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Research Article

Protective Effects of *Neptunia oleracea* Hydroethanolic Extracts Against Gentamicin Induced Liver and Kidney Oxidative Stress in Albino Rats

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ABSTRACT
This study evaluated the protective effects of *Neptunia oleracea* hydroethanolic extract against gentamicin induced Oxidative stress in albino rats. The qualitative phytochemical screening identified flavonoids, saponins, tannins, alkaloids, phenols, steroids are present while glycosides, and cardinolides are absent. Acute-toxicity test showed an LD_{50}> 5000 mg/kg with no mortality or behavioral changes in the animals, while sub-acute exposure (100, 200 and 400 mg/kg b.w, for 28 days) showed normal body weight gain. The 30 albino rats are used for the test and are divided in to six equal groups as:- Group-1, Positive Control (received distilled water); Group-2, Negative Control (received Gentamicin 80mg/kg I.p); Group-3, Standard Drug (received Gentamicin and silimarin 140mg/kg b.w orally); Group 4 to 6 received gentamicin and 100, 200 and 400 mg/kg b.w of *N. Oleracea* orally). Gentamicin administration led to marked elevations in biochemical markers of liver and kidney injury, including ALT, AST, bilirubin, creatinine, and urea, alongside decreased levels of albumin and total protein. It also caused histopathological damage and increased oxidative stress, confirming its toxic effects. Treatment with *N. Oleracea* hydroethanolic extract, especially at a dose of 400mg/kg, protected biochemical and histological parameters levels. The extract also significantly prevented oxidative stress markers and antioxidant enzyme activity, supporting its protective role. The efficacy of *N. Oleracea* hydroethanolic extract was comparable to that of the standard drug silymarin, indicating its potential as a natural therapeutic alternative. Therefore, it concluded that *N. Oleracea* hydroethanolic extract has hepatoprotective and nephroprotective effects, likely due to its antioxidant and anti-inflammatory properties.

Keywords: Anti-imflamation; Antioxidants; Gentamicin; Oxidative stress; Hepatotoxicity; Histopathology; Nephrotoxicity; *Neptunia oleracea*; Silybon; Silymarin

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INTRODUCTION

Gentamicin is an aminoglycoside antibiotic with a small therapeutic window that is currently used primarily as part of short-term empirical combination therapy (Hodiamont *et al*., 2022). Gentamicin dosing schemes still need refinement, especially for subpopulations where pharmacokinetics can differ from pharmacokinetics in the general adult population: obese patients, critically ill patients, paediatric patients, neonates, elderly patients and patients on dialysis (Hodiamont *et al.,* 2022). Several new population pharmacokinetic studies have focused on these subpopulations, providing insights into the typical values of the most relevant pharmacokinetic parameters, the variability of these parameters and possible explanations for this variability, although unexplained variability often remains high. A

gentamicin starting dose of 7 mg/kg based on total body weight (or on adjusted body weight in obese patients) appears to be the optimal strategy for increasing the probability of target attainment (PTA) after the first administration for the most commonly used PK/PD targets in adults and children older than 1 month, including critically ill patients. However, evidence that increasing the PTA results in higher efficacy is lacking; no studies were identified that show a correlation between estimated or predicted PK/PD target attainment and clinical success. Although it is unclear if performing therapeutic drug monitoring (TDM) for optimization of the PTA is of clinical value, it is recommended in patients with highly variable pharmacokinetics, including patients from all subpopulations that are critically ill (such as elderly, children and neonates) and patients on intermittent haemodialysis (Hodiamont *et al*., 2022).

Neptunia oleracea commonly known in English as water mimosa or water sensitive *Neptunia*, is pantropical nitrogen-fixing perennial legume. Genus and common name come from *Neptune,* god of the sea, in reference to the aquatic habit of some species in the genu (Bhunia and Mondal 2012). Primary leaf segments have 8-40 small oblong leaflets arranged in opposite pairs. Tiny greenish-yellow flowers are densely crowded into feathery orbicular inflorescences that bloom in summer. Fruits are flat pods (to 1-2" long) (Walia, 2014).

Oxidative stress occurs when there's an imbalance between the production of free radicals and the body's ability to neutralize them. This can lead to cell damage, inflammation, and various diseases (Chaudhary *et al*., 2023). Therefore, the aims of the present study is to prove the possibility of using a herbal medicine such as *Neptunia oleracea* protection of kidney and liver of albino rats from oxidative stress induced by Gentamicin toxicity and it is complications.

MATERIALS AND METHODS

Experimental Animals

Fifty albino 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. 36 rats were grouped into six groups of six equal rats (Positive control group (Normal control), Negative group (Gentamicin 80mg only), Gentamicin and Standard drug (slymarin 140mg), Gentamicin and the 100mg/kg b.w of the extract, Gentamicin and the 200mg/kg b.w of the extract, Gentamicin and 400mg/kg b.w of the extract). All they were for 14 days.

Collection and indentation 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 Kebbi State University of Science and Technology Aliero with the voucher number 239.

Preparation of Hydroethanolic Extract: 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, cardenolite, saponins, tannins, and steroids were carried out using the standard methods.

Blood sample collection procedures

Blood was collected using Hugo and Russel's method (Kihlstrom *et al.,* 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 haematological assays. Pancreas was excised, preserved in formalin for histopathology.

Qualitative Phytochemical Screening of the Extract

Phytochemical screening to test for the presence of alkaloids, anthraquinones, Carbohydrates, glycosides, flavonoids, cardenolite, saponins, tannins, and steroids were carried out using the standard methods.

Test for saponin

To 2 ml of the extract, 2 ml of distilled water was added and agitated in a test tube for 5 minutes. The formation of foams indicates the presence of saponin (Yekeen *et al.,* 2020).

Test for tannins

To 5 drops of 0.1% ferric chloride was added to 2 ml of the extract, brownish green or blue lack colouration indicates the presences of tannins (Shah and Yadav, 2015).

Test for glycosides

To 2 ml of acetic acid was added to 2 ml of the extract. The mixture was cool in cold water bath. 2 ml of concentrated H_2SO, was then added, colour development from blue to bluish green indicates the presence of glycosides (Rahman *et al*., 2013).

Test for flavonoids

To 2 ml of 10% Sodium hydroxide was added to 2 ml of the extract in a test tube. An intense yellow colour was form which turned to colourless upon addition of 2 ml of dilute hydrochloric acid indicating the presence of flavonoid (Shah and Yadav, 2015).

Test for antraquinones

To 2 ml of the extract was boiled with 5 ml of 10% hydrochloric acid for 3 minutes. 5 ml of chloroform was added. 5 drops of 10% ammonia was also added. A rose-pink colouration indicates the presence antraquinones (Ovonramwen, 2022).

Test for cardenolides

To 2 ml of benzene was added to 1 ml of the sample extract. The formation of a turbid brown colour indicates the presence of Cardenolides (Coker *et al.,* 2008).

Test for alkaloids

Alkaloids were determined as follows; 1.5 ml of 1% HCL was added to 2 ml methanol filtrates of samples. The solution was heated and six drops of Dragendroff reagent was added. Orange precipitate confirmed presence of alkaloids (Salamah and Ningsih, 2017).

Test for steroids

To 1 ml of the extract, 0.5 ml of acetic anhydride and 0.5 ml of chloroform was added, and concentrated sulphuric acid later added. Formation of a brownish green ring at the contact of the two liquids indicates the presence of steroid (Kiruthiga and Sekar, 2014).

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).

Protective 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 14 consecutive days to induce liver and kidney toxicity. 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 14 days.

Group 2 (Negative control): Received gentamicin (80 mg/kg/day, i.p.) for 14 days to induce liver and kidney toxicity.

Group 3 (Positive control): Received gentamicin (80 mg/kg/day, i.p.) And by oral administration of the standard drug silymarin (100mg/kg, p.o.) Daily for 14days

Group 4 (Treatment I): Received gentamicin (80 mg/kg/day, i.p.) And by *Neptunia oleracea* hydroethanolic extract (100 mg/kg, p.o.) Daily for 14days

Group 5 (Treatment II): Received gentamicin (80 mg/kg/day, i.p.) And by *Neptunia oleracea* hydroethanolic extract (200 mg/kg, p.o.) Daily for 14days

Group 6 (Treatment III): Received gentamicin (80 mg/kg/day, i.p.) And by *Neptunia oleracea* hydroethanolic extract (400 mg/kg, p.o.) Daily for 14days

At the end of the experimental period (day 15), all animals were sacrificed under light ether anaesthesia 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.

Measurement of Liver Function Markers

Determination of Aspartate Aminotransferase (AST)

Aspartate aminotransferase catalytic activity was determined by the method of Ansari (2019).

Determination of Alanine Aminotransferase (ALT)

Alanine aminotransferase activity was determined by the method of Okalebo (2002).

Determination of Alkaline Phosphatase (ALP)

Alkaline phosphatase activity was estimated using the method of Shivappa (2006).

Determination of Bilirubin (Total and Direct)

This was determined by the calorimetric method of Landis (1978).

Determination of Albumin

Albumin was determined using bromocresol green method as modified by Ueno *et al. (*2013).

Determination of Total Protein

Total protein was estimated using the Biuret reaction method by Gornall *et al.* (1949).

Kidney Function Assay

Serum creatinine estimation

Principle:

Creatinine reacts with alkaline picrate to form a red-orange complex measurable at 520 nm (Chapman, 1909). Method: Jaffe's colourimetric method.

Serum urea estimation

Principle:

Urea is hydrolysed by urease into ammonia and CO2. Ammonia reacts with diacetyl monoxime (DAM) to form a pink-coloured complex measurable at 540 nm (Reay *et al.,* 2019).

Method: DAM colourimetric method.

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

Principle:

Serum sodium and potassium were estimated using the flame photometric method as described by Garcia *et al. (*2019).

ANTIOXIDANT ACTIVITY MARKERS

Determination of Lipid peroxidation (Malondialdehyde) level

Lipid peroxidation will be determined by measuring spectrophotometrically the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Fauziah *et al.* (2018).

Assay for Superoxide Dismutase (SOD) Activity

This will be determined using the method of Sun *et al.* (1988).

Assay for Catalase Activity

The activity of catalase will be assayed according to the method of Hadwan (2018).

Determination of Glutathione Peroxidase (GPx)

This will be based on the method of Ahmed *et al. (*2021).

Hydrogen Peroxide (HP)

Principle

Hydrogen Peroxide (HP) reacts with molybdate to form a stable yellow complex measurable at 405 nm (Zhou *et al.,*2006).

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 haematoxylin and eosin, and examined under a light microscope for histopathological changes (Alhamedi, 2015).

Data Analysis

The data were presented as Mean ± Standard Error of Mean (SEM) 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 $P < 0.05$ (Belouafa *et al.,* 2017).

RESULTS

Qualitative Phytochemical Screening

Numerous phytochemicals were detected in *Neptunia oleracea* extract such as flavonoids, saponins, cardiac glycosides, tannins, steroids, alkaloids and cardenolides. While glycosides and anthraquinones were not detected as presented in Table 1.

The phytochemical screening of *Neptunia oleracea* extract revealed the presence of several bioactive compounds including flavonoids, phenolics, alkaloids, tannins, saponins, glycosides.

These constituents are well-known for their antioxidant, anti-inflammatory, and organs protective properties, supporting the therapeutic potential of the plant. The presence of flavonoids and phenolic compounds is particularly significant, as they are potent free radical scavengers and play a key role in mitigating oxidative damage to tissues.

Acute Toxicity Studies of *Neptunia oleracea* extract

The Table 2 indicated observation of physical signs in rats during acute toxicity study of *Neptunia oleracea hydroethanolic* extract.

The acute toxicity study revealed no mortality or significant behavioural changes in rats administered *Neptunia oleracea* hydroethanolic extract up to a dose of 5000 mg/kg. The gentamicin-only group, though not part of the acute toxicity assessment, it has exhibited signs of systemic toxicity, including weight loss, reduced activity, and clinical signs of discomfort, likely due to renal and hepatic damage.

Rats treated with *Neptunia oleracea* hydroethanolic extract at both low (100 mg/kg) and high doses (400 mg/kg) during the protective study phase showed no signs of toxicity, confirming that repeated administration of the extract is safe at therapeutic levels. The findings are in agreement with Olorunnisola *et al. (*2014).

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

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

(+) = Presence of phytochemicals (-) = Absence of phytochemicals

Table 2. Observation of physical signs in rats during acute toxicity study *of Neptunia 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)

Effect of *Neptunia oleracea* extract on body weight

The figure 1 indicated the body weight of animals administered with *Neptunia oleracea hydroethanolic* extract for 28 days and the increase in the body weight of the animals.

Rats treated with *Neptunia oleracea* hydroethanolic extract, particularly at 400 mg/kg, showed a noticeable showed a noticeable improvement in body weight and also improved appetite, nutrient utilization, and overall physiological status. The weight gain in 100mg, 200mg groups suggests that *Neptunia oleracea* hydroethanolic extract also showed a noticeable low improvement in body weight especially in 100mg group due to the low dose. The colour lines in the figure indicate the groups of the animals in which the blue line indicated the control group, the pinch line indicated the 100mg/kg body weight group, the greenish line indicated the 200mg/kg body weight group, and the yellow line indicated the 400mg/kg body weight group.

Liver Function Parameters

Table 3 indicated the effect of *Neptunia oleracea* hydroethanolic extract on biomarkers of liver function test (ALT, AST, ALB, T.P, And T.B) on albino rats

The Table 3 indicated Gentamicin administration group (Negative control group) which significantly elevated ($P< 0.05$) in serum ALT (81.19±0.13 mg/dl), AST (152.12±0.11 mg/dl), and Total Bilirubin levels (2.01±0.01 mg/dl) along with reduced Albumin (2.20±0.05 g/dl) and total protein level (4.86±0.20 g/dl), indicating hepatocellular damage. Protective with *Neptunia oleracea* extract, especially at 400mg/kg, resulted in normalization of the serum ALT (48.09±0.71 mg/dl), AST (81.02±0.43 mg/dl),

Total Bilirubin (0.19±0.01 mg/dl), Albumin (4.5±0.15 g/dl) and total protein level (9.96±0.08 g/dl) liver enzyme levels and improved protein synthesis parameters. This supports the work of Singh *et al. (*2017).

Protective with low dose of *Neptunia oleracea* hydroethanolic extract (100 mg/kg) group showed partial improvement, indicated dose dependency in the protective effect Protective with standard drug group (silymarin 140 mg/kg) also maintained serum ALT (45.74±0.31 mg/dl), AST (81.02±0.43 mg/dl), Total Bilirubin (0.19±0.01 mg/dl), Albumin (4.5±0.15 g/dl) and total protein level (9.96±0.08 g/dl) liver enzyme levels close to normal group**.**

Kidney Function Parameters

Table 4 indicated effect of *Neptunia oleracea* hydroethanolic extract on biomarkers of kidney function test (Na+, K+, Urea, Creatinine) on albino rats.

The table below indicated Gentamicin group (Negative group) significantly elevated serum creatinine (2.66±1.10 mg/dl) and urea (60.06±0.22 mg/dl), potassium (7.44±0.04 mmol/l) and sodium (180.00±0.11 mmol/l) compared with the normal control group, indicated renal impairment and loss of functional integrity. Protective with *Neptunia oleracea* hydroethanolic leaf extract produced a dose-dependent improvement in this parameter's levels, accompanied by disturbances in electrolytes such as hyponatremia and hyperkalemia hallmarks of nephrotoxicity.

Protective with *Neptunia oleracea* hydroethanolic extract, especially at 400 mg/kg group resulted in normalization of serum creatinine (0.70±0.16 mg/dl) and urea (28.90±0.16 mg/dl), potassium (143.25±0.50 mmol/l) and sodium (180.00±0.11[a] mmol/l), supporting its nephroprotective role. Protective with low-dose *Neptunia oleracea* hydroethanolic extract (100 mg/kg) group showed partial improvement, indicating dose-dependency in the protective effect.

Protective with standard drug group (silymarin 140 mg/kg) resulting in maintained of serum creatinine (0.74±4.10 mg/dl) and urea (28.90±0.16 mg/dl), potassium (4.94±0.05 mmol/l) and sodium (143.25±0.50 mmol/l) to normal range.

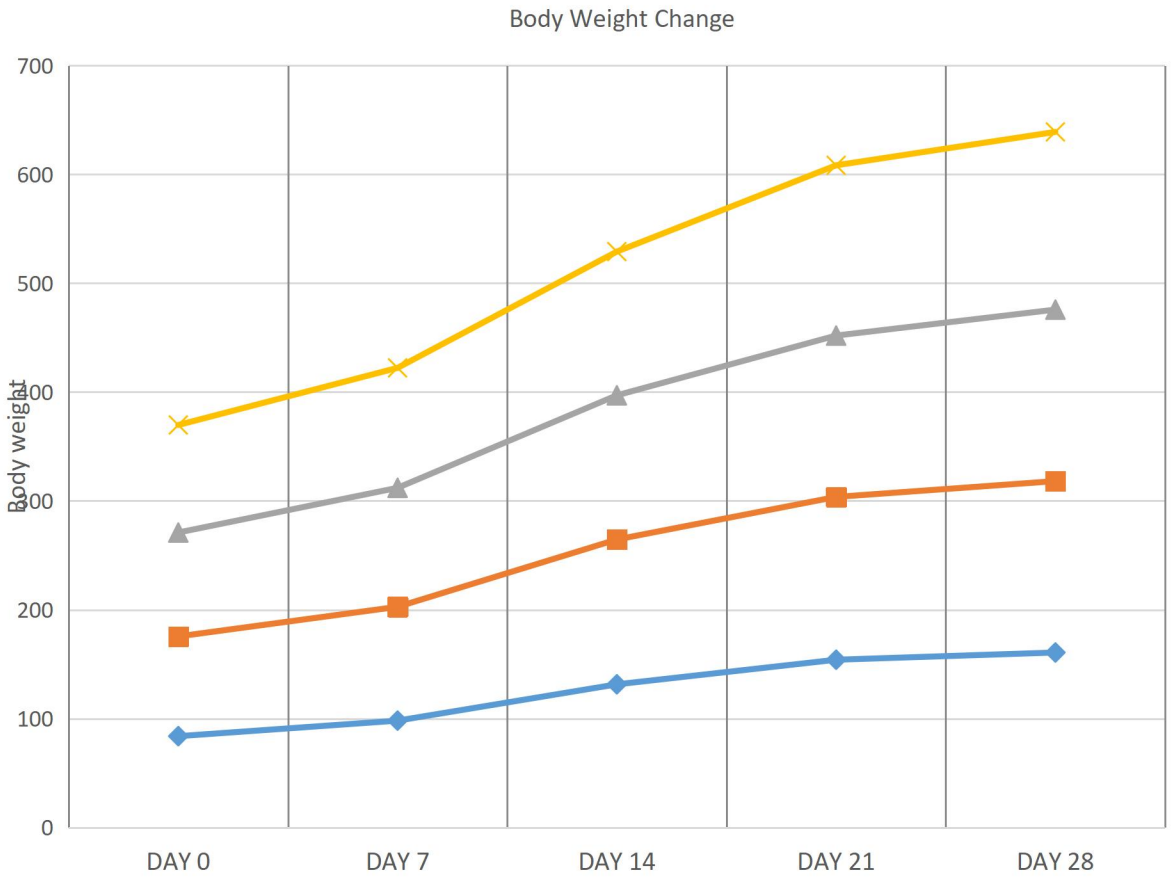


Figure 1. Effect of *Neptunia oleracea* on body weight change
Keys: 100mg/kg B.W, 200mg/kg B.W, 300mg/kg B.W, 400mg/kg B.W

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

Plant Extrats (mg/kg body weight)	ALT (unit/l)	AST (unit/l)	TP (g/dl)	TBIL (mg/dl)	ALB (g/dl)
Group ($D.H_2O$ 5ml/kg)	49.07 ± 0.10^{b}	81.45 ± 0.10^{b}	7.96 ± 0.80^{a}	0.15 ± 0.02^{f}	4.53 ± 0.05^{b}
Group2(Gentimicn 80mg/kg)	81.19 ± 0.13^{a}	152.12 ± 0.11^{a}	4.86 ± 0.20^{e}	2.01 ± 0.01^{a}	2.20 ± 0.05^{e}
Group3(Sylimarin 140mg/kg)	45.74 ± 0.31^{d}	75.30 ± 0.08^{c}	7.08 ± 0.50^{b}	0.21 ± 0.03^{d}	4.53 ± 0.02^{c}
Group4 (100mg/kg b.w of *N. oleracea*)	31.62 ± 0.05^{e}	71.16 ± 0.06^{e}	6.44 ± 0.03^{d}	0.33 ± 0.01^{b}	3.70 ± 0.02^{d}
Group5 (200mg/kg b.w of *N. oleracea*)	39.55 ± 0.04^{c}	75.12 ± 0.08^{d}	6.69 ± 0.01^{c}	0.25 ± 0.05^{c}	3.71 ± 0.03^{d}
Group6 (400mg/kg b.w of *N. oleracea*)	48.09 ± 0.71^{b}	81.02 ± 0.43^{b}	9.96 ± 0.08^{a}	0.19 ± 0.01^{e}	4.5 ± 0.15^{a}

Keys: Values are presented as mean ± SD. Values in the same column with completely different letters are significantly different at $p<0.05$
ALT= Alanine amino transferse, AST= Aspartate amino transferase, TP= Total protein, TBIL= Total bilirubin, ALB= Albumin

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

Plant Extrats (mg/kg body weight)	Creatine (mg/dl)	Sodium (mmol/l)	Urea (mg/dl)	Pottasium (mmol/l)
Group ($D.H_2O$ 5ml/kg)	0.76 ± 0.06^{b}	143.00 ± 0.50^{b}	29.09 ± 0.06^{b}	4.94 ± 0.09^{B}
Group2(Gentimicn 80mg/kg)	2.66 ± 1.10^{a}	180.00 ± 0.11^{a}	60.06 ± 0.22^{a}	7.44 ± 0.04^{a}

Group3 (Sylimarin 140mg/kg)	0.74 ± 4.10^{c}	140.03 ± 0.80^{e}	27.55 ± 0.14^{c}	4.22 ± 0.90^{d}
Group4 (100mg/kg b.w of *N. oleracea*)	0.44 ± 2.0^{e}	137.45 ± 0.70^{b}	18.64 ± 0.03^{e}	3.90 ± 0.10^{e}
Group5 (200mg/kg b.w of *N. oleracea*)	0.59 ± 3.01^{d}	140.00 ± 0.52^{d}	20.62 ± 0.05^{d}	4.01 ± 0.06^{c}
Group6 (400mg/kg b.w of *N. oleracea*)	0.70 ± 0.16^{b}	143.25 ± 0.50^{b}	28.90 ± 0.16^{b}	4.94 ± 0.05^{b}

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

***In vivo* Antioxidant Activity on liver**

Table 5 indicated the antioxidant activity of *Neptunia oleracea* hydroethanolic extract of the liver and kidney (SOD, H P, MDA, and GSH) organs. Gentamicin treated (negative control) group exhibited elevated MDA (4.54±0.74 nmol/mg) and hydrogen peroxides (3.50±0.03 µmol/mg), along with reduced activities of SOD (2.83±0.06 nmol/mg) and GSH (1.74±0.73 µmol/mg), indicating a state of oxidative stress. Protective with *Neptunia oleracea* hydroethanolic extract especially 400mg/kg significantly protect the MDA (2.40±0.07 nmol/mg) and hydrogen peroxides (1.55±0.07 µmol/mg), SOD (13.48±1.01 nmol/mg) and GSH (4.95±1.10 µmol/mg) oxidative stress markers and antioxidant enzyme levels. Protective with low-dose *Neptunia oleracea* hydroethanolic extract (100 mg/kg) group showed partial improvement, indicating dose-dependency in the protective effect. Protective with standard drug group (silymarin 140 mg/kg) also maintained significantly protect the MDA (2.40±0.07 nmol/mg) and hydrogen peroxides (1.66±0.22 µmol/mg), SOD (14.35±0.31 nmol/mg) and GSH (5.91±0.21 µmol/mg) oxidative stress markers and antioxidant enzyme levels to normal range.

***In vivo* Antioxidant Activity on kidney**

The Table 6 indicated the antioxidant activity of *Neptunia oleracea* hydroethanolic extract of the liver and kidney (SOD, H P, MDA, and GSH) organs.

Gentamicin treated (negative control) group exhibited elevated MDA (2.43±0.31 nmol/mg) and hydrogen peroxides (3.08±0.07 µmol/mg), along with reduced activities of SOD (2.83±0.07 nmol/mg) and GSH (1.74±0.73 µmol/mg), indicating a state of oxidative stress. Protective with *Neptunia oleracea* hydroethanolic extract especially 400mg/kg significantly protect the MDA (2.44±0.06 nmol/mg) and hydrogen peroxides (1.62±0.28 µmol/mg), SOD (13.64±0.09 nmol/mg) and GSH (5.58±0.06 µmol/mg) oxidative stress markers and antioxidant enzyme levels. Protective with low-dose *Neptunia oleracea* hydroethanolic extract (100 mg/kg) group showed partial improvement, indicating dose-dependency in the protective effect. Protective with standard drug group (silymarin 140 mg/kg) also maintained significantly protect the MDA (2.43±0.31 nmol/mg) and hydrogen peroxides (1.34±0.24 µmol/mg), SOD (13.24±0.08 nmol/mg) and GSH (5.05±0.12 µmol/mg) oxidative stress markers and antioxidant enzyme levels.

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

Plant Extract (mg/kg body weight)	SOD (nmol/mg)	HP (µmol/ml)	MDA (µmol/mg)	GSH (unit/mg)
Group (D.H_2O 5ml/kg)	14.47 ± 0.50^{a}	1.66 ± 0.02^{b}	2.45 ± 0.03^{b}	5.95 ± 0.10^{a}
Group2 (Gentimicn 80mg/kg)	2.83 ± 0.06^{e}	3.50 ± 0.03^{a}	4.54 ± 0.74^{a}	1.74 ± 0.73^{e}
Group3 (Sylimarin 140mg/kg)	14.35 ± 0.31^{b}	1.66 ± 0.22^{b}	2.40 ± 0.07^{c}	5.91 ± 0.21^{b}
Group4 (100mg/kg b.w of *N. oleracea*)	11.82 ± 0.32^{d}	1.22 ± 0.07^{e}	1.96 ± 0.03^{e}	3.84 ± 0.56^{d}
Group5 (200mg/kg b.w of *N. oleracea*)	11.95 ± 0.57^{c}	1.30 ± 0.05^{d}	1.99 ± 0.02^{d}	4.37 ± 0.48^{c}
Group6 (400mg/kg b.w of *N. oleracea*)	13.48 ± 1.01^{a}	1.55 ± 0.07^{c}	2.40 ± 0.07^{c}	4.95 ± 1.10^{a}

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 *Neptunia oleracea* on kidney *in-vivo* antioxidant activity

Plant Extract (mg/kg body weight)	SOD (nmol/mg)	HP (µmol/ml)	MDA (µmol/mg)	GSH (unit/mg)
Group (D.H_2O 5ml/kg)	13.68 ± 0.05^{a}	1.66 ± 0.24^{b}	2.48 ± 0.02^{b}	5.60 ± 0.70^{a}
Group2 (Gentimicn 80mg/kg)	2.83 ± 0.07^{e}	3.08 ± 0.07^{a}	4.62 ± 0.10^{a}	1.28 ± 0.09^{e}
Group3 (Sylimarin 140mg/kg)	13.24 ± 0.08^{b}	1.34 ± 0.24^{c}	2.43 ± 0.31^{c}	5.05 ± 0.12^{b}
Group4 (100mg/kg b.w of *N. oleracea*)	10.28 ± 0.12d	1.22 ± 0.07^{e}	1.22 ± 0.03^{e}	3.96 ± 0.11^{d}
Group5 (200mg/kg b.w of *N.oleracea*)	11.63 ± 0.03^{c}	1.30 ± 0.05^{d}	1.31 ± 0.09^{d}	4.20 ± 0.27^{c}

Group6 (400mg/kg b.w of *N.oleracea*)	13.64 ± 0.09[a]	1.62 ± 0.28[b]	2.44 ± 0.06[b]	5.58 ± 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

Histopathology of the liver

The Figure 2 indicated the histopathology Plates (1-6) of liver and tissues (H&E stain at x100). Which indicated positive control group, negative control group, standard drug group, 100mg/kg group, 200mg/kg group and 400mg/kg group.

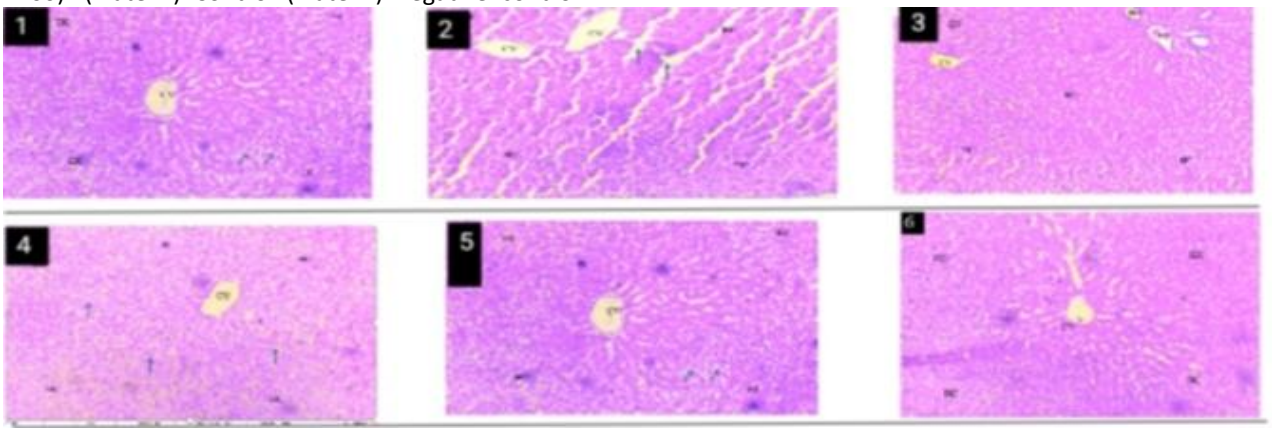
The gentamicin group (80 mg/kg) in Plate 2 showed severe histological alterations. The liver displayed signs of hepatocellular necrosis, sinusoidal dilation, and inflammatory cell infiltration. The kidney tissues showed tubular necrosis, glomerular shrinkage, and interstitial congestion hallmarks of gentamicin-induced hepatotoxicity. In the positive control group (Plate 1), standard drug group (Plate 3), 100mg/kg (Plate 4), 200mg/kg (Plate 5) and 400mg (Plate 6). Both liver tissues showed normal architecture with intact hepatocytes, well-defined central veins, and normal renal glomeruli and tubules, indicating healthy organ structure.

Histopathology of liver tissue (H and E stain at X100). (Plate 1) Control (Plate 2) negative control (Plate 3) standard drug (Plate 4) 100mg/kg (Plate 5) 200mg/kg (Plate 6) 400mg/kg.

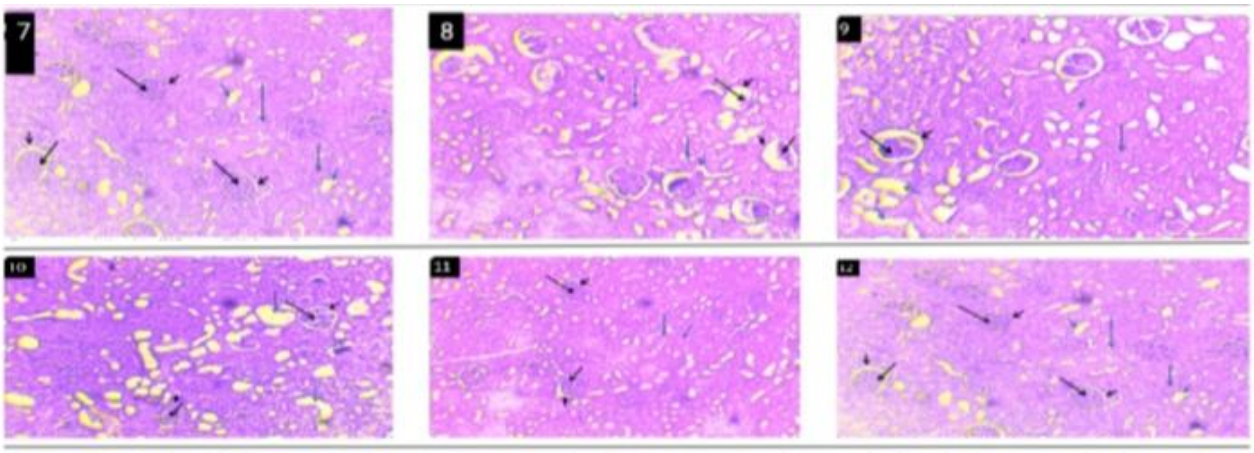
Histopathology of the kidney

The Plate 3 indicated the histopathology Plates (7-12) of kidney and tissues (H&E stain at x100). Which indicated positive control group, negative control group, standard drug group, 100mg/kg group, 200mg/kg group and 400mg/kg group. Gentamicin-only (negative) group (80 mg/kg) Photomicrograph of kidney tissue (H&E stains at X100) Showing Bowman's capsule, Glomerulus and Renal Tubule Glomerulus atrophy, degeneration, dilation of bowman space and Renal Tubules degeneration and vacuolation Hallmarks of gentamicin induced nephrotoxicity.

In the positive control group, standard drug group, 100mg/kg, 200mg/kg and 400mg both liver and kidney tissues showed the normal architecture of gloromolar and renal tubules with no deletion., indicating healthy organ structure.



Plates 1-6. Histopathology of Liver tissue (H and E stain at X100)



Plates 7-12. Histopathology of kidney tissue (HandE stain at X100). Plate 7 - Control, Plate 8 - negative control, Plate 9 - standard drug, Plate 10 - 100mg/kg, Plate 11 - 200mg/kg and Plate 12 - 400mg/kg

DISCUSSION

The phytochemical screening of *Neptunia oleracea* extract revealed the presence of several bioactive compounds including flavonoids, phenolics, alkaloids, tannins, saponins, glycosides.

These constituents are well-known for their antioxidant, anti-inflammatory, and organs protective properties, supporting the therapeutic potential of the plant. The presence of flavonoids and phenolic compounds is particularly significant, as they are potent free radical scavengers and play a key role in mitigating oxidative damage to tissues. This is consistent with the findings of Singh *et al. (*2017).

The acute toxicity study revealed no mortality or significant behavioural changes in rats administered *Neptunia oleracea* hydroethanolic extract up to a dose of 5000 mg/kg. This suggests a wide safety margin and classifies the extract as practically non-toxic according to OECD guidelines. In comparison, the gentamicin-only group, though not part of the acute toxicity assessment, it has exhibited signs of systemic toxicity, including weight loss, reduced activity, and clinical signs of discomfort, likely due to renal and hepatic damage. Rats treated with *Neptunia oleracea* hydroethanolic extract at both low (100 mg/kg) and high doses (400 mg/kg) during the protective study phase showed no signs of toxicity, confirming that repeated administration of the extract is safe at therapeutic levels. The findings are in agreement with Olorunnisola *et al. (*2014), aligns with the work of Singh *et al. (*2017).

Table 3 indicated the liver function test which showed Gentamicin administration group (Negative control group) which significantly elevated ($P< 0.05$) in serum ALT (81.19±0.13 mg/dl), AST (152.12±0.11 mg/dl), and Total Bilirubin levels (2.01±0.01 mg/dl) along with reduced Albumin (2.20±0.05 g/dl) and total protein level (4.86±0.20 g/dl), indicating hepatocellular damage. These findings are in agreement with Ali *et al. (*2019) and Olorunnisola *et al. (*2020).

Protective with *Neptunia oleracea* extract, especially at 400mg/kg, resulted in normalization of the serum ALT (48.09±0.71 mg/dl), AST (81.02±0.43 mg/dl), Total Bilirubin (0.19±0.01 mg/dl), Albumin (4.5±0.15 g/dl) and total protein level (9.96±0.08 g/dl) liver enzyme levels and improved protein synthesis parameters. This supports the work of Singh ***et al. (***2017).

Protective with low dose of *Neptunia oleracea* hydroethanolic extract (100 mg/kg) group showed partial improvement, indicated dose dependency in the protective effect. Protective with standard drug group (silymarin 140 mg/kg) also maintained serum ALT (45.74±0.31 mg/dl), AST (81.02±0.43 mg/dl), Total Bilirubin (0.19±0.01 mg/dl), Albumin (4.5±0.15 g/dl) and total protein level (9.96±0.08 g/dl) liver enzyme levels close to normal group.

Table 4. indicated the kidney function test which showed Gentamicin group (Negative group) significantly elevated serum creatinine (2.66±1.10 mg/dl) and urea (60.06±0.22 mg/dl), potassium (7.44±0.04 mmol/l) and sodium (180.00±0.11 mmol/l) compared with the normal control group,

indicated renal impairment and loss of functional integrity.

Protective with *Neptunia oleracea* hydroethanolic leaf extract produced a dose-dependent improvement in this parameter's levels, accompanied by disturbances in electrolytes such as hyponatremia and hyperkalemia hallmarks of nephrotoxicity. These results are consistent with Abdel-Raheem *et al. (*2010).

Protective with *Neptunia oleracea* hydroethanolic extract, especially at 400 mg/kg group resulted in normalization of serum creatinine (0.70±0.16 mg/dl) and urea (28.90±0.16 mg/dl), potassium (143.25±0.50 mmol/l) and sodium (180.00±0.11[a] mmol/l), supporting its nephroprotective role. Comparable outcomes were reported by Al-Harbi *et al. (*2018).

Protective with low-dose *Neptunia oleracea* hydroethanolic extract (100 mg/kg) group showed partial improvement, indicating dose-dependency in the protective effect. Protective with standard drug group (silymarin 140 mg/kg) resulting in maintained of serum creatinine (0.74±4.10 mg/dl) and urea (28.90±0.16 mg/dl), potassium (4.94±0.05 mmol/l) and sodium (143.25±0.50 mmol/l) to normal range.

The Table 5 indicated *In vivo* antioxidant activity on liver which showed Gentamicin treated (negative control) group exhibited elevated MDA (4.54±0.74 nmol/mg) and hydrogen peroxides (3.50±0.03 µmol/mg), along with reduced activities of SOD (2.83±0.06 nmol/mg) and GSH (1.74±0.73 µmol/mg), indicating a state of oxidative stress. These results align with the findings of Pedraza-Chaverri *et al. (*2000) and Yousef (2009).

Protective with *Neptunia oleracea* hydroethanolic extract especially 400mg/kg significantly protect the MDA (2.40±0.07 nmol/mg) and hydrogen peroxides (1.55±0.07 µmol/mg), SOD (13.48±1.01 nmol/mg) and GSH (4.95±1.10 µmol/mg) oxidative stress markers and antioxidant enzyme levels. This antioxidant action is comparable to the results of Chatterjee *et al. (*2012). Protective with low-dose *Neptunia oleracea* hydroethanolic extract (100 mg/kg) group showed partial improvement, indicating dose-dependency in the protective effect. Protective with standard drug group (silymarin 140 mg/kg) also maintained significantly protect the MDA (2.40±0.07 nmol/mg) and hydrogen peroxides (1.66±0.22 µmol/mg), SOD (14.35±0.31 nmol/mg) and GSH (5.91±0.21 µmol/mg) oxidative stress markers and antioxidant enzyme levels to normal range.

The Table 7 indicated *In vivo* Antioxidant Activity on kidney which showed Gentamicin treated (negative control) group exhibited elevated MDA (2.43±0.31 nmol/mg) and hydrogen peroxides (3.08±0.07 µmol/mg), along with reduced activities of SOD (2.83±0.07 nmol/mg) and GSH (1.74±0.73 µmol/mg), indicating a state of oxidative stress. These results align with the findings of Pedraza-Chaverri *et al. (*2000) and Yousef (2009).

Protective with *Neptunia oleracea* hydroethanolic extract especially 400mg/kg significantly protect the MDA (2.44±0.06 nmol/mg) and hydrogen peroxides (1.62±0.28 µmol/mg), SOD (13.64±0.09 nmol/mg) and GSH (5.58±0.06 µmol/mg) oxidative stress markers and antioxidant enzyme levels. This antioxidant action is comparable to the results of Chatterjee *et al. (*2012).

Protective with low-dose *Neptunia oleracea* hydroethanolic extract (100 mg/kg) group showed partial improvement, indicating dose-dependency in the protective effect. Protective with standard drug group (silymarin 140 mg/kg) also maintained significantly protect the MDA (2.43±0.31 nmol/mg) and hydrogen peroxides (1.34±0.24 µmol/mg), SOD (13.24±0.08 nmol/mg) and GSH (5.05±0.12 µmol/mg) oxidative stress markers and antioxidant enzymes to normal range level.

The Plate (1-6) indicated Histopathology of the liver which showed gentamicin group (80 mg/kg) in Plate 2 showed severe histological alterations. The liver displayed signs of hepatocellular necrosis, sinusoidal dilation, and inflammatory cell infiltration. The kidney tissues showed tubular necrosis, glomerular shrinkage, and interstitial congestion hallmarks of gentamicin-induced nephrotoxicity and hepatotoxicity. These observations are consistent with findings by Abdel-Raheem *et al.* (2010).

In the positive control group (Plate 1), standard drug group (Plate 3), 100mg/kg (Plate 4), 200mg/kg (Plate 5) and 400mg (Plate 6). Both liver tissues showed normal architecture with intact hepatocytes, well-defined central veins, and normal renal glomeruli and tubules, indicating healthy organ structure. These observations are consistent with findings by Chatterjee *et al. (*2012).

The Plate (7-12) indicated Histopathology of the liver which showed the gentamicin-only (negative) group (80 mg/kg) showed severe histological alterations. The liver displayed signs of hepatocellular necrosis, sinusoidal dilation, and inflammatory cell infiltration. The kidney tissues showed tubular necrosis, glomerular shrinkage, and interstitial congestion hallmarks of gentamicin-induced nephrotoxicity and hepatotoxicity. These observations are consistent with findings by Abdel-Raheem *et al. (*2010).

In the positive control group, standard drug group, 100mg/kg, 200mg/kg and 400mg both liver and kidney tissues showed normal architecture with intact hepatocytes, well-defined central veins, and normal renal glomeruli and tubules, indicating healthy organ structure. These observations are consistent with findings by Chatterjee *et al. (*2012).

CONCLUSION

The present study demonstrated that *Neptunia oleracea* hydroethanolic extract exhibits significant protective effects against gentamicin-induced kidney and liver damage in albino rats. Gentamicin administration led to marked elevations in biochemical markers of liver and kidney injury, including ALT, AST, bilirubin, creatinine, and urea, alongside decreased levels of albumin and total protein. Additionally, gentamicin caused histopathological damage and increased oxidative stress, confirming its toxic effects. Treatment with *Neptunia oleracea* hydroethanolic extract, especially at a dose of 400mg/kg, effectively protected biochemical and histological parameters levels. The extract also significantly prevented oxidative stress markers and antioxidant enzyme activity, supporting its protective role. The efficacy of *Neptunia oleracea* hydroethanolic extract was comparable to that of the standard drug silymarin, indicating its potential as a natural therapeutic alternative. Therefore, it concluded that *Neptunia oleracea* hydroethanolic extract has dose-dependent hepatoprotective and nephroprotective effects, likely due to its antioxidant and anti-inflammatory properties.

AUTTHORS STATEMENT

Further research involving molecular studies and clinical trials is Need to fully establish its therapeutic value and safety profile.

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