



RESEARCH ARTICLE

OPEN ACCESS

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

Temidayo Ogunmoyole^{a,*}, Omotola Grace Fatile^b, Olaitan Daniel Johnson^c,
Adeiwale Akeem Yusuff^d

^a Ekiti State University, College of Medicine, Department of Medical Biochemistry, P.M.B., 5363, Ado Ekiti, Ekiti State, Nigeria

^b Ekiti State University, Department of Science Laboratory Technology, Ado Ekiti, Ekiti State, Nigeria

^c Auchi Polytechnic, School of Applied Sciences, Department of Science Laboratory Technology, Auchi, Edo State, Nigeria

^d Osun State Primary Health Care Board, Department of Medical Laboratory Service, Oshogbo, Osun State, Nigeria

ARTICLE INFO

Article History:

Received: 03 April 2022

Revised: 09 May 2022

Accepted: 10 May 2022

Available online: 11 May 2022

Edited by: B. Tepe

Keywords:

Pergularia daemia

Liver

Kidney

Biomarkers

Toxicity

Rifampicin

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).

* Corresponding author:

E-mail address: temidayo.ogunmoyole@eksu.edu.ng (T. Ogunmoyole)

e-ISSN: 2791-7509

doi:

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-chlorobenzoyl-1-cyclohexoyl-5-tosylamino-1,4,12,3 zole, methyl ester pentadecanoic acid, 14-methyl-methyl ester ethyl 9-12-15-octodecatrienoate, 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) + <i>P. daemia</i> (50 mg/kg bwt)
IV	Rifampicin (250 mg/kg bwt) + <i>P. daemia</i> (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 20 min 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 μ l) of standard and 10 μ l 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 (\geq 0.5 U/ml), cholesterol esterase ion (\geq 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 μ l) and serum (10 μ l) were pipetted into labeled test tubes. One milliliter of the working reagents; R1a (buffer) containing pipes buffer (40 mmol/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 (\geq 150 U/ml), glycerol-kinase (\geq 0.4 U/ml), glycerol-3-phosphate oxidase (\geq 1.5 U/ml) and peroxidase (\geq 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 μ l of the serum, 200 μ l of the cholesterol standard, and 500 μ l 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 μ l) of the sample supernatant was added to 1 ml of cholesterol reagent and mixed in a test tube. The standard test tube contained 100 μ l 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:

$$\text{LDL cholesterol} = \text{Total cholesterol} - \text{Triglycerides}/5 - \text{HDL-cholesterol}$$

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 μ mol) 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.

$$\text{Catalase activity} =$$

$$H_2O_2 \text{ consumed} = 800 - \text{Concentration of } H_2O_2 \text{ remaining}$$

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 (Vaithyanathan 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).

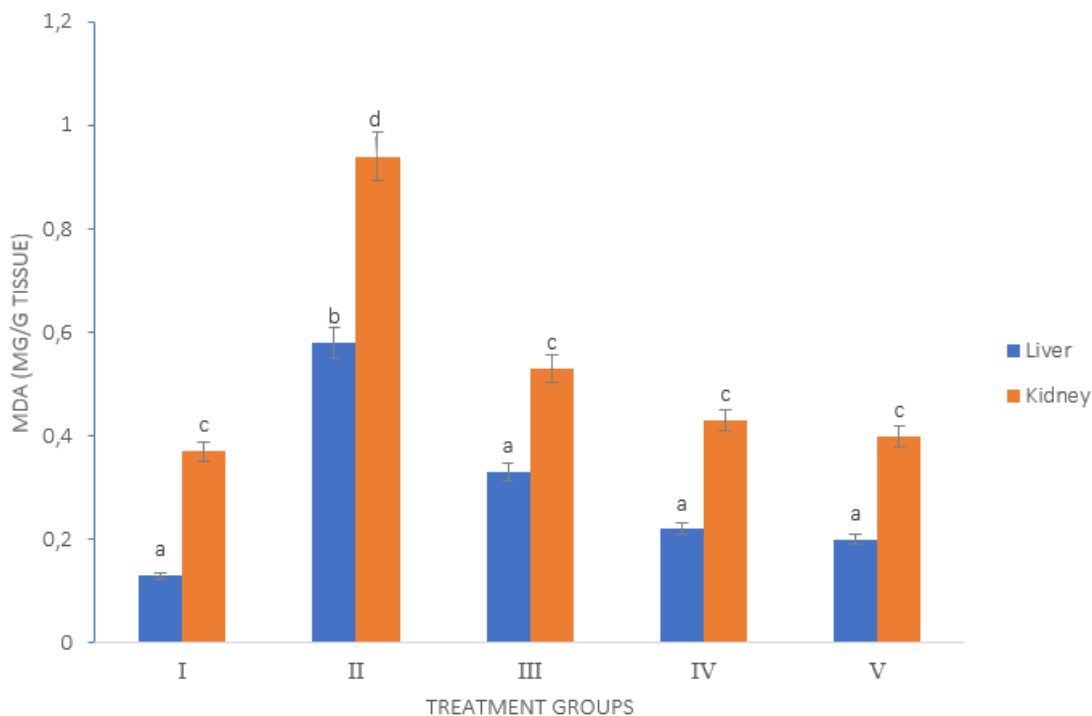


Figure 1. Effect of *P. daemia* on lipid peroxidation in the liver and kidney of rifampicin-exposed rats

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 $p = 0.05$.

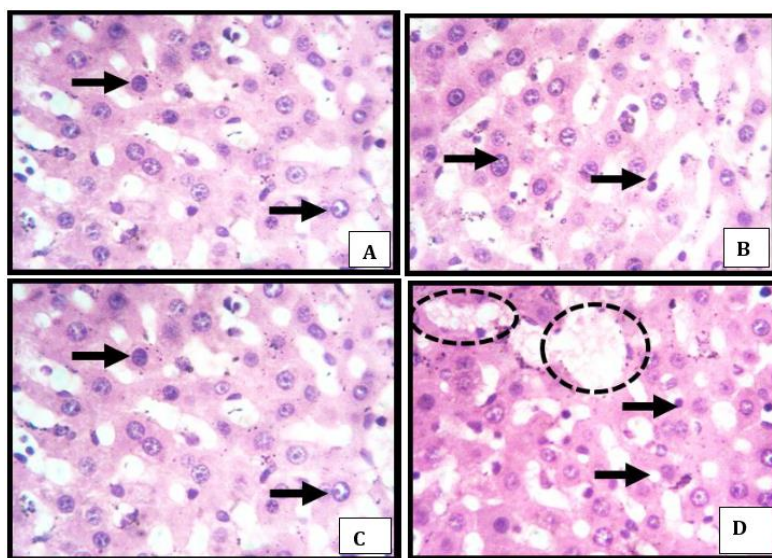


Figure 2. Histoarchitecture of the liver of experimental animals at a magnification (x400).

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).

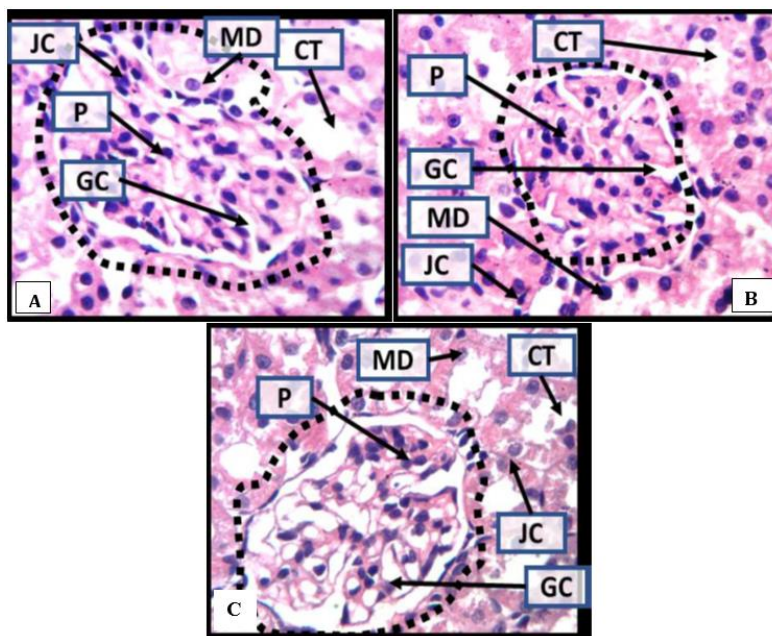


Figure 3. Histoarchitecture of the kidney tissue slice of experimental animals at a magnification (x400).

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 (Vaithyanathan, 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 (Vaithyanathan 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 (Vaithiyathan 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.

Acknowledgments

The authors wish to acknowledge the support of Mr. Oyelade of the Department of Science Laboratory Technology, The Federal Polytechnic, Ado Ekiti, Nigeria.

Conflict of interest

All authors declare that there is no conflict of interest.

CRedit authorship contribution statement

Temidayo Ogunmoyole: Conceptualization, Investigation, Writing - original draft, Supervision

Omotola Grace Fatile: Resources, Formal analysis, Investigation, Methodology

Olaitan Daniel Johnson: Resources, Formal analysis, Investigation

Adewale Akeem Yusuff: Resources, Formal analysis, Investigation

ORCID Numbers of the Authors

T. Ogunmoyole: 0000-0002-6185-0602

O.G. Fatile: 0000-0003-4224-1462

O.D. Johnson: 0000-0002-3492-0986

A.A. Yusuff: 0000-0001-7929-2111

Supplementary File

None.

References

- Abirami, A., Nagarani, G., Siddhuraju, P., 2014. *In vitro* antioxidant, anti-diabetic, cholinesterase and tyrosinase inhibitory potential of fresh juice from *Citrus hystrix* and *C. maxima* fruits. *Food Science and Human Wellness*, 3(1), 16-25.
- Antonyuk, S.V., Strange, R.W., Marklund, S.L., Hasnain, S.S., 2009. The structure of human extracellular copper-zinc superoxide dismutase at 1.7 Å resolution: insights into heparin and collagen binding. *Journal of Molecular Biology*, 388(2), 310-326.
- Balakrishnan, S., Khurana, B.S., Singh, A., Kaliappan, I., Dubey, G.P., 2012. Hepatoprotective effect of hydroalcoholic extract of *Cissampelos pareira* against rifampicin and isoniazid induced hepatotoxicity. *Continental Journal of Food Science and Technology*, 6(1), 30-35.
- Basheer, A.S., Siddiqui, A., Paudel, Y.N., Hassan, M.Q., Imran, M., Najmi, A.K., Akhtar, M., 2017. Hepatoprotective and antioxidant effects of fish oil on isoniazid-rifampin induced hepatotoxicity in rats. *PharmaNutrition*, 5(1), 29-33.
- Beutler, E., 1963. Improved method for the determination of blood glutathione. *Journal of Laboratory and Clinical Medicine*, 61, 882-888.
- Bhusari, S., Bhokare, S.G., Nikam, K.D., Chaudhary, A.N., Wakte, P.S., 2018. Pharmacognostic and Phytochemical investigation of stems of *Pergularia daemia*. *Asian Journal of Pharmacy and Pharmacology*, 4(4), 500-504.
- Biour, J.M., Tymoczko, J.L., Stryer, L., 2004. *Biochemistry*. W.H. Freeman. pp. 656-660. ISBN 978-0-7167-8724-2.
- Brehe, J.E., Burch, H.B., 1976. Enzymatic assay for glutathione. *Analytical Biochemistry*, 74(1), 189-197.
- Byrne, J.A., Strautnieks, S.S., Mieli-Vergani, G., Higgins, C.F., Linton, K.J., Thompson, R.J., 2002. The human bile salt export pump: characterization of substrate specificity and identification of inhibitors. *Gastroenterology*, 123(5), 1649-1658.
- Capelle, P., Dhumeaux, D., Mora, M., Feldmann, G., Berthelot, P., 1972. Effect of rifampicin on liver function in man. *Gut*, 13(5), 366-371.
- Chandak, R.R., 2010. Preliminary Phytochemical Investigation of *Pergularia daemia* linn. *Journal of Pharmaceutical Studies & Research*, 1(1), 11-16.
- Chandak, R.R., Dighe, N.S., 2019. A Review on Phytochemical & Pharmacological Profile of *Pergularia daemia* linn. *Journal of Drug Delivery and Therapeutics*, 9(4-s), 809-814.
- Dosumu, O.O., Ajetumobi, O.O., Omole, O.A., Onocha, P.A., 2019. Phytochemical composition and antioxidant and antimicrobial activities of *Pergularia daemia*. *Journal of Medicinal Plants for Economic Development*, 3(1), 1-8.
- Eminzade, N., Krance, S.M., Notenboom, S., Shi, S., Tieu, K., Hammond, C.L., 2008. Glutathione dysregulation and the etiology and progression of human diseases. *Biological Chemistry*, 390(3), 191-214.
- Englehardt, A., 1970. Measurement of alkaline phosphatase. *Aerztl Labor*, 16(42), 1.
- Espinosa-Diez, C., Miguel, V., Mennerich, D., Kietzmann, T., Sánchez-Pérez, P., Cadenas, S., Lamas, S., 2015. Antioxidant responses and cellular adjustments to oxidative stress. *Redox Biology*, 6, 183-197.
- Friedewald, W.T., Levy, R.I., Fredrickson, D.S., 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, 18, 499-502.
- Grosset, J., Leventis, S., 1983. Adverse effects of rifampin. *Reviews of Infectious Diseases*, 5(Supplement_3), S440-S446.
- Grove, T.H., 1979. Effect of reagent pH on determination of high-density lipoprotein cholesterol by precipitation with sodium phosphotungstate-magnesium. *Clinical Chemistry*, 25(4), 560-564.
- Heit, C., Marshall, S., Singh, S., Yu, X., Charkoftaki, G., Zhao, H., Vasilou, V., 2017. Catalase deletion promotes prediabetic phenotype in mice. *Free Radical Biology and Medicine*, 103, 48-56.
- Jaswal, A., Sinha, N., Bhadauria, M., Shrivastava, S., Shukla, S., 2013. Therapeutic potential of thymoquinone against anti-tuberculosis drugs induced liver damage. *Environmental Toxicology and Pharmacology*, 36(3), 779-786.
- Jaydeokar, A.V., Bandawane, D.D., Bibave, K.H., Patil, T.V., 2014. Hepatoprotective potential of *Cassia auriculata* roots on ethanol and antitubercular drug-induced hepatotoxicity in experimental models. *Pharmaceutical Biology*, 52(3), 344-355.

- Jelkmann, W., 2001. The role of the liver in the production of thrombopoietin compared with erythropoietin. *European Journal of Gastroenterology & Hepatology*, 13(7), 791-801.
- Kim, J.H., Nam, W.S., Kim, S.J., Kwon, O.K., Seung, E.J., Jo, J.J., Lee, S., 2017. Mechanism investigation of rifampicin-induced liver injury using comparative toxicoproteomics in mice. *International Journal of Molecular Sciences*, 18(7), 1417.
- Kohli, H.S., Bhaskaran, M.C., Muthukumar, T., Thenarasu, K., Sud, K., Jha, V., Sakhuja, V., 2000. Treatment-related acute renal failure in the elderly: a hospital-based prospective study. *Nephrology Dialysis Transplantation*, 15(2), 212-217.
- Kosanam, S., Boyina, R., 2015. Drug-induced liver injury: A review. *International Journal of Pharmacological Research*, 5(2), 24-30.
- Krishnaiah, D., Sarbatly, R., Nithyanandam, R., 2011. A review of the antioxidant potential of medicinal plant species. *Food and Bioproducts Processing*, 89(3), 217-233.
- Larrey, D., 2000. Drug-induced liver diseases. *Journal of Hepatology*, 32, 77-88.
- Lee, C.L., Sherman, P.M., 2000. Pediatric Gastrointestinal Disease. Connecticut: PMPH-USA. p. 751. ISBN 978-1-55009-364-3.
- Maheshwari, M., Vijayarangan, P., 2021. Phytochemical Evaluation, FT-IR and GC-MS Analysis of Leaf Extracts of *Pergularia daemia*. *Nature Environment and Pollution Technology*, 20(1), 259-265.
- Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*, 247(10), 3170-3175.
- Mohammed, S., Kaseera, P.K., Shukla, J.K., 2004. Unexploited plants of potential medicinal value from the Indian Thar desert. *Natural Product Radianc*, 3, 69-74.
- Muller, F.L., Song, W., Liu, Y., Chaudhuri, A., Pieke-Dahl, S., Strong, R., Van Remmen, H., 2006. Absence of CuZn superoxide dismutase leads to elevated oxidative stress and acceleration of age-dependent skeletal muscle atrophy. *Free Radical Biology and Medicine*, 40(11), 1993-2004.
- Naik, S.R., Panda, V.S., 2008. Hepatoprotective effect of Ginkgoselect Phytosome® in rifampicin induced liver injury in rats: Evidence of antioxidant activity. *Fitoterapia*, 79(6), 439-445.
- Nithyatharani, R., Kavitha, U., 2018. Phytochemical Studies on the Leaves of *Pergularia daemia* Collected from Villupuram District, Tamil Nadu, India. *IOSR Journal of Pharmacy*, 8(1), 9-12.
- Panich, U., Onksoong, T., Limsaengurai, S., Akarasereenont, P., Wongkajornsilp, A., 2012. UVA-induced melanogenesis and modulation of glutathione redox system in different melanoma cell lines: the protective effect of gallic acid. *Journal of Photochemistry and Photobiology B: Biology*, 108, 16-22.
- Rana, S.V., Pal, R., Vaiphie, K., Singh, K., 2006. Effect of different oral doses of isoniazid-rifampicin in rats. *Molecular and Cellular Biochemistry*, 289(1), 39-47.
- Reitman, S., Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, 28(1), 56-63.
- Renugadevi, J., Prabu, S.M., 2009. Naringenin protects against cadmium-induced oxidative renal dysfunction in rats. *Toxicology*, 256(1-2), 128-134.
- Renugadevi, J., Prabu, S.M., 2010. Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin. *Experimental and Toxicologic Pathology*, 62(2), 171-181.
- Santhosh, S., Sini, T.K., Anandan, R., Mathew, P.T., 2006. Effect of chitosan supplementation on antitubercular drugs-induced hepatotoxicity in rats. *Toxicology*, 219(1-3), 53-59.
- Saukkonen, J.J., Cohn, D.L., Jasmer, R.M., Schenker, S., Jereb, J.A., Nolan, C.M., 2006. On the behalf of ATS (American Thoracic Society). Hepatotoxicity of Antituberculosis Therapy. *American Journal of Respiratory and Critical Care Medicine*, 174(8), 935-952.
- Sedlak, T.W., Snyder, S.H., 2004. Bilirubin benefits: cellular protection by a biliverdin reductase antioxidant cycle. *Pediatrics*, 113(6), 1776-1782.
- Sentman, M.L., Granström, M., Jakobson, H., Reaume, A., Basu, S., Marklund, S.L., 2006. Phenotypes of mice lacking extracellular superoxide dismutase and copper-and zinc-containing superoxide dismutase. *Journal of Biological Chemistry*, 281(11), 6904-6909.
- Sharma, R., Sharma, V.L., 2015. Review: treatment of toxicity caused by anti-tubercular drugs by use of different herbs. *International Journal of Pharma Sciences and Research*, 6(10), 1288-1294.
- Shukla, S., Sinha, N., Jaswal, A., 2014. Anti Oxidative, Anti Peroxidative and Hepatoprotective Potential of *Phyllanthus amarus* Against Anti Tb Drugs. In: Pharmacology and Nutritional Intervention in the Treatment of Disease. *IntechOpen*, 2014, 283-294.
- Sidhu, D., Naugler, C., 2012. Fasting time and lipid levels in a community-based population: a cross-sectional study. *Archives of Internal Medicine*, 172(22), 1707-1710.
- Sinha, A.K., 1972. Colorimetric assay of catalase. *Analytical Biochemistry*, 47(2), 389-394.
- Tasduq, S.A., Kaiser, P., Sharma, S.C., Johri, R.K., 2007. Potentiation of isoniazid-induced liver toxicity by rifampicin in a combinational therapy of antitubercular drugs (rifampicin, isoniazid and pyrazinamide) in Wistar rats: A toxicity profile study. *Hepatology Research*, 37(10), 845-853.
- Tietz, N.W., 1995. Clinical Guide to Laboratory Tests, 3rd Edition, W.B. Saunders, Philadelphia.
- Trinder, P., 1969. A simple Turbidimetric method for the determination of serum cholesterol. *Annals of Clinical Biochemistry*, 6(5), 165-166.
- Ueno, Y., Kizaki, M., Nakagiri, R., Kamiya, T., Sumi, H., Osawa, T., 2002. Dietary glutathione protects rats from diabetic nephropathy and neuropathy. *The Journal of Nutrition*, 132(5), 897-900.
- Vaithyanathan, V., Mirunalini, S., 2015. Quantitative variation of bioactive phyto compounds in ethyl acetate and methanol extracts of *Pergularia daemia* (Forsk.) Chiov. *Journal of Biomedical Research*, 29(2), 169-172.
- Vaithyanathan, V., Mirunalini, S., 2016. Assessment of anticancer activity: A comparison of dose-response effect of ethyl acetate and methanolic extracts of *Pergularia daemia* (Forsk.). *Oral Science International*, 13(1), 24-31.
- Vanderlinde, R.E., 1981. Urinary enzyme measurements in the diagnosis of renal disorders. *Annals of Clinical & Laboratory Science*, 11(3), 189-201.
- Verma, P., Paswan, S., Singh, S.P., Shrivastava, S., Rao, C.V., 2015. Assessment of hepatoprotective potential of *Solanum xanthocarpum* (whole plant) Linn. against isoniazid & rifampicin induced hepatic toxicity in Wistar rats. *Indian Journal of Research in Pharmacy and Biotechnology*, 3, 373-379.
- Weichselbaum, C.T., 1946. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. *American Journal of Clinical Pathology*, 16(3_ts), 40-49.
- Yue-Ming, W., Sergio, C.C., Christopher, T.B., Taoshen, C., 2014. Pregnane X receptor and drug-induced liver injury expert Opin. *Journal of Drug Metabolism & Toxicology*, 10(11), 1521-1532.

Reviewed by:

Selami SELVI: Balikesir University, Balikesir, TURKEY
Bulent KIRKAN: Suleyman Demirel University, Isparta, TURKEY

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.



This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.