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# *Pergularia daemia* (Apocynaceae) mitigates rifampicin-induced hepato-renal injury: potentials in the management of liver and kidney diseases

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## ABSTRACT

Medicinal potentials of *Pergularia daemia* leaves in managing hepato-renal toxicity induced by rifampicin were investigated. Twenty-five (25) Wistar rats were randomly placed into five groups containing five animals each. All the animals, except group I, were orally exposed to 250 mg/kg bwt rifampicin and administered different treatments. Specific liver and kidney biomarkers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were determined. In addition, malondialdehyde (MDA), lipid profile, superoxide dismutase (SOD), catalase (CAT), as well as reduced glutathione (GSH) were determined in the serum, liver, and kidney homogenates of experimental animals. Results indicate that exposure to rifampicin caused significant depletion in SOD and CAT relative to the control animals. Lipid profile was deranged, while ALT, AST, ALP, urea, uric acid, bilirubin, creatine kinase, and MDA level were elevated by rifampicin exposure. All deranged biochemical indices, as well as distorted histoarchitecture, were restored dose-dependently after treatment with *P. daemia*. In conclusion, *P. daemia* ameliorated rifampicin toxicity on the liver and kidney as indicated in the restoration of all deranged biochemical and histopathological indices measured. Hence, it is a potential therapeutic agent that can be harnessed as panacea to the menace of liver and kidney diseases.

## 1. Introduction

Plants are enriched with phytochemicals that offer protection from stressors, thereby ensuring their survival within the ecosystem. These phytochemicals form the bulk of potential raw materials for rational drug synthesis in modern medicine (Muller et al., 2006). Over eighty percent of plants so far identified are rich in phytochemicals with therapeutic potentials to treat life-threatening diseases in humans. Unfortunately, the medicinal potential of these plants have not been fully exploited in treating human diseases (Krishnaiah et al., 2011). Hence, a deliberate research effort to investigate these plants for their potential benefits has been on top gear in recent times. The output of such research efforts could proffer a cheap therapeutic alternative for managing several pathological conditions, thereby strengthening the healthcare system (Jelkmann, 2001).

*Pergularia daemia* (Apocynaceae) is a plant popularly recognized by African traditional medical practitioners for its importance in treating several ailments (Chandak and Dighe, 2019). In folkloric medicine, *P. daemia* is useful in treating jaundice, aiding digestion, as an expectorant, analgesic, anticonvulsant, antiasthma, and antipyretic. Its root extract has been used to treat leprosy, mental derangement, anemia, and diarrhea (Chandak and Dighe, 2019).

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Oral administration of its leaf extract regulates menstruation and uterine function (Dosumu et al., 2019). In addition, reports suggest its potential antidiabetic, anticatarrhal, and antibronchitis properties (Chandak and Dighe, 2019). Its leaves contain active principles such as 4-4chlorobenzoyl-1-cyclohexoyl-5-tosylamino-1,H-123 zole, methyl ester pentadecoinic acid, 14-methyl-methyl ester ethyl 9-12-15-octodecotrionate, hexadecanoic acid, flavonoids, tannins, alkaloids, phenols and steroids (Chandak, 2010; Nithyatharani and Kavitha, 2018). Perhaps, these phytoconstituents are responsible for their medicinal relevance.

A bactericidal antibiotic drug, rifampicin is a semisynthetic component derived from streptomyces species. It is usually used as a first-line drug for treating tuberculosis globally (Eminzade et al., 2008). Drug-induced hepatotoxicity and nephrotoxicity are gradually assuming a global dimension in recent times. Specifically, reports have suggested that drug-induced hepatotoxicity is the leading cause of drug withdrawal or non-approval by the Food and Drug Administration (FDA) in the United States (US) (Kohli et al., 2000; Panich et al., 2012). Over a thousand drugs have predisposed their users to liver and kidney damage (Larrey, 2000; Biour et al., 2004; Ueno et al., 2002; Antonyuk et al., 2009). In all acute liver and kidney failures, 50% are caused by drugs administered in their management (Panich et al., 2012). Apart from its primary role as anti-tuberculosis, the metabolite of rifampicin metabolism has the potential to induce oxidative assault on the liver and kidney. Evidence suggests that rifampicin acts by causing derangement in heme biosynthesis, leading to the accumulation of hepatotoxic protoporphyrin (Yue-Ming et al., 2014).

Globally, the prevalence of liver and kidney diseases is a worrisome dimension of public health concern. Hence, a cheap but efficient therapeutic alternative must be sought to avoid further escalation. Considering the numerous medicinal relevance of *P. daemia* in folkloric medicine, it is necessary to investigate its hepatoprotective and nephroprotective potentials. This is the reason for the study.

## 2. Materials and methods

### *2.1. Plant materials*

*P. daemia* leaves were harvested from a farm within Ekiti State University, Campus, Iworoko Road, Ado Ekiti, in May 2021 and botanically identified at the Department of Plant Science, Ekiti State University, Ado Ekiti. Harvested leaves were rinsed with water, air-dried in the laboratory, pulverized, and then stored in an airtight container.

### *2.2. Reagents and chemicals*

All reagents and chemicals were of high analytical grade. All diagnostic kits used were products of Randox Chemical Ltd. England.

### *2.3. Extraction of the plant material*

*P. daemia* leaves were air-dried for 24 days and pulverized. One hundred and seventy-three (173) g of the powdered leaves were extracted in 1000 ml of distilled water for 72 hours. It was then filtered using cheesecloth and freeze-dried to obtain the dried extract. The extract was kept in a closed container and refrigerated at 4 °C for further studies.

### *2.4. Animals protocol*

Twenty-five (25) Wistar albino rats weighing 150 – 170 g were obtained from The Animal House, 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, and 12/12-hour light and dark cycle. They were given unrestricted access to food (commercial palletized diet from Vital Feed Mill) and drinking water ad libitum. Beddings for experimental rats were routinely changed and replaced every day throughout the experimental period (Table 1).

**Table 1.** Animal treatment/experimental design

| Groups | Treatment |
|---|---|
| I | Distilled water only |
| II | Rifampicin (250 mg/kg bwt) only |
| III | Rifampicin (250 mg/kg bwt) + *P. daemia* (50 mg/kg bwt) |
| IV | Rifampicin (250 mg/kg bwt) + *P. daemia* (100 mg/kg bwt) |
| V | Rifampicin (250 mg/kg body weight) + silymarin (100 mg/kg bwt |

bwt: body weight

### *2.5. Dissection of rats*

Experimental rats were decapitated and dissected after very mild ether anesthesia to obtain the liver and kidney. Blood was collected by cardiac puncture into EDTA sample bottles and allowed to stand for 1 hour. Serum was obtained by centrifugation at 3000 rpm for 15 min at 25 °C. The clear supernatant (serum) was collected and used to estimate serum biochemical parameters.

### *2.6. Preparation of homogenates*

Liver and kidney tissues were trimmed of fat, washed in distilled water, blotted with filter paper, and weighed. The kidney and liver were homogenized separately in ten volumes of the homogenizing phosphate buffer (pH 7.4) using a Teflon homogenizer. The resulting homogenates were centrifuged separately at 3000 rpm at 4 °C for 30 min. The supernatant obtained was stored in a refrigerator for further biochemical analyses.

### *2.7. Serum enzyme biomarkers*

#### *2.7.1. Creatine kinase (Ck-Mb) activity*

The level of creatine kinase was determined according to Vanderlinde (1981). One milliliter (1 ml) each of imidazole buffer (10 mM, pH 6.6), creatine phosphate (30 mM), glucose (20 mM), N-acetyl-cysteine (20 mM), magnesium acetate (10 mM), ethylenediaminetetraacetic 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 thermostatic cuvette. The mixture was then incubated at 37 °C, after which 50 µl of serum was added. The reaction components were thoroughly mixed, and absorbance was read immediately for 5 min at 30-sec intervals at a wavelength of 340 nm. Change in absorbance per minute was calculated (ΔAbs/min).

#### *2.7.2. Assay of aspartate aminotransferase (AST)*

The activity of AST in the serum and organs homogenates was determined as described by Reitman and Frankel (1957). One hundred microliters of serum and organ homogenates were mixed separately with phosphate buffer (100 mmol/l, pH 7.4), L-aspartate (100 mmol/l), and α-oxoglutarate (2 mmol/l). The mixture was incubated for exactly 30 min at 37 °C. 0.5 ml of 2,4-dinitrophenyl

hydrazine (2 mmol/l) was added to the reaction mixture and allowed to stand for 20min at 25 °C. After that, 5.0 ml of NaOH (0.4 mol/l) was added, and absorbance was read at 546 nm against the reagent blank after 5 min.

#### *2.7.3. Assay of alanine aminotransferase (ALT) activity*

The principle described by Reitman and Frankel (1957) was followed in the assay of ALT using a commercially available assay kit (Randox Laboratories, UK) according to the manufacturer's instructions. Reagent 1 (0.5 ml) containing phosphate buffer (100 mmol/l, pH 7.4), L-alanine (200 mmol/l), and α-oxoglutarate (2.0 mol/l) was added to 0.1 ml of serum and organs homogenates in a separate test tube, and the mixture was incubated at 37 °C for 30 min. Exactly 0.5 ml of reagent 2 (R2) containing 2, 4-dinitrophenylhydrazine (2.0 mmol/l) was added, and the solution and re-incubated at 20 °C for 20 min. Finally, 5 ml of NaOH was added, and the solution was allowed to stand for 5 min at 25 °C, and its absorbance was read at 546 nm.

#### *2.7.4. Assay of alkaline phosphatase (ALP) activity*

Assay of serum ALP was performed by the method of Englehardt (1970) using commercial assay kits (Randox Laboratories, UK) according to the instructions of the manufacturer. Exactly 1.0 ml of the reagent (1 mol/l diethanolamine buffer pH 9.8, 0.5 mmol/l $MgCl_2$; substrate: 10 mmol/l *p*-nitrophenol phosphate) was added to 0.02 ml of the serum sample and mixed. Absorbance was taken at 405 nm for 3 min at 1 min intervals.

### *2.8. Serum lipid profile analysis*

#### *2.8.1. Estimation of total cholesterol*

Total cholesterol level was determined based on the method of Trinder (1969) using commercially available kits (Randox Laboratories, UK). Ten microliters (10 µl) of standard and 10 µl serum samples were pipetted into labeled test tubes. One milliliter (1 ml) of working reagent containing; pipes buffer (80 mmol/l at pH 6.8), 4-amino antipyrine (0.25 mmol/l), phenol (6 mmol/l), 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 reaction mixtures were mixed thoroughly and incubated for 10 minutes. The absorbance of the sample ($A_{sample}$) was read at 500 nm against the reagent blank.

#### *2.8.2. Evaluation of concentration of triglyceride*

Triglycerides level in the serum and organs' homogenates was determined based on Tietz's (1995) method using commercially available kits (Randox Laboratories, UK). Triglyceride standard (10 µl) and serum (10 µl) were pipetted into labeled test tubes. One milliliter of the working reagents; R1a (buffer) containing pipes buffer (40mmol/l, pH 7.6), 4-chloro-phenol (5.5 mmol/l), magnesium-ion (17.5 mmol/l); R1b [(enzyme reagent containing 4-amino phenazone (0.5 mmol/l), ATP (1.0 mmol/l), lipase (≥ 150 U/ml), glycerol-kinase (≥ 0.4 U/ml), glycerol-3-phosphate oxidase (≥ 1.5 U/ml) and peroxidase (≥ 0.5 U/ml)] was added into all the tubes. The reaction components were thoroughly mixed and incubated for 10 min at room temperature. Absorbance was read at 546 nm against the blank.

#### *2.8.3. High-density lipoprotein (HDL-c)-cholesterol assay*

The method of Grove (1979) was adopted in estimating HDL-cholesterol in the serum. The precipitation reaction mixture contained 200 µl of the serum, 200 µl of the cholesterol standard, and 500 µl of the diluted precipitant R1 (0.55 mM phosphotungstic acid, 25 mM magnesium chloride) was mixed 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 mixture was decanted within 2 h to determine the cholesterol content by the CHOD-PAP reaction method.

##### *2.8.3.1. Cholesterol CHOD-PAP assay*

One hundred microliters (100 µl) of the sample supernatant was added to 1 ml of cholesterol reagent and mixed 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. The absorbance of the sample ($A_{sample}$) and standard ($A_{standard}$) was then measured at 500 nm against the reagent blank within 1 h.

#### *2.8.4. Low-density lipoprotein (LDL) - cholesterol*

The concentration of low-density lipoprotein in the serum was calculated using the formula of Friedewald et al. (1972) as given below:

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

### *2.9. Antioxidant assay*

#### *2.9.1. Determination of catalase activity*

Catalase activity was determined using the method described by Sinha (1972). Two hundred microliter each of the serum, liver, and kidney homogenates was mixed separately with 0.8 ml distilled water to give 1 in 5 dilutions of the sample. The assay mixture contained 2 ml of hydrogen peroxide solution (800 µmol) and 2.5 ml of phosphate buffer in a 10 ml flat bottom flask. Properly diluted enzyme preparation (0.5 ml) was rapidly mixed with the reaction mixture by a gentle swirling motion. The reaction was run at room temperature. A 1 ml portion of the reaction mixture was withdrawn and blown into a 1 ml dichromate/acetic acid reagent at 60 s intervals. The hydrogen peroxide content of the withdrawn sample was determined by the method described below.

Catalase activity =

$H_2O_2$ consumed = 800 – Concentration of $H_2O_2$ remaining

The concentration of $H_2O_2$ remaining was extrapolated from the standard curve for catalase activity

#### *2.9.2. Determination of superoxide dismutase (SOD)*

Superoxide dismutase activity was determined by the method of Misra and Fridovich (1972). One milliliter of serum, liver, and kidney homogenates was diluted separately in 9 ml of distilled water to make a 1 in 10 dilutions. An aliquot of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer. The reaction was initiated by adding 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 the substrate (adrenaline), and 0.2 ml of water. An increase in absorbance at 480 nm was monitored every 30 s for 150 s.

### 2.9.3. Determination of reduced glutathione (GSH) level

The amount of GSH was determined using the method of Beutler et al. (1963). Exactly 0.2 ml of serum, liver, and kidney was added to 1.8 ml of distilled water, followed by the addition of 3 ml of precipitating solution. The resulting solution was then mixed thoroughly, allowed to stand for 5 minutes, and filtered. One milliliter (1 ml) of the filtrate was added to 4 ml of 0.1 M phosphate buffer pH 7.4. Finally, 0.5 ml of Ellman reagent was added. A blank was prepared with 4 ml of the 0.1 M phosphate buffer, 1 ml of diluted precipitating solution (3 parts to 2 parts of distilled water), and 0.5 ml of the Ellman reagent. The absorbance of the resulting mixture was measured at 412 nm against a reagent blank.

### 2.9.4. Determination of total protein (TP) in serum

The amount of protein in the serum, liver, and kidney homogenates was measured according to the method of Weichselbaum (1946) using commercially available kits (Randox Laboratories, UK). One milliliter (1 ml) of Reagent R1 containing sodium hydroxide (100 mmol/l), Na-K-tartrate (18 mmol/l), potassium iodide (15 mmol/l), and cupric sulfate (6 mmol/l) was added to 0.02 ml of the serum sample. The mixture was incubated at 25 °C, and its absorbance was measured against the reagent blank at a wavelength of 546 nm.

**Table 2a.** Effects of aqueous extract of *P. daemia* on selected biomarkers of liver function in rifampicin-exposed rats

| Parameter | I | II | III | IV | V |
|---|---|---|---|---|---|
| ALT (U/l) | 40.71 ± 1.23$^a$ | 77.23 ± 1.42$^b$ | 69.23 ± 1.07$^b$ | 57.08 ± 1.00$^a$ | 51.33 ± 1.27$^a$ |
| AST (U/l) | 54.33 ± 1.71$^a$ | 96.14 ± 2.41$^b$ | 78.33 ± 1.88$^a$ | 62.54 ± 2.02$^a$ | 57.23 ± 1.72$^a$ |
| ALP (U/l) | 47.25 ± 0.72$^a$ | 73.84 ± 0.81$^b$ | 71.23 ± 0.61$^a$ | 57.07 ± 0.53$^a$ | 46.88 ± 1.39$^a$ |
| T. BIL (mg/dl) | 31.18 ± 0.67$^a$ | 56.18 ± 0.54$^b$ | 47.32 ± 0.30$^a$ | 40.20 ± 0.60$^a$ | 31.72 ± 3.82$^a$ |

**Table 2b.** Effects of aqueous extract of *P. daemia* on selected biomarkers of kidney function in rifampicin-exposed rats

| Parameter | I | II | III | IV | V |
|---|---|---|---|---|---|
| ALT (U/l) | 17.27 ± 2.13$^a$ | 40.29 ± 1.77$^b$ | 33.44 ± 1.40$^b$ | 26.42 ± 0.89$^a$ | 23.63 ± 1.03$^a$ |
| AST (U/l) | 21.60 ± 1.04$^a$ | 56.82 ± 1.73$^b$ | 39.22 ± 1.06$^a$ | 22.50 ± 1.31$^a$ | 25.06 ± 1.25$^a$ |
| ALP (U/l) | 31.21 ± 1.65$^a$ | 53.66 ± 1.98$^b$ | 41.86 ± 0.75$^a$ | 32.86 ± 0.65$^a$ | 29.32 ± 0.90$^a$ |
| Urea (mg/dl) | 47.82 ± 0.67$^a$ | 70.06 ± 0.83$^b$ | 62.08 ± 0.43$^a$ | 50.20 ± 0.52$^a$ | 53.76 ± 1.61$^a$ |
| U. acid (mg/dl) | 29.54 ± 0.26$^a$ | 54.23 ± 0.78$^b$ | 39.57 ± 0.55$^a$ | 28.32 ± 0.41$^a$ | 32.89 ± 1.62$^a$ |
| C. K. (U/l) | 27.61 ± 0.83$^a$ | 49.17 ± 0.94$^b$ | 36.42 ± 0.85$^a$ | 27.00 ± 0.53$^a$ | 25.73 ± 0.85$^a$ |

**Table 2c.** Effects of aqueous extract of *P. daemia* on selected serum biomarkers of liver and kidney function in rifampicin-exposed rats

| Parameter | I | II | III | IV | V |
|---|---|---|---|---|---|
| ALT (U/l) | 56.17 ± 3.04$^a$ | 88.45 ± 2.43$^b$ | 60.23 ± 2.63$^a$ | 55.13 ± 2.09$^a$ | 59.33 ± 1.34$^a$ |
| AST (U/l) | 71.26 ± 1.71$^a$ | 127.13 ± 2.60$^b$ | 96.51 ± 1.54$^a$ | 70.48 ± 1.60$^a$ | 75.22 ± 1.56$^a$ |
| ALP (U/l) | 62.13 ± 1.32$^a$ | 108.52 ± 1.32$^b$ | 75.39 ± 1.31$^a$ | 68.18 ± 1.53$^a$ | 67.11 ± 2.09$^a$ |
| Urea (mg/dl) | 38.77 ± 0.66$^a$ | 64.39 ± 0.62$^b$ | 54.20 ± 0.70$^a$ | 37.21 ± 0.65$^a$ | 45.26 ± 0.76$^a$ |
| U. acid (mg/dl) | 19.17 ± 0.43$^a$ | 35.43 ± 0.52$^b$ | 31.02 ± 0.34$^a$ | 24.88 ± 0.41$^a$ | 21.17 ± 0.68$^a$ |
| C.K. (U/l) | 20.33 ± 0.37$^a$ | 44.58 ± 0.44$^b$ | 33.41 ± 1.01$^a$ | 25.11 ± 0.58$^a$ | 23.66 ± 0.22$^a$ |
| T.BIL (mg/dl) | 42.25 ± 0.64$^a$ | 69.30 ± 1.02$^b$ | 50.26 ± 0.55$^a$ | 41.31 ± 0.86$^a$ | 45.37 ± 1.84$^a$ |

Data represent the mean ± SEM of the experiment performed in triplicate. I: Administered water only, II: Rats exposed to rifampicin (250 mg/kg bwt) only, III: Rats treated with *P. daemia* at 50 mg/kg bwt after exposure to rifampicin, IV: Rats treated with *P. daemia* at 100 mg/kg bwt after exposure to rifampicin, V: Rats treated with silymarin at 100 mg/kg bwt after exposure. 'b' represents a significant difference from the control 'a' at $p = 0.05$.

### 2.10. Statistical analysis

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

## 3. Results and discussion

Exposure of experimental rats to rifampicin at 250 mg/kg bwt caused a significant increase in hepatic and renal alanine aminotransferase, aspartate aminotransferase, and alkaline phosphate. Treatment of exposed rats with *P. daemia* leaf extract restored these biomarkers to the basal dose-dependent manner similar to animals treated with silymarin. The same trend was observed for urea, uric acid, total bilirubin, and creatine kinase in the serum, liver, and kidney homogenates (Table 2a-c). Similarly, oral administration of rifampicin at 250 mg/kg bwt resulted in marked derangement in lipid profile relative to the control. This caused a surge in cholesterol, triglyceride, and low-density lipoprotein, as well as marked depletion in high-density lipoprotein (HDL) in the liver, kidney, and serum (Table 3a-c). Treatment with *P. daemia* dose-dependently restored the deranged lipid profile to a level comparable with animals not exposed to rifampicin. Moreover, catalase and superoxide dismutase activities in the serum, kidney, and liver were significantly inhibited following exposure to rifampicin. Reduced glutathione was markedly diminished in the serum, and organs homogenated after exposure to rifampicin. However, treatment with *P. daemia* relieved the inhibition imposed on the enzymes and restored GSH to a level comparable to the control animals (Table 4a-c). Finally, there was an observed sharp increase in MDA levels in the liver and kidney of experimental animals following rifampicin exposure (Figure 1). This was brought back to normal dose-dependent when intoxicated animals were treated with *P. daemia*.

In recent times, the prevalence of kidney and liver diseases poses a serious threat to public health and must be addressed urgently. Developing nations, in particular, should pay attention to finding a cheap, locally available, and potent therapeutic alternative in the management of these diseases. Up to 40% of liver and kidney failure are traceable to side effects of conventional drugs administered in

the management of ailments (Kosanam and Boyina, 2015). Specifically, hepato-renal injuries have been linked to oxidative assaults caused by specific bioactivated intermediates produced in biotransformation (Sharma and Sharma, 2015; Basheer et al., 2017). The use of plants for medication is an age-long practice that has proven effective (Sentman et al., 2006; Abirami et al., 2014).

In the present study, there was a significant increase in AST, ALT, and ALP levels in the serum and organs' homogenates following exposure to rifampicin (Table 2a-c). Obviously, this is due to free radicals-induced leakage of these enzymes from their initial cellular compartments into the bloodstream. The leakage must have resulted from a compromise in membrane integrity via lipid peroxidation, as Rana et al. (2006) and Kim et al. (2017) suggested. Treatment with *P. daemia* extract reversed the surge in the level of these biomarker enzymes, perhaps via inhibition of lipid peroxidation. As a result, membrane integrity must have been restored, thereby blocking further leakage of these enzymes into the bloodstream. This healing ability of *P. daemia* extract can be attributed to its antioxidant potential, as earlier reported (Vaithiyanathan and Mirunalini, 2016).

**Table 3a.** Effects of aqueous extract of *P. daemia* on hepatic lipid profile of rifampicin-exposed rats

| Parameter | I | II | III | IV | V |
|---|---|---|---|---|---|
| CHOL (mg/dl) | 56.08 ± 1.86$^a$ | 97.23 ± 1.42$^b$ | 71.13 ± 1.01$^a$ | 64.43 ± 1.08$^a$ | 52.33 ± 1.34$^a$ |
| TRIG (mg/dl) | 41.33 ± 0.76$^a$ | 86.50 ± 0.42$^b$ | 69.26 ± 0.59$^a$ | 52.09 ± 0.94$^a$ | 39.43 ± 1.13$^a$ |
| HDL (mg/dl) | 24.72 ± 0.22$^a$ | 18.26 ± 0.24$^b$ | 18.76 ± 0.51$^a$ | 21.33 ± 0.33$^a$ | 26.31 ± 0.59$^a$ |
| LDL (mg/dl) | 33.76 ± 0.87$^a$ | 57.81 ± 0.11$^b$ | 51.25 ± 0.06$^a$ | 40.42 ± 0.15$^a$ | 33.04 ± 0.62$^a$ |

**Table 3b.** Effects of aqueous extract of *P. daemia* on renal lipid profile of rifampicin-exposed rats

| Parameter | I | II | III | IV | V |
|---|---|---|---|---|---|
| CHOL (mg/dl) | 30.07 ± 1.05$^a$ | 53.24 ± 1.03$^b$ | 47.23 ± 1.07$^b$ | 36.27 ± 0.76$^a$ | 38.63 ± 2.23$^a$ |
| TRIG (mg/dl) | 11.82 ± 0.14$^a$ | 26.48 ± 0.33$^b$ | 23.08 ± 0.49$^b$ | 17.80 ± 0.24$^a$ | 13.43 ± 0.29$^a$ |
| HDL (mg/dl) | 8.26 ± 0.95$^a$ | 6.08 ± 0.63$^b$ | 6.45 ± 0.56$^a$ | 7.03 ± 0.34$^a$ | 8.42 ± 0.68$^a$ |
| LDL (mg/dl) | 13.26 ± 0.77$^a$ | 31.27 ± 0.57$^b$ | 26.80 ± 0.38$^b$ | 16.29 ± 0.40$^a$ | 12.13 ± 0.48$^a$ |

**Table 3c.** Effects of aqueous extract of *P. daemia* on serum lipid profile of rifampicin-exposed rats

| Parameter | I | II | III | IV | V |
|---|---|---|---|---|---|
| CHOL (mg/dl) | 52.16 ± 1.14$^a$ | 87.46 ± 1.38$^b$ | 73.23 ± 1.05$^a$ | 66.20 ± 1.73$^a$ | 58.27 ± 1.56$^a$ |
| TRIG (mg/dl) | 37.51 ± 1.61$^a$ | 70.39 ± 1.45$^b$ | 63.71 ± 0.55$^a$ | 48.08 ± 1.09$^a$ | 40.14 ± 1.72$^a$ |
| HDL (mg/dl) | 9.42 ± 0.02$^a$ | 5.10 ± 0.07$^b$ | 5.63 ± 0.05$^a$ | 6.47 ± 0.01$^a$ | 8.79 ± 0.29$^a$ |
| LDL (mg/dl) | 22.36 ± 0.72$^a$ | 39.53 ± 0.68$^b$ | 34.48 ± 0.72$^b$ | 29.22 ± 0.63$^a$ | 24.67 ± 0.72$^a$ |

Data represent the mean ± SEM of the experiment performed in triplicate. I: Administered water only, II: Rats exposed to rifampicin (250 mg/kg bwt) only, III: Rats treated with *P. daemia* at 50 mg/kg bwt after exposure to rifampicin, IV: Rats treated with *P. daemia* at 100 mg/kg bw after exposure to rifampicin, V: Rats treated with silymarin at 100 mg/kg bwt after exposure. 'b' represents a significant difference from the control 'a' at $p = 0.05$.

**Table 4a.** Effects of aqueous extract of *P. daemia* on selected enzymic and non-enzymic antioxidant parameters in the liver of rifampicin-exposed rats

| Parameter | I | II | III | IV | V |
|---|---|---|---|---|---|
| SOD (U/mg protein) | 6.14 ± 0.14$^a$ | 2.36 ± 0.19$^b$ | 3.81 ± 0.12$^a$ | 5.92 ± 0.10$^a$ | 6.20 ± 1.23$^a$ |
| CAT (µmol/min) | 4.70 ± 0.13$^a$ | 1.63 ± 0.16$^b$ | 2.74 ± 0.22$^a$ | 3.82 ± 0.14$^a$ | 4.45 ± 1.20$^a$ |
| GSH (mmol) | 5.77 ± 0.03$^a$ | 2.93 ± 0.08$^b$ | 3.80 ± 0.05$^a$ | 4.63 ± 0.10$^a$ | 5.43 ± 0.08$^a$ |
| T. P (mg/ml) | 2.64 ± 0.13$^a$ | 1.04 ± 0.09$^b$ | 1.93 ± 0.10$^a$ | 2.45 ± 0.13$^a$ | 2.33 ± 0.78$^a$ |

**Table 4b.** Effects of aqueous extract of *P. daemia* on selected enzymic and non-enzymic antioxidant parameters in the kidney of rifampicin-exposed rats

| Parameter | I | II | III | IV | V |
|---|---|---|---|---|---|
| SOD (U/mg protein) | 3.48 ± 0.27$^a$ | 1.93 ± 0.19$^b$ | 2.63 ± 0.11$^a$ | 3.15 ± 0.13$^a$ | 3.15 ± 0.53$^a$ |
| CAT (µmol/min) | 2.77 ± 0.10$^a$ | 1.88 ± 0.26$^b$ | 2.04 ± 0.10$^a$ | 2.82 ± 0.16$^a$ | 2.58 ± 0.20$^a$ |
| GSH (mmol) | 3.23 ± 0.03$^a$ | 3.04 ± 0.02$^b$ | 3.17 ± 0.07$^a$ | 3.30 ± 0.04$^a$ | 3.19 ± 0.12$^a$ |
| T. P (mg/ml) | 1.73 ± 0.01$^a$ | 1.06 ± 0.01$^b$ | 1.24 ± 0.05$^a$ | 1.56 ± 0.07$^a$ | 1.44 ± 0.07$^a$ |

**Table 4c.** Effects of aqueous extract of *P. daemia* on selected enzymic and non-enzymic antioxidant parameters in the serum of rifampicin-exposed rats

| Parameter | I | II | III | IV | V |
|---|---|---|---|---|---|
| SOD (U/mg protein) | 4.29 ± 0.09$^a$ | 2.42 ± 0.07$^b$ | 3.61 ± 0.03$^a$ | 4.09 ± 0.07$^a$ | 4.34 ± 0.21$^a$ |
| CAT (µmol/min) | 3.06 ± 0.12$^a$ | 1.76 ± 0.14$^b$ | 2.39 ± 0.08$^a$ | 3.12 ± 0.10$^a$ | 2.93 ± 0.20$^a$ |
| GSH (mmol) | 2.66 ± 0.01$^a$ | 2.08 ± 0.02$^b$ | 2.26 ± 0.06$^a$ | 2.47 ± 0.07$^a$ | 2.49 ± 0.98$^a$ |
| T. P (mg/ml) | 2.08 ± 0.20$^a$ | 1.02 ± 0.18$^b$ | 1.39 ± 0.13$^a$ | 1.87 ± 0.14$^a$ | 2.17 ± 0.08$^a$ |

Data represent the mean ± SEM of the experiment performed in triplicate. I: Administered water only, II: Rats exposed to rifampicin (250 mg/kg bwt) only, III: Rats treated with *P. daemia* at 50 mg/kg bwt after exposure to rifampicin, IV: Rats treated with *P. daemia* at 100 mg/kg bwt after exposure to rifampicin, V: Rats treated with silymarin at 100 mg/kg bwt after exposure. 'b' represents a significant difference from the control 'a' at $p = 0.05$.

Serum bilirubin level was significantly elevated in the present study following exposure of experimental animals to rifampicin (Table 2a-c). It has been reported that rifampicin hinders bilirubin uptake, resulting in a subclinical level of unconjugated hyperbilirubinemia. Similarly, a blockage of the exporter pump specific for exporting bile could result in conjugated hyperbilirubinemia (Saukkonen et al.,

2006; Byrne et al. ,2002). Other reports also indicate that deficient bilirubin clearance or inefficient secretion at the canalicular level may trigger hyperbilirubinemia (Grosset and Leventis, 1983; Capelle et al., 1972). Treatment with *P. daemia* extract restored the bilirubin level to that comparable with animals not exposed at all. This is due to the antioxidant phytochemicals present in the extract, as earlier reported (Maheshwari and Vijayarengan, 2021).

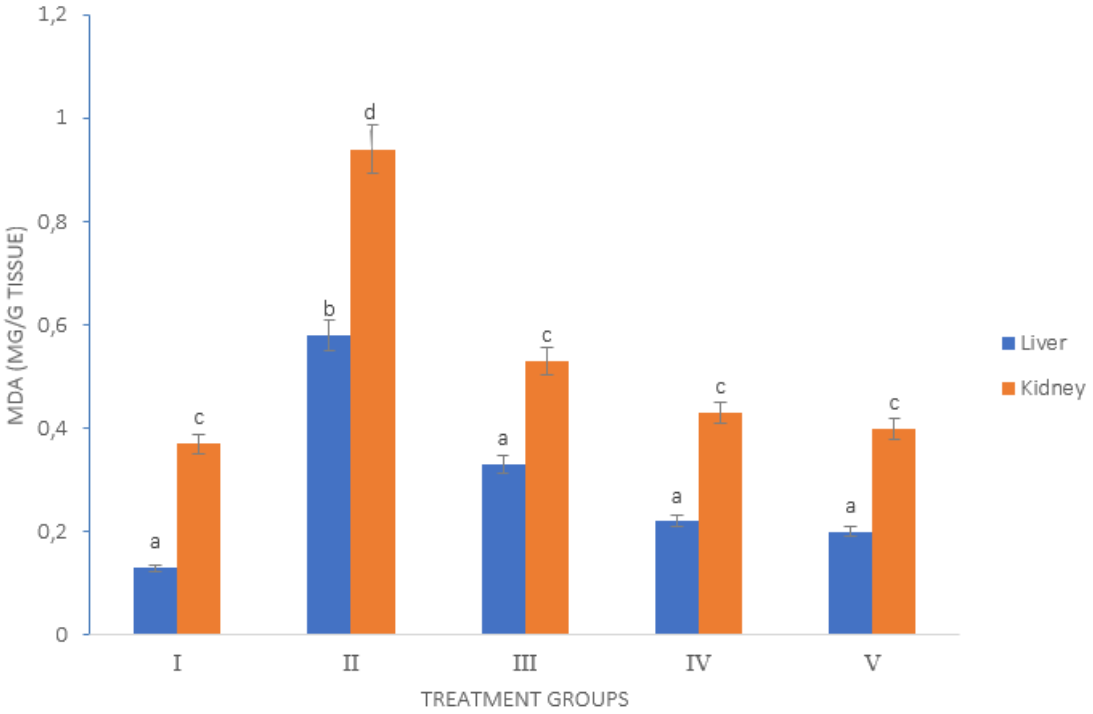


**Figure 1.** Effect of *P. daemia* on lipid peroxidation in the liver and kidney of rifampicin-exposed rats

Data represent the mean ± SEM of the experiment performed in triplicate. I: Administered water only, II: Rats exposed to rifampicin (250 mg/kg bw) only, III: Rats treated with *P. daemia* at 50 mg/kg bw after exposure to rifampicin, IV: Rats treated with *P. daemia* at 100 mg/kg bw after exposure to rifampicin, V: Rats treated with silymarin at 100 mg/kg bw after exposure. 'c' and 'd' represent a significant difference from the control 'a' and 'b', respectively at $p = 0.05$.

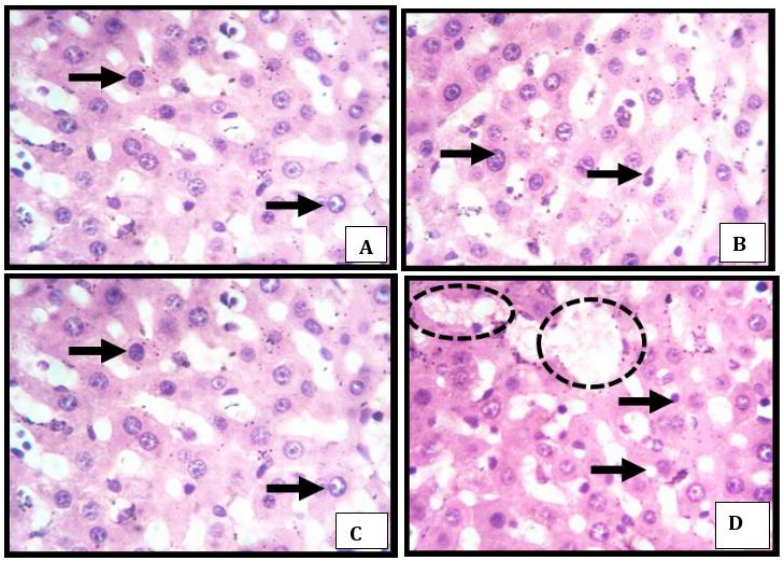


**Figure 2.** Histoarchitecture of the liver of experimental animals at a magnification (x400).

Black arrows represent inherent hepatocytes. Dotted black circles represent large vacuolation, while pink colorations represent fatty liver and bile plaques.

A: Liver of animals not exposed to rifampicin at all: It shows no histological distortion of the tissues. Liver histomorphology was normal, with hepatocytic nuclei well positioned in the cytoplasm.

B: The liver of animals exposed to rifampicin at 250 mg/kg bw without treatment showed cholestatic fatty liver.

C: The liver of animals exposed to rifampicin and treated with 100mg/kg bw of *P. daemia* showed unperturbed liver histomorphology with no histopathological distortion.

D: Liver of animals exposed to rifampicin and treated with silymarin at 100mg/kg bw showed intact liver tissue histomorphology with no histopathological distortion.

Serum level of creatine kinase, urea, and uric acid has been used as a diagnostic tool for measuring kidney integrity. In the present study, creatine kinase, urea, and uric acid increased significantly relative to control animals following exposure to rifampicin. This

observation suggests a free radical-induced upregulation in nitrogen metabolism. Consequently, there was an increase in the formation of urea and uric acid since the functional integrity of the glomerulus has been altered as a result of the oxidative attack on the kidney. These observations are consistent with the report of (Jaswal et al., 2013; Shukla et al., 2014). Treatment with graded doses of *P. daemia* caused restoration of creatine kinase, urea, and uric acid in a dose-dependent manner. The observed restoration can be linked to flavonoids and other antioxidant phytochemicals present in the extract, as earlier reported (Bhusari et al., 2018). It is also consistent with other reports on hepatoprotection (Renugadevi and Prabu, 2010; Renugadevi and Prabu, 2009).

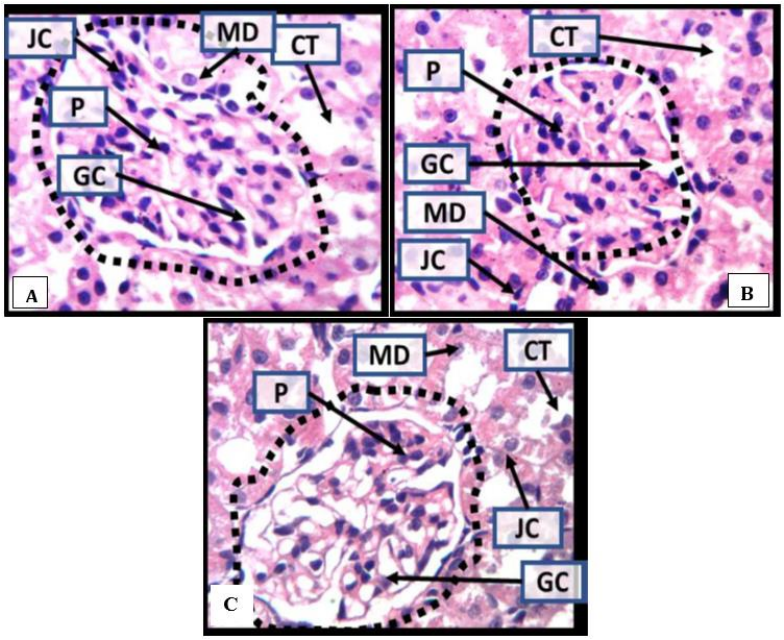


**Figure 3.** Histoarchitecture of the kidney tissue slice of experimental animals at a magnification (x400).
The black outline represents the renal corpuscle containing the glomerulus within the urinary space. Convoluted tubule (CT), glomerular capillaries (GC), and innate cells, including the intraglomerular podocytes (P) as well as the juxtaglomerular and macula densa cells.
A: Kidney slices of animals not exposed to rifampicin toxicity showed no noticeable histological distortion while the renal histoarchitecture was intact.
B: Kidney slice of animals exposed to rifampicin toxicity without treatment showed distorted renal histoarchitecture as well as glomerular atrophy. Degenerated renal tubules as well as intraluminal exfoliation, including nuclei pyknosis.
C: Kidney slice of animals exposed to rifampicin toxicity and treated with *P. daemia* at 100 mg/kg bw showed intact renal corpuscle having cellular delineation and appropriate distribution. There was no noticeable histopathological distortion.

Administration of rifampicin caused a significant derangement in lipid profile in experimental animals' liver, kidneys, and serum. Total cholesterol, triglycerides, and low-density lipoprotein (LDL) were markedly increased relative to control animals following exposure to rifampicin (Table 3a-c). This observation is in tandem with Tasduq et al. (2007), who showed that serum levels of triglyceride and cholesterol were significantly increased after administration of rifampicin to experimental rats. It also agrees with the earlier report of Santhosh et al. (2006), who observed a significant increase in serum LDL with a concomitant decrease in HDL-cholesterol following exposure of rats to rifampicin and isoniazid. When exposed animals were treated with graded doses of *P daemia*, there was a dose-dependent reversal of the toxicity imposed by rifampicin. In line with an earlier report, this reversal can be traced to antioxidant phytochemicals such as flavonoids and other polyphenols in the extract (Vaithiyanathan, 2015).

Moreover, rifampicin caused a significant decrease in the activities of SOD and CAT in the serum, liver, and kidney of experimental rats (Table 4a-c). Unarguably, SOD and CAT are the first lines of antioxidant enzymes' defense against oxidative stress. SOD is responsible for the catalytic dismutation of superoxide radicals to hydrogen peroxide. On the other hand, CAT is responsible for the catalytic decomposition of hydrogen peroxide into molecular oxygen and water (Lee and Sherman, 2000). Earlier reports suggested a decrease in antioxidant enzyme activity coupled with an increase in lipid peroxidation level after rifampicin intoxication (Sedlak and Snyder, 2003, Heit et al., 2017; Muller et al., 2006). Damage to renal tissue observed in the present study may be attributed to lipid peroxidation and inhibition of renal antioxidant enzymes following exposure to rifampicin.

Antioxidant capacity against reactive oxygen species is intrinsically linked to the enzymatic activity of SOD and catalase (Espinosa-Diez et al., 2015). Results from the present study indicate that rifampicin triggered a marked inhibition of SOD and CAT activity. This is probably due to the formation of potentially toxic intermediates. Inhibition of these enzymes led to an increase in the formation of superoxide anion radicals, eventually inactivating catalase and other hydrogen-peroxide-dependent enzymes (Naik and Panda, 2008). Oral administration of *P. daemia* leaf extracts markedly restored SOD and CAT activities. This observation can be linked to the free radical scavenging potentials of *P. daemia* due to flavonoids and other antioxidant phytochemicals in its leaf extract (Vaithiyanathan and Mirunalini, 2016). This observation is consistent with previous reports on *Solanum xanthocarpum* (Verma et al., 2015).

Reduced glutathione is a sulfhydryl tripeptide with potent antioxidant properties. Exposure to rifampicin caused a significant depletion in the GSH level. This observation partly suggests that the liver of exposed animals was vulnerable to free radicals attack caused by rifampicin. This also indicates the pivotal role of GSH in

cellular antioxidant defense. This observation is in agreement with Brehe and Burch (1976). Treatment with *P. daemia* led to an increase in GSH level, suggesting restoring antioxidant status. In agreement with an earlier report, this observation can be attributed to the antioxidant effect of the myriad of phytochemicals present in the leaf extract (Mohammed et al., 2004).

The significant decrease in the total protein of rifampicin-exposed rats agrees with Santhosh et al. (2006) and Eminzade et al. (2008). This observation could imply that toxic intermediates of rifampicin metabolism attacked the hepatocytes, thereby hampering the liver's ability to synthesize proteins such as albumin. Treatment with *P. daemia* significantly restored the protein level in a dose-dependent fashion. This observation agrees with Maheshwari and Vijayarengan (2021) and can be attributed to the antioxidant effect of phytochemicals present in the extract.

Lipid peroxidation was increased in the serum and organ homogenates of experimental rats exposed to rifampicin (Figure 1). This agrees with the report of Basheer et al. (2017), where administration of anti-tuberculosis drugs caused increased lipid peroxidation and the corresponding decrease in GSH levels. Perhaps, reactive intermediates of rifampicin metabolism bind covalently to critical macromolecules that overwhelm the endogenous antioxidant capacity, thereby triggering peroxidation of membrane lipids (Naik and Panda, 2008; Balakrishnan et al., 2012). The significant increase in lipid peroxidation suggests a compromise of the body's endogenous antioxidant capacity (Jaydeokar et al., 2014). The oral intervention of *P. daemia* leaf extract diminished the lipid peroxidation and restored the antioxidant status of the animals. Obviously, this is due to the array of polyphenols present in the extract. Specifically, this potential can be attributed to the condensed tannins and flavonoids present in *P. daemia* leaf extract, as earlier reported (Vaithiyanathan and Mirunalini, 2016).

Histological examination of liver and kidney of rifampicin–induced rats revealed many alterations such as tubular degeneration and atrophy of glomeruli (Figure 2A-D and 3A-C). Such observations were reported by Sidhu and Naugler (2012), who observed many histological and ultrastructural changes in the kidney of rats exposed to rifampicin.

## 4. Conclusions

The present study showed that aqueous extract of *P. daemia* leaf has an ameliorative effect on rifampicin-induced liver and kidney damage. All biochemical parameters, as well as histological evidence, established that the plant is a potential candidate that can be exploited in the management of liver and kidney diseases.

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## Conflict of interest

All authors declare that there is no conflict of interest.

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## Supplementary File

None.

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