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Evaluation of the Hepato-Renal Protective Activity of Ethanolic Mucuna pruriens Leaf Extract in Drug- and Chemical-Induced Toxicity in Wistar Rats

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Abstract

Given the extensive folkloric use of Mucuna pruriens (L.), the present work focuses on assessing the hepatoprotective and nephroprotective efficacy of its leaf extract, with the objective of determining its therapeutic relevance in the management of kidney and liver disorders. A total of forty male albino rats were used in this experiment and randomly distributed into eight groups, each consisting of five animals. The control group (Group I) received distilled water only. Hepatotoxicity and nephrotoxicity were induced in Groups II and VI using carbon tetrachloride (CCl_4) and rifampicin, respectively. Following CCl_4 administration, animals in Groups III and IV were treated with *Mucuna pruriens* leaf extract at doses of 50 and 100 mg/kg body weight (bw), respectively. Likewise, rats in Groups VII and VIII were subjected to rifampicin-induced toxicity and subsequently administered *M. pruriens* at 50 and 100 mg/kg bw, respectively. Group V served as the standard treatment group and received silymarin (100 mg/kg bw) orally after CCl_4 exposure. Indicators of hepatic and renal dysfunction were analyzed in serum samples and tissue homogenates. Furthermore, excised liver and kidney tissues were processed, stained, and examined microscopically for histopathological alterations. Administration of carbon tetrachloride and rifampicin produced pronounced alterations in lipid metabolism, suppression of endogenous antioxidant defenses, and significant elevations in alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea, uric acid, bilirubin, and creatine kinase levels. Conversely, intervention with *Mucuna pruriens* extract effectively ameliorated these biochemical disruptions and improved histological architecture in a dose-dependent fashion. The leaf extract of *Mucuna pruriens* effectively normalized altered biochemical indices and histological changes in both hepatic and renal tissues, demonstrating a protective efficacy comparable to that of silymarin. These findings suggest that *M. pruriens* leaf extract possesses significant hepatoprotective and nephroprotective potential and may serve as a promising therapeutic candidate for the treatment of liver and kidney disorders.

Keywords: Carbon tetrachloride, Mucuna pruriens, Oxidative stress, Rifampicin

Introduction

Oxidative stress has emerged as a major focus of contemporary biomedical research due to its central role in disease development [1]. It is defined as a condition arising from an imbalance between the generation of reactive oxygen species and the capacity of antioxidant defense systems to neutralize them within living organisms. Reactive free radicals are chemically unstable entities that readily interact with and damage essential cellular macromolecules, including lipids, proteins, nucleic acids, and carbohydrates [1, 2]. Extensive evidence now indicates that oxidative stress mediated by free radicals contributes significantly to the onset and progression of most pathological conditions. As a result, increasing research efforts have been directed toward the discovery and utilization of natural or synthetic compounds capable of attenuating oxidative damage and restoring redox homeostasis [3].

Carbon tetrachloride and rifampicin are commonly employed in experimental studies to induce liver and kidney toxicity, respectively [4]. The hepatotoxicity associated with carbon tetrachloride (CCl_4) is initiated through metabolic activation processes such as chloromethylation, saturation, and peroxidation, which ultimately lead to the formation of the trichloromethyl radical ($CCl_3•$). This highly reactive species initiates lipid

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peroxidation of the endoplasmic reticulum membrane in hepatocytes, resulting in progressive structural disintegration and impairment of liver function [4]. Conversely, rifampicin-induced toxicity has been attributed to the activation of the pregnane X receptor (PXR), which enhances the formation of reactive intermediates capable of inducing both hepatic and renal injury [5–7]. Additional studies suggest that rifampicin disrupts heme synthesis, causing accumulation of protoporphyrin, a metabolite with established hepatotoxic effects [7].

Since ancient civilizations, medicinal plants have played a critical role in healthcare and disease treatment [8]. In modern times, herbal medicine continues to gain acceptance worldwide as either an alternative or complementary approach to conventional therapies, particularly in both developing and industrialized nations. This widespread use is largely attributed to the natural origin of herbal products and their comparatively low incidence of adverse effects [9]. It has been estimated that more than half of currently marketed pharmaceutical agents are derived from, or modeled after, bioactive compounds originally isolated from medicinal plants [9, 10]. The global resurgence of interest in phytotherapy reflects a growing preference for nature-based therapeutic strategies in disease management [11, 12].

Mucuna pruriens (L.) is a tropical medicinal plant that has been extensively utilized in traditional medical systems across many regions of the world [13]. The seeds of the plant are particularly valued for their neuroprotective properties, which are largely attributed to their high L-DOPA content and form the basis for their long-standing use in the treatment of Parkinson's disease [14–17]. Beyond neuroprotection, seed extracts of *M. pruriens* have demonstrated antihyperglycemic, antihyperlipidemic, and antitumor activities in experimental animal models [18–20]. Additional pharmacological studies have shown that the plant exhibits antioxidant activity through inhibition of lipid peroxidation, as well as cognitive-enhancing effects related to learning and memory [21–23]. Its anti-inflammatory, analgesic, and antipyretic properties have also been well established [24–26]. Traditionally, *M. pruriens* seed preparations have been employed in the management of rheumatoid arthritis, diabetes mellitus, atherosclerosis, and sexual disorders [27, 28]. Furthermore, the immunomodulatory properties of the seed extract may explain its documented use as an anti-venom agent in folkloric medicine [29].

Despite the extensive documentation of the pharmacological benefits associated with *M. pruriens* seeds, limited attention has been given to the therapeutic potential of its leaves. Therefore, investigating the bioactivity of *M. pruriens* leaf extract, particularly in relation to hepatic and renal protection, is warranted. Such studies may contribute to the identification of effective plant-based alternatives to conventional drugs currently employed in the treatment of liver and kidney diseases.

Materials and Methods

Plant materials

Fresh leaves of Mucuna pruriens (L.) were collected from the university grounds and authenticated at the Department of Plant Science, Ekiti State University, Ado-Ekiti. A voucher specimen (UHAE2020070) was archived in the university herbarium for reference. The leaves were thoroughly air-dried and then ground into a fine powder using a Waring blender. The resulting powder was carefully weighed and stored in a sealed container until further use.

Chemicals and reagents

All chemicals used in this study were of analytical grade and sourced from reputable suppliers. Randox kits were utilized for the measurement of all biochemical parameters following the manufacturer's guidelines.

Preparation of ethanolic leaf extract

Air-dried Mucuna pruriens leaves were pulverized to a fine powder. About 32.5 g of this powder was mixed with 100 mL of 80% ethanol and allowed to stand for 72 hours at room temperature with occasional stirring. The mixture was then filtered using cheesecloth to remove particulate matter. The filtrate was covered with an insect-proof mesh and left to evaporate to dryness at ambient conditions. Weight measurements were taken periodically until no further loss in mass was observed. The dried crude extract was stored in a tightly sealed glass Petri dish in a refrigerator and reconstituted with distilled water before administration to the animals.

Experimental animals and ethics

The study protocol was approved by the Committee for the Care and Use of Experimental Animals, Office of Research and Development, Ekiti State University, Ado-Ekiti (Approval No: ORD/AD/EAC/19/0082). Forty

male Wistar albino rats, weighing 150–170 g, were obtained from the animal facility of the Department of Science Technology, Federal Polytechnic, Ado-Ekiti. The rats were housed in wire-mesh cages under controlled environmental conditions (temperature 24 ± 1 °C, relative humidity 40–60%, and a 12-hour light/dark cycle). They had unrestricted access to commercial pellet feed (Vital Feed Mill) and water. Cage bedding was replaced daily throughout the study to ensure hygiene.

To induce hepatotoxicity, rats received intraperitoneal injections of carbon tetrachloride at 3 mL/kg body weight. Nephrotoxicity was induced through oral gavage of rifampicin at 250 mg/kg body weight. Details of group allocation and treatment regimens are outlined in the experimental design below:

Organ homogenate preparation

After completing 14 days of treatment, the animals underwent a 24-hour fasting period. They were then humanely euthanized by decapitation under light ether anesthesia, followed by prompt dissection to remove the liver and kidneys. These organs were cleaned of excess fat, rinsed thoroughly in normal saline, dried gently with filter paper, and weighed accurately. The tissues were cut into fine pieces and homogenized in 10 times their volume of phosphate buffer (pH 7.4) with a Teflon pestle homogenizer. The homogenates were centrifuged at 3000 rpm for 30 minutes at 4 °C. The clear supernatant was carefully collected, maintained at 4 °C, and subsequently employed for various biochemical tests.

Serum preparation

Blood was drawn directly from the heart using cardiac puncture and transferred into uncoated tubes. It was left undisturbed for 1 hour to ensure complete clotting. The clotted samples were then spun at 3000 rpm for 15 minutes at 25 °C. The resulting clear serum layer was gently removed and stored on ice prior to analyzing serum biochemical markers.

Determination of creatine kinase (ck-mb) activity

Creatine kinase activity was evaluated using the protocol described by Mattenheimer [30]. In short, a 1.0 mL reaction mixture consisting of 10 mM imidazole buffer (pH 6.6) along with 30 mM creatine phosphate, 20 mM glucose, 20 mM N-acetyl-cysteine, 10 mM magnesium acetate, 2 mM ethylenediaminetetraacetic acid, 2 mM ADP, 2 mM NADP, 5 mM AMP, 10 µM DAPP, ≥2.0 ku/L glucose-6-phosphate dehydrogenase, and ≥2.15 ku/L hexokinase was placed in a temperature-regulated cuvette and warmed to 37 °C. Then, 50 µL of serum was added and mixed well. The increase in absorbance at 340 nm was tracked every 30 seconds for a total of 5 minutes.

Determination of aspartate aminotransferase (ast) activity

Aspartate aminotransferase activity was assessed according to the colorimetric method of Reitman and Frankel [31]. Briefly, 0.1 mL of either serum or tissue homogenate was combined with 100 mM phosphate buffer (pH 7.4), 100 mM L-aspartate, and 2 mM α-oxoglutarate. This mixture was incubated precisely at 37 °C for 30 minutes. Next, 500 µL of 2 mM 2,4-dinitrophenylhydrazine was introduced, and the reaction proceeded for an additional 20 minutes at 25 °C. Subsequently, 5.0 mL of 0.4 M sodium hydroxide was added, followed by a 5-minute incubation period. Absorbance readings were taken at 546 nm using a reagent blank for comparison. Enzyme activity levels were derived by referencing a calibration curve.

Measurement of alanine aminotransferase (alt) activity

Alanine aminotransferase activity was quantified using the colorimetric approach developed by Reitman and Frankel [31], with the aid of a Randox commercial kit. In test tubes, 0.1 mL of serum or organ homogenate was mixed with 500 µL of Reagent 1 (which included 100 mM phosphate buffer at pH 7.4, 200 mM L-alanine, and 2.0 mM α-oxoglutarate). The samples were then incubated at 37 °C for exactly 30 minutes. Following this, 0.5 mL of Reagent 2 (2.0 mM 2,4-dinitrophenylhydrazine) was added, and incubation continued at 20 °C for another 20 minutes. Afterward, 5.0 mL of 0.4 M NaOH was introduced, the mixtures were left at room temperature for 5 minutes, and absorbance was measured at 546 nm. Enzyme activity values were derived via interpolation from a calibration standard.

Measurement of alkaline phosphatase (alp) activity

The evaluation of alkaline phosphatase was carried out according to the procedure detailed by Englehardt *et al*. [32]. Separately, 0.02 mL of serum or tissue homogenate was combined with 1.0 mL of the assay reagent (composed of 1.0 M diethanolamine buffer at pH 9.8, 0.5 mM $MgCl_2$, and 10 mM p-nitrophenyl phosphate as the substrate), and the contents were mixed vigorously. Absorbance changes were recorded at 405 nm over a period of 3 minutes, with measurements taken every

minute. Activity levels were calculated by reference to standard values through interpolation.

Quantification of total cholesterol concentrations

Levels of total cholesterol were measured employing the enzymatic colorimetric technique reported by Trinder [33]. Labeled tubes received 10 µL aliquots of standard solution, serum, or tissue homogenate. Each tube was then supplemented with 1.0 mL of the enzyme reagent mixture (containing 80 mM Pipes buffer pH 6.8, 0.25 mM 4-aminoantipyrine, 6 mM phenol, peroxidase ≥0.5 U/mL, cholesterol esterase ≥0.15 U/mL, and cholesterol oxidase 0.10 U/mL). After thorough mixing, the tubes were incubated at room temperature for 10 minutes. Sample absorbance was read at 500 nm relative to the reagent blank, and cholesterol concentrations were determined based on the standard.

$$\text{Cholesterol concentration (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentrationof standard} \quad (1)$$

Evaluation of concentration of triglyceride

The concentration of triglycerides was determined following the method described by Tietz [34]. Ten microliters of triglyceride standard, serum, and organ homogenates were separately transferred into appropriately labeled test tubes. To each tube, 1.0 mL of the working reagent was added. The working reagent consisted of R1a (buffer) containing 40 mM Pipes buffer at pH 7.6, 5.5 mM 4-chloro-phenol, and 17.5 mM magnesium ions, and R1b (enzyme reagent) composed of 0.5 mM 4-amino phenazone, 1.0 mM ATP, lipase (≥150 U/mL), glycerol kinase (≥0.4 U/mL), peroxidase (≥0.5 U/mL) and glycerol-3-phosphate oxidase (≥1.5 U/mL). The mixtures were thoroughly mixed and incubated at room temperature for 10 minutes. Absorbance was then measured at 546 nm using a blank as reference.

$$\text{Triglyceride concentration} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard} \quad (2)$$

High-density lipoprotein (hdl-c) cholesterol assay

HDL-cholesterol levels were measured following the procedure outlined by Grove [35]. Briefly, 200 µL of serum or organ homogenate was mixed with 200 µL of cholesterol standard and 500 µL of diluted precipitant R1 (containing 0.55 mM phosphotungstic acid and 25 mM magnesium chloride) and allowed to stand at room temperature for 10 minutes. The mixture was then centrifuged at 4000 rpm for 10 minutes to obtain a clear supernatant, which was separated within two hours. Cholesterol concentration was determined using the CHOD-PAP method: 100 µL of the supernatant was combined with 1 mL of cholesterol reagent, and the mixture was thoroughly mixed and incubated at 25 °C for 10 minutes. A standard tube was prepared by mixing 100 µL of cholesterol standard supernatant with 1 mL of reagent. Absorbance of both sample (A_sample) and standard (A_standard) was measured at 500 nm against a reagent blank within one hour.

Low-density lipoprotein (ldl-c) determination

LDL-cholesterol levels in serum and organ homogenates were calculated using the formula described by Friedewald *et al*. [36].

$$\text{LDL cholesterol} = \text{Totalcholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL} - \text{cholesterol} \quad (3)$$

Assessment of catalase activity

Catalase activity was determined employing the technique reported by Sinha [37]. In summary, a five-fold dilution was achieved by combining 200 µL of serum or organ homogenate with 0.8 mL of distilled water in separate preparations. The reaction setup in a 10 mL flat-bottom flask included 2 mL of hydrogen peroxide solution (800 µmol) and 2.5 mL of phosphate buffer. The assay was initiated by quickly adding 500 µL of the diluted enzyme sample to the mixture, followed by gentle swirling at room temperature. At intervals of 60 seconds, 1.0 mL aliquots were rapidly withdrawn and transferred into 1 mL of dichromate/acetic acid reagent to terminate the reaction. The quantity of remaining hydrogen peroxide in each aliquot was subsequently quantified using the procedure outlined thereafter.

$$\text{Catalase activity} = \frac{\text{H}_2\text{O}_2\text{Consumed}}{\text{mg protein.}} \quad (4)$$

$$\text{H}_2\text{O}_2\text{Consumed} = 800 - \text{Concentration of H}_2\text{O}_2\text{ remaining} \quad (5)$$

The amount of H2O2 left was calculated using the catalase activity standard curve.

Evaluation of superoxide dismutase (sod) activity

SOD activity was quantified using the approach developed by Fridovich and Misra [38]. A portion of the ten-fold diluted serum or homogenate was introduced into 2.5 mL of 0.05 M carbonate buffer (pH 10.2) within a spectrophotometer cuvette and permitted to stabilize thermally. The assay was triggered by incorporating 0.3 mL of freshly made 0.3 mM adrenaline, after which the contents were inverted rapidly for mixing. For the blank, the cuvette held 0.3 mL adrenaline, 2.5 mL buffer, and 0.2 mL water. The rise in absorbance at 480 nm was followed for 150 seconds, recording values at 30-second intervals. Activity was computed from the extent to which the sample inhibited the auto-oxidation of adrenaline.

Assay for reduced glutathione (gsh) content

The levels of reduced glutathione (GSH) were quantified following the protocol described by Beutler *et al*. [39] Briefly, 0.2 mL of either serum or tissue homogenate was mixed with 1.8 mL of distilled water for deproteinization, after which 3.0 mL of precipitating mixture was introduced. The samples were thoroughly vortexed, left to incubate for 5 minutes, and subsequently filtered. To 1.0 mL of the clear filtrate, 4.0 mL of phosphate buffer (0.1 M, pH 7.4) was added, followed by 0.5 mL of Ellman's reagent to develop the chromophore. The blank consisted of 4.0 mL phosphate buffer, 1.0 mL of appropriately diluted precipitating mixture, and 0.5 mL Ellman's reagent. The intensity of the yellow color formed was measured spectrophotometrically at 412 nm, with readings taken against the blank.

Quantification of total protein (tp)

Total protein concentration was measured using the Biuret reaction as detailed by Weichselbaum.[40] Separate tubes containing 0.02 mL of serum or tissue homogenate received 1.0 mL of Biuret reagent (comprising 100 mM NaOH, 18 mM sodium-potassium tartrate, 15 mM KI, and 6 mM $CuSO_4$). After mixing, the reactions were allowed to proceed at 25 °C, and the resulting purple complex was quantified by absorbance at 546 nm relative to a reagent blank.

Assessment of lipid peroxidation

Lipid peroxidation was evaluated through the determination of thiobarbituric acid reactive substances (TBARS) in serum and tissue homogenates, employing the method of Ohkawa *et al*. [41] with commercial Randox kits. Each sample (100 µL) was combined with 2.5 mL of the provided reaction buffer and subjected to boiling for 1 hour. Following cooling, the tubes were centrifuged (3000 rpm, 10 min), and the absorbance of the pink supernatant was recorded at 532 nm. The malondialdehyde (MDA) content was derived and expressed in µmole MDA per mg protein, utilizing the extinction coefficient of the MDA-TBA adduct (1.56×10^5/M/cm).

Statistical analysis

Data are presented as mean ± SEM. Differences between groups were evaluated using one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) in SPSS version 11.09 for Windows. Statistical significance was defined as $p < 0.05$.

Results and Discussion

Administration of CCl_4 and rifampicin caused substantial elevations in liver cholesterol, increasing by 60% and 73%, respectively, relative to untreated **controls (Table 1)**. Similar trends were noted for LDL and triglycerides. In contrast, hepatic HDL levels decreased by 33% and 25% following CCl_4 and rifampicin exposure. These alterations were mirrored in both kidney and serum samples. Treatment with *M. pruriens* extract significantly improved the lipid profile, achieving 107% and 98% recovery in the livers of CCl_4 and rifampicin-exposed animals, respectively, compared to negative controls. Restoration by the standard drug reached 108%. The improvement in lipid parameters was dose-dependent **(Table 1).**

CCl_4 and rifampicin also induced marked increases in liver AST activity, by 52% and 78%, respectively, with ALP and ALT showing comparable elevations across liver, kidney, and serum **(Table 2).** Oral administration of *M. pruriens* extract at 100 mg/kg body weight restored AST levels in the liver by 102% and 92% for CCl_4 and rifampicin-treated animals, respectively. Silymarin at the same dose improved liver function by 95%, slightly less than the effect of the *M. pruriens* extract. The restorative effect was consistent across all tissues and independent of the toxicant used to induce oxidative stress.

Table 1. Animal treatment

Groups	Treatment
I: Negative Control	Distilled water only
II: Positive Control	Single dose of 3 mL CCl_4
III	3 mL CCl_4 + M. pruriens 50 mg/kg b.w.
IV	3 mL CCl_4 + M. pruriens 100 mg/kg b.w.
V	3 mL CCl_4 + Silymarin 100 mg/kg b.w.
VI	Rifampicin 250 mg/kg b.w. alone
VII	Rifampicin 250 mg/kg b.w. + M. pruriens 50 mg/kg b.w.
VIII	Rifampicin 250 mg/kg b.w. + M. pruriens 100 mg/kg b.w.

Table 2. Impact of *Mucuna pruriens* Extract on Liver and Kidney Lipid Profiles in Rats Subjected to CCl_4 and Rifampicin-Induced Toxicity

Parameter	Tissue	Normal Control	Toxin Control (CCl_4 alone)	CCl_4 + 50 mg/kg MP	CCl_4 + 100 mg/kg MP	CCl_4 + 100 mg/kg Silymarin	Toxin Control II (Rifampicin alone)	Rifampicin + 50 mg/kg MP	Rifampicin + 100 mg/kg MP
Total Cholesterol (mg/dL)	Liver	56.08 ± 2.09[a]	89.12 ± 1.73	64.22 ± 1.23[a]	53.25 ± 1.06[a]	52.33 ± 1.34[a]	97.23 ± 1.42	66.40 ± 2.15[a]	58.29 ± 1.76[a]
	Serum	52.16 ± 2.19[a]	72.44 ± 1.86	61.32 ± 1.36[a]	56.82 ± 1.45[a]	58.27 ± 1.56[a]	87.46 ± 1.38	64.03 ± 1.02[a]	59.34 ± 2.16[a]
	Kidney	30.07 ± 2.18[a]	41.86 ± 2.09	33.22 ± 2.03[a]	27.63 ± 1.97[a]	38.63 ± 2.23[a]	53.24 ± 1.03	46.32 ± 1.23[a]	34.04 ± 1.16[a]
Triglycerides (mg/dL)	Liver	41.33 ± 1.07[a]	70.25 ± 1.24	51.27 ± 1.39[a]	43.76 ± 1.22[a]	39.43 ± 1.13[a]	86.50 ± 0.42	71.49 ± 0.63[a]	60.08 ± 1.23[a]
	Serum	37.51 ± 1.39[a]	61.41 ± 1.28	50.14 ± 1.07[a]	42.66 ± 1.33[a]	40.14 ± 1.72[a]	70.39 ± 1.45	53.86 ± 2.09[a]	46.25 ± 1.77[a]
	Kidney	11.82 ± 0.34[a]	19.47 ± 0.42	14.06 ± 0.77[a]	12.93 ± 0.64[a]	13.43 ± 0.29[a]	26.48 ± 0.33	20.13 ± 0.29[a]	16.25 ± 0.20[a]
HDL-Cholesterol (mg/dL)	Liver	24.72 ± 0.44[a]	16.07 ± 0.53	20.15 ± 0.68[a]	22.43 ± 0.53[a]	26.31 ± 0.59[a]	18.26 ± 0.24	21.72 ± 0.66[a]	23.18 ± 0.52[a]
	Serum	9.42 ± 0.31[a]	6.37 ± 0.43	7.00 ± 0.81[a]	7.82 ± 0.70[a]	8.79 ± 0.29[a]	5.10 ± 0.07	8.15 ± 0.05[a]	8.86 ± 0.02[a]
	Kidney	8.26 ± 0.17[a]	5.31 ± 0.87	8.12 ± 0.56[a]	8.51 ± 0.53[a]	8.42 ± 0.68[a]	6.08 ± 0.63	6.83 ± 0.53[a]	7.27 ± 0.76[a]
LDL-Cholesterol (mg/dL)	Liver	33.76 ± 1.27[a]	41.27 ± 1.53	36.09 ± 1.31[a]	32.47 ± 0.78[a]	33.04 ± 0.62[a]	57.81 ± 0.11	48.06 ± 0.37[a]	39.16 ± 0.25[a]

	Serum	22.36 ± 0.32[a]	34.26 ± 0.53	26.42 ± 0.34[a]	22.14 ± 0.13[a]	24.67 ± 0.72[a]	39.53 ± 0.68	30.06 ± 0.51[a]	23.72 ± 0.44[a]
	Kidney	13.26 ± 0.73[a]	21.60 ± 0.82	16.01 ± 0.69[a]	14.07 ± 0.43[a]	12.13 ± 0.48[a]	31.27 ± 0.57	24.12 ± 0.54[a]	19.33 ± 0.66[a]

Results are reported as mean ± SEM from four separate experiments, each performed in triplicate ($n = 5$). Values labeled with 'a' indicate a statistically significant difference ($p < 0.05$) compared to the untreated control group.

Administration of CCl_4 and rifampicin significantly suppressed hepatic catalase activity, with reductions of 35% and 65%, respectively. Treatment with *Mucuna pruriens* extract at 100 mg/kg body weight substantially reversed this inhibition, restoring enzyme activity by 82% in CCl_4-exposed rats and 88% in rifampicin-exposed rats. Silymarin, used as a reference compound, restored catalase activity by 85%, showing slightly lower efficacy than the extract. A parallel protective effect was observed for superoxide dismutase (SOD), regardless of the tissue or toxicant involved **(Table 3).**

Exposure to these toxicants also caused marked depletion of reduced glutathione (GSH), a critical cellular antioxidant. In the liver, kidney, and serum, GSH levels dropped by 33%, 22%, and 41%, respectively, following toxin exposure. Specifically, CCl_4 induced a 49% decline, while rifampicin produced 10% and 22% decreases in liver, kidney, and serum GSH. Administration of *M. pruriens* extract restored GSH in a dose-dependent manner. In CCl_4-treated animals, hepatic GSH recovered by 73% at 50 mg/kg and 88% at 100 mg/kg; in rifampicin-treated animals, recovery was 55% and 73%, respectively. Silymarin at 100 mg/kg restored GSH by 94%, slightly higher than the extract **(Table 3).**

Renal function markers were also affected by toxicant exposure. CCl_4 and rifampicin increased urea concentrations by 60% and 47%, respectively. Treatment with silymarin (100 mg/kg) reduced urea to 12%, whereas *M. pruriens* extract at 50 and 100 mg/kg lowered urea from 60% to 28% and 2% in CCl_4-exposed rats, and from 47% to 29% and 14% in rifampicin-exposed rats. These results indicate that the extract provided stronger protection than silymarin. Similar trends were observed for uric acid and creatine kinase in kidney and serum samples **(Table 4).**

Indicators of oxidative stress, such as lipid peroxidation, increased substantially after toxicant administration. CCl_4 caused elevations of 83%, 108%, and 142% in liver, kidney, and serum, respectively, while rifampicin raised levels by 152%, 105%, and 63%. Silymarin completely prevented hepatic lipid peroxidation (100% inhibition). Treatment with *M. pruriens* extract at 50 and 100 mg/kg inhibited lipid peroxidation by 48% and 91% in CCl_4-treated rats, and by 43% and 91% in rifampicin-treated rats. Kidney and serum lipid peroxidation followed similar patterns **(Table 5).**

Table 3. summarizes the effects of *M. pruriens* extract on key biochemical markers, including AST, ALT, ALP, and total bilirubin, in the liver and kidney of rats exposed to CCl_4 and rifampicin-induced toxicity.

Biochemical Marker	Tissue	Normal Control Group I	CCl_4-Induced Damage Group	CCl_4 + 50 mg/kg Body Weight Plant Extract	CCl_4 + 100 mg/kg Body Weight Plant Extract	CCl_4 + 100 mg/kg Body Weight Silymarin (Reference)	Rifampicin-Induced Damage Group	Rifampicin + 50 mg/kg Body Weight Plant Extract	Rifampicin + 100 mg/kg Body Weight Plant Extract
AST (IU/L)	Liver	54.33 ± 2.31[a]	82.31 ± 2.47	62.13 ± 0.14[a]	52.93 ± 0.10[a]	57.23 ± 1.53[a]	96.14 ± 2.41	71.52 ± 2.15[a]	58.77 ± 1.87[a]

	Serum	71.26 ± 1.71[a]	115.63 ± 1.67	93.74 ± 1.88[a]	78.14 ± 0.98[a]	75.22 ± 1.56[a]	127.13 ± 2.60	112.31 ± 3.11[a]	92.14 ± 2.34[a]
	Kidney	21.60 ± 2.07[a]	37.81 ± 1.02	30.60 ± 1.12[a]	26.34 ± 1.08[a]	25.06 ± 1.20[a]	56.82 ± 1.73	38.62 ± 1.63[a]	28.26 ± 1.29[a]
ALT (IU/L)	Liver	40.71 ± 1.32[a]	64.21 ± 1.43	55.81 ± 1.16[a]	50.04 ± 2.01[a]	51.33 ± 1.1[a]	77.23 ± 1.42	62.49 ± 1.98[a]	42.03 ± 1.58[a]
	Serum	56.17 ± 1.98[a]	92.76 ± 1.23	72.10 ± 2.13[a]	64.40 ± 2.22[a]	59.33 ± 1.34[a]	88.45 ± 2.43	62.75 ± 2.00[a]	59.22 ± 1.96[a]
	Kidney	17.27 ± 0.25[a]	30.44 ± 0.97	24.16 ± 0.84[a]	20.03 ± 0.29[a]	23.63 ± 1.23[a]	40.29 ± 1.77	36.29 ± 1.23[a]	23.60 ± 2.01[a]
ALP (IU/L)	Liver	47.25 ± 1.92[a]	58.78 ± 1.63	51.06 ± 1.35	42.53 ± 1.07[a]	46.88 ± 0.49[a]	73.84 ± 0.81	64.67 ± 0.92[a]	49.22 ± 0.67[a]
	Serum	62.13 ± 3.12[a]	102.43 ± 4.20	82.42 ± 2.00[a]	71.38 ± 1.86[a]	67.11 ± 2.09[a]	108.52 ± 1.32	81.57 ± 2.07[a]	68.34 ± 1.82[a]
	Kidney	31.21 ± 1.25[a]	48.17 ± 0.84	33.09 ± 0.92[a]	26.04 ± 0.88[a]	29.32 ± 0.98[a]	53.66 ± 1.98	44.36 ± 1.72[a]	34.83 ± 1.45[a]
Total Bilirubin (mg/dL)	Liver	31.18 ± 4.04[a]	44.37 ± 3.54	37.25 ± 2.10[a]	30.34 ± 2.08[a]	31.72 ± 0.82[a]	56.18 ± 0.54	42.33 ± 0.61[a]	32.40 ± 0.50[a]
	Serum	42.25 ± 1.43[a]	72.18 ± 1.54	58.93 ± 1.43[a]	48.52 ± 1.37[a]	45.37 ± 1.84[a]	69.30 ± 1.02	53.26 ± 2.01[a]	43.17 ± 1.33[a]

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The data are presented as mean ± SEM from four independent experiments, each conducted in triplicate, with a sample size of five animals per group (n = 5). Values marked with 'a' indicate a statistically significant difference ($p < 0.05$) compared to the control group.

Table 4. Illustrates the impact of Mucuna pruriens extract on key antioxidant defenses, including superoxide dismutase (SOD), reduced glutathione (GSH), and catalase, in the kidney and liver of rats subjected to toxicity induced by CCl_4 and rifampicin.

Parameter	Tissue	Normal Control	Toxin Control (CCl_4 only)	CCl_4 + 50 mg/kg MP	CCl_4 + 100 mg/kg MP	CCl_4 + 100 mg/kg Silymarin (Standard)	Toxin Control II (Rifampicin only)	Rifampicin + 50 mg/kg MP	Rifampicin + 100 mg/kg MP
Superoxide Dismutase (SOD) (µmol/min/mg protein)	Liver	6.14 ± 0.31[a]	4.53 ± 0.22	4.72 ± 0.61[a]	4.93 ± 0.31[a]	6.20 ± 1.23[a]	2.36 ± 0.19	2.75 ± 0.12[a]	3.89 ± 0.15[a]

	Serum	4.29 ± 0.19[a]	3.17 ± 0.10	3.28 ± 0.18[a]	4.00 ± 0.33[a]	4.34 ± 0.21[a]	2.42 ± 0.07	2.89 ± 0.13[a]	3.76 ± 0.21[a]
	Kidney	3.48 ± 1.03[a]	2.46 ± 0.75	2.61 ± 0.54[a]	2.90 ± 0.44[a]	3.15 ± 0.53[a]	1.93 ± 0.19	2.76 ± 0.41[a]	3.08 ± 0.26[a]
Catalase (µmol/min/mg protein)	Liver	4.70 ± 0.56[a]	2.89 ± 0.21	3.15 ± 0.11[a]	4.15 ± 0.30[a]	3.97 ± 1.20[a]	1.63 ± 0.16	2.60 ± 0.41[a]	3.84 ± 0.23[a]
	Serum	3.06 ± 0.11[a]	2.38 ± 0.10	2.66 ± 0.14[a]	2.91 ± 0.10[a]	2.93 ± 0.20[a]	1.76 ± 0.14	2.53 ± 0.20[a]	3.01 ± 0.16[a]
	Kidney	2.77 ± 0.15[a]	2.02 ± 0.18	2.32 ± 0.20[a]	2.47 ± 0.11[a]	2.58 ± 0.20[a]	1.88 ± 0.26	2.16 ± 0.12[a]	2.53 ± 0.10[a]
Reduced Glutathione (GSH) (µmol/mg protein)	Liver	5.77 ± 0.06[a]	3.87 ± 0.01	4.20 ± 0.12[a]	5.13 ± 0.10[a]	5.43 ± 0.08[a]	2.93 ± 0.08	3.17 ± 0.02[a]	4.22 ± 0.06[a]
	Serum	2.66 ± 0.10[a]	1.57 ± 0.22	2.07 ± 0.64[a]	2.10 ± 0.52[a]	2.49 ± 0.98[a]	2.08 ± 0.02	2.30 ± 0.04[a]	2.52 ± 0.01[a]
	Kidney	3.23 ± 0.07[a]	2.53 ± 0.05	2.88 ± 0.08[a]	3.07 ± 0.09[a]	3.19 ± 0.12[a]	3.04 ± 0.02	3.15 ± 0.08[a]	3.19 ± 0.10[a]

Data are expressed as mean ± SEM from four separate experiments, each carried out in triplicate, with five animals per group (n = 5). Values labeled with 'a' indicate a statistically significant difference ($p < 0.05$) relative to the control.

Table 5. Presents the effects of Mucuna pruriens extract on key biochemical markers—specifically uric acid, urea, and creatine kinase—in the kidney and liver of rats exposed to toxicity induced by CCl_4 and rifampicin.

Parameter	Tissue	Normal Control	Toxin Control (CCl_4 only)	CCl_4 + 50 mg/kg MP	CCl_4 + 100 mg/kg MP	CCl_4 + 100 mg/kg Silymarin (Standard)	Toxin Control II (Rifampicin only)	Rifampicin + 50 mg/kg MP	Rifampicin + 100 mg/kg MP
Uric Acid (mg/dL)	Kidney	29.54 ± 1.52[a]	42.65 ± 0.82	33.16 ± 0.55[a]	32.02 ± 0.87[a]	32.89 ± 0.92[a]	54.23 ± 0.78	43.06 ± 1.08[a]	31.37 ± 1.13[a]
	Serum	19.17 ± 0.24[a]	33.51 ± 0.52	28.47 ± 0.65[a]	24.81 ± 0.59[a]	21.17 ± 0.68[a]	35.43 ± 0.52	28.76 ± 0.48[a]	24.08 ± 0.53[a]
Creatinine (IU/L)	Kidney	27.61 ± 1.07[a]	38.44 ± 0.87	30.22 ± 0.19[a]	28.31 ± 0.14[a]	25.73 ± 0.85[a]	49.17 ± 0.94	40.80 ± 0.64[a]	33.51 ± 0.50[a]
	Serum	20.33 ± 0.30[a]	31.70 ± 0.29	26.31 ± 1.00[a]	22.40 ± 0.81[a]	23.66 ± 0.22[a]	44.58 ± 0.44	31.49 ± 0.42[a]	28.60 ± 0.58[a]

Urea (mg/dL)	Kidney	47.82 ± 0.93[a]	76.32 ± 0.88	61.37 ± 1.34[a]	48.93 ± 1.17[a]	53.76 ± 0.78[a]	70.06 ± 0.83	61.82 ± 0.51[a]	54.70 ± 1.20[a]
	Serum	38.77 ± 0.83[a]	77.64 ± 1.36	59.26 ± 1.06[a]	51.26 ± 0.96[a]	45.26 ± 0.76[a]	64.39 ± 0.62	53.14 ± 0.76[a]	41.20 ± 0.81[a]

The results are presented as mean ± SEM from four independent experiments, each conducted in triplicate, with a sample size of five animals per group (n = 5). Values marked with 'a' indicate a statistically significant difference ($p < 0.05$) compared with the control group.

Oxidative stress plays a central role in the development, progression, and complications of numerous pathological conditions and can be effectively modeled in experimental settings for research purposes [1, 2, 42–44]. In this study **(Table 1),** administration of CCl_4 caused pronounced disturbances in the lipid profile of the experimental animals, including elevated total cholesterol, triglycerides, and LDL levels, accompanied by a significant decline in HDL, relative to the control group. These findings align with observations reported by Agbafor and Nwachukwu [45]. Mechanistically, CCl_4-induced liver injury involves the activation of transcription factors such as NF-κB, AP-1, and EGR-1, which promote inflammatory signaling and the release of proinflammatory cytokines responsible for hepatic inflammation [24, 46]. The lipid abnormalities observed are likely a consequence of oxidative stress. Treatment with *Mucuna pruriens* extract restored the lipid profile in a dose-dependent manner, demonstrating effects comparable to the standard hepatoprotective agent, silymarin. This supports previous findings by Enechi and Ozogwu [47], indicating the therapeutic promise of *M. pruriens* leaf extract.

Phytochemical investigations have identified anthraquinones, flavonoids, and cardiac glycosides in *M. pruriens* leaves through preliminary screening [48, 49], while GC-MS analyses have provided a detailed profile of bioactive constituents in the ethanolic extract [50]. The lipid-lowering and hepatoprotective properties of plant extracts are frequently attributed to such compounds [51]. Flavonoids, for example, reduce blood cholesterol and triglycerides, thereby lowering cardiovascular risks like atherosclerosis [52]. Saponins interact with cholesterol and bile acids, limiting their reabsorption and thus decreasing serum cholesterol [53]. Cardiac glycosides modulate myocardial contraction through effects on intracellular Ca^{2+} [54]. The anti-hypercholesterolemic activity of the extract may involve regulation of cholesterol biosynthesis, inhibition of LDL oxidation, and reduced intestinal absorption of cholesterol via complex formation.

Flavonoids and polyphenols also exhibit anti-inflammatory actions by suppressing transcription factors such as AP-1, transcription factor-3, and CREB-binding proteins [26]. Therefore, the hepatoprotective effects of *M. pruriens* leaf extract may be largely mediated by flavonoid-driven inhibition of NF-κB, contributing to the restoration of liver structure and function [26].

Rifampicin exposure significantly altered renal lipid profiles in the experimental animals **(Table 2).** Rifampicin toxicity involves lipid peroxidation and inhibition of detoxifying enzymes in the liver and kidney [55]. In the liver, rifampicin is metabolically converted to deacetyl rifampicin, which binds to critical macromolecules, causing tissue injury [55–57]. Administration of *M. pruriens* extract mitigated these changes in a dose-dependent manner, in agreement with Mohammed *et al*. [58]. The protective effect is attributed to flavonoids in the extract scavenging free radicals generated by the toxicants. Remarkably, the extract displayed effects comparable to silymarin, reflecting the synergistic action of its bioactive components [58].

Enzymes such as ALT, AST, and ALP serve as important biomarkers of liver function [58]. Organ injury leads to leakage of these enzymes into the bloodstream, elevating their serum levels. In this study, untreated toxicant-exposed animals showed significant increases in ALT, AST, and ALP **(Table 3).** Administration of graded doses of *M. pruriens* extract restored these enzyme levels in a dose-dependent manner, consistent with reports by Mohammed *et al*. [58] and Sylvester *et al*. [59]. This protective effect is linked to flavonoids, anthraquinones, and other antioxidant phytochemicals in the extract [49].

Antioxidant enzymes act as a defense system against free radical damage to cellular macromolecules. CCl_4 and rifampicin significantly reduced superoxide dismutase and catalase levels **(Table 4),** likely due to covalent binding of toxic metabolites to critical enzyme sites,

resulting in inhibition [60]. Treatment with *M. pruriens* extract restored these enzymes to levels comparable with silymarin-treated animals. The restoration is attributed to polyphenolic compounds that prevent toxic metabolite binding [58].

Serum bilirubin is a reliable indicator of liver health, with elevated levels signaling hepatic injury [61–64]. Toxicant exposure significantly increased bilirubin, whereas treatment with *M. pruriens* extract restored levels in a dose-dependent manner to near-normal values, confirming its hepatoprotective potential [47].

Renal function, reflected by urea concentration, was compromised following toxicant exposure **(Table 5).** CCl_4 and rifampicin elevated serum urea, indicating free radical-mediated glomerular damage and impaired filtration [65]. *M. pruriens* extract normalized urea levels in a dose-dependent manner, comparable to silymarin [47]. Similarly, uric acid, which plays a role in gout, kidney disorders, and type II diabetes [66], was elevated after toxicant exposure but restored to normal by extract treatment, likely through inhibition of purine catabolism by polyphenolic components.

Creatine kinase, a marker of cardiac injury, was significantly increased in toxicant-exposed animals, suggesting multi-organ toxicity **(Table 5).** Treatment with *M. pruriens* extract ameliorated this effect, restoring enzyme activity to levels similar to silymarin-treated animals, highlighting its systemic protective potential.

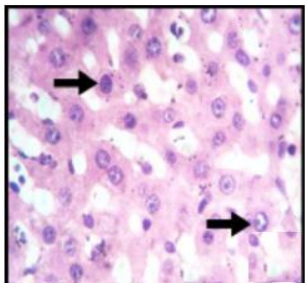
Lipid peroxidation, a major contributor to tissue damage, was significantly elevated in serum and tissue homogenates following CCl_4 and rifampicin exposure **(Table 6).** This is due to formation of reactive intermediates such as trichloromethyl and trichloroperoxyl radicals from CCl_4 metabolism [60]. Administration of *M. pruriens* extract inhibited lipid peroxidation in a dose-dependent manner, with potency comparable to silymarin. This effect likely arises from additive or synergistic actions of multiple antioxidant phytochemicals in the extract, supporting its potential as a therapeutic agent for liver and kidney disorders.

Table 6. summarizes the impact of *M. pruriens* extract on lipid peroxidation (MDA levels) and total protein content in liver and kidney tissues following CCl_4 and rifampicin-induced toxicity.

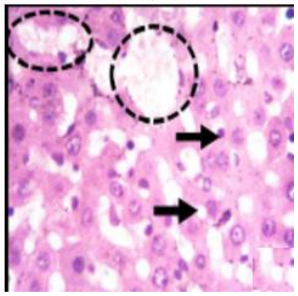
Parameters	Tissues	Normal Control	Toxin Control (CCl_4 only)	CCl_4 + 50 mg/kg MP	CCl_4 + 100 mg/kg MP	CCl_4 + 100 mg/kg Silymarin	Toxin Control II (Rifampicin only)	Rifampicin + 50 mg/kg MP	Rifampicin + 100 mg/kg MP
Malondialdehyde (MDA) (nmol/g tissue or serum)	Liver	0.23 ± 0.01[a]	0.42 ± 0.01	0.35 ± 0.01[a]	0.25 ± 0.01[a]	0.20 ± 0.01[a]	0.58 ± 0.01	0.36 ± 0.02[a]	0.25 ± 0.02[a]
	Serum	0.64 ± 0.02[a]	1.55 ± 0.01[a]	0.70 ± 0.02[a]	0.60 ± 0.07[a]	0.59 ± 0.04[a]	1.04 ± 0.09	0.63 ± 0.01[a]	0.58 ± 0.01[a]
	Kidney	0.89 ± 0.01[a]	1.76 ± 0.03	1.23 ± 0.01[a]	1.09 ± 0.02[a]	1.02 ± 0.01[a]	0.94 ± 0.01	0.52 ± 0.01[a]	0.47 ± 0.03[a]
Total Protein Content (mg/g tissue or serum)	Liver	2.64 ± 0.16[a]	1.47 ± 0.19	2.07 ± 0.42[a]	2.15 ± 0.17[a]	2.33 ± 0.78[a]	1.04 ± 0.09	1.63 ± 0.02[a]	2.53 ± 0.01[a]
	Serum	2.08 ± 0.08[a]	1.26 ± 0.06	1.81 ± 0.01[a]	1.74 ± 0.01[a]	2.17 ± 0.08[a]	1.02 ± 0.18	1.15 ± 0.13[a]	1.88 ± 0.20[a]
	Kidney	1.73 ± 0.020[a]	0.76 ± 0.06	0.89 ± 0.01[a]	1.24 ± 0.01[a]	1.44 ± 0.07[a]	1.06 ± 0.01	1.20 ± 0.01[a]	1.46 ± 0.02[a]

The results are expressed as mean ± SEM from four independent experiments performed in triplicate, with 'a' denoting a significant difference ($p < 0.05$) relative to the control ($n = 5$). Group I served as the positive control, while Group II was the CCl4-treated negative control. Groups III and IV received M. pruriens extract at 50 and 100 mg/kg body weight, respectively, following CCl4 exposure, whereas Group V was treated with silymarin (100 mg/kg body weight) post-CCl4 administration. Group VI acted as the negative control for rifampicin, and Groups VII and VIII were given M. pruriens at 50 and 100 mg/kg body weight, respectively, after rifampicin exposure.

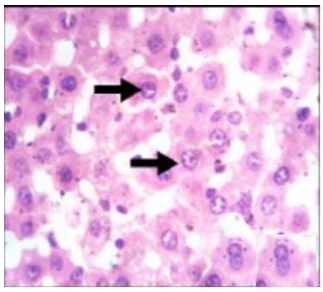
Histopathological examination **(Figures 4a–4g)** showed marked disruption of the liver tissue in CCl4-treated animals, including extensive hepatocellular necrosis with vacuolation, inflammatory infiltration, and congestion of the central vein, likely resulting from oxidative stress induced by free radicals [67]. Treatment with M. pruriens extract effectively restored liver tissue structure, producing effects comparable to silymarin. These findings suggest that M. pruriens leaf extract mitigates drug-induced histopathological damage and stabilizes membrane integrity, in agreement with previous studies [68–70].



a)



b)



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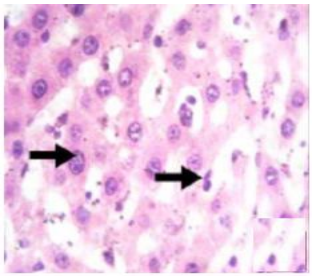

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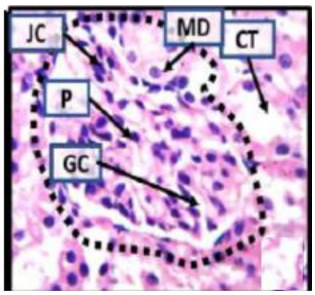
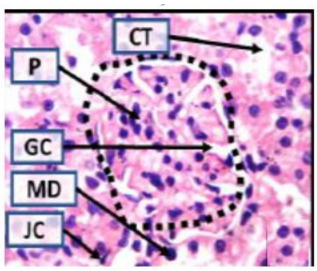
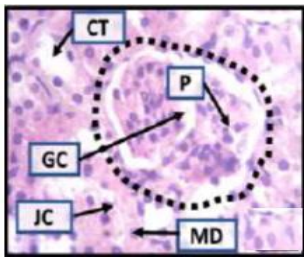


e)



f)



g)

Figure 4. Illustrative histological images of liver tissue (a–d) and kidney tissue (e–g) obtained from the study animals.

Images **Figures 4a–4d** are captured at high magnification (×400) and highlight typical hepatocytes

(denoted by black arrowheads). These sections reveal various histological aspects of the liver cells, including cell density, arrangement, staining characteristics, central vein diameter, material within central veins, and notable vacuolar changes (marked by dotted black circles). Prominent vacuoles with pink staining reflect lipid accumulation and bile deposits, pointing to cholestatic changes. Similarly, images **Figures 4e–4g** are taken at ×400 magnification and focus on the renal corpuscle (black outline), containing the glomerulus in the Bowman's space, with blood supply via the afferent arteriole and outflow through the efferent arteriole. Visible structures include convoluted tubules (CT), glomerular capillaries (GC), and key cellular components such as podocytes within the glomerulus (P), along with juxtaglomerular cells and macula densa at the vascular pole. The urinary pole connects to the proximal convoluted tubules.

Figure 4a Liver tissue from animals given only standard feed and distilled water; **Figure 4b** Liver tissue from animals receiving 3 ml/kg CCl_4 with no further treatment; **Figures 4c**Liver tissue from animals exposed to 3 ml/kg CCl_4 and subsequently treated with 100 mg/kg M. pruriens; **Figuren 4d** Liver tissue from animals given 3 ml/kg CCl_4 and treated with 200 mg/kg silymarin; e) Kidney tissue from animals receiving distilled water alone; **Figures 4f** Kidney tissue from animals given 250 mg/kg rifampicin with no further treatment; **Figure 4g**Kidney tissue from animals administered 250 mg/kg rifampicin and treated with 100 mg/kg body weight M. pruriens.

Conclusion

M. pruriens leaf extract effectively inhibited lipid peroxidation, normalized altered lipid profiles, enhanced liver and kidney function markers, restored the activity of antioxidant enzymes, and improved the structural integrity of liver and kidney tissues. Both biochemical and histopathological findings indicate that M. pruriens leaf extract exhibits comparable efficacy to silymarin at the same dosage. Therefore, it has the potential to serve as a viable alternative to conventional therapies for liver and kidney disorders. Future research should focus on detailed characterization of the extract's phytochemical components, identification of its active constituents, and elucidation of their mechanisms of action.

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Conflict of Interest: None

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Ethics Statement: None

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