Hepato-protective activity of ethanol extract of *Moringa oleifera* leaves in acute rifampicin-induced hepatotoxicity

Elijah O. Oyinloye[1], Abdullahi A. Murtala[2], Farouk A. Oladoja[1], Theophilus A. Jarikre[3], Uwaiz O. Abdullah[1], Ajewole Y. Hassan[2], Adejolajesu O. Adeniji[1]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Olabisi Onabanjo University, Nigeria.
[2]Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Nigeria.
[3]Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan, Nigeria

Corresponding author: Oladapo E. Oyinloye
Email: oyinloye.oladapo@oouagoiwoye
Telephone: +2348039196240, 09020531914

ABSTRACT

Background: Drugs are an important cause of liver injury. The use of natural remedies for the treatment of liver diseases has a long history, and medicinal plants are still used all over the world for this purpose. For their medicinal and nutritional values, the *Moringa oleifera* plant is of particular interest.

Objective: In the current study, we aimed to explore the hepato-protective effects of ethanol extract of *Moringa oleifera* leaves in acute rifampicin-induced hepatotoxicity.

Methods: Thirty mice (18-20 g) were randomly assigned into 6 groups (n = 5). Group I received normal saline only; groups II, III, IV, V, and VI were administered with single oral rifampicin 400 mg/kg, treated with normal saline, different doses of ethanol extract of *Moringa oleifera* (200, 400, and 800 mg/kg), and ascorbic acid, respectively. Serum levels of liver enzymes Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), catalase and superoxide dismutase (SOD) were measured in the serum. Furthermore, phytochemical constituents were also measured in the *Moringa oleifera* leaves extract.

Results: Treatment with an ethanol extract of *Moringa oleifera* decreased liver enzyme levels and improved oxidative stress status in hepatotoxic mice in a dose-dependent manner.

Conclusion: Normal levels of liver enzymes, an increase in total antioxidant capacity, and a decrease in lipid peroxidation level uncovered the hepatoprotective effects of the ethanol extract of *Moringa oleifera*. It seems the antioxidant and hepatoprotective effects of botanical extracts are basically linked with their phenol and flavonoid properties that neutralize oxidant agents. However, more studies are required to implement our strategy.

Keywords: Hepatotoxicity, Rifampicin, *Moringa oleifera,* ethanol, antioxidant.

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Activité hépatoprotectrice de l'extrait éthanolique de feuilles de *Moringa oleifera* dans l'hépatotoxicité aiguë induite par la rifampicine

Elijah. O. Oyinloye[1], Abdullahi. A. Murtala[2], Farouk. A. Oladoja[1], Theophilus A. Jarikre[3], Uwaiz. O. Abdullah[1], Ajewole. Y. Hassan[2], Adejolajesu. O. Adeniji[1]

[1]Département de pharmacologie et de toxicologie, Faculté de pharmacie, Olabisi Onabanjo Université, Nigéria.
[2]Département de pharmacologie et de thérapeutique, Faculté des sciences médicales de base, Collège Obafemi Awolowo des sciences de la santé, Université Olabisi Onabanjo, Nigéria.
[3]Département de pathologie vétérinaire, Faculté de médecine vétérinaire, Université d'Ibadan, Nigeria

Auteur correspondant: Oladapo E. Oyinloye
Email: oyinloye.oladapo @oouagoiwoye
Numéro de téléphone: +2348039196240, 09020531914

RÉSUMÉ

Contexte: Les médicaments sont une cause importante de lésions hépatiques. L'utilisation de remèdes naturels pour le traitement des maladies du foie a une longue histoire et les plantes médicinales sont encore utilisées à cette fin dans le monde entier. Pour ses valeurs médicinales et nutritionnelles, la plante *Moringa oleifera* présente un intérêt particulier.

Objectif: Dans la présente étude, nous avons cherché à explorer les effets hépatoprotecteurs de l'extrait éthanolique de feuilles de Moringa oleifera dans l'hépatotoxicité aiguë induite par la rifampicine.

Méthodes: Trente souris (18-20 g) ont été réparties au hasard en 6 groupes (n = 5). Le groupe I a reçu uniquement une solution saline normale ; les groupes II, III, IV, V et VI ont reçu une seule dose orale de rifampicine de 400 mg/kg, traités avec une solution saline normale, différentes doses d'extrait éthanolique de *Moringa oleifera* (200, 400 et 800 mg/kg) et de l'acide ascorbique, respectivement. Les taux sériques des enzymes hépatiques alanine transaminase, aspartate transaminase, lactate déshydrogénase et phosphatase alcaline, glutathion réduit (GSH), catalase, superoxyde dismutase et peroxydation lipidique sont mesurés dans le tissu hépatique à l'aide de kits spécifiques à la fin de la période expérimentale. En outre, les constituants phytochimiques ont également été mesurés dans l'extrait de feuille de *Moringa oleifera.*

Résultats: Le traitement avec un extrait éthanolique de *Moringa oleifera* a diminué les niveaux d'enzymes hépatiques et amélioré l'état de stress oxydatif chez des souris hépatotoxiques de manière dose-dépendante.

Conclusion: Des niveaux normaux d'enzymes hépatiques, une augmentation de la capacité antioxydante totale et une diminution du niveau de peroxydation lipidique ont mis en évidence les effets hépatoprotecteurs de l'extrait éthanolique de *Moringa oleifera*. Il semble que les effets antioxydants et hépatoprotecteurs des extraits botaniques soient essentiellement liés à leurs propriétés phénoliques et flavonoïdes qui neutralisent les agents oxydants. Cependant, des études supplémentaires sont nécessaires pour mettre en œuvre notre stratégie.

Mots clés: Hépatotoxicité, Rifampicine, *Moringa oleifera*, éthanol, antioxydant.

INTRODUCTION

The liver plays a crucial role in drug metabolism, situated between absorption and systemic circulation, and serves as the primary site for metabolizing and eliminating external substances. Due to these functions, the liver is also highly susceptible to drug toxicity. Drug-induced liver injury (DILI) is the most frequent side effect causing the failure of new drug candidates or their withdrawal from the market, making it a significant clinical challenge.[1] Liver cell damage is caused by toxic chemicals, antibiotics, carbon tetrachloride, thioacetamide, excessive alcohol intake, and microbial infections. Hepatotoxicity is a prevalent condition that can lead to serious disorders and potentially fatal outcomes in both animals and humans. Studies have demonstrated that oxidative biomarkers and pro-inflammatory cytokines produced during liver injury contribute to the progression of tissue damage.[2]

Tuberculosis is one of the leading causes of death among treatable infectious diseases. According to World Health Organization (WHO) data published in 2016, 9.6 million people were affected by tuberculosis, with 1.5 million deaths recorded in 2014.[3] The four primary drugs used in tuberculosis treatment are rifampicin isoniazid, pyrazinamide, and ethambutol. The usual treatment protocol for adult respiratory tuberculosis involves administering rifampicin, isoniazid, and pyrazinamide for two months, followed by rifampicin and isoniazid for an additional four months.[4] However, anti-tuberculosis medications like rifampicin, isoniazid, and pyrazinamide are often linked to frequent hepatotoxicity.[1]

Rifampicin (RIF) is known to cause hepatocellular dysfunction during the initial phases of treatment, although these issues generally resolve once the medication is stopped.[5] Additionally, rifampicin can impair bilirubin excretion, leading to short-term hyperbilirubinemia.[6] Because it can induce liver damage through cholestasis, it is associated with unique hepatic lesions characterized by hepatocellular alterations and centrilobular necrosis.[7] Medicinal plants play a valuable role in managing various disorders. Numerous medicinal herbs have been documented to have successful hepatoprotective effects in various animal models.[8,9]

Moringa oleifera (MO) is a well-known species of the Moringa genus, noted for its rapid growth and ability to withstand harsh conditions.[10,11] In ancient times, the Egyptians utilized *Moringa oleifera* in cosmetics and for treating skin conditions. It was also recognized by both the Romans and Greeks.[12] *Moringa oleifera* leaf extract is renowned for its numerous medicinal and cytoprotective benefits, contributing to its broad use as both a nutritional supplement and therapeutic remedy. Native to northwestern India, Moringa is now cultivated globally. It is rich in antioxidants.[13] and polyphenols,[14] exhibiting antifungal, antimicrobial, and antidiabetic properties.[15]

Additionally, it enhances organ function by regulating oxidative stress and preventing damage and enhances organ function by serving as a modulator of oxidative stress and preventing damage.[15] *Moringa oleifera* is considered to have minimal toxicity and is safe for human consumption, even in high doses.[16] However, its therapeutic effects and the underlying hepato-renal signaling pathways remain poorly understood. Thus, we concentrated on exploring its role in alleviating rifampicin-induced liver damage, specifically analyzing its influence on liver enzymes, antioxidant levels, and histological alterations

METHODS

Preparation of plant material

Moringa oleifera leaves were collected from Ibadan located in Oyo State, southwest Nigeria. At the Department of Botany Herbarium, University of Ibadan, Oyo State, Nigeria, and air-dried at room temperature (27-30^{o}C) for 7 days until they reached a stable weight, then ground into a coarse powder using a mechanical grinder.

Extraction of *Moringa oleifera* leaves material

The powdered plant material was soaked in a solution of 80 % ethanol and 20 % water (v/v) for 72 hours at room temperature. The plant residues were then filtered, and the extract was concentrated using a water bath at 60^{o}C, the crude extract was stored at -20^{o}C.

Phytochemical screening:

The phytochemical components of the ethanol extract from *Moringa oleifera* leaves were analyzed following the protocols outlined by Trease and Evans[17] and De Silva *et al*.[18], which were used for both qualitative and quantitative assessments.

Fourier-transform infrared spectroscopy (ft-ir) analysis

FT-IR analysis of the plant was performed using established methods from Liu and Kim *et al*.[19] and Bolade *et al*.[20] The Moringa oleifera extract was analyzed with an Agilent Cary 630 FTIR spectrometer, which was equipped

with Microlab PC software and an attenuated total reflectance (ATR) accessory. The instrument had a resolution of 8 cm^{-1} and a scanning range from 4000 cm^{-1} to 400 cm^{-1}.

Treatment protocol

Mice were divided into six groups of five mice each.
Group 1: Control (mice received standard diet and Normal saline)
Group 2: Single oral RIF 400 mg/kg + treated with Normal saline for 4 days
Group 3: Single oral RIF 400 mg/kg + treated with MO 200mg/kg for 4 days
Group 4: Single oral RIF 400 mg/kg + treated with MO 400mg/kg for 4 days
Group 5: Single oral RIF 400 mg/kg + treated with MO 800mg/kg for 4 days
Group 6: Single oral RIF 400 mg/kg + treated with Ascorbic acid (ASC) 200mg/kg for 4 days

Preparation of serum samples and organs
The animals were sacrificed under ether anesthesia 24 hours after getting the last dosage of Rifampicin and ethanol extract of *Moringa oleifera*. Blood samples obtained by cardiac puncture were placed in plain tubes and allowed to coagulate. After centrifuging the clotted blood samples at 3000 rpm for 15 minutes, the serum samples aspirated were used to evaluate the liver function tests. Livers were harvested after dissection of the animals and rinsed in ice-cold 1.15 % KCl, dried and weighed. The liver samples were homogenised in 4 volumes of 50 mM phosphate buffer, pH 7.4 using a Potter Elvehjem homogeniser and centrifuged at 10,000 g for 15 minutes to obtain post-mitochondrial supernatant fraction (PMF) which was used to determine malondialdehyde (MDA) and reduced glutathione (GSH) levels. Meanwhile, catalase (CAT), super oxide dismutase (SOD activities) was determined in serum. The liver samples were excised, rinsed in normal saline, blotted on filter paper and fixed in 10 % formalin for histological assessment.

Biochemical analysis: Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), was determined in serum, using Randox Laboratories Ltd commercial kit.

Histological analysis: Each group's liver tissue fixed in 10% formalin was embedded in paraffin. A light microscope was used to view 5-6 mm sections stained with Haematoxylin and Eosin (H&E) (Olympus CH02). Differences between the liver of the control and treated mice were noted.

Statistical Analysis:
The experimental data was presented as Mean ± standard error of the mean (SEM). Data were analyzed using One-way analysis of variance (ANOVA) and followed by Dunnett's/Tukey's post-hoc tests using Graph Prism 6 Software (Graph Pad Soft Inc., CA, USA). The results were considered significant at $p<0.05$.

RESULTS

Phytochemical screening results

Qualitative and quantitative phytochemical screening of *Moringa oleifera* leaves
The preliminary qualitative and quantitative screening of ethanol extract of *Moringa oleifera* was carried out and the results are presented in table 1. In the qualitative study, tannin, phenol, alkaloids, saponin, flavonoid and Terpenoid were present. The quantitative screening of the extract showed that Flavonoid (5.14 %) was the most abundant, followed by Alkaloid (1.65 %), phenols (1.56%) and Tannins (1.05), while Saponin (0.90 %) was the least abundant.

Table 1: Photochemical Screening of ethanol extract of *Moringa oleifera*

S/N	Qualitative screening	*Moringa oleifera* extract	Quantitative Screening (% w/w)
1	Tannin	+	1.05±0.011
2	Phenol	+	1.56±0.010
3	Alkaloid	++	1.65±0.001
4	Saponin	++	0.90±0.002
5	Flavonoid	++	5.14±0.002
6	Terpenoid	+	0.60±0.001

+: Present; ++: Abundantly Present. Statistical data were indicated as Mean ± Standard Error Mean.

FT-IR Spectrum of Ethanol Extract of *Moringa oleifera* leaves

The FTIR spectrum of *Moringa oleifera* leaves extract exhibited prominent peaks at 33126 cm^{-1}, 2928.1 cm^{-1}, 1689.28 cm^{-1}, 1611.25 cm^{-1}, 1208.05 $cm^{-1}$and 600.845 cm^{-1}. Figure 1 showed that the spectrum of ethanol extract of *Moringa oleifera* leaves revealed the presence of the strong C-H stretching at 2928.1 cm^{-1} conjugated by C=C stretching at 1611.25 cm^{-1} confirms Alkane's and Alkene's presence. The C-C stretching at 1450.34 cm-1 suggested aromatic compounds, while the C-O bands at 1346.98 cm^{-1} confirmed the presence of carboxylic acids. The C-N bands at 1268.28 cm^{-1} revealed the presence of Aromatic amines, while the C-O stretching at 1208.05 cm^{-1} confirmed the presence of alcohol and carboxylic acids.

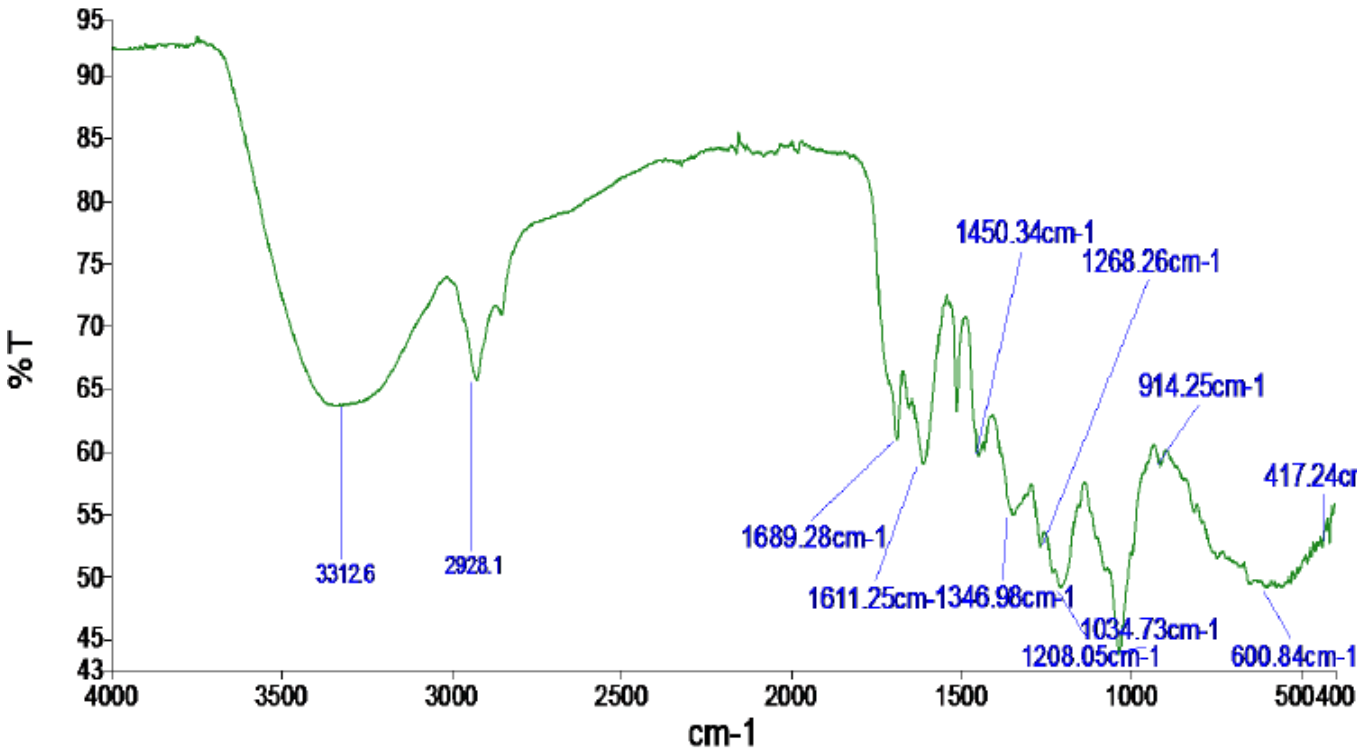


Figure 1: FT-IR Spectrum of Ethanol Extract of *Moringa oleifera* leaves

Effect of Curative effect of *Moringa oleifera* effect on liver biomarkers in Rifampicin (RIF) induced liver toxicity in mice.

MO at all dose levels (200, 400 and 800 mg/kg) exhibit a dose dependent decrease in ALT, AST and ALP levels, which was significant (P<0.05) in RIF + MO 800 mg/kg and ascorbic acid, for AST when compared with RIF+ Normal saline (Table 2).

Table 2: - Effect of Ethanol extract of *Moringa oleifera* activity on liver enzymes in acute Rifampicin induced liver toxicity in mice.

Groups	AST (I.U/L)	ALT (I.U/L)	ALP (I.U/L)
Normal saline	23.00±0.2	6.48±2.05	31.50±1.16
RIF+ Normal saline	59.21±1.58$^{\#}$	10.79±1.80	55.36±2.45
RIF + MO 200 mg	57.31±1.65	9.60±0.29	50.67± 0.51
RIF+ MO 400 mg	51.20±1.95	9.60±0.45	50.20±2.72
RIF + MO 800 mg	29.62±2.04*	8.50±1.15	45.90±2.70
RIF+ ASC 200 mg	28.67 ±1.62*	8.28±1.05	40.55±1.44

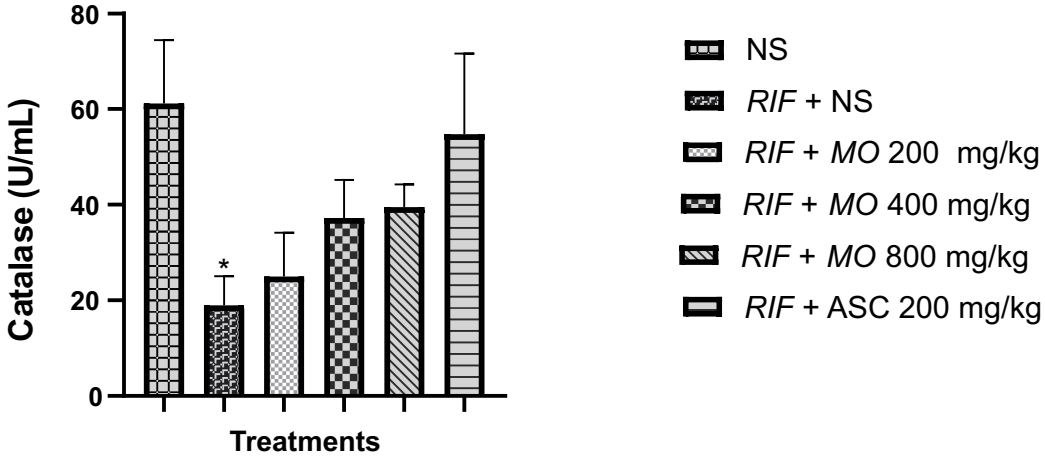
Legend: MO= *Moringa oleifera;* RIF=Rifampicin; ASC= Ascorbic Acid

$^{\#}$ = p<0.05 significant against Normal saline

***= p<0.05 significant against RIF+ Normal saline**

Effect of *Moringa oleifera* on catalase activities in Rifampicin (RIF) induced liver toxicity in mice.

The result in figure 2 showed a significant (P<0.05) reduction in catalase activity in RIF+ Normal saline in comparison with group that received normal saline only. A dose-dependent increase in catalase activities were observed in all groups administered with rifampicin plus *Moringa oleifera* extract.



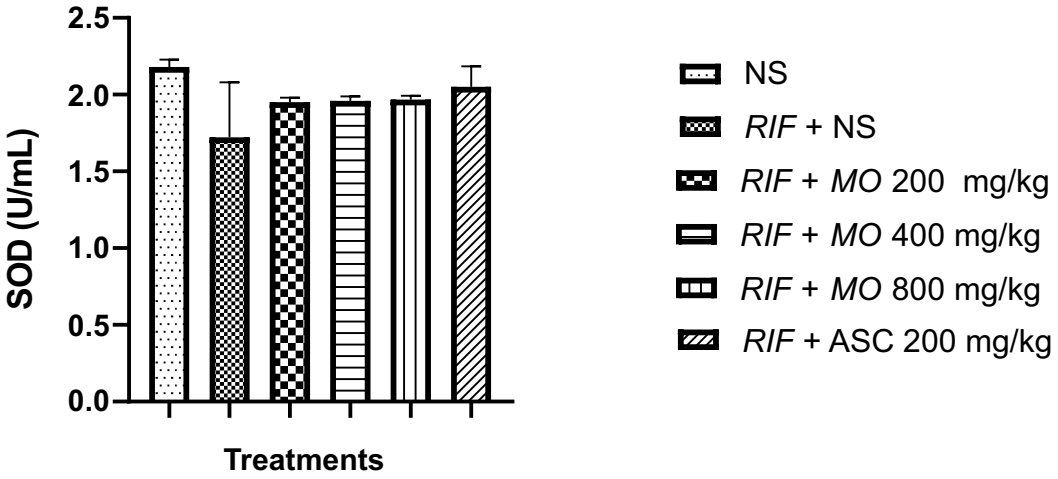
Legend: RIF - Rifampicin, MO - *Moringa oleifera*, ASC- Ascorbic acid

Figure 2: Effect of *Moringa oleifera* on catalase activities in Rifampicin (RIF) induced liver toxicity in mice.

***= p<0.05 significant against Normal saline**

Effect of *Moringa oleifera* on super oxide dismutase (SOD) activities in Rifampicin (RIF) induced liver toxicity in mice.

A reduction in SOD activity was exhibited in group treated with RIF+ Normal saline when compared with that received normal saline only. A dose dependent increase in SOD activities were observed in all groups administered with rifampicin plus Moringa oleifera extract (Figure 3).

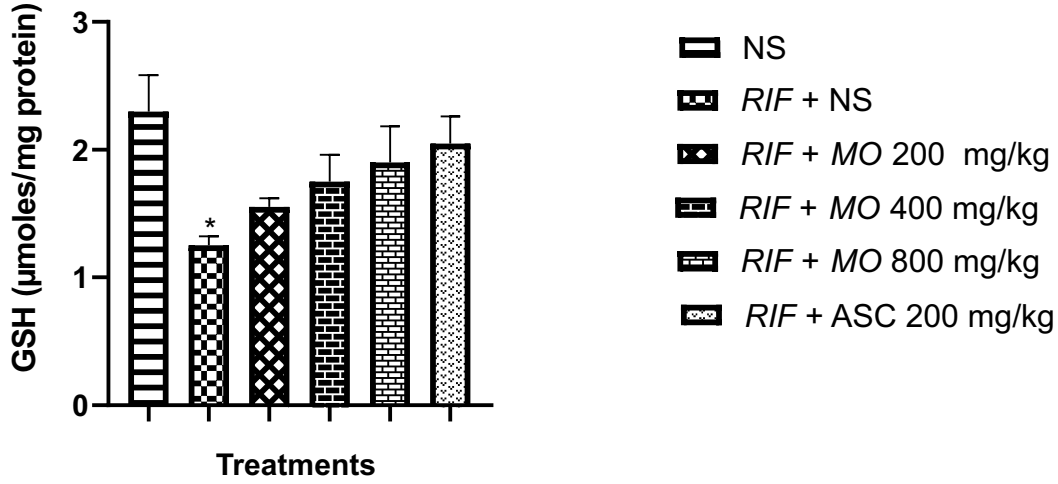


Legend: RIF - Rifampicin, MO - *Moringa oleifera*, ASC- Ascorbic acid

Figure 3: Effect of *Moringa oleifera* on superdioxide dismutase (SOD) activities in rifampicin (RIF) induced liver toxicity in mice.

Effect of *Moringa oleifera on* reduced glutathione (GSH) levels in Rifampicin (RIF) induced liver toxicity in mice.

The result showed a significant ($p<0.05$) reduction in GSH level relative to Normal saline treated group. All the groups administered with *Moringa oleifera* extract showed an increase in GSH levels in a dose dependent fashion relative to RIF+ NS (Figure 4).

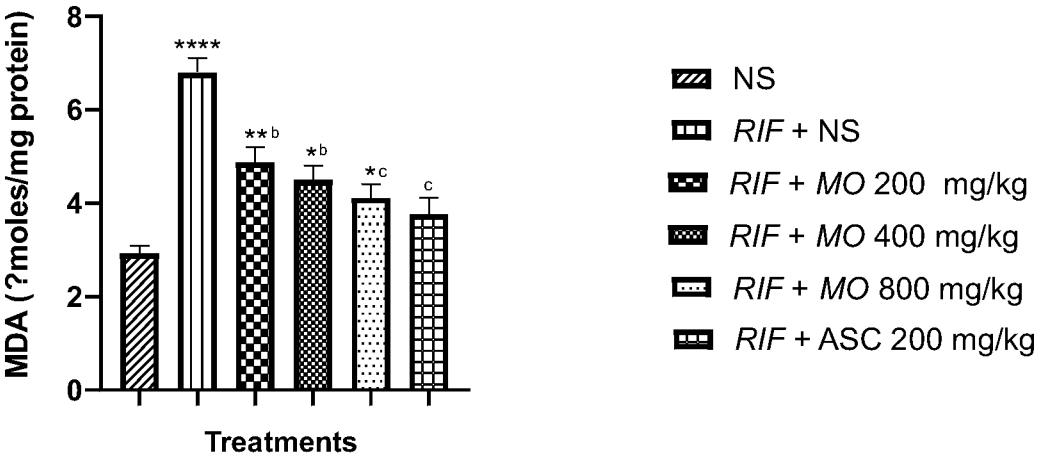


Legend: RIF - Rifampicin, MO - *Moringa oleifera*, ASC- Ascorbic acid

Figure 4: Effect of Curative action of *Moringa oleifera* on reduced glutathione (GSH) levels in Rifampicin (RIF) induced liver toxicity in mice.

***= $p<0.05$ significant against Normal saline**

Effect of *Moringa oleifera* on malondialdehyde (MDA) levels in Rifampicin (RIF) induced liver toxicity in mice.
Figure 5 exhibited a significant (P<0.001) increase in MDA levels in RIF + NS relative to group that was administered with normal saline only. In contrary, a dose dependent (P<0.05, bP<0.01, cP<0.001) reduction in MDA levels were exhibited all groups treated with *Moringa oleifera* extract.



Legend: RIF - Rifampicin, MO - *Moringa oleifera*, ASC- Ascorbic acid

Figure 5: Effect of Curative action of *Moringa oleifera* on malondialdehyde (MDA) levels in Rifampicin (RIF) induced liver toxicity in mice.

******= p<0.0001 significant against Normal saline, *b=0.1, **b=P<0.01, *c=P<0.001 vs RIF + NS (One way ANOVA followed by Tukey's Multiple Comparison test).**

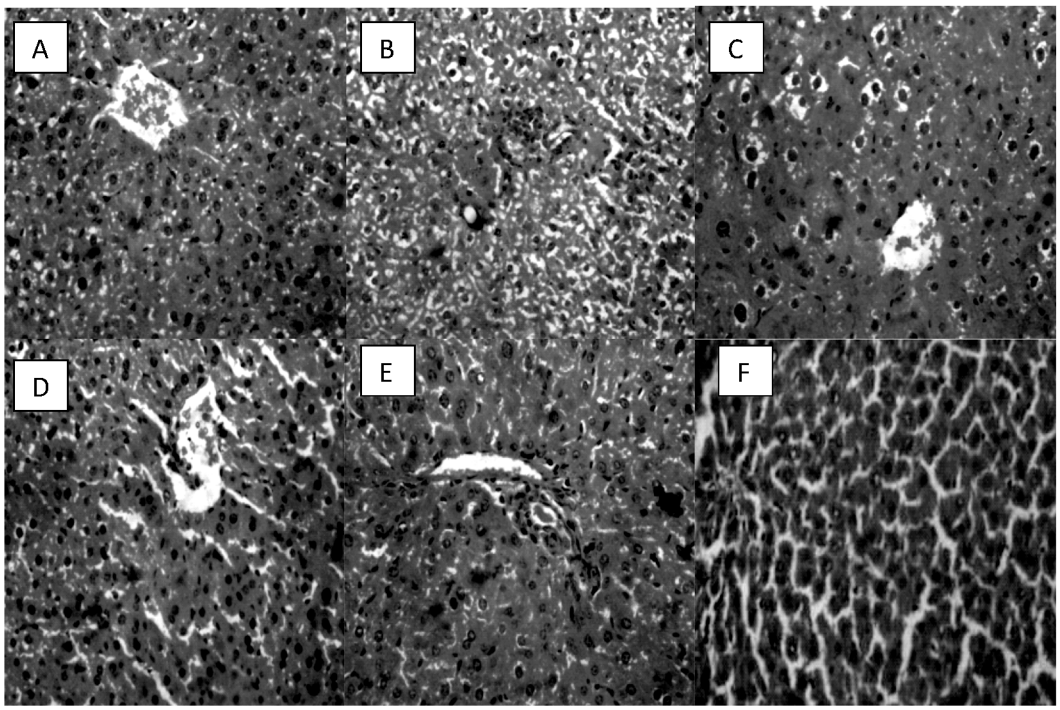


Figure 6: Effect of ethanol extract of *Moringa oleifera* on the liver in Rifampicin induced liver toxicity in mice, HE x400.

(A) Control animals (Normal Saline) show no observable lesion; (B) RIF +Normal Saline animals show focal necrotizing hepatitis; (C) RIF + MO 200 mg/kg, show centrilobular hepatocellular swelling; (D) RIF + MO 400 mg/kg, show no observable lesion; (E) RIF + MO 800 mg/kg, show no observable lesion ; (F) RIF + ASC 200 mg/kg, show no observable lesion.

DISCUSSION

Over the years, numerous experimental studies and intervention trials have demonstrated that traditional herbs play a crucial role in the treatment and prevention of liver diseases.[21] Overdoses of rifampicin have been shown to cause acute liver damage and severe hepatic necrosis by inducing oxidative stress or reducing overall antioxidant capacity.[22,23] Since rifampicin is extensively metabolized by the liver and increases several hepatic enzymes, liver injury may result from idiosyncratic metabolic byproducts that are either directly toxic or trigger an immunological response.[23] Many studies have indicated that antioxidants from natural products help protect cells from oxidative stress and enhance liver health.[24]

In the present study, we evaluated the hepato-protective effects of ethanol extracts of *Moringa oleifera* in acute rifampicin-induced hepatotoxicity. Research has demonstrated that *Moringa oleifera* offers significant protection against drug-induced liver damage, positioning it as a potential therapeutic solution for xenobiotic-related liver injury.[25] The plant's phytochemicals possess antioxidant, anti-inflammatory, and immune-modulating properties, which contribute to its liver-protective effects.[26] These compounds help restore liver function and alleviate oxidative stress, which is essential for mitigating the toxic impact of rifampicin.[27,26]

Furthermore, *Moringa oleifera* has been shown to effectively reduce liver damage caused by drugs, particularly by lowering levels of liver enzymes such as ALT, AST, and ALP. This is corroborated by multiple studies that emphasize its effectiveness in reducing liver damage induced by various hepatotoxic agents.[28] In this study, treatment with ethanol extract of *Moringa oleifera* in mice with rifampicin-induced liver toxicity led to a dose-dependent decrease in liver enzyme levels. The extract at 800 mg/kg had the most pronounced effect in reducing liver enzymes (ALT, AST, and ALP) (Table 2). These results align with earlier findings by Abd-elhameed *et al*.[25], which demonstrated that *Moringa oleifera* extract significantly lowered liver enzyme levels, reflecting reduced liver damage, when administered with rifampicin. Al-Sultan and Al-Sowayan[29], previously observed a notable reduction in ALT, AST, and ALP levels in rats with paracetamol-induced liver injury, underscoring the *Moringa oleifera* extract's protective effect against hepatotoxicity. Additionally, another study revealed that *Moringa oleifera* leaf extract lowered AST and ALT levels in rats exposed to bisphenol-A, demonstrating its efficacy in counteracting oxidative stress and inflammation related to liver damage.[30]

The hepatoprotective effect of *Moringa oleifera* is attributed to its ability to neutralize free radicals generated by rifampicin metabolism, which leads to glutathione depletion and subsequent damage to hepatocyte membranes. Additionally, in this study, the extract was found to boost antioxidant activity by elevating levels of reduced glutathione, catalase, and superoxide dismutase, while decreasing malondialdehyde, a marker of oxidative stress. These findings corroborate earlier research by Abd-elhameed *et al*.[25], which demonstrated the protective effects of *Moringa oleifera* extract when used alongside isoniazid and rifampicin.

Based on prior research and our findings on antioxidant activity, it appears that the antioxidant potential of the ethanol extract of *Moringa oleifera* leaves may be attributed to its flavonoid and phenolic compounds, which neutralize reactive oxygen species generated by rifampicin. Notably, the highest levels of flavonoids, followed by phenols, in our phytochemical analysis align with the objectives of our study. The results corroborate our assertion that *Moringa oleifera* leaves exhibit significant hepatoprotective effects. It is hypothesized that the antioxidant and hepatoprotective properties of plant extracts are primarily associated with their phenolic and flavonoid content.[31,32] Significant advancements have been achieved in comprehending how herbal plants and their metabolites interact with the liver. However, our understanding of plant extracts remains incomplete, and further research is needed to fully elucidate their significance and mechanisms. The histology results in the groups treated with rifampicin and varying doses of ethanol extract of *Moringa oleifera* (400 and 800 mg/kg), show no lesion. Hepatoprotective effect observed in animals administered with ascorbic acid is consistent with the earlier findings by Abd-elhameed *et al*.[25]

CONCLUSION

Our study revealed that the ethanol extract of *Moringa oleifera* leaves substantially lowered serum liver enzyme levels in rifampicin-induced hepatotoxicity, in dose-dependent effects. Therefore, the ethanol extract of *Moringa oleifera* leaves appears to be a promising hepatoprotective agent and could be considered a potential new treatment for rifampicin-induced liver diseases.

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Authors declare that there are no competing interests.

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