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Prevention of Hepato-renal Toxicity with *Moringa oleifera* in Gentamicin -treated Rats

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Abstract

The purpose of this investigation was to ascertain if orally administered *Moringa oleifera* (MO) extract had any protective effects on several biochemical markers in the kidney and liver in gentamicin (GNT)-induced hepato-renal toxicity in rats. Forty male albino rats were divided into four groups: the control group, the MO treated group, the GNT administered group, and the (MO+ GNT) group. The MO+ GNT group received GNT (100 mg/kg b.wt, i.p.) together with *Moringa oleifera* (400 mg/kg b.wt) for 20 consecutive days. Rats were put to death at the conclusion of the experiment, and blood samples were taken to measure serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total proteins, albumin, and globulin, as well as serum urea, creatinine, and uric acid. Catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), superoxide dismutase (SOD), total antioxidant capacity (TAC), 8-hydroxy-2'-deoxyguanosine (8OHdG), and malondialdehyde (MDA) were measured in isolated kidneys and liver. The liver and kidneys were divided into pieces for histology and a few immunohistochemistry tests. Following administration of GNT, there was a significant decrease in the activities of the hepatic and renal CAT, GPX, GSH, SOD, and TAC while there was a significant increase in the levels of MDA, 8OHdG, serum AST, ALT, ALP, urea, creatinine, and uric acid. Treatment with MO significantly lessened the histopathological abrasions in the liver and kidney tissue brought on by GNT and restored the levels of renal and hepatic BAX and TNF. It also restored the evaluated criteria to normal values. According to the results, MO has a protective effect against GNT-induced hepato-nephrotoxicity in rats. This effect may be explained by the fact that MO prevents free radical generation and restores antioxidant activity, which reduces the negative effects of GNT.

KEYWORDS
Gentamicin, *Moringa oleifera*, Hepato-renal toxicity, Antioxidant activity.

INTRODUCTION

The kidney is well recognized as a crucial organ that regulates body homeostasis and excretes harmful chemicals. Oxidative stress, which causes cellular death, lipid peroxidation, and a decrease in antioxidant levels, coexists with renal damage (Negin *et al*., 2022). In addition, hazardous substances, xenobiotics, and various pharmaceuticals are mostly detoxified and eliminated by the liver (Soliman *et al*., 2022).

A bactericidal antibiotic belonging to the aminoglycoside class called gentamicin (GNT) is used to treat gram-negative bacterial infections (Yarijani *et al*., 2021). According to Prayle *et al*. (2010) and Noorani *et al*. (2010), the main adverse effects of aminoglycosides are kidney damage, nephrotoxicity, hearing loss, vestibular toxicity, and hepatotoxicity. The exact mechanism by which GNT causes the development of hepatorenal toxicity is still unknown, but evidence suggests that GNT increases oxidative stress, lipid peroxidation, and the production of reactive oxygen species (ROS) like the hydroxyl radical, hydrogen peroxide, and superoxide anion while decreasing the activity of antioxidant enzymes like CAT and SOD (Najafian *et al*., 2014).

Due to their advantages over chemical drugs, such as little or zero toxicity, availability naturally, and excellent features as feed additives, herbal medicine has gained popularity in recent years (Gholami-Ahangaran *et al*., 2021).

A drought-resistant tree with a wide distribution in tropical and subtropical regions of Asia and Africa is the *Moringa oleifera* (MO) family *Moringaceae*. Due to its health advantages, it is referred to as the "miracle tree". Numerous nutrients are abundant in MO leaves (Hamed and El-Sayed, 2019). They have been used extensively as an antifungal, antibacterial, antipyretic, antioxidant, anticonvulsant, anti-inflammatory, antiulcer, antihypertensive, anti-tumor reagent, as well as a treatment for haematological and cardiovascular disorders, and they were shown to have a hepatoprotective effect (Abdel Fattah *et al*., 2020; Ahmed *et al*., 2020).

The goal of this investigation was to determine whether *Moringa oleifera* aqueous extract can protect male Albino rats from gentamicin-induced hepato-renal damage.

MATERIALS AND METHODS

Drug

Gentamicin: Product of El-Nasr Pharmaceutical Chemicals Co. Egypt. Each ampoule contains GNT (as sulphate) 80 mg. Dose in

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rats is 100 mg/kg b.wt i.p injection once daily for 10 successive days (Khan *et al*., 2011).

Moringa oleifera

Preparation of Aqueous extract of *Moringa oleifera*

Before the extraction, fresh leaves of MO were washed in water, allowed to dry naturally, and then ground to a powder and dried in an airtight container. One gram of dried and powdered leaves was combined with 10 ml of boiling water in the laboratory to create the plant-derived aqueous extract that was tested in this study. The liquid was then run twice through sterile filter paper with a 2 m pore size before being allowed to cool. As previously disclosed (Berkovich *et al*., 2013), the aqueous extract stock solution (100 mg/ml) was freshly made every day before to animal administration. According to the previously reported approach, 400 mg/kg body weight of the aqueous extract of MO is administered orally once daily for 30 consecutive days to rats (Bassey *et al*., 2013).

Experimental animals

In the Faculty of Veterinary Medicine at Zagazig University, Egypt, forty mature male albino rats weighing 150–200 g were procured. Before beginning the experiment, rats were kept under observation and given a week to get used to the lab setting. They were then housed in metal cages under sanitary conditions, fed barley and milk during the trial, and given access to water as needed. The Ethics Committee of the Faculty of Veterinary Medicine at Zagazig University gave its approval to the study methodology and the use of rats with the approval code ZU-IACUC/2/F/21/2022.

Experimental protocol

Forty adult male albino rats were allocated into four groups and each group contains 10 rats as follows:

The 1st group (Control): Rats received 0.5 ml distal water orally by stomach tube once daily for 30 successive days.

The 2nd group (MO treated animals): Rats received 400 mg/kg b.wt of aqueous extract of MO orally via stomach tube once daily for 30 successive days (Bassey *et al*., 2013).

The 3rd group (GNT administrated animals): Rats received 100 mg/kg b.wt of GNT I/P injected once daily for 10 successive days (Khan *et al*., 2011).

The 4th group (MO +GNT treated animals): Rats received MO aqueous extract before GNT administration for 20 successive days then simultaneously with GNT for another 10 successive days with their respective doses.

Collection of samples

At the end of the experiment (24 hours after the last dose), rats were sacrificed and the following samples were collected:

Kidneys and liver samples from all groups were immediately removed and kept in deep freezer (-70°C) for assay of antioxidant enzymes activities; CAT according to the method described before (Aebi, 1984), SOD according to the method described (Nishikimi *et al*., 1972), GPX (Paglia and Valentine, 1967), GSH (Moron *et al*., 1979), TAC (Koracevic *et al*., 2001), 8OHdG (Korkmaz *et al*., 2018), and determination of MDA concentration (Ohkawa *et al*., 1979).

For histological examination, 3 g of additional kidney and liver tissue were placed in liquid paraffin (Suvarna *et al*., 2018).

For liver and kidney function tests, blood was drawn, allowed to clot, and then the serum was separated by centrifugation at 3000 rpm for 15 minutes. These tests included estimation of ALT and AST activities according to Tietz (1976), ALP activity according to Belfeild and Goldberg (1971), total proteins level according to Okutucu *et al*. (2007), albumin level according to Peters Jr, (1985), globulin was calculated mathematically according to Kapale *et al*. (2008), serum creatinine level according to Young *et al*. (1975), serum urea level according to Fawcett and Scott, (1960) and serum uric acid according to Fossati *et al*. (1980). All measurements were performed using diagnostic kits procured from BioMed, Egypt.

Statistical analysis

Using the statistical software programme SPSS, version 16.00, USA, the data were statistically analysed in one direction (ANOVA). Results were statistically significant at $P < 0.05$ when the data were reported as the mean SE.

RESULTS AND DISCUSSION

A versatile, evergreen tree with excellent nutritional and economic value is called *Moringa oleifera* (Padayachee and Baijnath, 2020). Protein, vitamins, minerals, and phytonutrients like carotenoids, tocopherols, polyphenols, flavonoids, alkaloids, and tannins are all found in MO, making it a good source of functional components. The plant is found all over the world in tropical regions. The leaves of MO have a variety of medicinal benefits, including antioxidant, anti-inflammatory, hepatoprotective, and anti-cancer activities (Ariani *et al*., 2023).

To treat severe infections brought on by aerobic gram-negative bacteria, gentamicin (GNT), an aminoglycoside antibiotic, is utilized (Meka Kedir *et al*., 2022). Nephrotoxicity and hepatotoxicity are the most serious adverse effects of gentamycin, which might happen in 10–20% of patients (Mahi-Birjand *et al*., 2020; Khaksari *et al*., 2021). Therefore, the aim of the current research

Table 1. The effect of *Moringa oleifera* aqueous extract (400 mg/kg b.wt orally) once daily for 30 successive days on renal CAT, GPX, GSH, SOD, TAC, MDA and 8-OHdG in adult male rats treated with gentamicin (100 mg/kg b.wt) once daily for 10 successive days.

Groups	Antioxidants/Oxidant status						
	GPx (mol /g tissue)	SOD (U/g tissue)	CAT (U/g tissue)	GSH (nmol/g tissue)	MDA (nmol/g tissue)	TAC (µmol /g tissue)	8-OHdG (ng/ml)
Control	22.00±10[a]	34.7±1.45[a]	39.00±2.60[a]	36.00±1.15[a]	29.00±0.57[c]	40.66±0.88[a]	41.00±0.58[c]
Moringa	24.00±1.15[a]	35.3±0.88[a]	41.00±0.58[a]	35.00±1.15[a]	24.00±1.15[d]	41.00±1.70[a]	39.00±0.58[c]
Gentamycin	9.7±0.88[c]	16.7±1.20[c]	21.3±0.33[b]	21.00±0.58[c]	84.3±2.30[a]	20.00±1.15[c]	96.7±1.20[a]
Moringa + Gentamycin	18.3±0.30[b]	28.7±0.88[b]	37.3±1.20[a]	30.7±0.88[b]	41.3±1.45[b]	35.33±2.18[b]	59.33±2.18[b]

Means within the same column carrying different superscripts are significant at $P \leq 0.05$

is to evaluate the potential hepato renal protective effects of *Moringa oleifera* watery extract on gentamicin induced toxicities in male Albino rats.

Intraperitoneal administration of GNT at a dose of (100 mg/kg b.wt) once daily for 10 successive days resulted in a significant (p< 0.05) decrease in renal CAT, GPX, GSH, SOD and TAC (21.3±0.33 U/g, 9.7.3±0.88 mol/g, 21.00±0.58 nmol/g, 16.7±1.2 U/g, , 20.00±1.15 mmol/g respectively) versus 39.00±2.6 U/g, 22.00±1 mol/g, 36.00±1.15 nmol/g, 34.7±1.45 U/g, 40.66±0.88 μmol/g respectively, for control group. Meanwhile, a significant (p< 0.05) increase in both renal MDA and 8-OHdG (84.3±2.3 nmol/g, 96.7±1.2 ng/ml respectively) compared with 29.00±0.57 nmol/g, 41.00±0.58 ng/ml respectively, for control group (Table1).

Oxidative stress is triggered by an imbalance between reactive oxygen species (ROS) production and their elimination by the antioxidant system. This imbalance promotes damage of DNA, lipids, and proteins if oxidative stress is not controlled. Our findings affirmed by Mohammed *et al*. (2022) who found that administration of GNT induced a significant decrease in the renal CAT and SOD activity and a significant increase in the renal MAD content compared with control group.

In the same line, Laorodphun *et al*. (2022) who reported that administration of GNT resulted in elevation in renal MDA levels and decrease in CAT, GSH, SOD activity and elevation in MDA level compared with control group.

Further reports by Subhi *et al*. (2022) reinforced our results as they found that administration of gentamicin caused high significant increase in renal malondialdehyde (MDA) levels, also showed a highly significant decrease in renal catalase (CAT) and glutathione activities as compared to the control group. Besides, El-Azeem *et al*. (2023) who found that administration of GNT showed a highly significant decrease in SOD and TAC activity. In parallel, the renal tissue concentration of MDA was significantly elevated in GNT group relative to the control group.

Concurrent administration of MO aqueous extract at a dose of (400 mg/kg b.wt, orally) once daily for 30 successive days with GNT sulphate (100 mg/kg b.wt) for 10 successive days induced significant (p< 0.05) increases in renal CAT, GPX, GSH, SOD, and TAC (37.3±1.2 U/g, 18.3±0.3 mol/g, 30.7±0.88 nmol/g, 28.7±0.88 U/g, 35.33±2.18 μmol/g respectively) versus 21.3±0.33 U/g, 9.7±0.88 mol/g, 21.00±0.58 mol/g, 16.7±1.2 U/g, 20.00±1.15 μmol/g respectively, of gentamicin treated group. And significant (p< 0.05) decreases in renal MDA and 8-OHdG (41.3±1.45 nmol/g, 59.33±2.18 ng/ml respectively) compared with 84.3±2.3 nmol/g, 96.7±1.2 ng/ml respectively, for GNT administrated group. Our results strengthened by Arafat *et al*. (2018) who found that supplementation of GNT administrated groups with MO extract induced antioxidant activity through restoring and improving GSH level and reducing oxidative stress. In the same line, Lukiswanto *et al*. (2023) recorded that the administration of MO extract at a dose of 600 mg/kg in GNT- administrated rats induced a decrease in the MDA levels in the liver and kidney.

Administration of GNT resulted in significant (p< 0.05) decreases in hepatic CAT, GPX, GSH, SOD and TAC (18.3±2.02 U/g, 12.00±1.15 mol/g, 10.7±0.88 nmol/g, 28.00±2 U/g, 23.00±2.5 μmol/g) versus 38.3±0.66 U/g, 31.00±0.6 mol/g, 26.7±1.4 nmol/g, 42.00±1.15 U/g, 43.66±2.7 μmol/g respectively, for the control group. Meanwhile, significant (p< 0.05) increases in both renal MDA and 8-OHdG (104.00 ±2.6 nmol/g, 107.7±1.45 ng/ml respectively) compared with 35.00±1.15 nmol/g, 51.00±0.58 ng/ml respectively, for the control group (Table 2). These results agreed with Laaroussi *et al*. (2021) who found that intraperitoneal injection of GNT (120 mg/kg b.wt/day i.p) induced remarkable decreases in hepatic CAT, GSH, GPX activities and a high elevation in MDA level in comparison with the non-treated group. Likely, Yarijani *et al*. (2021) who mentioned that administration of GNT reduce the activity of both hepatic CAT and SOD compared with control group.

Co-administration of MO aqueous extract at a dose of with GNT induced significant (p< 0.05) increases in hepatic CAT, GPX, GSH, SOD, and TAC (29.7±0.88 U/g, 24.7±2.4 mol/g, 20.3±0.88 nmol/g, 35.00±2.5 U/g, 38.33±0.88 μmol/g respectively) versus 18.3±2.02 U/g, 12.00±1.15 mol/g, 10.7±0.88 nmol/g, 28.00±2 U/g, 23.00±2.5 μmol/g respectively, of GNT administrated group. And significant (p< 0.05) decreases in renal MDA and 8-OHdG

Table 2. The effect of *Moringa oleifera* aqueous extract (400 mg/kg b.wt orally) once daily for 30 successive days on hepatic CAT, GPX, GSH, SOD, TAC, MDA and 8-OHdG of adult male rats treated with gentamicin (100 mg/kg b.wt) once daily for 10 successive days.

Groups	Antioxidants/Oxidant status						
	GPx (mol /g tissue)	SOD (U/g tissue)	CAT (U/g tissue)	GSH (nmol/g tissue)	MDA (nmol/g tissue)	TAC (μmol/g tissue)	8-OHdG (ng/ml)
Control	31.00±0.6^{a}	42.00±1.15^{a}	38.3±0.66^{a}	26.7±1.4^{a}	35.00±1.15^{c}	43.66±2.7^{ab}	51.00±0.58^{c}
Moringa	33.7±2.4^{a}	38.7±0.88^{ab}	39.7±0.88^{a}	27.00±1.15^{a}	32.67±1.76^{c}	49.66±0.88^{a}	52.00±1.52^{c}
Gentamycin	12.00±1.15^{c}	28.00±2^{c}	18.3±2.02^{c}	10.7±0.88^{c}	104.00±2.6^{a}	23.00±2.5^{c}	107.7±1.45^{a}
Moringa + Gentamycin	24.7±2.4^{b}	35.00±2.5^{b}	29.7±0.88^{b}	20.3±0.88^{b}	44.00±3.05^{b}	38.33±0.88^{b}	66.7±1.8^{b}

Means within the same column carrying different superscripts are significant at P≤0.05

Table 3. The effect of *Moringa oleifera* aqueous extract (400 mg/kg b.wt orally) once daily for 30 successive days on serum creatinine, urea and uric acid of adult male rats treated with gentamicin (100 mg/kg b.wt) once daily for 10 successive days.

Groups	Parameters		
	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
Control	1.09±0.06^{c}	35.66±2.3^{c}	5.13±0.18^{c}
Moringa	0.93±0.04^{c}	35.00±3.05^{c}	5.1±0.17^{c}
Gentamycin	3.66±0.28^{a}	83.3±2.02^{a}	7.9±0.23^{a}
Moringa + Gentamycin	1.73±0.18^{b}	47.66±1.85^{b}	6.1±0.11^{b}

Means within the same column carrying different superscripts are significant at P≤0.05

levels (44.00±3.05 nmol/g, 66.7±1.8 ng/ml respectively) compared with 104.00±2.6 nmol/g, 107.7±1.45 ng/ml respectively, for GNT administrated group.

The results of the current study were confirmed by those of Lukiswanto *et al*. (2023) who found that administration of MO extract at a dose of 600 mg/kg in GNT-induced rats resulted in decrease in MDA levels in the liver and kidneys. The preventive role of MO leaves extract for GNT induced toxicity could be attributed to the antioxidant properties of MO. Similarly, Lukiswanto *et al*. (2023) revealed that co-administration of MO with GNT induced a decrease in the levels of hepatic and renal MDA in rats.

Administration of GNT at a dose of 100 mg/kg b.wt, i.p once daily for successive 10 days induced significant ($p< 0.05$) increases in serum creatinine, urea and uric acid levels (3.66±0.28 mg/dL, 83.3±2.02 mg/dL, 7.9±0.23 mg/dL respectively) compared with 1.09±0.06 mg/dL, 35.66±2.3 mg/dL, 5.13±0.18 mg/dL respectively, for the control group (Table 3).

The obtained results of the current study were hand in hand with Laaroussi *et al*. (2021) who stated that intraperitoneal injection of GNT (120 mg/kg bwt /da y,i.p) induced an elevation in serum creatinine, urea and uric acid levels. Besides, Youssef *et al*. (2022) who observed that administration of GNT at dose of 80 mg/kg once daily for eight consecutive days showed a highly significant increase in serum urea, creatinine and uric acid when compared to control rats. On similar ground, Ibrahim *et al*. (2022) reported that nephrotoxicity was induced in male Wistar rats by injection of GNT (80 mg/kg/day, i.p.) and evidenced by increase in serum creatinine and urea. Likely, Abouzed *et al*. (2021) who found that serum levels of creatinine, urea and uric acid were significantly elevated in the GNT group compared with the control group.

The mechanism underlying the GNT nephrotoxicity is unclear. However, investigations have shown that GNT induced nephrotoxic damage is related to the triggering of oxidative stress, inflammatory cascades, transcriptional factors activation, apoptosis, and necrosis linked to lipid peroxidation and protein alterations (Ahmed and Mohamed, 2019).

On the other hand, a significant decrease in serum creatinine, urea and uric acid were recorded in MO treated group (400 mg/ kg b.wt orally once daily) for 30 successive days before and simultaneously with GNT (100 mg/kg b.wt ip) for 10 successive days (1.73±0.18 mg/dL, 47.66±1.85 mg/dL, 6.1±0.11 mg/dL respectively) compared with 3.66±0.28 mg/dL, 83.3±2.02 mg/dL, 7.9±0.23 mg/dL respectively, for GNT administrated group. These changes may be due to inhibition of lipid peroxidation in kidney of GNT intoxicated animal combined treatment with MO (Ouédraogo *et al*., 2013).

Our findings are supported by Ouédraogo *et al*. (2013) who reported that serum urea and creatinine levels were reduced in the MO (150-300 mg/kg) plus GNT (80 mg/kg) treated groups. Moreover, our data agreed with Ismaiel *et al*. (2019) who stated that co-administration of MO before GNT injection improve serum creatinine and BUN when compared with GNT group. Similarly, Lukiswanto *et al*. (2023) stated that supplementation of GNT administrated groups with MO extract induced significant nephro-protective effects through restoring levels of urea and creatinine. Along this line Nafiu *et al*. (2019) mentioned that concurrent treatment of MO (100-200 and 400 mg/kg b. wt) orally for 28 days along GNT (100 mg/kg/day) i.p for 8 days significantly ameliorated the alterations caused by GNT in the plasma urea and creatinine levels.

The current study stated that intraperitoneal administration of GNT at a dose of (100 mg/kg b.wt) once daily for successive 10 days resulted in significant ($p< 0.05$) increases in serum ALT, AST and ALP activities (89.3±5.5 U/L, 110.00±4.6 u/L, 237.7±16.18 U/L respectively) compared with 26.7±1.8 U/L, 25.00±3.5 U/L, 109.3±5.2 U/L respectively, for the control group (Table 4). Significant decreases ($p< 0.05$) in both total protein and albumin levels (4.5±0.18 g/dL, 2.99±0.1 g/dL respectively) compared with 7.4±0.36 g/dL, 4.4±0.33 g/dL respectively, for the control group (Table 5). Likely, Elgazzar *et al*. (2022) found that administration of GNT induced an elevation in serum activities of ALT, AST and ALP. On the same ground, Babaeenezhad *et al*. (2021) reported that the serum activities of AST, ALT, and ALP in GNT administrated rats were significantly higher when compared to those in healthy rats. The present results were in accordance with Khaksari *et al*. (2021) who noticed that GNT increases liver enzymes (ALT, AST and ALP) activities. Regarding serum total protein and albumin; our findings are in agreement with Galaly *et al*. (2014) who found that serum total protein and albumin levels were decreased with administration of GNT.

Meanwhile, simultaneous administration of MO aqueous extract (400 mg/kg b.wt) once daily for 30 successive days with GNT (100 mg/kg b.wt) once daily for 10 successive days provoked sig-

Table 4. The effect of *Moringa oleifera* aqueous extract (400 mg/kg b.wt orally) once daily for 30 successive days on ALT, AST and ALP activities of adult male rats treated with gentamicin (100 mg/kg b.wt) once daily for 10 successive days.

Groups	Parameters		
	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Control	26.7±1.8^{c}	25.00±3.5^{c}	109.3±5.2^{c}
Moringa	24.7±2.2^{c}	23.7±3.4^{c}	108.00±1.53^{c}
Gentamycin	89.3±5.5^{a}	110.00±4.6^{a}	237.7±16.18^{a}
Moringa + Gentamycin	43.7±2.6^{b}	58.00±1.53^{b}	146.00±3.5^{b}

Means within the same column carrying different superscripts are significant at $P\leq0.05$

Table 5. The effect of *Moringa oleifera* aqueous extract (400 mg/kg b.wt orally) once daily for 30 successive days on total protein, albumin and globulin of adult male rats.

Groups	Parameters		
	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Control	7.4±0.36^{a}	4.4±0.33^{a}	2.96±0.6^{a}
Moringa	8.1±0.54^{a}	4.8±0.2^{a}	3.2±0.7^{a}

Means within the same column carrying different superscripts are significant at $P\leq0.05$

nificant (p< 0.05) decreases in serum activities of ALT, AST and ALP (43.7±2.6 U/L, 58.00±1.53 U/L, 146.00±3.5 U/L respectively) compared with 89.3±5.5 U/L, 110.00±4.6 U/L, 237.7±16.18 U/L respectively, for GNT administrated group, a significant increase (p< 0.05) in total protein (6.2±0.17 g/dL) compared with 4.5±0.18 g/dL for GNT administrated group and non-significant increase (p<0.05) in both albumin and globulin (3.5±0.15 g/dL, 2.7±0.32 g/dL respectively) compared with 2.99±0.1 g/dL, 1.54±0.27 g/dL respectively, for GNT administrated group.

Like manner, it was mentioned that combined treatment of MO and GNT induced significant hepato- protective effects through restoring levels of AST, ALT, ALP, Albumin and total protein to normal levels compared with GNT administrated group. *Moringa oleifera* reversed the severity of GNT drawbacks; hep-

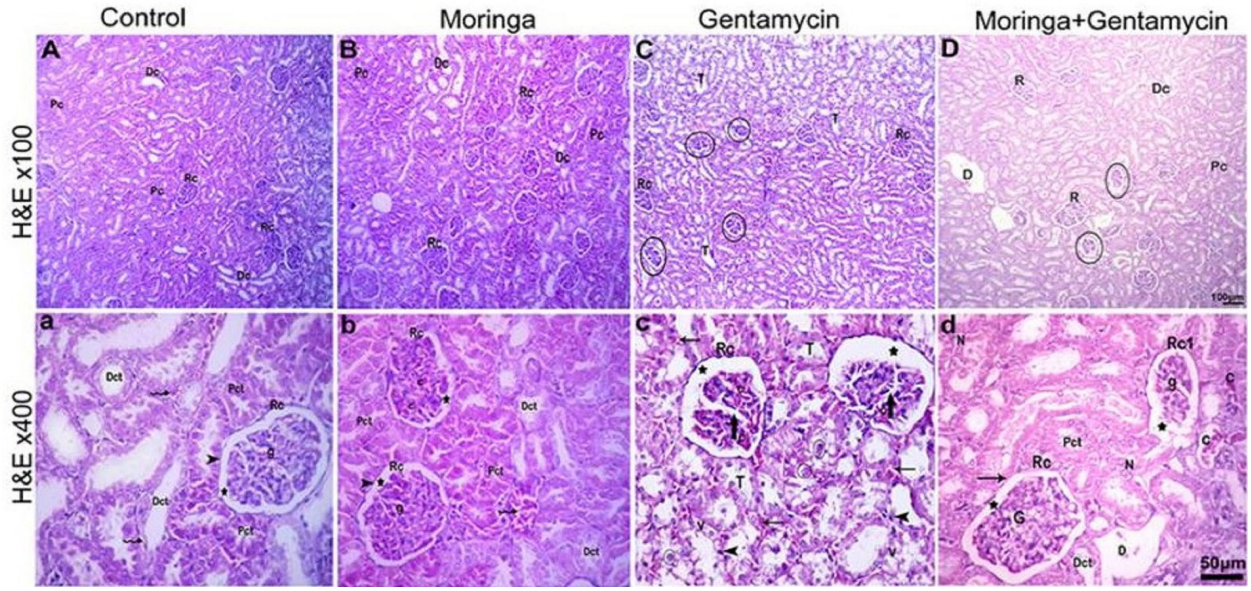

Fig. 1. Representative photomicrographs of hematoxylin and eosin stained cross sections in the renal cortex of control (A, a), *Moringa oleifera* aqueous extract (B, b), gentamycin (C, c) and moringa+ gentamycin (D, d) treated rats. The cytoarchitecture for (A, B) groups showed nearly all renal corpuscles with normal round shape (Rc), Proximal convoluted tubules (PCT) with narrow lumens (Pc), and distal tubules (DCT) with wide lumens (Dc). A higher magnification (a, b) shows a renal corpuscle (Rc) with a dense rounded glomerulus (g) surrounded by a partial layer of Bowman's capsule (arrowhead) with narrow urinary space (star). PCT and DCT show cuboidal cells with rounded pale nuclei and acidophilic cytoplasm. Peritubular blood capillaries (tailed arrows) can also be seen. Gentamycin treated group (C) showed numerous renal corpuscles with abnormal shapes (circle) while few others with normal round shapes (Rc). Most renal tubules show luminal dilation (T). A higher magnification (c) shows renal corpuscles (Rc) with lobulation of their glomeruli (thick arrow), congestion of the glomerular capillary tufts (c), and extensive wide urinary space (star). Some renal tubules (T) show pale stained nuclei with acidophilic cytoplasm while others show darkly stained pyknotic nuclei (arrowhead), vacuolated cytoplasm (v), exfoliated nuclei (circle) and numerous congested peritubular capillaries (arrow) are noticed. Moringa+ Gentamycin treated group (D) shows some renal corpuscles with abnormal shapes (circle) while numerous corpuscles with normal round shapes (Rc). Normal apparent PCT with narrow lumens (Pc), DCT with wide lumens (Dc) and few extensive dilated distal tubules (D) can be noticed. A higher magnification (d) shows a normal renal corpuscle (Rc) with its glomerulus (G), urinary space (star), and Bowman's capsule (arrow). Another corpuscle (Rc1) with wide urinary space (star) and hypocellular glomerulus (g) is also observed. Most renal tubules show pale stained nuclei (N) and acidophilic cytoplasm. Dilated distal tubule (D) and some congested peritubular capillaries (c) are noticed. (Scale bar; 100 µm for A, B, C, D and 50 µm for a, b, c, d).

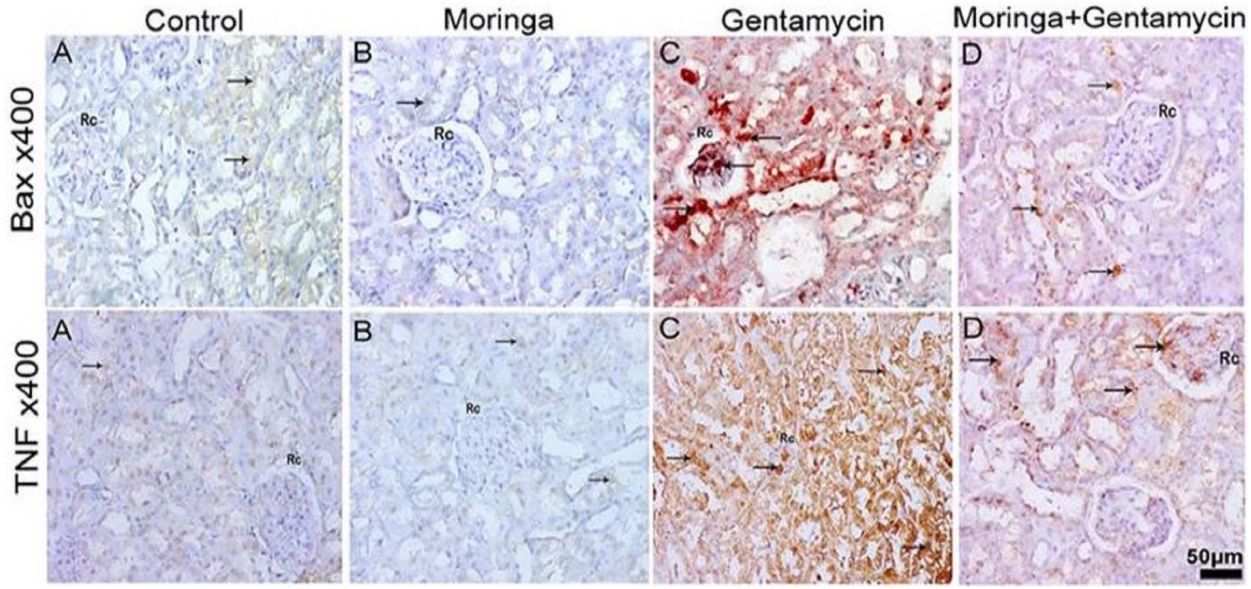

Fig. 2. Representative photomicrographs of immunohistochemical stained cortical renal cross-sections, in control (A), Moringa-treated (B), gentamycin-treated (C), and Moringa+ Gentamycin treated (D) rats. Bcl-2 Associated X-protein (Bax) in the control and moringa-treated rats (A, B) shows weak or faint positive immunoreaction in the cytoplasm of some tubular epithelium (arrow) while a negative immunoreactivity in renal corpuscle (Rc). Gentamycin-treated rat (C) shows strong positive immunoreaction in the cytoplasm of the tubular epithelium (arrow) and also in the renal corpuscle (Rc). Moringa+ Gentamycin treated group (D) shows positive immunoreaction in the cytoplasm of a few tubular epithelia (arrow) while a negative immunoreactivity in the renal corpuscle (Rc). Tumor Necrotic Factor (TNF) in the control and moringa-treated rats (A, B) show faint positive immunoreaction in the cytoplasm of some tubular epithelium (arrow) while a negative immunoreactivity in renal corpuscle (Rc). Gentamycin-treated rats (C) show extensive positive immunoreaction in the cytoplasm of the tubular epithelium (arrow) and also in the renal corpuscle (Rc). Moringa+ Gentamycin-treated rats (D) show positive immunoreaction in the cytoplasm of a few tubular epithelia (arrow) and also in the renal corpuscle (Rc). (Scale bar; 50 µm for A, B, C, D).

atotoxicity as it reduced hepatic tissue oxidative stress that promotes tissue injury (Arafat *et al*., 2018).

Regarding kidney architecture, GNT administrated group revealed a wide array of nephrotoxic changes (Fig. 1C and c). These changes included glomerulopathy as glomerular congestion, extensive wide urinary space, and lobulation and necrosis, vasculopathy as congestion, tubular cell cytotoxicity as epithelial cell vacuolation, pyknosis, and necrosis, tubular luminal dilatations, and interstitial nephritis as interstitial mononuclear cell infiltrates.

The prophylactic effects of MO aqueous extract were noticeable where the reduction in the lesion severities and frequencies of the GNT -induced nephrotoxic changes were observed in the MO+ GNT treated rats compared to the GNT -administrated group only. The tissue sections of these groups exhibited nearly the same histological picture, and the basic alterations were mild glomerular shrinkage with a widening of the urinary space and some vascular congestion (Fig. 1D and d).

Regarding BAX, the renal tissue sections of GNT administrated group were immune stained with anti-BAX and resulted in overexpression of BAX+ in the cytoplasm of the tubular epithelium and also in the renal corpuscle of the GNT administrated group. In the MO+GNT treated group, only a few tubular cells showed BAX+ reactivity. Tumor necrotic factor (TNF), positive brownish immune expressions of inflammatory markers Tumor Necrotic Factor (TNF) strongly increased in the GNT intoxicated group compared to the control group. Treatment with MO extracts moderately decreased the expression of TNF compared to the GNT intoxicated group with comparable levels in control and Moringa extract-treated groups (Fig. 2).

Our findings were confirmed by Lukiswanto *et al.* (2023) who found that GNT administration increased the damage in the kidney as glomerular damage, degeneration, and necrosis of tubules score.

Further reports confirmed our findings as they found that when GNT enters the body, it is reabsorbed in the proximal tubule and causes acute tubular necrosis through a process that takes a quite long time. The damage is characterized by loss of brush border integrity, severe degeneration, necrosis of tubular epithelial cells, and mononuclear cell infiltration in the interlobular area (Raju *et al*., 2011; Liu *et al*., 2016; Veljković *et al*., 2016).

Meanwhile other reports were in accordance with our results as they found that co-administration of MO leaf extract with GNT has a nephroprotective effect as indicated by the low degeneration and necrosis levels in the renal tubular cells due to the presence of antioxidant compounds that have proved to reduce the risk of and kidney injury by GNT (Wang *et al*., 2004; Ouédraogo *et al*., 2013).

Regarding BAX and TNF α, Balaha *et al.* (2023) confirmed our

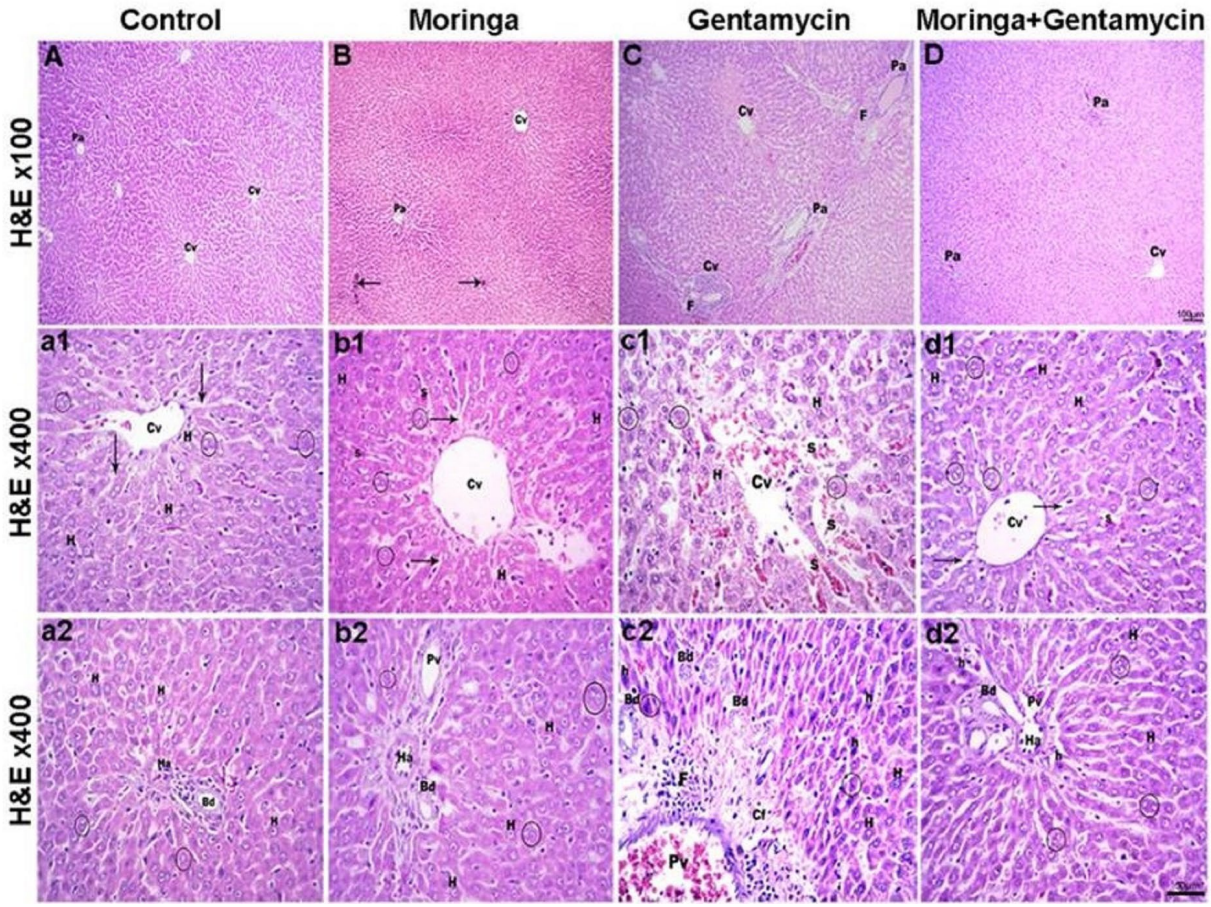

Fig. 3. Representative photomicrographs of hematoxylin and eosin stained liver cross sections of the control(A,a1,2), *Moringa oleifera* aqueous extract (B,b1,2), gentamycin (C,c1,2) and Moringa+ gentamycin (D,d1,2) treated rats. The cytoarchitecture for (A, B) groups shows normal hepatic lobules with Central veins (Cv) and portal area (Pa). A higher magnification (a1,2-b1,2) shows the classic hepatic lobules with the central vein (Cv), radiating blood sinusoids (arrow), the portal area with the hepatic artery (Ha), and small bile ducts (Bd) with simple cuboidal epithelium. Nearly all hepatocytes in their cords have rounded pale nuclei and acidophilic cytoplasm (H). Some bi-nucleated hepatocytes (circle) can be noticed. Gentamycin-treated group (C) shows abnormal appearance for both central veins (Cv) and portal areas (Pa). An extensive mononuclear cellular infiltration (F) around the central vein and portal area. A higher magnification (c1) shows an irregular dilated central vein with disrupted endothelial lining (Cv), and congested blood sinusoids (s). In c2 showing congested dilated portal vein (Pv), mononuclear cellular infiltration (F), collagen fibers (Cf), and proliferated bile ducts (Bd). The hepatocytes (H) with rounded pale small nuclei and acidophilic cytoplasm, others with darkly stained pyknotic nuclei (h), and also a few bi-nucleated hepatocytes (circle) can be observed. Moringa+ Gentamycin treated group (D) shows the liver restores its normal architecture where hepatic lobules with Central veins (Cv) and portal area (Pa). A higher magnification (d1) shows a regular slightly dilated central vein with radiating blood sinusoids (arrow) a few congested blood sinusoids (s). The hepatocytes have rounded pale nuclei and acidophilic cytoplasm (H) and numerous bi-nucleated hepatocytes (circle) can be noticed. A higher magnification (d1) shows portal vein (Pv), hepatic artery (Ha), single bile duct (Bd), and, a few inflammatory cells (arrow). Most hepatocytes (H) have rounded pale nuclei and acidophilic cytoplasm, few with darkly stained pyknotic nuclei (h), and also numerous bi-nucleated hepatocytes (circle) can be observed.

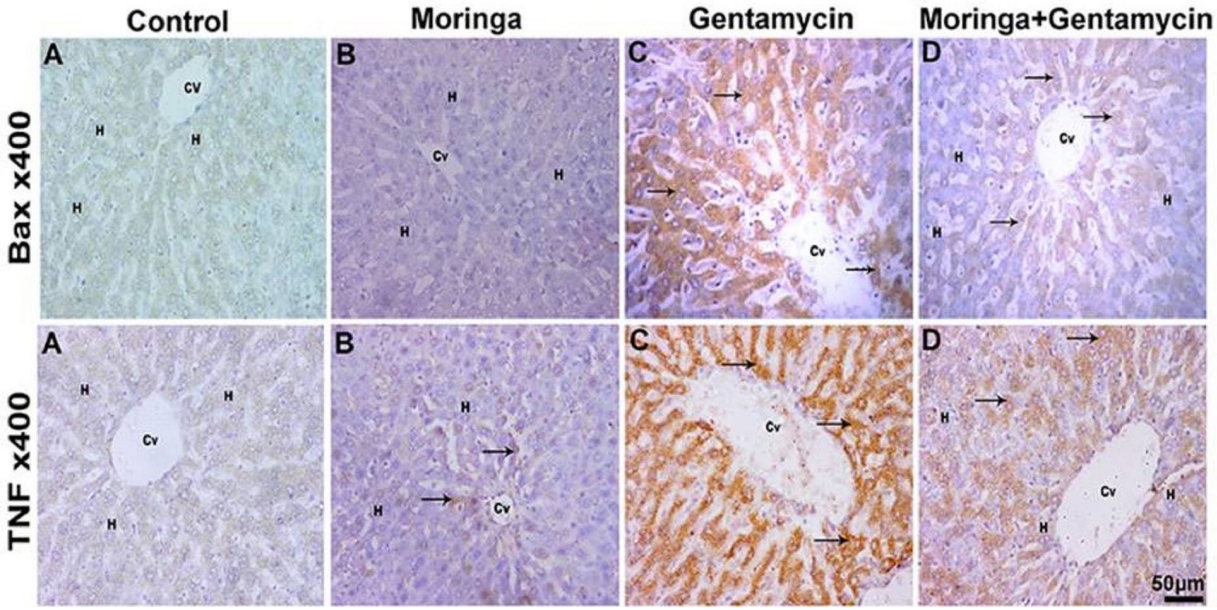

Fig. 4. Representative photomicrographs of immunohistochemically stained liver cross-sections, in control (A), Moringa-treated (B), gentamycin-treated (C), and Moringa+ Gentamycin-treated (D) rats. Bcl-2 Associated X-protein (Bax) in the control and Moringa-treated rats (A, B) shows negative immunoreaction in the cytoplasm of all hepatocytes (H) around the central vein (Cv). Gentamycin-treated rat (C) shows strong positive immunoreaction in the cytoplasm of nearly all hepatocytes (arrow) around the central vein (Cv). Moringa+ Gentamycin treated group (D) shows negative immunoexpression in numerous hepatocytes (H) while, a faint positive immunoreaction in the cytoplasm of some hepatocytes (arrow). Tumor Necrotic Factor (TNF) in the control and moringa-treated rats (A, B) show negative immunoreaction in the cytoplasm of hepatocytes (H) around the central vein (Cv) while Moringa treated group shows weak or faint immunoreaction around the central vein (Cv). Gentamycin-treated rat (C) showing strong positive immunoreaction in the cytoplasm of hepatocytes (arrow) around the central vein (Cv). Moringa+ Gentamycin treated group (D) shows weak positive immunoreaction in the cytoplasm of some hepatocytes (arrow) while others show negative immunoexpression (H) around the central vein (Cv).

work as they found that GNT injections resulted in a significant increase in Bax protein levels and TNF-α level in GNT treated group's kidneys compared with control group.

In the same line, Nadeem *et al*. (2023) found that GNT exhibited a notable amplification in renal TNF-α level, elevation in renal BAX expression and histopathological abnormalities as inflammation, degradation, and necrosis in GNT administrated group compared with control group.

On the other hand, Edeogu *et al*. (2020) stated that MO possesses marked nephroprotective effect against GNT-induced renal damage via modulating oxidative stress, inflammation, and apoptosis in Wistar rats. And induce an amelioration of histopathological abrasions induced by GNT with restoration the content of TNF-α.

Regarding liver architecture, GNT administrated group revealed a wide array of hepatotoxic changes (Fig. 3C and c). These changes included necrotic degeneration in a considerable number of hepatic cells, sinusoidal congestion, and hemorrhage was evident. Concerning portal areas, there was extensive mononuclear inflammatory cell infiltration, bile duct hyperplasia, and congestion of portal blood vessels, also there was portal fibrosis.

The prophylactic effects of MO aqueous extract were noticeable where the reduction in the lesion severities and frequencies of the GNT -induced hepatotoxic changes were observed in the MO+ GNT treated rats compared to the GNT administrated group only. The tissue sections of these groups exhibited nearly the same histological picture, and the basic alterations were a regular slightly dilated central vein with radiating blood sinusoids and a few congested blood sinusoids. a few inflammatory cells in the portal area with darkly stained pyknotic nuclei hepatocytes (Fig. 3D and d).

Regarding BAX, the hepatic tissue sections of GNT- administrated group were immune-stained with anti-BAX and resulted in overexpression of BAX+ in the cytoplasm of nearly all hepatocyte around the central vein from GNT administrated group, meanwhile in MO and GNT treated group only a few hepatocyte showed a faint BAX+ reactivity (Fig. 4).

Regarding tumor necrotic factor (TNF), positive brownish immune expressions of inflammatory markers Tumor Necrotic Factor (TNF) strongly increased in the GNT intoxicated group compared to the control group (Fig. 4).

Treatment with MO extracts moderately decreased the expression of TNF compared to the GNT intoxicated group with comparable levels in control and Moringa extract-treated groups (Fig. 4).

Our findings are confirmed by Lukiswanto *et al*. (2023) who demonstrated that GNT administration induced liver damage as degeneration, necrosis, and fibrosis score by the process of oxidative stress due to excessive ROS production by GNT.

On the other hand, Arafat *et al*. (2018), reported that co-administration of MO with GNT reduced the histopathological lesions in liver that induced by GNT due to the increase in GSH level and decrease in NO level induced by MO.

Similarly, Junqueira and Carneiro (2015) reported that liver histopathology of the white rats in T2 and T3 groups that received MO by 300 and 600 mg/kg bwt respectively along with GNT show that the hepatocytes provide the ability to regenerate or repair cells due to the high dose of MO leaf extract in these groups which is high enough to enable the antioxidant levels that enter to rebalance the oxidative stress conditions which result from the exposure to GNT. The regeneration ability can occur by means of the hepatocyte division mechanism and continues until tissue mass repair is achieved.

Regarding hepatic BAX and TNF, Ali *et al*. (2020) stated that Ursodeoxycholic acid (UDCA) remarkably ameliorated the histopathological changes in liver that induced by GNT with down regulation of both hepatic BAX and TNF that elevated by GNT administration in rats.

CONCLUSION

The results of this study suggested that administration of *Moringa oleifera* might be beneficial in preventing gentamicin-induced hepato-renal toxicity and the related oxidative stress by inhibiting free radicals generation and by restoration of the antioxidant systems. The simultaneous treatment of *Moringa oleif-*

era can avert both the functional and histological hepato-renal changes induced by the aminoglycoside antibiotic in rats.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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