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Research Article

Curative Effects of *Neptunia oleracaea* Hydroethanol Leaf Extract on Gentamacin-Induced Liver and Kidney Oxidative Stress in Wister Rats

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ABSTRACT
This study investigated the curative potential of *Neptunia oleracea* hydroethanolic leaf extract against gentamicin-induced oxidative stress in the liver and kidney of albino rats. Thirty-six rats were divided into six groups: normal control, negative control, standard control (gentamicin + silymarin 100 mg/kg), and three treatment groups receiving *N. oleracea* extract (100, 200, and 400 mg/kg). Acute toxicity testing showed no adverse effects up to 5000 mg/kg, indicating the extract is practically non-toxic (LD_{50} > 5000 mg/kg). Gentamicin administration significantly increased serum AST, ALT, bilirubin, creatinine, urea, potassium, MDA, and hydrogen peroxide levels, while decreasing albumin, total protein, sodium, SOD, and GSH—confirming hepatic and renal oxidative damage. Treatment with *N. oleracea* extract produced dose-dependent protection, with the 400 mg/kg dose showing the most pronounced effects. The extract significantly restored liver function markers (AST, ALT, bilirubin, albumin, and total protein) and improved renal parameters (creatinine, urea, and electrolyte balance). Antioxidant enzyme activities (SOD, GSH) were enhanced, while lipid peroxidation markers (MDA, hydrogen peroxide) were reduced. Histopathological examination supported these biochemical results, showing preservation of normal liver and kidney structures in treated rats compared with the gentamicin-only group. In conclusion, *Neptunia oleracea* hydroethanolic leaf extract exhibits potent hepatocurative and nephrocurative effects against gentamicin-induced toxicity, likely due to its antioxidant mechanisms. These results validate its traditional medicinal use and highlight its potential as a natural therapeutic agent for managing drug-induced liver and kidney oxidative damage.

Keywords: Antioxidants; Gentamicin; Hepatotoxicity; Nephrotoxicity; Oxidatives stress; *Neptunia oleracea*

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INTRODUCTION

Gentamicin, an aminoglycoside antibiotic, is a widely used for treatment severe Gram-negative bacterial infections, particularly in resource-limited settings where cost-effective therapies are critical (Nagai *et al.,* 2014). Despite its efficacy, gentamicin's clinical utility is hampered by its propensity to induce nephrotoxicity and hepatotoxicity, primarily through oxidative stress mechanisms (Lopez-Novoa *et al.,* 2011). These toxicities, affecting up to 25% of patients, manifest as renal tubular damage and hepatic dysfunction, leading to elevated biomarkers such as creatinine, urea, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) (Balakumar *et al.,* 2010). The oxidative stress induced by gentamicin results from an overproduction of reactive oxygen species (ROS), which disrupts cellular homeostasis, causes lipid peroxidation, and depletes endogenous antioxidants like superoxide dismutase (SOD) and glutathione (GSH) (Sahu *et al.,* 2014).

The global burden of antibiotic-induced organ damage has spurred interest in natural remedies, particularly plant-based antioxidants, which offer a cost-effective and accessible approach to mitigating toxicity (Negrette-Guzmán *et al.,* 2013). *Neptunia oleracea* commonly known as water mimosa, is an aquatic legume prevalent in tropical regions, including parts of Africa and Southeast Asia, where it is traditionally used for its anti-inflammatory and restorative properties (Sarmah *et al.,* 2017). Preliminary studies suggest that its hydroethanol extract, rich in flavonoids, phenolics, and alkaloids, exhibits potent antioxidant activity, making it a promising candidate for counteracting oxidative stress (Phang *et al.,* 2019). However, the plant remains understudied in the context of drug-induced toxicity, particularly gentamicin-related damage, presenting a unique opportunity for scientific exploration.

In Nigeria and other developing nations, where gentamicin is widely used due to its affordability, the prevalence of drug-induced organ damage underscores the need for locally sourced protective agents. *Neptunia oleracea's* abundance in wetland ecosystems and its ethnomedicinal use for kidney and liver ailments make it a culturally and economically relevant subject for investigation (*Rahman et al.,* 2020). This study seeks to evaluate the curative potential of *Neptunia oleracea* hydroethanol extract in albino rats, a well-established model for studying gentamicin toxicity, to provide evidence that could inform safer therapeutic strategies.

MATERIALS AND METHODS

Experimental Animals

The albino (Wistar) rats used in this study were purchased from the Animal House of Ahmadu Bello University, Zaria, and were transported in ventilated plastic cages to the Animal House, Faculty of Science, Federal University Birnin Kebbi. The rats were allowed to acclimatize for two (2) weeks prior to the commencement of the experiment. They were fed with standard rodent pellets and given access to water ad libitum.

Collection, Identification and Preparation of Plant Samples

A fresh leaf of *Neptunia oleracea* plant were collected at Birnin Kebbi, Kebbi State of Nigeria, the plant sample was identified and authenticated at the Department of Plant Science and Biotechnology, Abdullahi Fodiyo University of Science and Technology Aleiro with the voucher number 239. The leaves were washed, shade-dried at room temperature, and pulverized into fine powder using a laboratory mill. Approximately 500g of powdered material was macerated in 70% ethanol (ethanol: water, 70:30 v/v) for 72 hours with intermittent shaking. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure at 40–45 °C using a rotary evaporator. The concentrated extract was dried to yield a hydroethanolic crude extract, stored at 4 °C until use.

Qualitative Phytochemical Screening of the Extract

Phytochemical screening to test for the presence of alkaloids, anthraquinones, Carbohydrates, glycosides, flavonoids, Cardenolides, saponins, tannins, and steroid was carried out using the standard methods as descrive by valsala *et al*. (2002) and Harborne, (1998)

Acute Toxicity Study of *Neptunia oleracea extract*

The acute toxicity study of *Neptunia oleracea* hydroethanolic extract was carried out according to the procedures described by Lorke (1983) and the OECD guideline 423 (2008). The study was conducted through the oral route of administration in two phases.

Curative Activity Studies

Thirty-six (36) healthy albino rats were randomly divided into six (6) groups, each consisting of six animals. Gentamicin (80 mg/kg/day, i.p.) was administered for 10 consecutive days to induce liver and kidney toxicity, as previously reported by Tavafi and Ahmadvand (2011). The experimental groups were designed as follows:

- Group 1 (Normal control): Received 0.1 ml i.p. of normal saline (0.9% w/v NaCl) for 10 days.
- Group 2 (Negative control): Received gentamicin (80 mg/kg/day, i.p.) for 10 days to induce liver and kidney toxicity.
- Group 3 (Positive control): Received gentamicin (80 mg/kg/day, i.p.) for 10 days followed by oral administration of the standard drug silymarin (100mg/kg, p.o.) from the 10th to the 24th day of the study, as described by Venkatesan *et al*. (2012).
- Group 4 (Treatment I): Received gentamicin (80 mg/kg/day, i.p.) for 10 days followed

by *Neptunia oleracea* hydroethanolic extract (100 mg/kg, p.o.) from the 10th to the 24th day.

- Group 5 (Treatment II): Received gentamicin (80 mg/kg/day, i.p.) for 11days followed by *Neptunia oleracea* hydroethanolic extract (200 mg/kg, p.o.) from the 11th to the 24th day.
- Group 6 (Treatment III): Received gentamicin (80 mg/kg/day, i.p.) for 10 days followed by *Neptunia oleracea* hydroethanolic extract (400 mg/kg, p.o.) from the 11th to the 24th day.

At the end of the experimental period (day 24), all animals were sacrificed under light ether anesthesia after blood collection. The liver and kidneys were excised, washed in ice-cold saline, and preserved for biochemical estimations, lipid peroxidation analysis, and histopathological examination.

Blood Sample Collection Procedures

Blood was collected using Hugo and Russel's method (Hugo and Russel, 2001). Rats were anesthetized with chloroform in a glass chamber, avoiding lethality to ensure blood flow. Each rat was secured on a workbench with pins, and a surgical blade incised the chest dorsoventrally. Blood was drawn from the beating heart via heparinized capillary tubes into sample bottles. A portion was centrifuged at 3000 rpm for 10 minutes, and the supernatant was used for biochemical and hematological assays. Pancreas was excised, preserved in formalin for histopathology.

Liver Function Assay

Determination of serum alanineaAmino transferase (ALT) activity

ALT activity was measured using Reitman and Frankel's (1957) method. ALT catalyzes alanine and α-ketoglutarate to pyruvate and glutamate; pyruvate reacts with 2,4-dinitrophenylhydrazine (DNPH) to form a red-brown hydrazone at 546 nm.

Determination of aspartate amino transferase (AST) activity

AST activity followed Reitman and Frankel (1957). AST catalyze the transfer of amino groups from aspartate to α-ketoglutarate, forming oxaloacetate and pyruvate. These keto acids react with 2,4-dinitrophenylhydrazine (DNPH) to produce a brown-colored hydrazone measurable at 546 nm. The intensity of the color is directly proportional to enzyme activity (Reitman & Frankel, 1957).

Bilirubin estimation

Bilirubin was quantified using Jendrassik and Grof's (1938) colorimetric method. Bilirubin couples with diazotized sulfanilic acid to form a red-colored azo-bilirubin complex. The absorbance is measured at 546 nm and is proportional to bilirubin concentration (Jendrassik and Grof, 1938).

Total protein determination

Total protein was determined using Gornall *et al*.'s (1949) Biuret method. Proteins form a purple complex with copper in alkaline conditions, measured at 546 nm.

Serum albumin estimation

Serum albumin concentration was determined using the bromocresol green (BCG) dye-binding colorimetric method (Doumas *et al*., 1971). Albumin binds with bromocresol green (BCG) at acidic pH to form a green-colored complex, measurable at 628 nm.

Kidney function Assay

Serum creatinine estimation

Serum creatinine was determined using Jaffe's colorimetric method. Creatinine reacts with alkaline picrate to form a red-orange complex measurable at 520 nm (Jaffe, 1886).

Serum urea estimation

Serum urea was determined using DAM colorimetric method. Urea is hydrolyzed by urease into ammonia and CO2. Ammonia reacts with diacetyl monoxime (DAM) to form a pink-colored complex measurable at 540 nm (Fawcett and Scott, 1960).

Determination of Serum Sodium (Na^+) and Potassium (K^+)

Serum sodium and potassium were estimated using the flame photometric method as described by Cheesbrough (1991). The principle is based on the fact that sodium and potassium ions emit characteristic wavelengths of light when aspirated into a non-luminous flame. The emitted light intensity is directly proportional to the concentration of the ions in the serum.

Antioxidant activity markers

Malondialdehyde (MDA)

Malondialdehyde was determined using TBARS assay. MDA reacts with thiobarbituric acid (TBA) under acidic and high temperature conditions to form a pink colored complex measurable at 532 nm (Ohkawa *et al.,* 1979).

Superoxide dismutase (SOD)

Superoxide dismutase was determined using Misra and Fridovich method. SOD inhibits the autoxidation of epinephrine or the reduction of nitroblue tetrazolium. The extent of inhibition is proportional to SOD activity (Misra and Fridovich, 1972).

Reduced glutathione (GSH)

GSH was determined using Ellman's colorimetric method. GSH reduces 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) to produce a yellow-colored compound measurable at 412 nm (Ellman, 1959).

Hydrogen peroxide (H_2O_2)

Hydrogen Peroxide was determined using Colorimetric method. H_2O_2 reacts with molybdate to form a stable yellow complex measurable at 405 nm (Pick and Keisari, 1980).

Histopathological Procedure (Liver and Kidney)

Liver and kidney tissues were excised and immediately fixed in 10% neutral buffered formalin for 24–48 h, dehydrated through ascending grades of ethanol, cleared in xylene, embedded in paraffin, sectioned at 4–5 µm thickness using a rotary microtome, mounted on slides, stained with hematoxylin and eosin, and examined under a light microscope for histopathological changes (Bancroft & Gamble, 2008).

Data Analysis

The data were presented as Mean ± Standard deviation of Mean (SDM) and were subjected to one-way analysis of variance (ANOVA). Statistical differences between means were separated using Duncan's multiple comparison test with the Statistical Package for Social Sciences (SPSS) version 20. Values were considered statistically significant at $P < 0.05$ (Field, 2013)

RESULTS

Qualitative Phytochemical Content of *Neptunia oleracea* extract

The results for the qualitative determination of some phytochemical constituents of screening of *Neptunia oleracea* demonstrated the presence of flavonoids, saponins, cardiac glycosides, tannins, steroids, alkaloids and cardenolides. However, glycosides and anthraquinones were not detected, as detailed in Table 1.

Acute Toxicity Studies of *Neptunia oleracea* extract

The acute toxicity evaluation of *Neptunia oleracea* hydroethanolic extract revealed no observable changes in physical or behavioral signs and no mortality in any of the experimental animals at doses up to 5000 mg/kg body weight, compared with the control group, during both Phase I and Phase II (Table 4.5). The median lethal dose (LD_{50}) was therefore estimated to be greater than 5000 mg/kg, classifying the extract as practically non-toxic.

Effect of *Neptunia oleracea* extract on body weight

The body weight changes in all experimental groups from baseline to week 4 showed that untreated (Negative) Control experienced a significant reduction in body weight, while treatment with *Neptunia oleracea* extract at doses ranging from 100mg/kg, 200mg/kg and 400mg/kg(shown in Figure 1) dependently improved body weight, suggesting a curative effect against gentamicin induced weight loss.

Table 1. Qualitative Phytochemical constituent of *Neptunia oleracea* extract

Phytochemicals	Presence
Flavonoids	+
Saponins	+
Cardiac glycosides	+
Tannins	+
Steroids	+
Alkaloids	+
Cardenolides	+
Glycosides	-
Anthraquinones	-

(+) = Detected, (-) = Not detected

Table 2. Observation of physical signs in rats during acute toxicity study *of N. oleracea* extract

	Phase I			Phase II			
	Control	10	100	1000	1600	3000	5000
Feeding	N	N	N	N	N	N	N
Drowsiness	N	N	N	N	N	N	N
Aggressiveness	N	N	N	N	N	N	N
Alive/Death	A	A	A	A	A	A	A

(N, Normal; A, Alive)

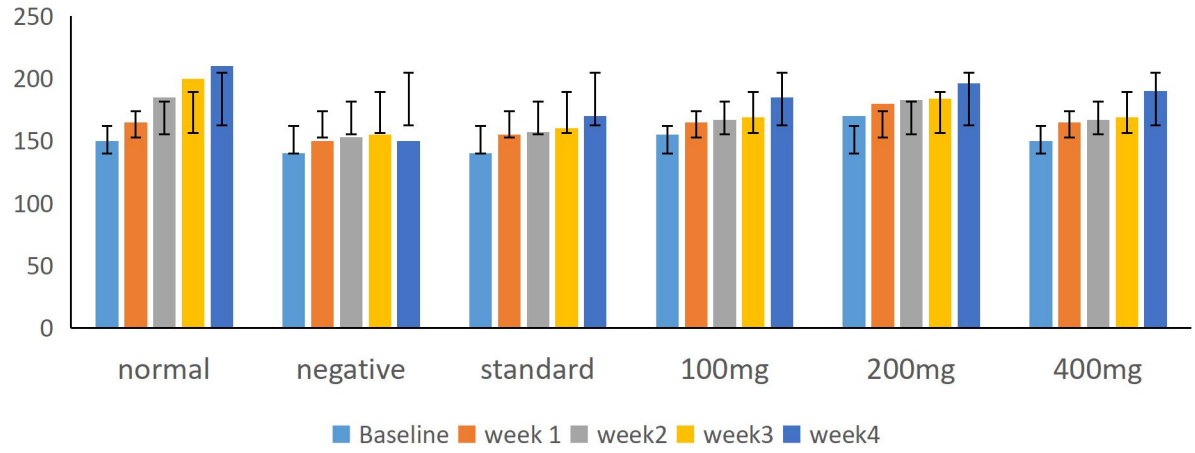

Figure 1. Effect of *N. oleracea* on body weight changes

Liver Function Parameters

The results in Table 3 showed that administration of gentamicin alone (Negative control) caused significant elevation ($P < 0.05$) in serum ALT (25.5±0.58 unit/l), AST (27.13±0.33 unit/l), and total bilirubin levels (2.01±0.01 mg/dl) along with reduced albumin (7.69±1.64 g/dl) and total protein level (2.81±0.07 g/dl) compared with the normal control group, indicating hepatic injury and impaired liver function. Treatment with *Neptunia oleracea* hydroethanolic leaf extract produced a dose-dependent improvement in these parameters. Particularly, rats administered 400 mg/kg of the extract demonstrated a significant reduction in ALT (7.95±0.32 unit/l), AST (7.69±0.87 unit/l) and bilirubin levels (0.19±0.01mg/dl), alongside a restoration of albumin (25.71±0.24g/dl) and total protein values (9.69±0.16 g/dl) towards normal. These findings suggest that *N. oleracea* possesses hepatocurative effects, mitigating gentamicin-induced oxidative damage and preserving liver function integrity.

Kidney Function Parameters

The results in Table 4 showed that administration of gentamicin alone (Negative control) caused significant elevation ($P < 0.05$) in serum creatinine(3.01±0.01mg/dl), urea(14.10±0.03mg/dl), and potassium levels (5.01±0.61 mmol/l) along with reduced sodium (17.77±0.08 mmol/l) compared with the normal control group, indicating renal impairment and loss of functional integrity. Treatment with *Neptunia oleracea* hydroethanolic leaf extract produced a dose-dependent improvement in these parameters. Notably, rats administered 400 mg/kg of the extract demonstrated a significant reduction in creatinine (0.29±0.08 mg/dl), urea (4.99±0.45 mg/dl), and potassium(1.78±0.02mmol/l) levels, along with restoration of sodium (68.82±0.15 mmol/l) concentration towards normal. These findings suggest that *N. oleracea* possesses nephrocurative effects, counteracting gentamicin-induced kidney damage and preserving renal function.

Table 3. The effect of *N. olearacea* extracts administration on liver function

Plant Extracts (mg/kg body weight)	Parameters				
	ALT (unit/l)	AST (unit/l)	TP (g/dl)	TBIL (mg/dl)	ALB (g/dl)
Normal control	5.34±0.15^{e}	6.11±0.12^{e}	10.59±0.01^{a}	0.15±0.02^{f}	31.11±0.27^{a}
Negative control	25.5±0.58^{a}	27.13±0.33^{a}	2.81±0.07^{e}	2.01±0.01^{a}	7.69±1.64^{e}
Stnd drug	8.14±0.31^{d}	7.91±0.90^{d}	9.76±0.13^{b}	0.21±0.03^{d}	25.46±0.24^{b}
100	18.13±0.58^{b}	11.21±0.58^{b}	6.76±0.04^{d}	0.33±0.01^{b}	14.07±0.59^{d}
200	13.16±0.87^{c}	9.89±0.956^{c}	8.11±0.62^{c}	0.25±0.05^{c}	19.65±0.43^{c}
400	7.95±0.32^{d}	7.69±0.87^{d}	9.69±0.16^{b}	0.19±0.01^{e}	25.71±0.24^{b}

Keys: Values are presented as mean ± SD. Values in the same column with completely different letters are significantly different at $p<0.05$

ALT= Alanine aminotransferse, AST= Aspartate aminotransferase, TP= Total protein, TBIL= Total bilirubin, ALB= Albumin.

Table 4. The effect of *N. olearacea* hydroethanol leaf extract administration on kidney function parameter

Plant Extracts (mg/kg body weight)	Parameters			
	CREATINE (mg/dl)	POTTASIUM (mmol/l)	UREA (mg/dl)	SODIUM (mmol/l)
Normal control	0.29±0.21^{e}	1.89±0.02^{c}	3.11±0.63^{e}	68.24±0.18^{a}
Negative control	3.01±0.01^{a}	5.01±0.61^{a}	14.10±0.03^{a}	17.77±0.08^{e}
Stnd drug	0.31±0.08^{d}	1.88±0.01^{c}	5.10±0.45^{d}	68.14±0.30^{b}
100	1.11±0.07^{b}	2.47±0.01^{b}	8.09±0.20^{b}	52.24±0.13^{d}
200	0.81±0.06^{c}	2.01±0.01^{b}	6.87±0.20^{c}	60.92±0.20^{c}
400	0.29±0.08^{d}	1.78±0.02^{c}	4.99±0.45^{d}	68.82±0.15^{a}

Keys: Values are presented as mean ± SD. Values in the same column with completely different letters are significantly different at $p<0.05$

***Invivo* Antioxidant Activity on liver**

The results in Table 5 showed that administration of gentamicin alone (Negetive control) caused a significant increase ($P < 0.05$) in hepatic MDA levels(10.17±0.43nmol/mg), and HP (2.01±0.11 µmol/mg) along with reduced SOD (1.98±0.10 unit/mg) and GSH (12.46±0.43µmol/ml) compared with the normal control group, indicating enhanced lipid peroxidation and oxidative damage in the liver. Treatment with *Neptunia oleracea* hydroethanolic leaf extract produced a dose-dependent improvement in these antioxidant parameters. Notably, rats treated with 400 mg/kg extract showed a significant reduction in hepatic MDA (2.98±0.09 nmol/mg) and HP (0.68±0.01 µmol/mg) levels, along with restoration of SOD (11.19±0.06 unit/mg) and GSH (86.48±0.55µmol/ml) values towards normal. These findings suggest that *N. oleracea* confers strong hepatocurative effects through its antioxidant potential, counteracting gentamicin-induced oxidative stress in liver tissue.

***Invivo* Antioxidant Activity on kidney**

The results in Table 6 showed that administration of gentamicin alone (Negative control) caused a significant elevation ($P < 0.05$) in renal MDA (13.01±0.28) and HP (3.11±0.06) levels along with reduced SOD (2.97±0.04) and GSH (12.61±0.68) compared with the normal control group, indicating increased lipid peroxidation and oxidative stress in kidney tissue. Treatment with *Neptunia oleracea* hydroethanolic leaf extract produced a dose-dependent restoration of these parameters. Notably, rats administered 400 mg/kg of the extract showed a significant decrease in MDA (2.01±0.03) and HP (0.51±0.01) levels and a concurrent increase in SOD (13.01±0.03) and GSH (85.35±0.53) values towards normal. These findings suggest that *N. oleracea* exerts strong nephrocurative activity by enhancing renal

antioxidant defenses and mitigating gentamicin-induced oxidative stress.

Table 5. Effect of the *N.Oleracea* on liver *in-vivo* antioxidant activity

Plant Extract (mg/kg body weight)	Parameters			
	MDA (nmol/mg)	GSH (µmol/mg)	HP (µmol/mg)	SOD (unit/mg)
Normal control	2.73±0.18^{e}	89.57±1.46^{a}	0.5±0.02^{e}	12.1±0.04^{a}
Negative control	10.17±0.43^{a}	12.46±0.43^{e}	2.01±0.11^{a}	1.98±0.10^{e}
Stnd drug	3.11±0.09^{d}	85.30±0.56^{b}	0.71±0.01^{d}	10.01±0.08^{c}
100	7.01±0.06^{b}	48.94±0.73^{d}	1.11±0.01^{b}	6.99±0.03^{b}
200	5.23±0.01^{c}	81.48±1.24^{c}	0.95±0.06^{c}	8.11±0.03^{d}
400	2.98±0.09^{d}	86.48±0.55^{b}	0.68±0.01^{d}	11.19±0.06^{b}

Keys: Values are presented as mean ± SD. Values in the same column with completely different letters are significantly different at $p<0.05$

MDA= Malondialdehyde, GSH= Glutathione peroxidase, HP= Hydroperoxides, SOD= Superoxide dismutase

Table 6. Effect of *N. oleracea* on kidney *in-vivo* antioxidant activity

Plant Extract (mg/kg body weight)	Parameters			
	MDA (nmol/mg)	GSH (µmol/mg)	HP (µmol/mg)	SOD (unit/mg)
Normal control	1.91±0.03^{e}	92.59±0.41^{a}	0.51±0.01^{d}	14.02±0.08^{a}
Negative control	13.01±0.28^{a}	12.61±0.68^{e}	3.11±0.06^{a}	2.97±0.04^{e}
Stnd drug	2.98±0.02^{d}	85.82±0.56^{b}	0.61±0.01^{d}	12.91±0.03^{b}
100	9.07±0.8^{b}	43.87±0.49^{d}	1.01±0.01^{b}	5.04±0.02^{d}
200	7.11±0.37^{c}	69.13±1.43^{c}	0.8±0.04^{c}	8.91±0.01^{c}
400	2.01±0.03^{d}	85.35±0.53^{b}	0.51±0.01^{d}	13.01±0.03^{b}

Keys: Values are presented as mean ± SD. Values in the same column with completely different letters are significantly different at $p<0.05$

MDA= Malondialdehyde, GSH= Glutathione peroxidase, HP= Hydroperoxides, SOD= Superoxide dismutase

Histopathology of the liver

Histopathological examination of liver tissue was performed using hematoxylin and eosin (H&E) staining at 100× magnification across the experimental groups. The photomicrographs of the normal control (A) as well as the treatment groups administered *Neptunia oleracea extract* at 100 mg/kg (C), 200 mg/kg (D), and 400 mg/kg (E) revealed normal hepatocyte (HC) architecture radiating from the central vein (CV), with no observable pathological alterations, thereby establishing a baseline for healthy liver histology. In contrast, the photomicrograph of the negative control group (B) displayed degenerative changes in the architecture of hepatocytes and distortion of the central vein (Figure 2).

Histopathology of the kidney

Histopathological examination of kidney tissue was carried out using hematoxylin and eosin (H&E) staining at 100× magnification across the experimental groups. The photomicrographs of the normal control (A) as well as the treatment groups administered *Neptunia oleracea extract* at 100 mg/kg (C), 200 mg/kg (D), and 400 mg/kg (E) revealed normal renal histoarchitecture with intact glomeruli (G) and renal tubules (RT), showing no observable pathological alterations. In contrast, the photomicrograph of the negative control group (B) displayed degenerative changes, including glomerular shrinkage and tubular necrosis, indicating gentamicin-induced renal injury (Figure 3).

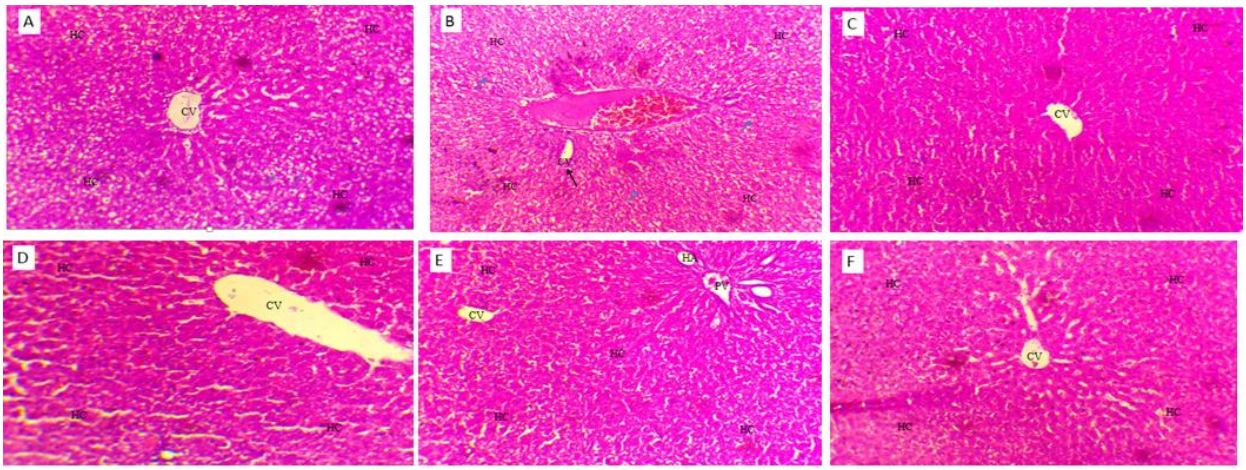

Figure 2. **Histopathology of Liver tissue (HandE stain at X100). (a) Control, (b) negative control, (c) 100mg/kg, (d) 200mg/kg, (e) 400mg/kg**

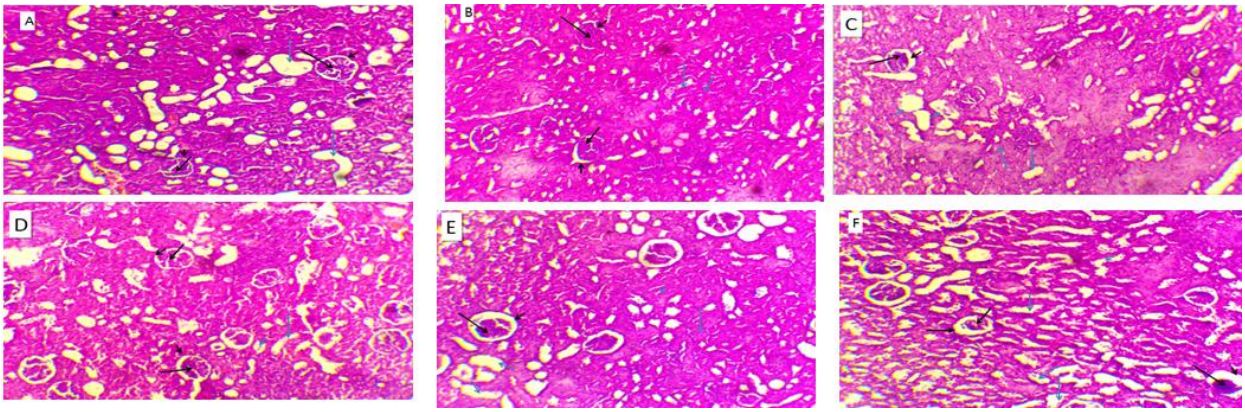

Figure 3. **Histopathology of kidney tissue (HandE stain at X100). (a) Control, (b) negative control, (c) 100mg/kg, (d) 200mg/kg, (e) 400mg/kg**

DISCUSSION

Phytochemical screening of the *Neptunia oleracea* hydroethanolic extract revealed the presence of flavonoids, tannins, saponins, glycosides, alkaloids, and steroids. These bioactive compounds are known to possess antioxidant, anti-inflammatory, and organ-protective properties (Valsala and Karpagaganapathy, 2002; Harborne, 1998; Tiwari *et al*., 2011; Rasool *et al.,* 2010). The presence of these secondary metabolites provides a pharmacological basis for the hepatocurative and nephrocurative effects observed in this study, since flavonoids and phenolic compounds have been widely reported to scavenge free radicals and prevent lipid peroxidation (Nguyen *et al.,* 2019).

Acute toxicity evaluation showed that administration of *N. oleracea* extract at doses up to 5000 mg/kg produced no observable adverse effects or mortality, indicating an LD_{50} greater than 5000 mg/kg. According to OECD (2008) and Lorke (1983), substances with LD_{50} above 5000 mg/kg are considered practically non-toxic. This suggests that *N. oleracea* extract is safe and could be developed as a therapeutic agent with minimal risk of acute toxicity.

Gentamicin administration caused a progressive loss of body weight compared with the normal control, consistent with systemic toxicity and metabolic stress induced by aminoglycosides (Singh *et al.,* 2017). Treatment with *N. oleracea* extract improved body weight in a dose dependent manner, particularly at 400 mg/kg. This effect could be attributed to the ability of the extract to mitigate hepatic and renal dysfunction and restore metabolic balance, as similarly observed by Olorunnisola *et al.* (2020) in gentamicin-treated rats receiving plant-based antioxidants.

Liver function tests showed that gentamicin significantly elevated serum AST, ALT, and bilirubin levels while reducing albumin and total protein, indicating hepatocellular damage. These findings are consistent with Ali *et al*. (2019), who reported that gentamicin-induced hepatic injury is associated with oxidative membrane damage and leakage of intracellular enzymes. Olorunnisola *et al*. (2020) also observed increased liver enzyme activities in gentamicin-treated rats, confirming its hepatotoxic effects. Treatment with *N. oleracea* extract, particularly at 400 mg/kg, significantly restored these parameters towards normal, suggesting hepatocurative activity. This aligns with the findings of Singh *et al.* (2017), who demonstrated that antioxidant-rich plant extracts attenuate aminoglycoside-induced liver injury by reducing oxidative damage.

Similarly, kidney function parameters were markedly altered by gentamicin administration. Elevated serum creatinine and urea, together with electrolyte imbalance (low sodium and high potassium), confirmed nephrotoxicity due to impaired glomerular filtration and tubular dysfunction (Lopez-Novoa *et al.,* 2011; Abdel-Raheem *et al*., 2010). Administration of N. oleracea extract significantly normalized these renal indices, indicating nephroprotective potential. Al-Harbi *et al.* (2018) similarly demonstrated that plant extracts with antioxidant activity ameliorated gentamicin-induced kidney injury by restoring renal function markers. Thus, the ability of *N. oleracea* to restore creatinine, urea, and electrolyte balance supports its therapeutic promise.

Oxidative stress markers further corroborated the biochemical findings. Gentamicin treatment significantly elevated MDA and hydroperoxides, while antioxidant defenses such as SOD and GSH were markedly reduced. These alterations reflect enhanced lipid peroxidation and depletion of endogenous antioxidants, as previously reported by Pedraza-Chaverri *et al.* (2000) and Yousef (2009). Treatment with *N. oleracea* extract significantly reduced MDA and hydroperoxides while restoring SOD and GSH levels in a dose-dependent manner. Comparable antioxidant effects were described by Chatterjee *et al*. (2012), who showed that flavonoid-rich extracts protect tissues against ROS-mediated damage by enhancing antioxidant enzyme activities. The observed antioxidant activity of *N. oleracea* therefore likely underlies its hepatorenal curative effects.

Histopathological observations provided further confirmation. Gentamicin produced hepatocellular degeneration, congestion, and loss of normal liver architecture, along with tubular necrosis and glomerular distortion in the kidney. These lesions are consistent with previous reports of gentamicin-induced hepatorenal damage (Bancroft and Gamble, 2008; Abdel-Raheem *et al.,* 2010). In contrast, tissues from rats treated with *N. oleracea* extract, particularly at 400 mg/kg, showed near-normal histological features with preserved hepatocytes and renal tubules. These findings substantiate the biochemical evidence and indicate structural protection offered by the extract.

Collectively, the results of this study demonstrate that *N. oleracea* hydroethanolic extract exerted potent hepatocurative and nephrocurative effects against gentamicin-induced toxicity. The curatives mechanisms are most likely mediated by its rich phytochemical composition and antioxidant properties, which scavenged free radicals, prevented oxidative damage, and preserved cellular and tissue integrity

CONCLUSION

The acute toxicity (LD_{50}) test indicated that the hydroethanolic leaf extract of *Neptunia oleracea* was safe at the tested doses, as no mortality or adverse clinical signs were observed. The extract significantly improved liver and kidney function parameters that were altered due to gentamicin-induced oxidative stress, restoring them toward normal values in a dose-dependent manner. Antioxidant assays demonstrated that the extract reduced lipid peroxidation (measured by MDA and hydroperoxides) and enhanced the activity of endogenous antioxidant enzymes (SOD and GSH) in both liver and kidney tissues. Histopathological analysis confirmed a marked recovery of normal liver and kidney architecture in the treated groups compared to the negative control, which supports the biochemical findings. Overall, the hydroethanolic leaf extract of *Neptunia oleracea* shows strong curative antioxidant activity and has potential as an affordable phytotherapeutic agent for managing liver and kidney damage induced by gentamicin.

Authors Statements

The authors declare no conflict of interest.

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