Concomitant Administration of Aloe Vera Gel and Rifampicin Protects Against Rifampicin Hepatorenal Toxicity in Male Wistar Rats

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Concomitant Administration of *Aloe Vera* Gel and Rifampicin Protects Against Rifampicin Hepatorenal Toxicity in Male Wistar Rats

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ABSTRACT

Background: Rifampicin, an antibiotic used in the treatment of tuberculosis has raised concerns about its potential liver and kidney toxicity. **Aim:** This study aimed to evaluate the protective effects of *Aloe vera* against hepatorenal toxicity induced by rifampicin in male Wistar rats. **Methods:** Thirty rats were divided into six groups (n = 5): group A (control), group B treated with rifampicin, groups C–E treated with varying doses (50, 100, and 200 mg/kg) of *Aloe vera* alongside rifampicin as well as a group F treated with furosemide and rifampicin for a total of 30 days. Alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), creatinine, urea, and histopathological changes were evaluated. One-way analysis of variance and Tukey's *post hoc* tests were applied with a significance level of 5%. **Results:** Results showed 98.28%, 107.66%, and 334.66% increase in ALT, AST, and ALP levels of group B (Rifampicin only) compared with the control group. In contrast, groups treated with *Aloe vera* showed significantly lower ALT, AST, and ALP levels as the dose increased from 50–200 mg/kg. A value of 2.23 Mg/dL content as a lipid peroxidation marker was observed in group B in comparison to the control group indicating oxidative stress while animals treated with *Aloe vera* at 50, 100, and 200 mg/kg showed decreased levels of MDA (1.53, 1.13, and 0.80 Mg/dL respectively) in comparison to group B. A decrease in CAT and SOD levels in the rifampicin-only treated animals was observed while there was an increase in CAT and SOD levels in animals treated with *Aloe vera* and furosemide concomitantly with rifampicin. Creatinine and urea levels increased significantly in group B and reduced as *Aloe vera* was introduced at 50, 100, and 200 mg/kg respectively. Histopathological analysis confirmed liver and kidney tissue damage in rifampicin only and progressive regeneration in groups treated with *Aloe vera* as the dose increased to 200 mg/kg. **Conclusion:** The results of this study indicate that *Aloe vera* has a protective effect against rifampicin-induced hepatorenal toxicity in a dose-dependent manner by mitigating oxidative stress and improving liver and kidney function markers.

Keywords: *Aloe vera, hepatorenal toxicity, oxidative stress, rifampicin, Wistar rats*

Introduction

The liver is an important organ of the body involved in the regulation of the internal chemical environment[1] and plays crucial roles in the control of metabolism, detoxification, and regulation of various substances including drugs, and in ensuring the overall homeostasis of the body. Because of its wide range

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of activities, the liver is the main target of drugs and xenobiotic toxicity resulting in dysfunction, disorders, and severe health complications. On the other hand, the kidneys which are essential in maintaining the body's internal environment and responsible for regulating body fluids, electrolyte concentrations, blood pressure, osmolality, erythropoietin production, and toxin removal are vulnerable to drug-induced toxicity, leading to conditions like chronic kidney disease, acute renal failure, and kidney stones.[2]

Rifampicin, sometimes referred to as rifampin, is a first-line antibiotic drug used to treat a variety of infectious diseases such as tuberculosis (TB), brucellosis, and some staphylococcal infections, including infectious endocarditis and bone and joint infections.[3] Rifampicin exerts antimicrobial properties by inhibiting DNA-dependent RNA polymerase (RNAP). This inhibition occurs either by lowering the affinity of RNAP for short RNA transcripts or by serially blocking the elongating RNA's route at its 5′ end.[4] Rifampicin is known to increase the activity of peroxisome proliferator-activated receptor gamma (PPARγ), leading to the accumulation of lipids in liver cells[5,6] This results in oxidative stress, as indicated by an increase in markers of lipid peroxidation such as malondialdehyde (MDA).[6] The overproduction of reactive oxygen species (ROS) (RO2, OH, and H2O2) causes damage to cell membranes through lipid peroxidation (LPO). The presence of MDA indicates tissue damage and an imbalance in redox homeostasis.[8] Additionally, rifampicin has been found to cause kidney damage, although patients may recover kidney function after discontinuing the medication.[9]

Aloe vera (*Aloe barbadensis miller*), a perennial succulent plant belonging to the *Liliaceal* family is said to contain several compounds including vitamins, glycoproteins, minerals, amino acids, and phytonutrients.[10] *Aloe vera* gel also contains many glycosides (anthraquinones), the most significant of which are aloin A and aloin B. Anthraquinones and its derivatives are powerful antibacterial and analgesic agents.[11] *Aloe vera* is abundant in antioxidants such as α-tocopherol (vitamin E), carotenoids, ascorbic acid (vitamin C), flavonoids, and tannins. *Aloe gel* scavenges free radicals 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and nitric oxide.[12] A portion of the antioxidant activity of *Aloe* vera can also be attributed to anthraquinones as they possess the ability to reduce and scavenge peroxyl radicals.[13] *Aloe vera* gel has a therapeutic potential to reduce cholesterol levels and the risk of cardiovascular disease.[14] *Aloe vera* is also said to possess pharmacological properties such as anti-inflammatory, anti-cancer, anti-diabetic, and anti-microbial properties.[12,13,15] Their phytochemicals have been recorded to repair the antioxidant defense mechanism, limit oxidative stress, prevent tissue inflammation, and reduce necrotic cell death and mitochondrial malfunction leading to its adoption as an alternative therapy for various ailments.[16-18] Based on these properties, this study aims to investigate the protective effects of Aloe vera on rifampicin-induced toxicity.

Materials and Methods

Aqueous *Aloe vera* gel extraction

Thick succulent *Aloe vera* plants were obtained from a herbal garden at the University of Nigeria, Enugu Campus, Nigeria. To obtain *Aloe vera* gel extract, the leaves were rinsed 5 times with water and mild chlorine solution. The back was peeled leaving the gel which was then homogenised. The homogenized suspension was filtered through a cotton cloth.

Procurement of drugs

The drugs rifampicin (Lupin Limited, India) and furosemide (Fredun Pharmaceutical Limited India) were procured from a local pharmacy in the University of Nigeria Enugu Campus (UNEC), Enugu State.

Experimental animals

Thirty (30) healthy male adult Wistar rats weighing 180 ± 20g were obtained from the animal house at the University of Nigeria, Nsukka. They were acclimatized under standard laboratory conditions for 14 days with a temperature of 22–24°C, humidity 50-55%, 12 hours of dark and light cycle, and given free access to rat chow and clean water *ad libitum*. Proper care for the experimental animals was carried out according to the National Research Council "Guide for the Care and Use of Laboratory Animals", Washington, DC. Ethical Clearance was obtained from the Health Research Ethics Committee of the University of Nigeria Teaching Hospital, Enugu (NHREC/05/01/2008B-FWA00002458-1RB00002326).

Experimental design

After acclimatization, the animals were weighed and grouped into six (6) groups with each group having five rats (n = 5). Group A (the control group) received only a normal diet and water *ad libitum*, and Group B (The negative control group) received a normal diet, water *ad libitum*, and rifampicin 450 mg/70 kg daily. Group C–E received a normal diet, water *ad libitum,* and 450 mg/70 kg body weight of rifampicin as well as low (50 mg/kg), medium (100 mg/kg), and

high (200 mg/kg) doses of Aloe vera respectively daily. Group F (Standard drug group) received a normal diet, water *ad libitum*, and 450 mg/70 kg of rifampicin together with 20 mg/70kg of furosemide [Table 1]. The dosage and treatment which lasted for 30 days were based on the previous study.[19,20] The weight of the animals was measured every day during this study to adjust the dosage taking into account changes in body weight.

Sacrifice of animals and blood and tissue collection

Twenty-four hours after the 30th day of drug administration all animals were anesthetized (100 mg/kg ketamine and 5 mg/kg xylazine intraperitonealy). The blood sample was collected through the orbital sinus. The liver and kidney were surgically removed using dissection kits. The organs were then rinsed in 10% buffered formal saline and preserved in 10% neutral formaldehyde solution to preserve the tissue's structural integrity and prevent tissue degradation till processed.

Serum separation and preparation of liver homogenate

Blood samples were allowed to stand undisturbed at room temperature for 30 minutes and then subjected to refrigerated centrifugation at 1500 × g for 10 minutes to separate serum, which was then stored at −20°C for biochemical analysis of various parameters. To prepare tissue homogenate, 1.5 g of hepatic tissue was first washed in 0.9% normal saline and then homogenized in ice-cold buffer containing 0.25 M sucrose, 1 mM EDTA, and 1 mM Tris-HCl (pH 7.4). The homogenate was then centrifuged at 6000 × g for 10 minutes at 4°C, and the resulting supernatant was collected and stored at −20°C for biochemical analysis.

Assessment of liver and kidney biomarkers

Serum alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) were determined as important hepatic biomarkers. For kidney analysis blood urea and serum creatinine were carried out. All these assays were carried out using assay kits of Randox Laboratories Ltd., United Kingdom.

Determination of Malondialdehyde level

The determination of the level of lipid peroxidation in tissue was carried out by measuring the amount of malondialdehyde (MDA) present. The tissue MDA content was determined using the thiobarbituric acid reactive substance assay, which was described by Buege and Aust.[21] To perform the assay, 100 µL of tissue lysate was mixed thoroughly with a stock solution of 15.00% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid, and 0.25M hydrochloric acid. The resulting solution was then left to stand for 15 minutes in a boiling water bath, after which it was cooled and the precipitate was removed by centrifugation at 5,000 RPM for 10 minutes. The quantification of thiobarbituric acid reactive substances was determined by comparing the absorption with the standard curve of MDA equivalents generated by acid-catalyzed hydrolysis of 1,1,3,3-tetramethoxypropane.

Determination of superoxide dismutase activity

Superoxide dismutase activity was determined using the nitroblue tetrazolium (NBT) method.[22] The assay involves the production of superoxide from O_2 using reduced β-nicotinamide adenine dinucleotide (NADH) as a reductant and phenazine methosulphate (PMS) as a catalyst in the presence of an indicator, NBT, which turns blue when reduced by superoxide. The color change during the reaction was monitored spectrophotometrically in the visible range at 560 nm. When the SOD enzyme is added to the reaction, it competes with NBT to react with superoxide. The NBT reduction inhibition percentage was used to quantify the superoxide-scavenging level. The tissue homogenate (10 µL) was mixed with sodium pyrophosphate buffer (Merck) (100 mM; pH: 8.30), 1.28 mM PMS (Sigma–Aldrich), and 0.18 mM NBT (Sigma–Aldrich). The reaction was started by the addition of 12.72 mM NADH. The reaction mixture was then incubated at 30.00 ˚C for 90 sec and stopped by the addition of 1.00 mL glacial acetic acid (Merck). The absorbance was measured at 560 nm. One unit of SOD activity is defined as the enzyme concentration required in inhibiting chromogen production by 50.00% in 1 min under the assay conditions.

Determination of catalase (CAT) activity

The spectrophotometric method of Koroliuk *et al*.[23] was used to determine catalase activity. To perform the test, 10.00 µL of the sample was incubated with 100 µmol mL-1 of H_2O_2 (Merck, Kenilworth, USA) in 0.05 mmol L-1 Tris-HCl (Sigma-Aldrich) buffer at pH 7.00 for 10 minutes. The reaction was stopped by adding 50.00 µL of 4.00% ammonium molybdate (Sigma–Aldrich) which resulted in the formation of a yellow complex of ammonium molybdate and H_2O_2. The yellow complex was then measured at 410 nm using the multimode plate reader (BioTek). The amount of enzyme required to decompose 1.00 µmol H_2O_2 per minute was defined as one unit of CAT activity.

Histological study

Hepatic and renal tissues were washed in ascending grades of ethanol (50%, 70%, and absolute), each for 30 minutes or more depending on the thickness of the tissue to prevent tissue damage. The tissue was immersed in xylene for 15 minutes and a second emersion

was done in fresh xylene for another 15 minutes rendering the tissue almost transparent. Haematoxylin and Eosin (H and E) stains were used to observe the histo-architecture of the hepatic and renal tissues and viewed under a microscope using × 400 magnification.[24] An experienced histopathologist who was blinded to the study design performed the histological assessments.

Statistical analysis

The data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 20 program. All data were expressed as mean ± (SEM). Data sets were compared using a one-way analysis of variance (ANOVA) followed by *Tukey's* post hoc test. Values of $P < 0.05$ were considered statistically significant.

Results

The results of this study [Table 2] indicate a significant increase in the mean value of serum ALT AST and ALP in group B (Rifampicin only) by 98.28%, 107.66%, and 334.66% respectively when compared with the control group A. Group C (Rifampicin 450 mg/70 kg and *Aloe vera* 50 mg/kg), recorded a non-significant decrease in AST and ALP by 35.86% and 54.72.87% respectively compared to the Rifampicin-only group. However, group E (Rifampicin 450 mg/70 kg and *Aloe vera* 200 mg/kg) showed a significant decrease in ALT, and ALP by 41.32%, and 79.31% respectively when compared with the negative control group (Rifampicin only) [Figures 1-3]. The mean serum enzyme activity of ALT (26.34 u/l), AST (40.20 u/l), and ALP (294.66 u/l) in group E (Rifampicin 450 mg/70 kg and *Aloe vera* 200 mg/kg) shows no statistically significant difference when compared with the control group A. Group Rifampicin (450 mg/70 kg) and furosemide (20 mg/70 kg) showed statistically significant differences in AST (26.35 u/L) when compared to the control group while ALT (24.38 u/L) and ALP (229.95 u/L) showed no statistically significant difference when compared to the control group.

Table 1: Grouping of animals and treatments

Group	Treatment
A	Rat pellet and distilled water (Control Group)
B	Rifampicin (450 mg/70 kg body weight/day)
C	Rifampicin (450 mg/70 kg body weight/day) + *Aloe vera* (50 mg/kg body weight/day)
D	Rifampicin (450 mg/70 kg body weight/day) + *Aloe vera* (100 mg/kg body weight/day)
E	Rifampicin (450 mg/70 kg body weight/day) + *Aloe vera* (200 mg/kg body weight/day)
F	Rifampicin (450 mg/70 kg body weight/day) + Furosemide (20 mg/70 kg body weight/day)

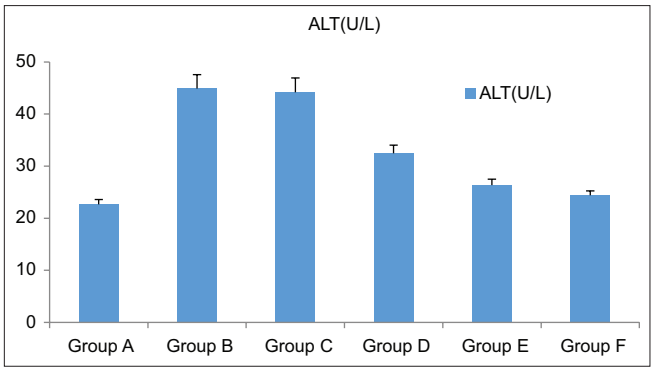

Figure 1: Means Plot of ALT levels in groups A to F experimental animals. Group A-Control group, Group B- Rifampicin (450 mg/70 kg body weight/day), Group C- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (50 mg/kg body weight/day) Group D- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (100 mg/kg body weight/day), Group E- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (200 mg/kg body weight/day), Group F- Rifampicin (450 mg/70 kg body weight/day)+ Furosemide (20 mg/70 kg body weight/day)

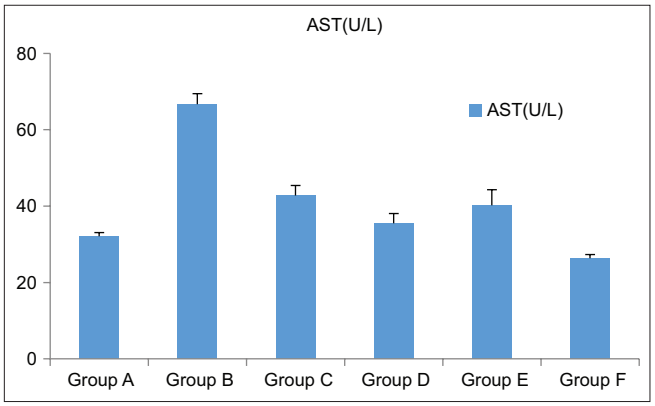

Figure 2: Means Plot of AST levels in groups A to F experimental animals. Group A-Control group, Group B- Rifampicin (450 mg/70 kg body weight/day), Group C- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (50 mg/kg body weight/day) Group D- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (100 mg/kg body weight/day), Group E- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (200 mg/kg body weight/day), Group F- Rifampicin (450 mg/70 kg body weight/day) + Furosemide (20 mg/70 kg body weight/day)

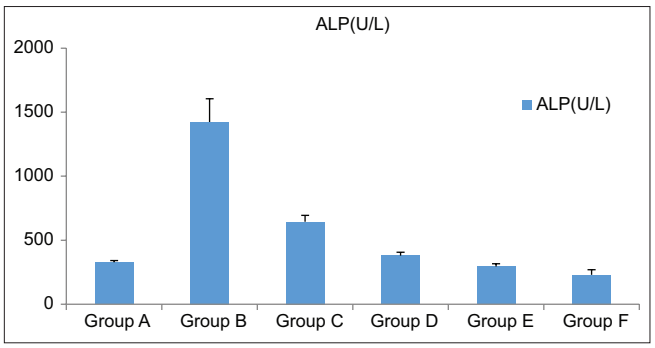

Figure 3: Means Plot of ALP levels in groups A to F experimental animals. Group A-Control group, Group B- Rifampicin (450 mg/70 kg body weight/day), Group C- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (50 mg/kg body weight/day) Group D- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (100 mg/kg body weight/day), Group E- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (200 mg/kg body weight/day), Group F- Rifampicin (450 mg/70 kg body weight/day)+ Furosemide (20 mg/70 kg body weight/day)

The effect of rifampicin and *Aloe vera* on oxidative stress markers (MDA, CAT, SOD) of experimental animal groups after 30 days are given in Table 3. In this study, MDA contents were measured to evaluate lipid peroxidation and membrane damage. The results show a statistically significant increase in the MDA values by 156.32% in experimental group B administered rifampicin only when compared with the control group A. In experimental groups Rifampicin (450 mg/70 kg) and *Aloe vera* 50 mg/kg, Rifampicin (450 mg/70 kg) and *Aloe vera* 100 mg/kg, Rifampicin (450 mg/70 kg) and *Aloe vera* 200 mg/kg where the Wistar rats were administered rifampicin concomitantly with *Aloe vera* in 50 mg/kg, 100 mg/kg, 200 mg/kg the results show a gradual decrease [Figure 4] in MDA levels from 1.53 (group C) to 0.80 (group E) while CAT and SOD level of group C–F increased gradually (non-significantly) moving closer to the value of the control group [Figures 5 and 6].

The result of renal biomarkers given in Table 4 depicts a significant increase in creatinine (3.10) of the negative control group (Rifampicin 450 mg/70 kg only) in comparison to the control group (0.41). The results for Group Rifampicin (450 mg/70 kg) and *Aloe vera* 50 mg/kg administered rifampicin concomitantly with a 50 mg/kg of *Aloe vera* shows a slight decrease [Figure 7] in comparison to the negative control group while statistically significant creatinine decrease was observed from Rifampicin (450 mg/70 kg) and *Aloe vera* 100 mg/kg

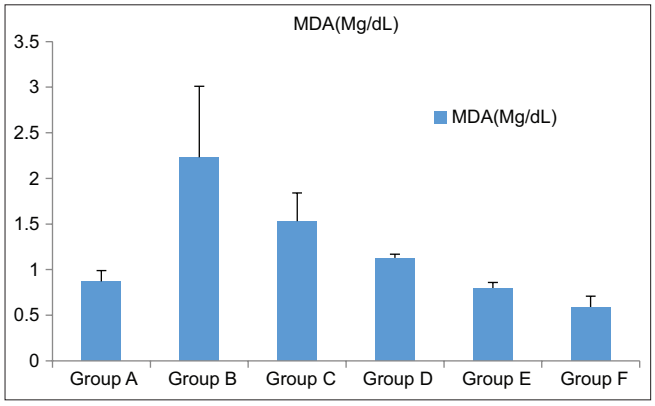

Figure 4: Means Plot of MDA levels in groups A to F experimental animals. Group A-Control group, Group B- Rifampicin (450 mg/70 kg body weight/day), Group C- Rifampicin (450 mg/70 kg body weight/day) + *Aloe vera* (50 mg/kg body weight/day) Group D- Rifampicin (450 mg/70 kg body weight/day) + *Aloe vera* (100 mg/kg body weight/day), Group E- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (200 mg/kg body weight/day), Group F- Rifampicin (450 mg/70 kg body weight/day) + Furosemide (20 mg/70 kg body weight/day)

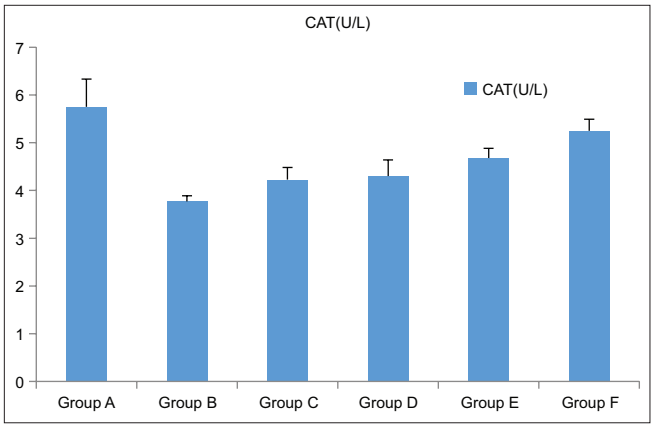

Figure 5: Means Plot of CAT levels in groups A to F experimental animals. Group A-Control group, Group B- Rifampicin (450 mg/70kg body weight/day), Group C- Rifampicin (450 mg/70kg body weight/day)+ *Aloe vera* (50 mg/kg body weight/day) Group D- Rifampicin (450 mg/70kg body weight/day)+ *Aloe vera* (100 mg/kg body weight/day), Group E- Rifampicin (450 mg/70 kg body weight/day) + *Aloe vera* (200 mg/kg body weight/day), Group F- Rifampicin (450 mg/70 kg body weight/day)+ Furosemide (20 mg/70 kg body weight/day)

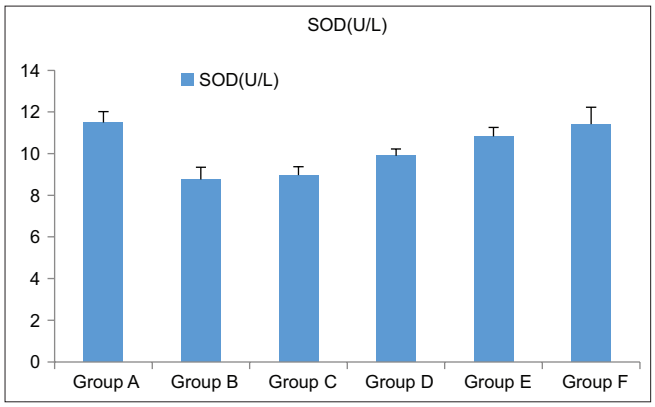

Figure 6: Means Plot of SOD levels in groups A to F experimental animals. Group A-Control group, Group B- Rifampicin (450 mg/70 kg body weight/day), Group C- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (50 mg/kg body weight/day) Group D- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (100 mg/kg body weight/day), Group E- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (200 mg/kg body weight/day), Group F- Rifampicin (450 mg/70 kg body weight/day)+ Furosemide (20 mg/70 kg body weight/day)

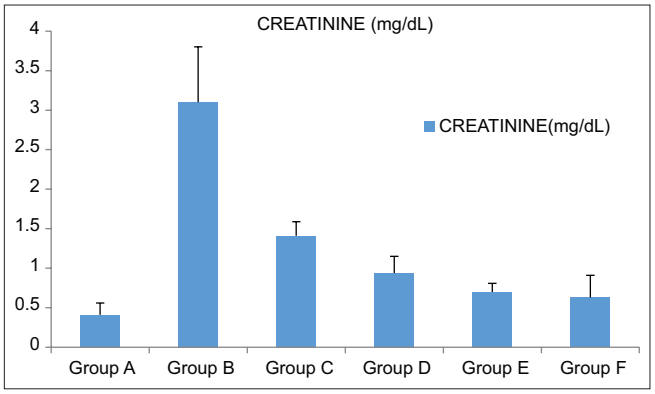

Figure 7: Means Serum Creatinine levels in groups A to F experimental animals. Group A-Control group, Group B- Rifampicin (450 mg/70 kg body weight/day), Group C- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (50 mg/kg body weight/day) Group D- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (100 mg/kg body weight/day), Group E- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (200 mg/kg body weight/day), Group F- Rifampicin (450 mg/70 kg body weight/day)+ Furosemide (20 mg/70 kg body weight/day)

group (0.940), Rifampicin (450 mg/70 kg) and *Aloe vera* 200 mg/kg group (0.696) to Rifampicin (450 mg/70 kg) and furosemide (80 mg/70 kg) group (0.626). There is an obvious increase in the urea level (76.32) in the negative control group in comparison to the control group. In groups Rifampicin (450 mg/70 kg) and *Aloe vera* 100 mg/kg, Rifampicin (450 mg/70 kg) and *Aloe vera* 200 mg/kg, Rifampicin (450 mg/70 kg) and furosemide (20 mg/70 kg), there is a marked significant decrease in serum urea when compared with the negative control as their values are moving closer to the values of the positive control group [Figure 8].

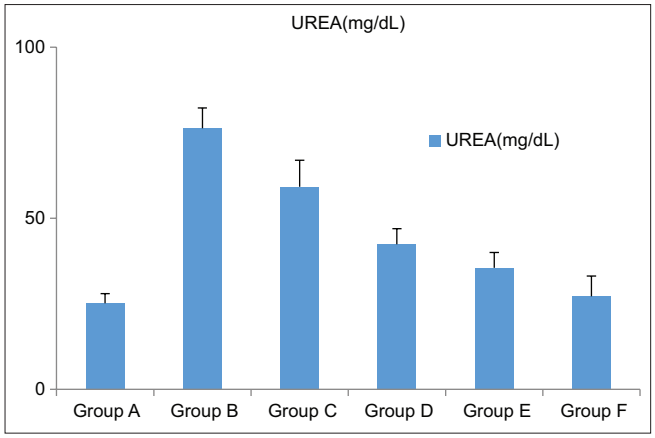

Figure 8: Means Serum Urea levels in groups A to F experimental animals. Group A-Control group, Group B- Rifampicin (450 mg/70 kg body weight/day), Group C- Rifampicin (450 mg/70 kg body weight/day) + *Aloe vera* (50 mg/kg body weight/day) Group D- Rifampicin (450 mg/70 kg body weight/day) + *Aloe vera* (100 mg/kg body weight/day), Group E- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (200 mg/kg body weight/day), Group F- Rifampicin (450 mg/70 kg body weight/day) + Furosemide (20 mg/70 kg body weight/day)

Histological analysis of the liver

The liver sections of the rifampicin-treated group exhibited lipid accumulation, massive cellular necrosis, central vein enlargement, and portal vein disruption, indicating the loss of cellular architecture due to excessive intracellular lipid deposition. Compared to the control group, the group that was treated with rifampicin only (group B) showed severe degeneration of the hepatic tissue with a portal aggregate of inflammatory cells and focal area hemorrhage, the overall features consistent with chronic hepatitis. On the other hand, administering

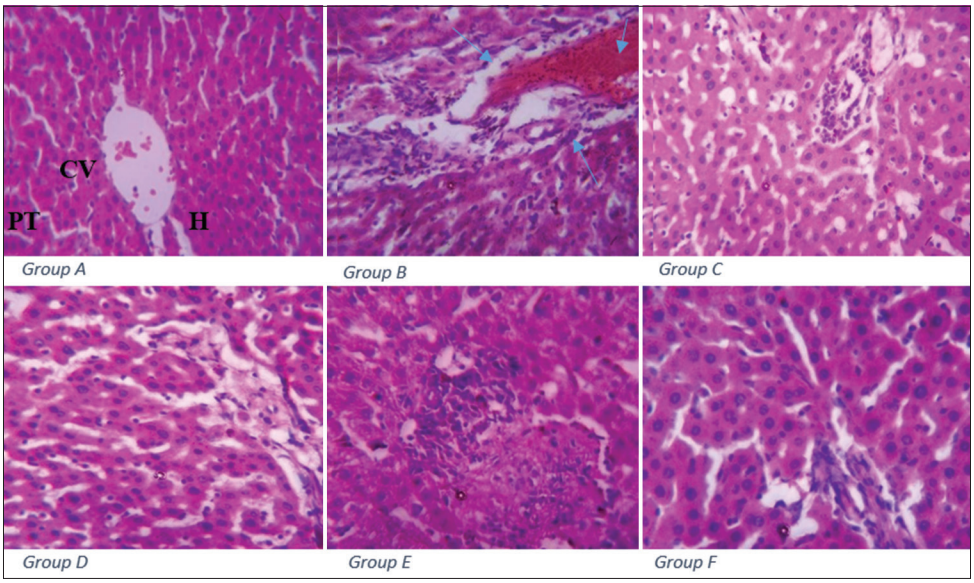

Figure 9: Photomicrograph of liver tissue from rats (H and E, ×400) Group A-Control group, Group B- Rifampicin (450 mg/70 kg body weight/day), Group C- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (50 mg/kg body weight/day) Group D- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (100 mg/kg body weight/day), Group E- Rifampicin (450 mg/70 kg body weight/day) + *Aloe vera* (200 mg/kg body weight/day), Group F- Rifampicin (450 mg/70 kg body weight/day)+ Furosemide (20 mg/70 kg body weight/day)

Table 2: Mean concentration of ALT, AST, and ALP in the experimental groups

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
A: Control group	22.64±0.95	32.11±0.96	327.66±13.13
B: Rifampicin (450 mg/70 kg) only	44.89±2.67*	66.68±2.78*	1424.21±180.71*
C: Rifampicin (450 mg/70 kg) and *Aloe vera* 50 mg/kg	44.17±2.78*	42.77±2.67	644.82±50.00*
D: Rifampicin (450 mg/70 kg) and *Aloe vera* 100 mg/kg	32.45±1.57*	35.51±2.56	379.79±27.03
E: Rifampicin (450 mg/70 kg) and *Aloe vera* 200 mg/kg	26.34±1.15	40.20±4.09	294.66±21.17
F: Rifampicin (450 mg/70 kg) and furosemide (20 mg/70 kg)	24.38±0.88	26.35±1.00*	229.95±39.68

The values are expressed as mean±SEM, n=5. *P<0.05 is significant when experimental groups are compared with the control group

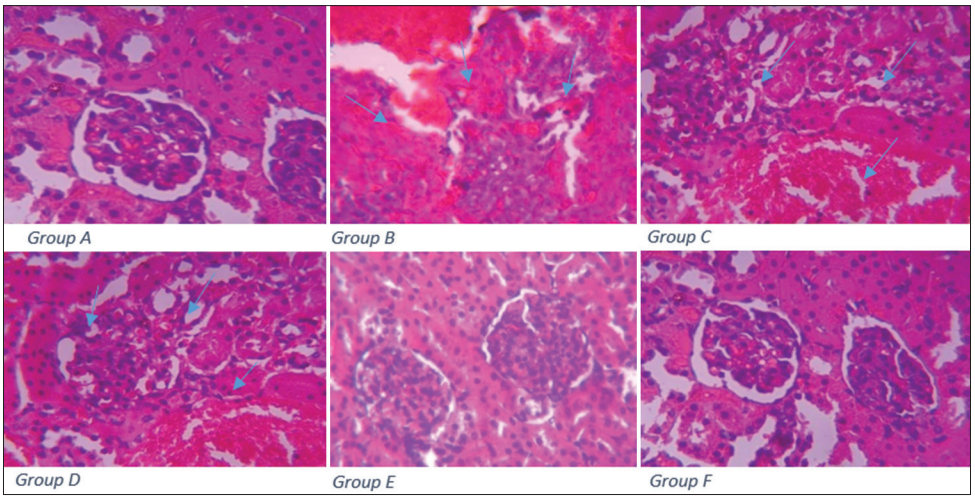

Figure 10: Photomicrograph of Kidney tissue from rats (H and E, ×400) Group A-Control group, Group B- Rifampicin (450 mg/70 kg body weight/day), Group C- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (50 mg/kg body weight/day) Group D- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (100 mg/kg body weight/day), Group E- Rifampicin (450 mg/70 kg body weight/day)+ *Aloe vera* (200 mg/kg body weight/day), Group F- Rifampicin (450 mg/70 kg body weight/day)+ Furosemide (20 mg/70 kg body weight/day)

Table 3: Effects of rifampicin and Aloe vera on MDA, CAT, and SOD activities in experimental groups

Groups	MDA (Mg/dL)	CAT (U/L)	SOD (U/L)
A: Control group	0.87±0.12	5.75±0.58	11.49±0.53
B: Rifampcin (450 mg/70 kg) Only	2.23±0.78*	3.77±0.12	8.75±0.60
C: Rifampicin (450 mg/70 kg) and *Aloe vera* 50 mg/kg	1.53±0.31	4.23±0.25	8.97±0.40
D: Rifampicin (450 mg/70 kg) and *Aloe vera* 100 mg/kg,	1.13±0.04	4.30±0.34	9.90±0.32
E: Rifampicin (450 mg/70 kg) and *Aloe vera* 200 mg/kg	0.80±0.06	4.68±0.20	10.83±0.43
F: Rifampicin (450 mg/70 kg) and furosemide (20 mg/70 kg)	0.59±0.12	5.25±0.24	11.41±0.82

The values are expressed as mean±SEM, n=5. *P<0.05 is significant when experimental groups are compared with the control group

Table 4: Results for the statistical comparison of renal biomarkers (urea and creatinine) of experimental animal groups after 14 days

Groups	Creatinine (mg/dL)	Urea (mg/dL)
A: Control group	0.41±0.15	25.18±2.77
B: Rifampicin (450 mg/70 kg) only (Negative control group)	3.10±0.70*	76.32±5.89*
C: Rifampicin (450 mg/70 kg) and *Aloe vera* 50 mg/kg	1.41±0.18	59.19±7.79
D: Rifampicin (450 mg/70 kg) and *Aloe vera* 100 mg/kg	0.94±0.21*	42.37±4.61*
E: Rifampicin (450 mg/70 kg) and *Aloe vera* 200 mg/kg	0.70±0.11*	35.49±4.48*
F: Rifampicin (450 mg/70kg) and furosemide (20 mg/70 kg)	0.63±0.28*	27.16±5.99*

The values are expressed as mean±SEM, n=5. *P<0.05 is significant when experimental groups are compared with the control group

Aloe vera to animals concomitantly with rifampicin at varying doses resulted in progressive regeneration of hepatic tissues as the dose increased [Figure 9].

Histological analysis of the kidney

The photomicrograph shows normal renal architecture with glomeruli, bowman space, renal tubules, and tubular cells in the control group while the section of the kidney of rifampicin-treated animals shows moderate degeneration with intra-renal inflammation and severe coagulative necrosis of renal tubules and glomeruli with severe closure of bowman space. Groups C and D show moderate to severe degeneration with moderate focal area hemorrhage, moderate infiltration of inflammatory cells and abnormal renal architecture of glomeruli. Groups E and F show normal renal architecture with glomeruli, bowman space, and renal tubules with distinct tubular cells [Figure 10].

Discussion

Drug-induced hepatorenal toxicity poses a significant challenge in clinical practice, often leading to hepatorenal dysfunction and ultimate failure. Rifampicin, a potent antibiotic used in the treatment of tuberculosis, is known to induce hepatorenal toxicity in some patients.[5] Rifampicin is said to cause liver toxicity by inducing the overexpression of PXR increasing the

amount of the CYP3A4 subset of the cytochrome P450 enzyme, which results in increased amounts of reactive metabolites that can covalently bind to proteins and stress cellular organelles.[25] The reactive metabolite may target endoplasmic reticulum (ER) or mitochondrial proteins, causing ER or mitochondrial stress.[7]

In this present study, elevated levels of Alanine Transaminase (ALT), Aspartate Transaminase (AST), and Alkaline Phosphatase (ALP) in rats administered only rifampicin indicate hepatocellular injury, hepatotoxicity, or liver dysfunction and this is supported by histological analysis of the negative control group which reveals severe hepatocyte degeneration, portal inflammatory cell aggregation, and focal hemorrhage, characteristic of chronic hepatitis. These findings align with previous studies reporting similar increases in liver biomarkers due to rifampicin exposure.[7,19] In contrast, experimental groups receiving *Aloe vera* concurrently with rifampicin show reduced ALT, AST, and ALP activity. *Aloe vera* contains potent antioxidants like vitamins C and E, flavonoids, and polyphenols which scavenge reactive oxygen species (ROS) and free radicals which are known to cause oxidative stress and liver damage.[26] By reducing oxidative stress, *Aloe vera* helps prevent hepatocyte damage, which in turn lowers the release of liver enzymes like AST, ALT, and ALP into the bloodstream. Furthermore, the protective effect of *Aloe vera* appears to be dose-dependent, with 100 mg/kg and 200 mg/kg doses yielding greater reductions in ALT, AST, and ALP levels. This observation is supported by previous studies demonstrating *Aloe vera's* protective effects against various hepatotoxic agents.[27-30] Additionally, co-administration of rifampicin and furosemide demonstrates a protective effect of furosemide against hepatic injury, as evidenced by significant decreases in hepatic biomarkers.

The impact of rifampicin administration on renal function is indicated by changes in creatinine and urea levels in experimental groups compared to the control. The significant increase in creatinine and urea levels in the negative control group suggests renal impairment and dysfunction, which aligns with previous literature on rifampicin-induced nephrotoxicity.[9] Interestingly, the administration of rifampicin concomitantly with *Aloe vera* at 50 mg/kg, 100 mg/kg, and 200 mg/kg resulted in a decrease in creatinine and urea levels with 100 mg/kg and 200 mg/kg doses of *Aloe vera* yielding greater reductions in creatinine and urea levels. This suggests a dose-dependent trend in the protective effects of Aloe vera on renal function. Co-administration of rifampicin with furosemide also led to a gradual decrease in serum creatinine as well as urea levels. This observation supports a potential protective effect of furosemide against rifampicin-induced nephrotoxicity, consistent with previous studies, highlighting the nephroprotective properties of furosemide.[31] Histopathological analysis of hepatorenal tissues from animals administered high doses of *Aloe vera* alongside rifampicin reveals hepatorenal regeneration. This could be due to the presence of polysaccharides and glycoproteins in *Aloe vera* which helps in tissue repair and regeneration.[32]

Animals treated with rifampicin alone exhibited a significant increase in malonaldehyde (MDA) levels, indicative of lipid peroxidation, and a decrease in catalase (CAT) and superoxide dismutase (SOD) levels, suggesting oxidative stress and tissue damage. This agrees with previous studies and reports[7,33] on the association between rifampicin administration and oxidative stress markers. Meanwhile, animals receiving *Aloe vera* concomitantly with rifampicin showed a gradual decrease in MDA levels and an increase in CAT and SOD levels as *Aloe vera* dosage increases, indicating a potential antioxidant effect of *Aloe vera*. The results of this study is consistent with previous findings[34,35] and reinforce the antioxidant properties of Aloe vera in restoring CAT and SOD levels in hepatotoxicity models induced by various agents. Co-administration of furosemide with rifampicin led to a significant decrease in MDA levels and an increase in CAT and SOD levels, aligning them closer to the control group, suggesting furosemide's ability to help eliminate free radicals, which is consistent with the findings of Rogóż *et al*.[36] However, a recent study by Olcay *et al.*[37] suggests that furosemide may cause further structural and functional impairment to the kidney following ischemic injury and should be administered with caution. According to Philomena[38] a good percentage of people admitted to the hospital are results of adverse or side effects of synthetic drugs. Furthermore, synthetic drugs address symptoms caused by specific diseases while herbal medicine is directed toward aiding the body's own repair mechanism.[39] Therefore, during rifampicin administration, the concomitant use of *Aloe vera* could provide hepatorenal protective effects mitigating potential drug-induced injury during rifampicin administration.

Conclusion

The study demonstrates that *Aloe vera* exhibits protective effects against rifampicin-induced hepatorenal toxicity in male Wister rats. The administration of *Aloe vera*, particularly at higher doses of 100 mg/kg and 200 mg/kg, significantly reduced liver and kidney function markers, suggesting its dose-dependent efficacy.

Additionally, *Aloe vera's* antioxidant properties offered protection against lipid peroxidation as demonstrated by a significant reduction in MDA activity, effectively mitigating oxidative stress induced by rifampicin administration. Furthermore, co-administration of furosemide with rifampicin exhibited similar protective effects, reducing oxidative stress and hepatorenal damage. These findings provide promising insights into the potential of *Aloe vera* as a therapeutic agent in managing drug-induced toxicity. While *Aloe vera* demonstrated stronger protective effects at 200 mg/kg, the specific bioactive compounds responsible for its hepatorenal protective actions were not identified. Further research would need to focus on the isolation and characterization of the bioactive compounds in *Aloe vera* responsible for its hepatorenal protective effects as well as conduct long-term studies to assess the sustained efficacy and safety of *Aloe vera* in managing chronic rifampicin-induced toxicity.

Authors contributions

All authors contributed equally to the manuscript. The manuscript has been read and approved by all the authors.

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Conflicts of interest

There are no conflicts of interest.

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