Ethanolic extract of *Mucuna pruriens* ameliorates carbon tetrachloride and rifampicin-induced hepatotoxicity and nephrotoxicity in wistar albino rat

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Abstract

The present study investigates the hepatoprotective and nephroprotective potentials of *Mucuna pruriens* leaf extract with a view to providing a potent alternative in the management of liver and kidney diseases. Forty male albino rats were randomly placed into eight groups comprising five animals each. Animals in group I were administered with the distilled water, while groups II and VI were exposed to CCl_4 and rifampicin respectively. Animals in groups III and IV were initially exposed CCl_4 and treated with 50 and 100 mg/kg bw *M. pruriens* respectively. Similarly, groups VII and VIII animals were exposed to rifampicin and treated with 50 and 100 mg/kg bw *M. pruriens* respectively. Animals in group V were treated with 100 mg/kg bw silymarin by oral gavage after an initial exposure to CCl_4. Selected biomarkers of liver and kidney damage were determined in the serum and organs homogenate. Liver and kidney slices of experimental animals were also stained for histopathological examination. Exposure to CCl_4 and rifampicin respectively resulted in marked distortion in lipid profile, inhibition of antioxidant enzymes and a surge in ALT, AST, ALP, urea, uric acid, bilirubin and creatine kinase. Treatment with *M. pruriens* extract reversed all deranged biochemical and histopathological parameters in a dose-dependent manner.

Restoration of both biochemical and histopathological alterations established the fact that *M. pruriens* is a potent hepatoprotective and nephroprotective plant, thereby giving credence to the potential usefulness of its leaf extract in the management of liver and kidney diseases.

1.0 Introduction

In recent times, oxidative stress concept has become an object of vast research attention [1]. It depicts deleterious alteration in the delicate balance between free radicals and antioxidants in the physiological system. Free radicals are highly reactive chemical species with an unpaired electron in the outermost shell. Being very reactive, free radicals can initiate oxidative damage to critical macromolecules such as proteins, nucleic acids, lipids and carbohydrates [1,2]. This damage is often triggered by abstracting electron from such macromolecules thereby oxidizing them. Endogenous free radicals (reactive oxygen species (ROS)) are produced majorly during aerobic respiration in the mitochondrial electron transport chain [3,4]. At the molecular level, oxidative stress has been traced to an upregulation of specific redox signaling pathways [4,5]. Its mechanism appears to vary from cell to cell. Generally, cells that are enrich with mitochondria are more vulnerable to oxidative stress than others [1]. Organelles such as mitochondria, microsomes, and peroxisomes of hepatocytes which are directly involved in free radicals' production could modulate specific signaling pathways. Peroxisome proliferator-activated receptor alpha (PPAR), mitogen-activated protein kinases (MAPK) and other protein kinases are said to upregulated during oxidative stress [6]. Specifically, upregulation of inflammatory cytokines such as interleukin-1 superfamily is the major molecular basis of hepatic damage [6–9]. Pathogenesis of hepatic injury has been linked to complex molecular interplay between oxidative stress and immune system [10,11]. Interestingly, a robust antioxidative system exists in the liver and kidney of all mammals including humans, to maintain the redox status of all critical macromolecules in the reduced form [1]. However,

when the redox homeostasis in the liver, kidney and other vital organs is perturbed, oxidative stress sets in. Consequently, vital intracellular targets such as DNA, proteins, lipids are oxidatively attacked resulting in compromise of their functions. Signaling pathways of inflammations are then upregulated with its attendant devastating effects [6,12]. Oxidative stress can also be triggered by lipid peroxidation [13]. Oxidation of polyunsaturated fatty acids present in membranes can initiate a chain of free radicals which can in turn attack proteins leading to protein carbonylation [6,14]. Lipid peroxidation can be detrimental to critical membrane functions as a result of compromise in integrity and fluidity. Recently, free radical implicated peroxidation of membrane phospholipids has been linked to some chronic diseases including cancer, atherosclerosis, Parkinson diseases and aging[14]. Hence, a major strategy to mitigate the effects of these diseases is amelioration of oxidative damage by medicinal plants intervention [14].

M. pruriens Linn. (Fabaceae), has been used extensively as medicaments in the Ayurvedic and Indian system of medicine [15,16]. Its powdered seeds extract has been used to manage several oxidative stress implicated diseases, such as rheumatoid arthritis, diabetes, atherosclerosis, nervous disorders and sexual dysfunctions [17–19]. Its high level of (L-3,4 dihydroxyphenyl alanine (L-DOPA) have been suggested for its usage in the management of Parkinson's disease [16,18]. Presence of several phytochemicals with established antioxidant properties in *M. pruriens* extract has been linked to its numerous medicinal uses [20,21]. Considering, the medicinal potentials of *M. pruriens*, there is a dire need to investigate the therapeutic potential of its leaves as a viable alternative to conventional drugs used in the management of liver and kidney diseases.

2.0 Materials And Methods

2.1 Plant Materials

Fresh *M. pruriens* leaves were obtained within the University campus, botanically identified at the Department of Plant Science, Ekiti State University, Ado-Ekiti. The voucher specimen of the leaf with number UHAE2020070 was deposited at the University herbarium. The air-dried leaves were then powdered using a Warring blender. The weight of the powdered leaves was measured and stored in an airtight container.

2.2 Reagents and Chemicals

All reagents and chemicals were of high analytical grade obtained from standard commercial suppliers.

2.3 Extraction of the extract

M. pruriens leaves were air-dried for thirty (30) days at room temperature. The air-dried samples were ground to fine powder using a blender. 32.5 g of the powdered leaves was soaked in 100 ml of 80% ethanol and left for 72 h. It was then filtered using a cheese cloth to obtain a clear filtrate which was tightly covered with an insect-proof net and allowed for evaporation to dryness, which was monitored by

weighing until a constant weight was obtained. The crude extract was kept airtight in a glass petri-dish inside a refrigerator and subsequently reconstituted with distilled water for animal treatment.

2.4 Animals protocol

Forty (40) wistar albino rats weighing 150 g – 170 g were obtained from the animal house of the Department of Science Technology, The Federal Polytechnic, Ado-Ekiti and housed in clean wire meshed cages under standard conditions temperature (24 ± 1°C), relative humidity (40-60%), and 12 / 12-hour light and dark cycle. They were allowed to have free access to food (commercial palletized diet from Vital Feed Mill) and drinking water *ad libitum* daily. The rat beddings were changed and replaced every day throughout the experimental period.

2.4.1 Preparation of organs homogenate

Animals were decapitated under cold-ether anesthesia and quickly dissected to obtain the liver, heart and kidney. They were trimmed of fatty tissue, washed in saline, blotted with filter paper and weighed. They were then chopped into bits and homogenized in ten volumes of the homogenizing phosphate buffer (pH 7.4) using a Teflon homogenizer. The resulting homogenates were centrifuged at 3000 rpm at 4°C for 30 min. The supernatant obtained was collected and stored under 4°C and then used for biochemical analyses.

2.4.2 Preparation of serum

Whole blood collected from the heart into samples bottles and allowed to stand for 1 h to allow for coagulation. The coagulated blood was then centrifuged at 3000 rpm for 15 min at 25°C to obtain a clear supernatant which was carefully decanted and placed on ice for the estimation of serum biochemical parameters.

2.5 Assay for Creatine Kinase (Ck-Mb) Activity

Creatine kinase was assayed following the method previously described by Mattenheimer [22]. Briefly, 1.0 ml of imidazole buffer (10 mM, pH 6.6) containing: creatine phosphate (30 mM), glucose (20 mM), N-acetyl-cysteine (20 mM), magnesium acetate (10 mM), ethylene diaminetetraacetic acid (2 mM), ADP (2 mM), NADP (2 mM), AMP (5 mM), DAPP (10 µM), G6PDH (≥2.0 ku/L) and HK(≥2.15 ku/L)) was pipetted into a thermostated cuvette and incubated at 37°C. Thereafter, 50 µl of serum was added and thoroughly mixed. Absorbance of the resulting mixture at 340 nm was monitored for 5 min at 30 sec interval.

2.6 Assay of Aspartate Aminotransferase (AST) Activity

Aspartate aminotransferase activity was determined as earlier described by Reitman and Frankel [23]. Briefly, 0.1 ml each of serum and organ homogenates respectively was mixed with phosphate buffer (100 mM, pH 7.4), L-aspartate (100 mM), and α-oxoglutarate (2 mM) and the mixture incubated for exactly 30 min at 37°C. Five hundred microliters (500 µl) of 2,4-dinitrphenylhydrazine (2mM) was added to the

reaction mixture and allowed to stand for exactly 20 min at 25 °C. Thereafter, 5.0 ml of NaOH (0.4 M) was added and left to stand for 5 min. Absorbance was then read at 546 nm against the reagent blank.

2.7 Assay of Alanine Amino transferase (ALT) Activity

The principle previously described Reitman and Frankel [23] was followed in the assay of ALT using Randox kit. Five hundred microliters (500 µl) of reagent 1 containing, phosphate buffer (100 mM, pH 7.4), L-alanine (200 mM) and α-oxoglutarate (2.0 M) was added to 0.1ml of serum and each of the organ homogenates in a test tube and the mixture was incubated at 37°C for 30 min. Exactly 0.5 ml of reagent 2 containing, 2, 4-dinitrophenylhydrazine (2.0 mM) was added and the solution incubated again at 20°C for 20 min. Finally, 5.0 ml of NaOH was added and the solution was allowed to stand for 5 min at room temperature and the absorbance measured at 546 nm.

2.8 Assay of Akaline Phosphatase (ALP) Activity

Assay for ALP was based on the protocol described by Englehardt *et al.* [24]. Exactly 1.0 ml of the reagent (1.0 M diethanolamine buffer pH 9.8, 0.5 mM $MgCl_2$; substrate: 10 mM p-nitrophenol phosphate) was added separately to 0.02 ml of serum and organs homogenates and mixed. The absorbance was taken at 405 nm for 3 min at 1 min interval.

2.9 Estimation of Total Cholesterol Level

Total cholesterol level was determined based on established method of Trinder [25]. Ten microliter each of standard, serum and organs homogenates were pipetted into labeled test tubes. Thereafter, 1.0 ml of working reagent containing; Pipes buffer (80 mM pH 6.8), 4-aminoantipyrine (0.25 mM), phenol (6 mM), peroxidase (≥ 0.5 U/ml), cholesterol esterase ion (≥0.15 U/ml) and cholesterol oxidase (0.10 U/ml) was added into all the tubes. The test tubes were mixed thoroughly and incubated for 10 min at room temperature. Absorbance of the sample (Asample) was read at 500 nm against the reagent blank.

Cholesterol concentration (mg/dl) = Absorbance of sample/Absorbance of standard X Concentration of standard

2.10 Evaluation of Concentration of Triglyceride

Triglycerides level was measured as previously described by Tietz [26]. Ten microliters each of triglyceride standard, serum and organs homogenates were pipetted separately into labeled test tubes. Exactly 1.0 ml of the working reagents; R1a (buffer) containing Pipes buffer (40mM, pH 7.6), 4-chloro-phenol (5.5 mM), magnesium-ion (17.5 mM); R1b (enzyme reagent containing 4-amino phenazone (0.5 mM), ATP (1.0 mM), lipase ((≥150 U/ml), glycerol-kinase ((≥0.4U/ml), glycerol-3-phosphate oxidase ((≥1.5 U/ml) and peroxidase (≥0.5 U/ml) was added into all the tubes. The reaction mixtures were mixed thoroughly and incubated for 10 min at room temperature. Absorbance was measured at 546 nm against the blank.

Triglyceride concentration (mg/dl) = Absorbance of sample/Absorbance of standard X Concentration of standard

2.11 High Density Lipoprotein (HDL-c)-Cholesterol Assay

Estimation of HDL-cholesterol was done as described by Grove [27]. Reaction mixture containing 200 µl each of the serum and organs homogenates, 200 µl of the cholesterol standard, 500 µl of the diluted precipitant R1 (0.55 mM phosphotungstic acid, 25 mM magnesium chloride) were mixed together and allowed to stand for 10 min at room temperature. It was then centrifuged for 10 min at 4000 rpm to obtain a clear supernatant. The clear supernatant was separated off within 2 h and the cholesterol content was determined by the CHOD-PAP reaction method as follows:

One milliliter cholesterol reagent was added separately to 100 µl each of serum and organs homogenates and mixed together in a test tube. The standard test tube contained 100 µl of the cholesterol standard supernatant and 1 ml of cholesterol reagent. The reagent mixture was mixed thoroughly and incubated for 10 min at 25°C. Absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was then measured at 500 nm against the reagent blank within 1 h.

2.12 Low Density Lipoprotein (LDL) - Cholesterol Determination

Concentration of low-density lipoprotein in the serum and organs homogenates was calculated as described as described by Friedwald *et al.* [28]:

LDL cholesterol = Total cholesterol – Triglycerides/5 – HDL-cholesterol

2.13 Determination of Catalase Activity

Catalase activity was measured using the method previously described by Sinha [29]. Briefly, 200 µl each of serum and organs homogenates was mixed separately with 0.8 ml distilled H_2O to obtain a five-fold dilution. The assay mixture contained 2 ml of solution (800 mmol) and 2.5 ml of phosphate buffer in a 10 ml flat bottom flask. Five hundred microliters of appropriate dilution of the enzyme was rapidly mixed with the reaction mixture by a gentle swirling motion at room temperature. Thereafter, 1.0 ml portion of the reaction mixture was withdrawn and blown into 1 ml dichromate/acetic acid reagent at 60 s intervals. Hydrogen peroxide content of the withdrawn sample was determined by the method described below.

Catalase activity = H2o2 Consumed/mg protein

H_2O_2 consumed = 800 – Concentration of H_2O_2 remaining

Concentration of H_2O_2 remaining was extrapolated from the standard curve for catalase activity.

2.14 Determination of Superoxide Dismutase (SOD) Activity

Superoxide dismutase activity was determined following the method of Misra and Fridovich [30]. An aliquot of a ten-fold dilution each of serum and organs homogenates was added separately to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) and allowed to equilibrate in a spectrophotometer. Reaction was initiated by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of water. Absorbance at 480 nm of the resulting solution was monitored for 150 s at 30 s interval.

2.15 Determination of Reduced Glutathione (GSH) Level

Previously established method of Beutler *et al.* [31] was followed in estimating the level of reduced glutathione (GSH). Exactly 0.2 ml each of serum and organs homogenates was added separately to 1.8 ml of distilled water followed by the addition of 3.0 ml of the precipitating solution and then shaken thoroughly. The mixture was then allowed to stand for 5 min and then filtered. One milliliter of filtrate was added of 4.0 ml of 0.1 M phosphate buffer pH 7.4. Finally, 0.5 ml of the Ellman's reagent was added to the mixture. A blank was prepared with 4.0 ml of the 0.1 M phosphate buffer, 1.0 ml of diluted precipitating solution and 0.5 ml of the Ellman's reagent. Absorbance of the resulting solution was then measured at 412 nm against reagent blank.

2.16 Determination of Total Protein (TP) in Serum

The Biuret method described by Weichselbaum [32] was employed in the determination of total protein. One milliliter of reagent R1 containing sodium hydroxide (100 mM), Na-K-tartrate (18 mM), potassium iodide (15 mM) and cupric sulphate (6 mM) was added separately to 0.02 ml each of serum and organs homogenates. The resulting mixture was incubated at 25°C and absorbance measured at 546 nm against the reagent blank.

2.17 Lipid Peroxidation

Thiobarbituric acid reactive substances (TBARS) content in the serum and organs homogenates were measured as previously described by Okhawa *et al*. [33] using Randox kits. One hundred microliters each of serum and organs homogenates were mixed separately with 2.5 ml reaction buffer and boiled for 1 h. The resulting mixture was centrifuged at 3,000 rpm for 10 min. Absorbance of the supernatant obtained for each tube was then measured at 532 nm. Malonidialdehyde (MDA) level in the supernatant was expressed as µmole MDA/mg protein using molar extinction coefficient of MDA-thiobarbituric chromophore (1.56×10^5/M/cm).

2.18 Statistical Analysis

Data are expressed as mean ± SEM. Statistical evaluation was done using One-Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) by using SPSS 11.09 for windows. The significance level was set at $p < 0.05$.

3.1 Results

Animals exposed to CCl_4 and rifampicin developed significant derangement in hepatic, renal and serum lipid profile (Table 1). This derangement was restored (in a dose-dependent manner) to a level comparable to animals that were not exposed to toxicants following treatment with *M. pruriens* extract (Table 1). Alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) activities were significantly depleted in the liver and kidney homogenates of experimental animals exposed to CCl_4 and rifampicin (Table 2). However, oral treatment with *M. pruriens* extract restored the enzymes dose-dependently. Its effect was comparable to animals treated with silymarin (Table 2). Antioxidant enzymes (superoxide dismutase and catalase) were significantly inhibited in animals administered with CCl_4 and rifampicin. Administration of *M. pruriens* extract resulted in a dose-dependent restoration of the antioxidant enzymes' activity (Table 3). This restoration was comparable with animals treated with silymarin (Table 3). Similar trend was observed regardless of organ (liver, kidney or serum) involved. (Table 3). Similarly, reduced glutathione (GSH) was significantly decreased in the serum and other tissue homogenates of experimental animals administered with CCl_4 and rifampicin without treatment (Table 3). Administration of *M. pruriens* extract caused a dose-dependent amelioration and restoration of GSH level back to levels comparable with negative control and animals treated with the standard drug (Table 3). There was significant increase in total bilirubin (T. bil), urea, creatine kinase (CK) and uric acid in the serum and other organs homogenates following the administration of rifampicin and CCl_4 without treatment (Table 4). Treatment with graded doses of *M. pruriens* extract resulted in a dose-dependent restoration of these parameters to values comparable with the negative control and animals treated with the standard drug (Table 4).

Exposure to CCl_4 and rifampicin respectively resulted in a significant increase in hepatic, renal and serum lipid peroxidation (Table 5). Treatment with *M. pruriens* markedly inhibited lipid peroxidation and restored it (in a dose dependent manner) to a level comparable with animals treated with silymarin (Table 5).

Table 1. Effects of *M. pruriens* extract on lipid profile in the liver and kidney of rats after CCl_4 and rifampicin induced toxicity.

Data shows mean SEM values of four independent experiments performed in triplicate 'a' represents significant difference ($p<0.05$) from the control, (n= 5).

Table 2. Effects of *M. pruriens* extract on selected biomarkers (AST, ALT and ALP) in the liver and kidney of rats after CCl_4 and rifampicin induced toxicity.

Data shows mean SEM values of four independent experiments performed in triplicate 'a' represents significant difference ($p<0.05$) from the control, (n= 5).

Table 3. Effects of *M. pruriens* extract on selected antioxidant enzymes (superoxide dismutase and Catalase) and reduced glutathione (GSH) in the liver and kidney of rats after CCl_4 and rifampicin induced

toxicity.

Data shows mean SEM values of four independent experiments performed in triplicate ‘a’ represents significant difference ($p<0.05$) from the control, (n= 5).

Table 4. Effects of *M. pruriens* extract on selected biomarkers (urea, uric acid, creatine kinase, total bilirubin) in the liver and kidney of rats after CCl_4 and rifampicin induced toxicity

Data shows mean SEM values of four independent experiments performed in triplicate ‘a’ represents significant difference ($p<0.05$) from the control, (n= 5).

Table 5. Effects of *M. pruriens* extract on lipid peroxidation (MDA) level and total protein in the liver and kidney of rats after CCl_4 and rifampicin induced toxicity

Data shows mean SEM values of four independent experiments performed in triplicate ‘a’ represents significant difference ($p<0.05$) from the control, (n= 5).

Group I- Positive control; Group II- negative control (CCl_4); Group III – Animals treated with M. pruriens extract at 50 mg/kg bw after exposure to CCl_4; Group IV – Animals treated with M. pruriens (100 mg/kg bw); Group V- Animals treated with silymarin (200 mg/kg bw) after exposure to CCl_4. Group VI- Negative control (rifampicin). Group VII- Animals treated with M. pruriens (50 mg/kg bw) extract after exposure to rifampicin; Group VIII- Animals treated with M. pruriens (100 mg/kg bw) after exposure to rifampicin.

Figures (A-D) are representative photomicrograph of the liver slices of experimental animals showing a high-power magnification (x400 mag) of the inherent hepatocytes (black arrow head). Photomicrographs show the histomorphological manifestation of the hepatocytes, density of hepatocytes, distribution of hepatocytes, staining intensity of hepatocytes, size of central veins, content of central veins and expression of large vacuolations (dotted black circles). Large vacoulations with pick colouration are fatty livers with bile plaques which suggests cholestasis. Similarly, representative photomicrographs of the kidney slices (Figures E-G) of experimental animals showing a high-power magnification (x400mag) of renal corpuscle (black outline) which houses the glomerulus within the urinary space that is supplied by the afferent arteriole and drained by the efferent arteriole. The histomorphology presents with the convoluted tubule (CT), glomerular capillaries (GC) and inherent cells which include the intraglomerular podocytes (P) as well as the juxtaglomerular cells and macula densa cells in the vascular poles of the renal corpuscles. The urinary pole continues out as the proximal convoluted tubules. *A- liver slice of animals fed with animal feed and distilled water only; B- liver slice of animals administered with 3ml/kg CCl_4, without treatment; C- liver slice of animals induced with 3ml/kg CCl_4 and treated with 100mg/kg M. pruriens; D - liver slice of animals administered with 3ml/kg CCl_4 and treated with 200mg/kg Silymarin; E - kidney slice of animals administered with distilled water only; F - kidney slice of animals administered with 250mg/kg rifampicin only without treatment; G- kidney slice of animals administered with 250mg/kg rifampicin and treated with 100 mg/kg bw of M. pruriens*

4.0 Discussion

Carbon tetrachloride and rifampicin have been routinely used in animal model of hepatotoxicity and nephrotoxicity respectively [34]. However, in the present study, each of CCl_4 and rifampicin exhibited toxicity to both kidney and liver tissues. This indicates that both toxicants are multiorgan specific.

Oxidative stress is the major culprit in the pathophysiology of CCl_4-induced hepatotoxicity [35]. During metabolism, CCl_4 is bioactivated to trichloromethyl free radical (CCl_3) in a serial process involving chloromethylation, saturation and peroxidation. Consequently, there is a progressive deterioration of membrane phospholipid of the hepatic endoplasmic reticulum. These processes eventually result to structural and functional disruption of hepatocytes [36,37]. In the present study, exposure to CCl_4 resulted in a significant derangement in lipid profile of experimental animals. Total cholesterol, triglycerides and LDL were significantly elevated while HDL level was markedly depleted compared to negative control animals. The molecular event of CCl_4-induced hepatic damage has been reported [38]. It involves the activation of several transcription factors such as NF-kB, activator protein 1 (AP-1) and early growth response 1 (EGR-1). Upon its activation, NF-kB triggers an upregulation of the inflammatory cascade by activating the release of specific proinflammatory cytokines [38]. Accumulation of inflammatory cytokines in hepatic cells then culminates in increased blood flow thereby resulting in liver inflammation ([38]. CCl_4-induced hepatic inflammation has also been suggested to be linked to an alteration in the transcriptional expression of IL-6/STAT3 genes. Other mechanisms underlying CCl_4-induced hepatic fibrosis has been reported [39]. The key feature of CCl_4-induced fibrosis in the liver is the accumulation of extracellular matrix. Exposure to CCl_4 increases tissue inhibitors of metalloproteinases (TIMP-1), activates transforming growth factor (TGF)-β and inhibits nitric oxide formation. The consequence of these events is hepatic steatosis, fibrosis and cirrhosis [39]. Sympathetic nervous system (SNS) can also play a key role in CCl_4 induced inflammatory responses that trigger hepatic injury. SNS has been found a culprit in the upregulation of ILs, monocyte chemoattractant protein − 1 (MCP-1/CCL2) and TNFα. These cytokines are the major inflammatory triggers of hepatic injury [39]. In the present study, CCl_4 must have caused the derangement in lipid profile via these mechanisms. Treatment with *M. pruriens* extract, caused a dose-dependent restoration of triglycerides, cholesterol, HDL and LDL back to a level comparable to animals treated with silymarin. Anti-inflammatory potentials of flavonoids and other polyphenols have been extensively studied [40]. Flavonoids act by down regulating inflammatory transcriptional factors via the activation of transcription factor-3, activator protein-1 and CREB binding proteins. Hepatoprotective effect of *M. pruriens* can be attributed to the activation of TNF-induced NF-kB inhibition by its polyphenols [39]. In the present study, rifampicin exposure caused a significant alteration in renal lipid profile of experimental animals. Rifampicin acts by enhancing renal lipid peroxidation as well as inhibiting key detoxification enzymes [40]. Biotransformation of rifampicin in the liver leads to formation of a reactive toxic intermediate, deacetyl rifampin, which binds to critical macromolecules causing liver injury [41,42]. Treatment with *M. pruriens* extract reversed the toxicity imposed on renal lipid profile in a dose dependent manner. This effect can be attributed to the inhibition of ROS production by the polyphenolic content of the extract.

Biomarker enzymes are important biochemical indicators of tissues functionality [43]. During organs damage, cellular enzymes such as AST, ALT, and ALP and bilirubin leak into the serum resulting in an elevation of their serum concentrations. Marked elevation in serum levels of ALP, AST and especially ALT are specific indicators of liver injury [44]. In the present study, there was a surge in serum level of ALT, AST and ALP following exposure to rifampicin and CCl_4. This indicated toxicant-induced hepatocellular damage [45]. Studies have shown that rifampicin-induced hepatotoxicity and nephrotoxicity involves the activation of pregnane X receptor (PXR). Activation of PXR triggers the upregulation of specific phase I and II enzymes such as GSTs, cytochrome P_{450} as well as ATP binding cassette (ABC) transporters [46]. Rifampicin has been suggested to induce the expression of hydrolases, and other enzymes, leading to an increased generation of hepatotoxic intermediates from isoniazid [46]. Similarly, rifampicin has been reported to contribute in no small measure to PXR-mediated derangement of heme biosynthesis resulting in the accumulation of hepatotoxic protoporphyrin IX [47]. Restoration of ALT, AST and ALP levels following *M. pruriens* treatment is indicative of its vast antioxidant potential. The restorative mechanism must involve inhibition of the activation of PXR receptor by polyphenolic component of the extract.

The devastating role of lipid peroxidation on human health has been reported [48]. Exposure to CCl_4 and rifampicin resulted in a significant increase in malonidialdehyde (MDA) content in the serum and tissue homogenates of experimental animals. CCl_4 has been identified with oxidative stress via inflammation. Biotransformation of CCl_4 via the cytochrome P_{450} enzyme system results in the generation of hepatotoxic intermediates such as trichloromethyl and trichloroperoxyl radicals. These metabolites triggers lipid peroxidation with the production of hepatotoxic aldehydes responsible for liver injury [48]. *M. pruriens* treatment inhibited lipid peroxidation in a dose-dependent manner with potency comparable to the standard drug, irrespective of the organ involved. This suggests that *M. pruriens* leaves extract is a potential therapeutic alternative in the management of multi-organ disorders.

Activities of antioxidant enzymes are routinely used to monitor the health status of experimental animals. Marked decrease in SOD, CAT, creatine kinase and GSH levels was observed in animals challenged with CCl_4 and rifampicin. In the liver, hepatotoxic metabolites of CCl_4 and rifampicin metabolism bind to critical functional groups of antioxidant enzymes leading to their inhibition [48]. Treatment of exposed animals with *M. pruriens* extract restored these parameters in the serum and selected organs. The restorative mechanism is suggested to involve prevention of the toxic metabolites of rifampicin and CCl_4, by polyphenols in the extract, from binding to critical groups on antioxidant enzymes.

Bilirubin, a product of hemoglobin degradation is usually excreted into the bile via conjugation involving UDP-glucuronyltransferase. Hence, an increase in bilirubin level in the blood is a marker of toxicity. Exposure of animals to CCl_4 and rifampicin respectively, caused a significant increase in serum bilirubin. However, treatment with *M. pruriens* extract, caused a dose-dependent restoration of bilirubin. This observation adduced to the therapeutic potentials of the plant in the management of liver and kidney disorders.

Urea has been used as a diagnostic tool to monitor the efficiency of dialysis procedure in patients with chronic kidney disease is urea [49]. Following exposure to CCl_4 and rifampicin, there was a significant increase in serum urea level of animals. This suggests a free radical-induced dysfunction in ultrafiltration leading to urea retention. However, when treated with graded doses of *M. pruriens*, serum urea level was restored, in a dose-dependent manner. This is an indication that *M. pruriens* offers a promising potential in the management of kidney diseases.

Uric acid has been suggested as a culprit in the formation of gout and intricately linked with renal insufficiency and several other diseases including type II diabetes [50]. Exposure to CCl_4 and rifampicin caused a significantly higher uric acid levels relative to the negative control. This may be related to free radical-induced up-regulation of purine metabolism. Treatment with *M. pruriens* extract restored the uric acid in the serum and organs homogenates to a level similar to the positive control animals. The restorative potential of *M. pruriens* was due to the prevention of an upregulation of purine catabolism by its phytochemicals.

Histopathological observation showed a distortion in the liver parenchymal histoarchitecture of animals administered with CCl_4. This distortion was due to oxidative damage by CCl_4. It manifested as severe hepatocellular necrotic vacuolation, cellular inflammation and congestion of central vein. These findings are in agreement with previous report on rodents [51]. Treatment with *M. pruriens* revealed a restoration of the hepatic histoarchitecture in a manner similar to silymarin-treated animals. Hence, *M. pruriens* protects against membrane permeability and alter drug-induced histopathological distortions. Similarly, exposure to rifampicin caused a severe distortion in the renal histoarchitecture. The onset progression and complications of kidney diseases has been linked to oxidative stress [52,53]. However, treatment with *M. pruriens* restored the renal histoarchitecture. This observation is consistent with earlier reports [54].

Conclusion

Biochemical and histopathological observations gave credence to the fact that *M. pruriens* is therapeutically potent. It can favorably compete with conventional drugs available for the treatment of liver and kidney diseases.

Declarations

All experimental protocols were approved by The Committee on Care and Use of Experimental Animals, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria.

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Authors declare that there is no conflict of interest of any kind

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Data availability

Data will be made available on request by the corresponding author.

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Tables

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Figures

Figures A-G were not provided with this version of the manuscript.

Figure 1

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