmann E, Correa DM, Romano M, Trevelin SC, Peng Q, *et al*. Dysregulated antioxidant signalling and compromised mitochondrial integrity negatively influence regulatory T cell function and viability in liver disease. EBioMedicine. 2023; 95:104778. https://doi.org/10.1016/j.ebiom.2023.104778 PMid:37657135 PMCid:PMC10480539
10. Kim SH, Oh DS, Oh JY, Son TG, Yuk DY, Jung YS. Silymarin prevents restraint stress-induced acute liver injury by ameliorating oxidative stress and reducing inflammatory response. Molecules. 2016; 21(4):443. https://doi.org/10.3390/molecules21040443 PMid:27043523 PMCid:PMC6274117
11. Chen HC, Yip T, Lee JK, Juliani J, Sernia C, Hill AF, *et al*. Restraint stress alters expression of glucocorticoid bioavailability mediators, suppresses Nrf2, and promotes oxidative stress in liver tissue. Antioxidants (Basel). 2020; 9(9):853. https://doi.org/10.3390/antiox9090853 PMid:32932938 PMCid:PMC7554900
12. Thakur S, Kumar V, Das R, Sharma V, Mehta DK. Biomarkers of hepatic toxicity: An overview. Curr Ther Res Clin Exp. 2024; 100:100737. https://doi.org/10.1016/j.curtheres.2024.100737 PMid:38860148 PMCid:PMC11163176
13. Aswathy S, Jmanjunathan J. Phytochemical profiling of *Pleurotus pulmonarius* from paddy straw substrate. Int J Pharm Qual Assur. 2024; 15:1275-1280. https://doi.org/10.25258/ijpqa.15.3.28

14. Bhambri A, Srivastava M, Mahale VG, Mahale S, Karn SK. Mushrooms as potential sources of active metabolites and medicines. Front Microbiol. 2022; 13:837266. https://doi.org/10.3389/fmicb.2022.837266 PMid:35558110 PMCid:PMC9090473
15. Klausen SJ, Falck-Ytter AB, Strætkvern KO, Martin C. Evaluation of the extraction of bioactive compounds and the saccharification of cellulose as a route for the valorization of spent mushroom substrate. Molecules. 2023; 28(13):5140. https://doi.org/10.3390/molecules28135140 PMid:37446802 PMCid:PMC10343705
16. Sangthong S, Pintathong P, Pongsua P, Jirarat A, Chaiwut P. Polysaccharides from *Volvariella volvacea* mushroom: Extraction, biological activities and cosmetic efficacy. J Fungi (Basel). 2022; 8(6):572. https://doi.org/10.3390/jof8060572 PMid:35736055 PMCid:PMC9225106
17. Zakaria N, Fadzil N, Harith Z, Wahab I, Idris A, Rahman K, *et al*. Macronutrient composition, antioxidant and antibacterial properties of *Pleurotus pulmonarius* fruiting body vs. stem waste. BIO Web Conf. 2024; 131:105033. https://doi.org/10.1051/bioconf/202413105033
18. Okonkwo O, Afieroho O, Ahanonu E, Okwubie L, Abo K. *Pleurotus pulmonarius* (Fr.) Quel. (Pleurotaceae): *In vitro* antioxidant evaluation and the isolation of a steroidal isoprenoid. J Appl Biol Biotechnol. 2019; 7:32-38. https://doi.org/10.7324/JABB.2019.70406
19. Ogidi CO, Ubaru AM, Ladi-Lawal T, Thonda OA, Aladejana OM, Malomo O. Bioactivity assessment of exopolysaccharides produced by *Pleurotus pulmonarius* in submerged culture with different agro-waste residues. Heliyon. 2020; 6(12):05685. https://doi.org/10.1016/j.heliyon.2020.e05685 PMid:33336098 PMCid:PMC7734227
20. Arromsava A, Chuchawankul S, Amornsupak K. LC-MS/MS profiling and *in silico* molecular docking analysis of water-soluble bioactive compounds from *Pleurotus pulmonarius* as potential immunomodulators on monocyte immune response. J Appl Pharm Sci. 2025; 15(4):162-178. https://doi.org/10.7324/JAPS.2025.217871
21. Balaji P, Madhanraj R, Rameshkumar K, Veeramanikandan V, Eyini M, Arun A, *et al*., Evaluation of antidiabetic activity of *Pleurotus pulmonarius* against streptozotocin-nicotinamide induced diabetic wistar albino rats. Saudi J Biol Sci. 2020; 27(3):913-924. https://doi.org/10.1016/j.sjbs.2020.01.027 PMid:32127771 PMCid:PMC7042672
22. Owolabi GO, Lawal OA, Offiong IE, Olanrewaju AN. Effects of restraint stress and cadmium chloride on the liver function of female Wistar rats (*Rattus norvegicus*). Asian J Med Health. 2024; 22(12):1-13. https://doi.org/10.9734/ajmah/2024/v22i121131
23. Türkez H, Tozlu ÖÖ, Saraçoğlu M, Yıldız E, Baba C, Bayram C, *et al*. Colemanite and biological disruptions: Behavioral, neurological, and physiological findings. Regul Toxicol Pharmacol. 2025; 161:105840. https://doi.org/10.1016/j.yrtph.2025.105840 PMid:40324558
24. Charkiewicz AE, Omeljaniuk WJ, Nowak K, Garley M, Nikliński J. Cadmium toxicity and health effects: a brief summary. Molecules. 2023; 28(18):6620. https://doi.org/10.3390/molecules28186620 PMid:37764397 PMCid:PMC10537762
25. Liu Y, Sun Z, Sun Q. The effects of restraint stress on ceramide metabolism disorders in the rat liver: The role of CerS6 in hepatocyte injury. Lipids Health Dis. 2024; 23:68. https://doi.org/10.1186/s12944-024-02019-x PMid:38431645 PMCid:PMC10908211
26. Datta S, Aggarwal D, Sehrawat N, Yadav M, Sharma V, Sharma A, *et al*. Hepatoprotective effects of natural drugs: Current trends, scope, relevance and future perspectives. Phytomedicine. 2023; 121:155100. https://doi.org/10.1016/j.phymed.2023.155100 PMid:37801892
27. Hayat M, Zohaib M, Ijaz MU, Batool M, Almutairi B, Atique U. Ameliorative potential of eriocitrin against cadmium-instigated hepatotoxicity in rats via regulating Nrf2/Keap1 pathway. J Trace Elem Med Biol. 2024; 84:127445. https://doi.org/10.1016/j.jtemb.2024.127445 PMid:38613902
28. Jiang H, Gao L, Hu X, Fu J, Zhang J. Identification and nutrient composition of a wild *Pleurotus pulmonarius* strain from Tibet, and the antioxidant and cytotoxic activities of polysaccharides from this fungus. Foods. 2025; 14(7):1198. https://doi.org/10.3390/foods14071198 PMid:40238420 PMCid:PMC11989227
29. Im KH, Baek SA, Choi J, Lee TS. *In vitro* and *in vivo* medicinal value of culinary-medicinal lung oyster mushroom *Pleurotus pulmonarius* var. stechangii (Agaricomycetes). Int J Med Mushrooms. 2020; 22(8):763-774. https://doi.org/10.1615/IntJMedMushrooms.2020035566 PMid:33389870
30. Bai Y, Li K, Li X. Effects of oxidative stress on hepatic encephalopathy pathogenesis in mice. Nat Commun. 2023; 14:4456. https://doi.org/10.1038/s41467-023-40081-8 PMid:37488119 PMCid:PMC10366183
31. Asgharzadeh J, Derakhshan L, Asgharzadeh N. N-acetylcysteine reduces the hepatic complications of social isolation stress through modulation of interleukin 1 and 6 gene expression and liver enzymes in mice. Sci Rep. 2025; 15:23166. https://doi.org/10.1038/s41598-025-01557-3 PMid:40603325 PMCid:PMC12222526
32. Ahmed KA-A, Shareef SH, Nanakaly HT. Mangiferin: A novel hepatoprotective for liver cirrhosis via modulation of histological, cellular, biochemical, inflammatory markers and oxidative stress. BMC Pharmacol Toxicol. 2026; 27:26. https://doi.org/10.1186/s40360-025-01061-4 PMid:41476305 PMCid:PMC12866065
33. Sun S, Wang Y, Du Y, Sun Q, He L, Zhu E, *et al*. Oxidative stress-mediated hepatotoxicity in rats induced

by ethanol extracts of different parts of *Chloranthus serratus*. Pharm Biol. 2020; 58(1):1286-1298. https://doi.org/10.1080/13880209.2020.1859552 PMid:33355514 PMCid:PMC7759245

34. Atia MM, Mahmoud HAA, Wilson M, Abd-Allah EA. A comprehensive survey of warfarin-induced hepatic toxicity using histopathological, biomarker, and molecular evaluation. Heliyon. 2024; 10:26484. https://doi.org/10.1016/j.heliyon.2024.e26484 PMid:38440292 PMCid:PMC10909775

Author Contribution

Tudi Mani: Conceptualization, methodology, investigation, data collection, experimentation, data analysis, interpretation of results, and preparation of the initial manuscript.

Neha Vanjre Honnurapp: Review of methodology, validation of experimental procedures, interpretation of findings, critical review and editing of the manuscript

Kavya Manjunath: Conceptualization, study design, supervision, methodology, interpretation of results, critical revision of the manuscript, overall project administration and final approval of the manuscript.

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