J Herbmed Pharmacol. 2022; 11(2): 226-237.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2022.27

Journal of Herbmed Pharmacology

The methanol leaf extract of *Picralima nitida* mitigated cisplatin-induced toxicities in rats through nuclear factor kappa beta, cardiac troponin, mineralocorticoid receptor, and Nrf2 signaling pathways

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ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 17 August 2021
Accepted: 26 October 2021

Keywords:
Anti-inflammation
Antioxidant
Histopathology
Immunohistochemistry
Phytotherapy

ABSTRACT

Introduction: Cisplatin (CP)-induced toxicity involves oxidative stress and *Picralima nitida* is rich in natural antioxidants hence its methanol leaf extract was used to mitigate the toxic effect of CP.

Methods: Forty rats divided into four groups of 10 rats per group were used as follows: group A (normal saline), group B (CP 10 mg/kg), group C [Methanol Leaf Extract of *Picralima nitida* (MLEPN), 100 mg/kg and CP 10 mg/kg], and group D (MLEPN 200 mg/kg and CP 10 mg/kg). All administrations were done by oral gavage with the volumes of the treatments administered determined by the average weight of the rats in each group except CP, which was given intraperitoneally. Administration of normal saline and MLEPN lasted for seven consecutive days after which a single dose of CP was given on day 8. All animals were sacrificed 72 hours after CP administration. On day 9, blood pressure measurement was taken, and changes in body weight were determined. On day 10, blood samples were taken for serum chemistry, and kidneys, liver, and heart were harvested from the animals for Serum assay, histopathology, and immunohistochemistry, respectively.

Results: The extract improved weight changes caused by CP and reversed the toxic changes produced by CP on serum chemistry, oxidative stress, and histopathology. The extract caused a significant decrease in the levels of nuclear factor kappa beta, cardiac troponin, and mineralocorticoid receptors (MCRs). However, it increased the protein expression of Nrf2 compared to the toxicant group.

Conclusion: The extract exhibited anti-inflammatory, antioxidant, and anti-renin properties.

Implication for health policy/practice/research/medical education:
Cisplatin (CP) toxicities involve oxidative stress and substances rich in anti-oxidants could mitigate this effect. *Picralima nitida* is rich in phenol and other phytochemical substances that could mitigate the effects of oxidative stress, especially the toxic effect of CP. Hence, the administration of this plant alongside CP therapy may go a long way in mitigating the side effects of CP administration.

Please cite this paper as: Adedapo AA, Yusuf AA, Falayi OO, Ogunmiluyi IO, Ogunpolu BS, Omobowale TO, et al. The methanol leaf extract of *Picralima nitida* mitigated cisplatin-induced toxicities in rats through nuclear factor kappa beta, cardiac troponin, mineralocorticoid receptor, and Nrf2 signaling pathways. J Herbmed Pharmacol. 2022;11(2):226-237. doi: 10.34172/jhp.2022.27.

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Introduction

One of the major side effects of chemotherapy with various drugs is nephrotoxicity, and nephrotoxicity is associated with considerable morbidity and mortality. There are several mechanisms responsible for the development of nephrotoxicity, including oxidative stress, direct cytotoxicity to the tubular epithelial cells, DNA adducts, inflammation, and mitochondrial dysfunction (1). Acute kidney injury (AKI) as a clinical syndrome is characterized by a rapid decrease in renal function in conjunction with the accumulation of waste products such as urea in the body. About 7% of all hospital admissions are on account of AKI complication. As a matter of fact, the risk of death due to AKI is increased by 10- to 15-fold resulting in a mortality rate of 50% (2).

Drug-induced AKI is a frequent entity in clinical medicine because kidneys are the major targets for the toxic effects of various chemical agents. Hence, AKI is commonly caused by an injury to kidney tissue resulting in acute tubular necrosis (3). Administration of the chemotherapeutic agent, cisplatin (CP) leads to AKI, and this CP-induced AKI has a complex pathophysiological map, which has been linked to cellular uptake and efflux, oxidative stress, ER stress, inflammation, apoptosis, and even vascular injury. Unfortunately, even though many research efforts have been made, a consistent and stable pharmacological treatment option to reduce AKI in patients receiving CP remains unavailable.

Various molecular pathways and mechanisms have been investigated to unravel the unknown pathological events caused by this type of AKI. However, the key molecular mechanisms involved in this AKI include cellular uptake and accumulation, inflammation, oxidative stress, vascular injury, ER stress, and necrosis and apoptosis (3).

The involvement of oxidative stress in the pathogenesis of CP-induced AKI is very interesting. Oxidative stress results from the disproportionate formation of reactive oxygen species (ROS) and regular antioxidant defense mechanisms. One of the major mechanisms of CP-induced AKI is the generation of ROS. Other features are the accumulation of lipid peroxidation products in kidneys and suppression of the antioxidant systems (4). In the cell, CP is said to be converted into a highly reactive form, thus rapidly reacting with thiol-containing antioxidant molecules such as glutathione leading to the depletion of glutathione hence increased oxidative stress within the cells. It has also been thought that through impaired respiratory chain, CP may also cause mitochondrial dysfunction and increased ROS production (5). Furthermore, CP as a nephrotoxic agent may induce ROS formation via the cytochrome P450 (CYP) system, especially in CYP2E1-null mice, CP-induced ROS accumulation was attenuated, and CYP2E1- null mice were protected against CP-induced AKI (6).

Cisplatin (dichloro-di-amino platinum) is an inorganic platinum-based agent widely used to treat a variety of solid malignant tumors. As a result of its inhibitory action on the growth of *Escherichia coli,* the antibacterial effects of CP were first elucidated; however, the drug was later discovered to have potent anti-neoplastic effects on tumor cells (7). CP, oxaliplatin, and carboplatin are still being used as first-line treatments for patients who have been diagnosed with various types of malignancies, such as lymphomas, sarcomas, as well as breast, testicular, ovarian, cervical, head, and neck cancers (7). Nephrotoxicity and resistance by tumor cells to CP are the limiting factors to CP therapy. However, this nephrotoxicity could be ameliorated by free radical scavenging agents for as much as the toxicity of these classes of drugs is related to ROS (8). The major side effect that limits the dose of CP is nephrotoxicity. In patients with chronic kidney disease (CKD) there is an increased risk of developing AKI when this agent is used in chemotherapy (9). As a matter of fact, it has been shown that AKI is often seen in patients with CKD (10). Therefore, these side effects of CP have remarkably decreased the use of CP despite its outstanding anticancer activity. As already discussed, CP induces oxidative stress; it is also known to suppress the NQO1 gene in mice kidneys (11). Therefore, research focusing on the validation of natural products, as sources of antioxidants, is vital because intake of plant-derived dietary antioxidants such as flavonoids, carotenoids, and phenolic compounds may lead to protection against cardiovascular diseases and diabetes cataracts, and even cancer (12). One of such plants to be evaluated for this purpose is *Picralima nitida*.

Picralima nitida (Stapf.) Th. & H. Durand is a shrub plant widely distributed in tropical Africa, including Nigeria, and it belongs to the Apocynaceae family. It has varied applications as a folk medicine for such ailments as antipyretic, antihypertensive, gastro-intestinal disorders, antimalarial, aphrodisiac, antitrypanocidal, and a remedy against hyperglycemia (13). While the root of *P. nitida* is traditionally used to relieve fever, malaria, and pneumonia, the seeds are used to treat stomach aches (14). On the other hand, the leaf has been reported to have antitussive and antidiabetic properties. Furthermore, many studies have shown that this plant has potential antioxidant, anti-inflammatory, and hypoglycemic activities (15). Research has also shown that the aqueous extract of the seed has hypotensive activity (16). In terms of phytoconstituents, both the aqueous and ethanolic extracts of *P. nitida* leaves have been reported to contain alkaloids, cardiac glycosides, saponins, and terpenes (17). The nephroprotective, cardioprotective, and hepatoprotective effects of the methanol leaf extract of *P. nitida* were evaluated in this study.

Materials and Methods

Plant collection and extract preparation

Fresh leaves of *P. nitida* were sourced from and identified at the School of Forestry, Jericho, Ibadan, Oyo State,

Nigeria, where herbarium specimen was deposited. The leaf extracts of *P. nitida* have been reported to contain alkaloids, cardiac glycosides, saponins, and terpenes. The leaves of the plant were cleaned with distilled water and air-dried in a well-ventilated shady room. The dried leaves were ground to powder using a blender, extracted in cold methanol in a screw-capped flask, and shaken at room temperature. The solvent was filtered, squeezed off, and evaporated under reduced pressure in a rotatory evaporator at 40°C to obtain semi-solid crude extract stored at 4°C. The stored methanol extract of the plant was then used for the studies.

Experimental animals

Throughout this study, 40 male albino rats weighing between 160 and 275 g were obtained and kept at the experimental animal house of the Department of Veterinary Pharmacology and Toxicology, the University of Ibadan. They were housed in rat cages and fed standard livestock pellets produced by Ladokun and Son Livestock Feed, Nigeria Limited. They were also given access to clean water *ad libitum* and left in a clean, well-ventilated polyethylene cage under hygienic conditions to acclimatize for one week before the onset of the experiment. All experimental procedures were in conformity with the University of Ibadan Ethics Committee on Research in Animals and internationally accepted principles for laboratory animal upkeep and use.

Experimental design

Rats were randomly divided into four groups with ten rats in each group, as follows: group A (saline), group B (CP 10 mg/kg), group C (MLEPN 100 mg/kg and CP 10 mg/kg) and group D (MLEPN 200 mg/kg and CP 10 mg/kg). All administrations were done by oral gavage, with the volumes of the treatment administered determined by the average weight of the rats in each group except CP which was given intraperitoneally. Administration of normal saline and MLEPN lasted for seven consecutive days, after which a single dose of CP was given on day eight. All animals were sacrificed 72 hours after CP administration. Blood samples, liver, heart, and kidneys were collected.

Measurement of blood pressure

Indirect blood pressure parameters (systolic, diastolic, and mean blood pressure, heart rate, blood flow, and tail blood volume) were determined without anaesthesia by tail plethysmography using an electrosphyngmanometer (CODA, Kent scientific, USA). The average of at least nine readings, taken in the quiescent state following acclimatization, was taken per animal.

Serum collection

About 5 mL of blood was collected from the retro-orbital venous plexus into sterile plain tubes and left for about 30 minutes to clot. The clotted blood was centrifuged at 4000 rpm for 10 minutes. Finally, serum was decanted into Eppendorf tubes and stored at -4°C till the time of analysis.

Tissue preparation

The rats were sacrificed 72 hours after CP administration. The heart, liver, and kidneys were removed, rinsed in normal saline, and immediately kept on ice to prevent denaturation of biomolecules. Subsequently, tissue samples were homogenized in ice-cold phosphate buffer (0.1M, pH = 7.4) with the Teflon homogenizer. The resulting homogenates were centrifuged at 10 000 g for 15 minutes in a cold centrifuge which operates at 4°C to obtain the post mitochondrial fractions. The supernatants from each of these organs were used as the samples for the biochemical analyses.

Chemicals

O-dianisidine, sodium potassium tartrate, ethanol, sodium azide, 2- dichloro-4-nitrobenzene (CDNB), thiobarbituric acid (TBA), hydrogen peroxide (H_2O_2), hydrochloric acid, sulphuric acid, xylenol orange, sodium hydroxide, potassium iodide, reduced glutathione (GSH), trichloroacetic acid, Ellman's reagent (DTNB), ferrous ammonium sulphate, sorbitol were purchased from Sigma (St. Louis, MO, USA). All other chemicals used were of analytical grade and obtained from British Drug Houses (Poole, Dorset, UK).

Biochemical analyses

The glutathione S-transferase (GST) estimation, activity of xanthine oxidase, renal nitric oxide (NO) measurement, and hydrogen peroxide generation were all determined in the study. In addition, thiobarbituric acid reactive substance (TBARS) was quantified as malondialdehyde (MDA) in the renal post mitochondrial fraction and reduced glutathione content determination. Furthermore, glutathione peroxidase (GPx) activity measurement, protein concentration determination, and superoxide dismutase (SOD) assay were all carried out in the study. Also carried out were serum myeloperoxidase (MPO) activity and liver enzymes such as alanine transaminase (ALT) and aspartate transaminase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH), all in line with standard biochemical assay procedures (18).

Histopathology

Renal tissues were collected in 10% formal saline buffer for proper fixation. These tissues were processed and embedded in paraffin wax. For histopathological examinations, sections of 5–6 µm in thickness were made and stained with haematoxylin and eosin (19).

Immunohistochemistry

The immunolocalization of renal mineralocorticoid receptor (MCR), nuclear factor kappa beta (NF-kB), cardiac troponin I (CTnI), and nuclear factor erythroid

2-related factor 2 (Nrf2) in the kidney (MCR, NF-kB), heart (CTn1, NF-kB), and liver (nrf2, NF-kB) respectively was determined as earlier reported by Oyagbemi et al (18). Fixed tissues (heart and kidney) in 10% buffered formalin were embedded in paraffin and sectioned at a thickness of 5 microns. The immunoreactive positive expressions of MCR, NF-kB, Nrf2, and CTnI anti-rabbit intensive regions were observed with a light microscope (Leica) equipped with a digital camera.

Statistical analysis

All values are expressed as mean ± SD. The test of significance between two groups was estimated by Student's *t* test. One-way ANOVA with Tukey's post-test was also performed using GraphPad Prism version 4.00. The level of statistical significance was considered as $P < 0.05$.

Results

The results of the CP-induced toxicities are as shown in tables 1-7 as well as in Figures 1-9. Table 1 shows that the toxicant group caused 34% weight loss in the animals while the extract at both doses mitigated this effect by reducing the weight loss to 14.3 and 15.5%, respectively. Table 2 shows the effect of the extract on blood pressure measurement in CP-induced toxicities in rats where CP caused a significant decrease in systolic and diastolic values of the animals, but the extract reversed these effects. The dose of 100 mg/kg appeared to be more effective than the 200 mg/kg dose.

The effect of the extract on the serum markers of inflammation and oxidative stress (NO, MPO, and advanced oxidative protein products) in CP-induced toxicities in rats showed that there was no significant change in the level of NO, but for MPO and advanced oxidation protein product (AOPP), the extract caused a significant decrease in their levels compared to the toxicant group (Table 3).

The effect of the extract on the liver enzymes (ALT, AST, ALP, and LDH) in CP-induced toxicities in rats was that

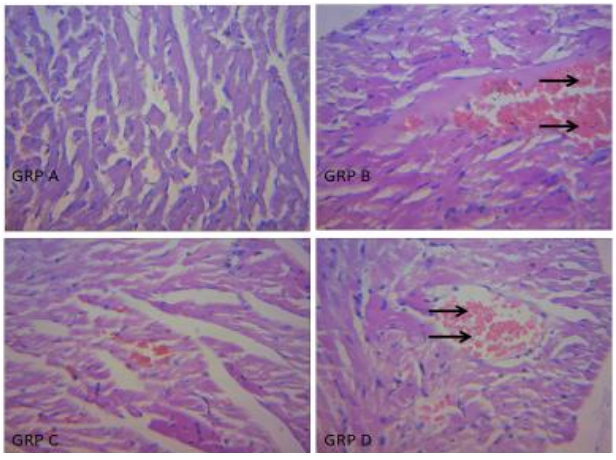

Figure 1. Photomicrographs of heart plates at X400. Group A (Normal rats), group B (Cisplatin group), group C (cisplatin + *Picralima nitida* 100 mg/kg), group D (cisplatin + *Picralima nitida* 200 mg/kg). Group A shows no visible lesion. Group B shows mild congestion of the coronary vessels (arrows). Group C shows no visible lesion. Group D shows moderate congestion of the coronary vessels (arrows).

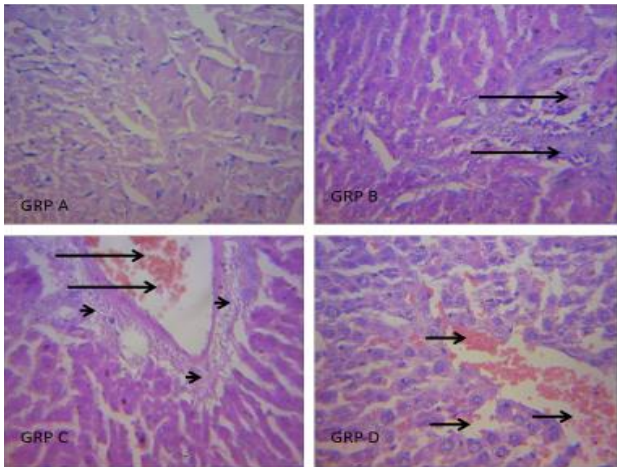

Figure 2. Photomicrograph of liver plates at X400. Group A (Normal rats), group B (Cisplatin group), group C (cisplatin + *Picralima nitida* 100 mg/kg), group D (cisplatin + *Picralima nitida* 200 mg/kg). Group A shows no visible lesion. Group B shows severe portal and central venous congestion, with mild periportal cellular infiltration/fibrosis (arrows). Group C shows mild to moderate portal congestion (arrows), with mild fibroplasia of the portal area (arrowheads). Finally, group D shows moderate portal and sinusoidal congestion (arrows).

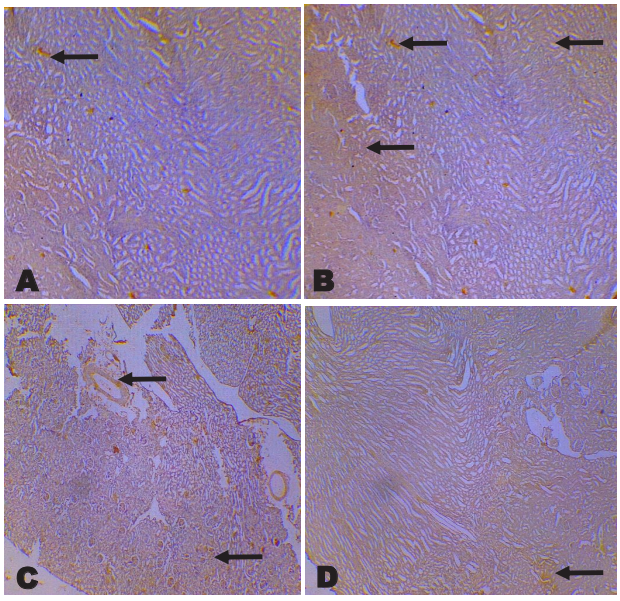

Figure 3. Photomicrograph of kidney plates at X400. Group A (Normal rats), group B (Cisplatin group), group C (cisplatin + *Picralima nitida* 100 mg/kg), Group D (cisplatin + *Picralima nitida* 200 mg/kg). Group A shows no visible lesion. Group B shows moderate portal and central venous congestion (black arrows), with very mild diffuse hydropic degeneration of the nephrons (arrowheads). Group C shows no visible lesions, and Group D shows moderate to severe interstitial congestion (black arrows).

of a significant decrease in the levels of the four enzymes compared to the toxicant group (Table 4).

The effect of the extract on cardiac enzymatic antioxidants (SOD, GPx, and GST) in CP-induced toxicities in rats was that of a significant increase in the levels of SOD and GST but not that of GPx when compared to the toxicant group. The results for renal enzymatic antioxidants are similar to that of cardiac enzymes. However, in the case of hepatic enzymatic antioxidants, only GPx experienced a significant increase relative to the toxicant; the other two experienced a significant decrease (Table 5). The result also showed that the extract at 200 mg/kg dose caused a significant increase in the reduced glutathione in the kidney and liver but no changes in the heart (Table 6). Table 7 shows that the extract caused a significant decrease in hydrogen peroxide and protein carbonyl levels in cardiac, renal, and hepatic tissues.

Figures 1-3 show the effect of the methanol leaf

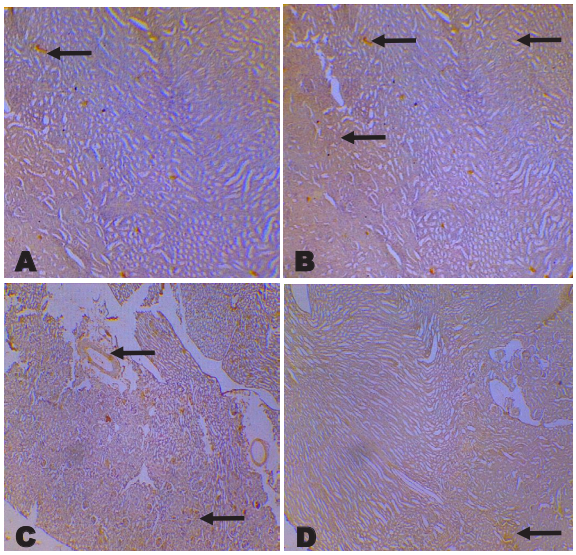

Figure 4. The immunohistochemistry of Mineralocorticoid receptor (MCR). A (Control), B (10 mg/kg cisplatin), C (10 mg/kg cisplatin + *Picralima nitida* 100 mg/kg), and D (10 mg/kg cisplatin + *Picralima nitida* 200 mg/kg). Slides stained with high definition heamtoxylin (magnification at X100).

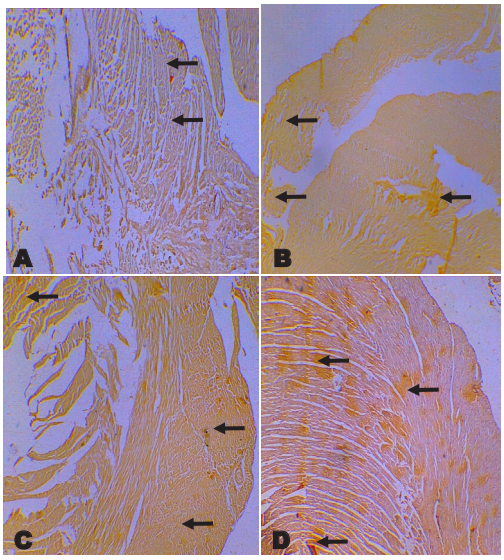

Figure 6. The immunohistochemistry of cardiac troponin. A (Control), B (10 mg/kg cisplatin), C (10 mg/kg cisplatin + *Picralima nitida* 100 mg/kg), and D (10 mg/kg cisplatin + *Picralima nitida* 200 mg/kg). Slides stained with high definition Heamtoxylin (Magnification at X100).

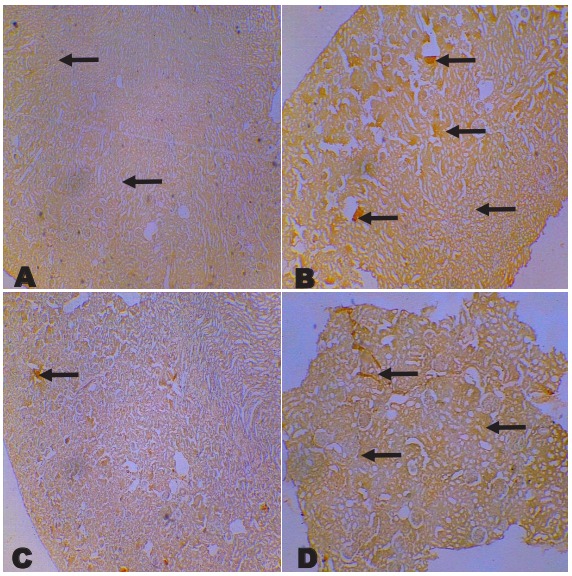

Figure 5. The immunohistochemistry of nuclear factor kappa B (NF-κB). A (Control), B (10 mg/kg cisplatin), C (10 mg/kg cisplatin + *Picralima nitida* 100 mg/kg), and D (10 mg/kg cisplatin + *Picralima nitida* 200 mg/kg). Slides stained with high definition heamtoxylin (Magnification at X100).

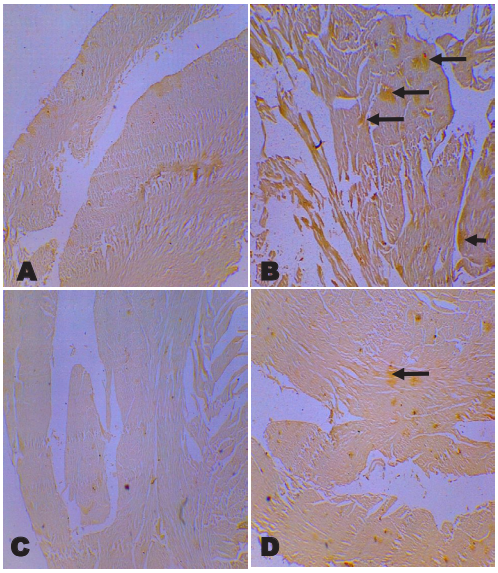

Figure 7. The immunohistochemistry of nuclear factor kappa B (NF-κB). A (Control), B (10 mg/kg cisplatin), C (10 mg/kg cisplatin + *Picralima nitida* 100 mg/kg), and D (10 mg/kg Cisplatin + *Picralima nitida* 200 mg/kg). Slides stained with high definition Heamtoxylin (Magnification at X100.

Table 1. Effect of methanol leaf extract of *Picralima nitida* on body weight changes in cisplatin-induced toxicities in rats (g)

Group	Initial Weight	Final Weight	% Gain/Loss
A	197.5 ± 20.35	224 ± 17.82	26.5
B	220 ± 30.36	186 ± 25.10	-34
C	241.25 ± 24.60	227 ± 20.80	-14.25
D	237.5 ± 39.46	222 ± 35.99	-15.5

Values are presented as mean ± SD, n=6. Group A (Control given distilled water only), group B (Toxicant group given 10 mg/kg of cisplatin), Group C (Cisplatin + 100 mg/kg of *Picralima nitida* leaf extract), group D (Cisplatin + 200 mg/kg of *Picralima nitida* leaf extract).

extract of *P. nitida* in mitigating the various pathological changes in the heart, liver, and kidney. Figures 4 to 9, on the other hand, show the effects of the extract on immunohistochemical protein expression in the kidney, liver, and heart. These proteins are NF-kB, Nrf2, CTnI, and MCR.

Discussion

The results from this study showed that the plant extracts improved weight changes caused by CP and reversed the toxic changes produced by CP on serum chemistry, oxidative stress, and histopathology. In immunohistochemistry, the extract caused a significant decrease in the levels of nuclear factor kappa beta, cardiac troponin, and MCR, but an increase in the protein expression of Nrf2 when compared to the toxicant group.

Changes in body weight are some of the basic parameters to evaluate the experimental animals' physiological or pathological condition. From this study, we observed a significant decrease in the bodyweight of CP alone (toxicant group) compared with the control. Also, there was a significant increase in body weight of the treated groups when compared with the CP alone group. This is in accordance with Pani et al results (20), which studied the protective effect of *Bauhinia variegata* Linn against CP-induced nephrotoxicity in plants. The significant decrease in body weight in the toxicant group when compared with the control may be associated with alteration in lipid metabolism with resultant fat and muscle loss (lipodystrophy commonly associated with cachexia) (21). The significant increase in body weight of the treated group when compared with the toxicant group

Table 2. Effect of methanol leaf extract of *Picralima nitida* on blood pressure in cisplatin-induced toxicities in rats

Parameter	GroupA	Group B	Group C	Group D
Systolic BP	215 ± 10.5	125.9 ± 2.6[a]	179.8 ± 24.4[a,b]	114.2 ± 6.8[a]
Diastolic BP	181.5 ± 17.3	91.9 ± 8.6[a]	155.8 ±40.4[b]	96.0 ± 16.3[a]
Mean arterial BP	196.0 ± 13.5	107.1 ± 3.7[a]	168.0 ± 29.7[a,b]	125.89 ± 2.62[a]

Alphabet (a) indicates a significant difference when groups B, C, and D were compared with group A. Alphabet (b) indicates a significant difference when groups C and D were compared with group B. Values presented as mean ± SD, n=6. Group A (Control given saline), group B (Toxicant group given 10 mg/kg of cisplatin), group C (cisplatin + 100 mg/kg of *Picralima nitida* leaf extract), group D (cisplatin + 200 mg/kg of *Picralima nitida* leaf extract). BP: Blood pressure.

Table 3. Effect of methanol leaf extract of *Picralima nitida* on the serum markers of inflammation and oxidative stress

Parameter	GroupA	Group B	Group C	Group D
NO	0.44 ± 0.01	0.46 ± 0.04	0.45 ± 0.02	0.42 ± 0.04
MPO	23.1 ± 11.2	28.0 ± 3.9	9.3 ± 2.1[a,b]	8.9 ± 5.0[a,b]
AOPP	119.8 ± 15.7	181.2 ± 39.0 [a]	144.7 ± 50.1	129.7 ± 16.3

Alphabet (a) indicates a major difference when groups B, C, and D were compared with group A. Alphabet (b) indicates a significant difference when groups C and D were compared with group B. Values presented as mean ± SD, n=6. Group A (Control given saline), group B (Toxicant group given 10 mg/kg of cisplatin), group C (cisplatin + 100 mg/kg of *Picralima nitida* leaf extract), group D (cisplatin + 200 mg/kg of *Picralima nitida* leaf extract). MPO: Myeloperoxidase, AOPP: Advanced oxidation protein product, NO: Nitric oxide.

Table 4. Effect of methanol leaf extract of *Picralima nitida* on the liver function tests in cisplatin-induced toxicities in rats

Parameter	Group A	Group B	Group C	Group D
ALT (U/L)	7.1 ± 0.3	4.7 ± 1.3[a]	4.2 ± 2.2 [a]	3.7 ± 0.8 [a]
AST (U/L)	16.3 ± 1.5	13.9 ± 2.9	3.4 ± 0.7 [a]	8.4 ± 1.4 [b]
ALP (U/L)	4.1 ± 2.0	18.4 ± 12.8 [a]	12.9 ± 1.6 [b]	12.4 ± 5.9 [b]
LDH (U/L)	89.1 ± 22.9	145.7 ± 18. 0 [a]	86.4 ± 38.3[b]	74.9 ± 18.0 [b]

Alphabet (a) indicates a significant difference when groups B, C, and D were compared with group A. Alphabet (b) indicates a significant difference when groups C and D were compared with group B. Values present as mean ± SD, n=6. Group A (Control given saline), group B (Toxicant group given 10 mg/kg of cisplatin), group C (cisplatin + 100 mg/kg of *Picralima nitida* leaf extract), group D (cisplatin + 200 mg/kg of *Picralima nitida* leaf extract). Note: ALT: Alanine transaminase; AST: Aspartate transferase; ALP: Alkaline phosphatase and LDH: Lactate dehydrogenase.

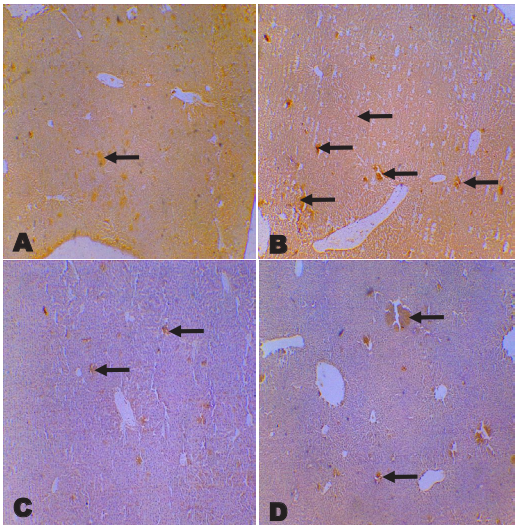

Figure 8. The immunohistochemistry of hepatic Nuclear factor kappa B (NF-κB). A (Control), B (10 mg/kg cisplatin), C (10 mg/kg cisplatin + *Picralima nitida* 100 mg/kg), and D (10 mg/kg cisplatin + *Picralima nitida* 200 mg/kg). Slides stained with high definition Heamtoxylin (Magnification at X100).

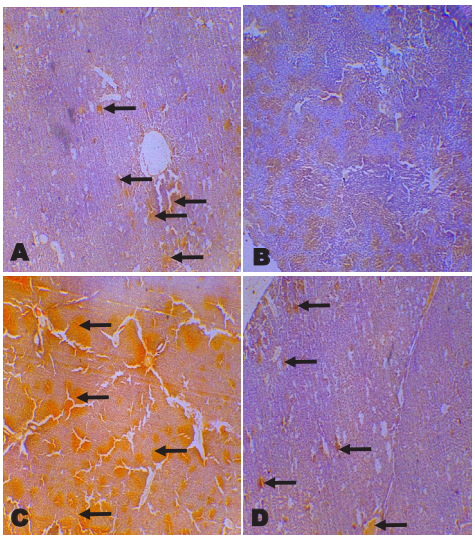

Figure 9. The immunohistochemistry of hepatic nuclear factor erythroid 2-related factor 2 (Nrf2). A (Control), B (10 mg/kg cisplatin), C (10 mg/kg cisplatin + *Picralima nitida* 100 mg/kg), and D (10 mg/kg cisplatin + *Picralima nitida* 200 mg/kg). Slides stained with high definition Heamtoxylin (Magnification at X100).

may be due to its high nutritional values and antimicrobial properties characterized by the presence of antioxidants such as alkaloids, saponins, and tannins (16).

The major side effects of CP therapy are nephrotoxicity, hypotension, and vascular injury, including vasoconstriction leading to reduction of renal blood flow and GFR (22). From this study, a significant decrease in systolic, diastolic, and mean arterial blood pressure of the toxicant group (CP alone group) was observed when compared with the control, thus a decrease in blood pressure. Also, a significant increase in systolic, diastolic, and arterial blood pressure was observed following treatment with *P. nitida* leaves extract. The result of this study is similar to that of Komaki et al results (22), which demonstrated that CP could lower blood pressure in experimental animal and this was as a result of increased sodium excretion in urine and polyuria following CP administration, which induced further reduction of blood volume. The subsequent decrease in renal blood flow was due to the damaging effect of CP accumulation on renal tubular epithelial cells and vascular injury. Saleh et al (23) reported that the increased blood pressure following treatment with losartan (angiotensin receptor blocker) was due to the activity of losartan on CP-induced

Table 5. Effect of methanol leaf extract of *Picralima nitida* on enzymatic antioxidants in cisplatin-induced toxicities in rats

Parameter	Group A	Group B	Group C	Group D
Cardiac enzymatic antioxidants				
SOD	7.3 ± 4.2	1.3 ± 0.2[a]	6.8 ± 3.4[b]	5.5 ± 2.3[b]
GST	8.0 ± 2.4	3.6 ± 1.3[a]	6.4 ± 2.6	7.0 ± 1.3[b]
GPx	161.2 ± 17.1	143.8 ± 3.6	159.2 ± 15.8	162.8 ± 15.0
Renal enzymatic antioxidants				
SOD	15.0 ± 2.0	14.3 ± 1.9[a]	15.0 ± 3.1[a]	14.7 ± 2.4[a,b]
GST	17.5 ± 2.0	12.9 ± 1.0[a]	14.4 ± 1.0	16.9 ± 3.2[b]
GPx	77.1 ± 6.1	64.7 ± 10.0	70.7 ± 7.1	73.8 ± 6.5
Hepatic enzymatic antioxidants				
SOD	3.0 ± 0.8	1.1 ± 0.5[a]	0.8 ± 2.0[a]	0.5 ± 0.4[a]
GST	40.5 ± 5.6	25.4 ± 5.3[a]	18.7 ± 1.7[b]	15.9 ± 3.7[a,b]
GPx	66.8 ± 3.9	36.9 ± 5.3[a]	42.1 ± 1.7[a]	43.3 ± 4.8[a]

Abbreviations: SOD, Superoxide dismutase; GST, Glutathione S-transferase; GPx, Glutathione peroxidase.
Alphabet (a) indicates a significant difference when groups B, C, and D were compared with group A. Alphabet (b) indicates a substantial difference when groups C and D were compared with group B. Values presented as mean ± SD, n=6. Group A (Control given saline), Group B (Toxicant group given 10 mg/kg of cisplatin), Group C (Cisplatin + 100 mg/kg of *Picralima nitida* leaf extract), Group D (cisplatin + 200 mg/kg of *Picralima nitida* leaf extract).

Table 6. Effect of methanol leaf extract of *Picralima nitida* on reduced glutathione (GSH) content in cisplatin-induced toxicities in rats

Parameter	Group A	Group B	Group C	Group D
Cardiac GSH	91.5 ± 2.7	87.6 ± 3.4	88.6 ± 7.2	90.7 ± 3.4
Renal GSH	100.0 ± 10.0	80.4 ± 5.8[a]	87.7 ± 7.6	93.4 ± 3.2[b]
Hepatic GSH	129.7 ± 9.5	113.6 ± 3.7[a]	121.2 ± 8.9	135.7 ± 13.6[b]

Alphabet (a) indicates a significant difference when groups B, C, and D were compared with group A. Alphabet (b) indicates a significant difference when groups C and D were compared with group B. Values presented as mean ± S.D., n=6. Group A (Control given saline), group B (Toxicant group given 10 mg/kg of cisplatin), group C (cisplatin + 100 mg/kg of *Picralima nitida* leaf extract), Group D (cisplatin + 200 mg/kg of *Picralima nitida* leaf extract).

Table 7. Effect of methanol leaf extract of *Picralima nitida* on cardiac markers of oxidative stress (hydrogen peroxide and protein carbonyl) in cisplatin-induced toxicities in rats

Parameters	GroupA	Group B	Group C	Group D
Renal markers of oxidative stress				
PCO	9.5 ± 8.4	26.9 ± 14.7[a]	9.9 ± 0.4[b]	9.2 ± 0.9[b]
H_2O_2	41.9 ± 3.7	55.6 ± 4.1[a]	44.3 ± 6.7[b]	45.7 ± 4.2[b]
Cardiac markers of oxidative stress				
PCO	9.2 ± 1.2	20.3 ± 8.3	12.7 ± 3.9	10.8 ± 2.3
H_2O_2	35.9 ± 4.8	52.5 ± 11.0[a]	34.7 ± 11.3[b]	37.2 ± 3.6[b]
Hepatic markers of oxidative stress				
PCO	6.4 ± 2.0	17.2 ± 2.8[a]	9.8 ± 0.4[b]	6.9 ± 5.0[b]
H_2O_2	38.8 ± 9.8	77.8 ± 14.8[a]	70.8 ± 13.1[a]	81.1 ± 16.4[a]

Abbreviation: PCO, Protein carbonyl.
Alphabet (a) indicates a significant difference when groups B, C, and D were compared with group A. Alphabet (b) indicates a significant difference when groups C and D were compared with group B. Values presented as mean ± S.D., n=6. Group A (Control given saline), group B (Toxicant group given 10 mg/kg of cisplatin), group C (cisplatin + 100 mg/kg of *Picralima nitida* leaf extract), group D (cisplatin + 200 mg/kg of *Picralima nitida* leaf extract).

lipid peroxidation and glutathione depletion. *P. nitida* leaf extract used in this study may have affected blood pressure changes due to its antioxidative effect in CP-induced nephrotoxicity.

A biomarker is used as an indicator of pharmacological responses to therapeutic intervention and has applications in diagnosis and prognosis in various systemic diseases (24). In the case of oxidative stress and inflammation, these biomarkers or molecules are modified by interactions with ROS in the microenvironment (25). CP-induced nephrotoxicity mediated by oxidative stress is indicated by increased oxidative stress markers, especially the ROS (26). A decrease in serum NO level of the treated groups when compared with the toxicant group was observed in our study. Though not significant, it is in accordance with the works of Razo-Rodríguez et al (27), which showed that garlic powder ameliorated CP-induced nephrotoxicity and oxidative stress. The increased NO level in the toxicant group may be due to CP accumulation in the renal tubular cells that can bind to reduced glutathione, decreasing the endogenous antioxidant system (28) and resulting in renal damage due to inflammation of the nephron (nephritis). The decreased level of NO in the treated group may be due to the antioxidant (free radical scavenging activity) of *P. nitida*. MPO is a good marker of oxidative stress, inflammation, and cardiac damage. It is involved in damaging cell components by adhering and causes degradation of the basement membrane at the site of attachment (29).

In this study, CP caused a significant increase ($P<0.05$) in the MPO level. It is observed that treatment with *P. nitida* obviously contributed to reducing CP-induced renal oxidative stress due to its enzymatic and non-enzymatic antioxidants activities. This result agrees with the findings of Salih and Al-Baggou (30), which showed that MPO increased significantly in CP only group and significantly decreased in groups treated with different doses of Memantine hydrochloride. AOPP is another marker of oxidative stress, inflammation, and renal damage (31). In this study, CP caused a significant increase ($P<0.05$) in the AOPP level compared to the control group but a non-significant decrease in the AOPP level in the treated groups. This result is similar to Adeoye et al (32), which studied the protective effect of the methanol leaf extract of *Andrographis paniculata* on CP-induced AKI in rats. This study proved that an increased level of AOPP in the toxicant group is associated with oxidative stress that is known to generate ROS and free radicals leading to decreased antioxidant defence system, which is made up of antioxidant enzymes and non-enzymatic glutathione.

Protein carbonyl (PCO), hydrogen peroxide (H_2O_2), and MDA are the other markers of oxidative stress evaluated in this study. CP has been found to induce tissue damage via the generation of free radicals and a subsequent increase in the levels of PCO, H_2O_2, and MDA following its administration (33). In tissues (cardiac, hepatic, and renal) of CP administered rats, the level of hydrogen peroxide and protein carbonyl increased

significantly ($P<0.05$) compared with the normal control group. In this study, a significant decrease ($P<0.05$) in the level of PCO and H_2O_2 was observed in the cardiac and renal tissues of *P. nitida* extract-treated groups. Still, only a significant decrease in the level of PCO was observed in hepatic tissues of the treated group when compared with the toxicant groups. The significant decrease in the levels of these markers may be due to the presence of flavonoids, phenols, and others that have reducing effects on the PCO and H_2O_2 activities in *P. nitida*. This study is in line with the work of Afsar et al (34), indicating that phytochemicals in *Acacia hydaspica* inhibited lipid peroxidation and enhanced endogenous antioxidants via its antioxidative and anti-inflammatory potentials. Shahid et al (35) also observed that polyphenolic in the *Acacia catechu* fraction might be responsible for the protective effect via diminishing oxidative stress and increasing the antioxidant enzyme status.

Cisplatin-induced nephrotoxicity has been studied more than CP-induced hepatotoxicity (36). Nevertheless, CP-induced toxicities have been attributed to the ability of CP to bring about ROS formation hence oxidative stress, one of the primary pathogenic mechanisms by which CP induced damage to kidney and liver (37). The liver enzymes ALT, AST, ALP, and LDH are markers usually employed to determine the level of damage caused to the liver cells in any hepatotoxicity study. An increase in the level of these liver enzymes in most cases indicates damage to the hepatocytes (37). In this study, the ALT and AST in the CP-alone group experienced a significant decrease when compared to the control group. A further decrease in the level of ALT and AST was also observed in the treated group when compared with the toxicant (CP only group) except for the 200 mg/kg treated group in AST assay where a slight increase in its level was observed. The decreased levels of ALT and AST in the CP-only group implied little or no damage caused by the toxicant to the liver. The decrease in the membrane enzyme activities could be due to the oxidative modification and consequent inactivation of enzymes by CP-generated free radicals and ROS. This study is in contrast to the work done earlier by Farooqui et al (38). They found that single administration of CP caused a significant increase in serum ALT and AST, but administration of *Nigella sativa* oil to CP-induced toxicity led to decrease levels of ALT and AST. The decreased levels of ALT and AST in the treated group may be due to the antioxidant activity of *P. nitida*. Also, in this study, there was a significant increase ($P<0.05$) in the level of ALP and LDH in the toxicant group when compared with the control group and a significant decrease ($P<0.05$) in ALP and LDH level in the treated groups in a dose-dependent manner when compared to the toxicant group. This work was in agreement to results of Farooqui et al (38), in which the extract of *N. sativa* blunted CP toxicities on membrane enzymes, hence damaging rat liver. The anti-inflammatory and antioxidant effects of the plant extract might be responsible for the significant decrease in ALP and LDH levels (39).

Antioxidants serve as a body defense mechanism reducing the adverse effect of toxicants in the body as they counter free radicals in any biological system. The antioxidant body defense mechanisms might be negatively impacted by CP by reducing the intracellular concentration of GST, SOD, and GPx in tissues (40). The toxic changes of CP were observed on the cardiac, renal, and hepatic antioxidants in our work. Reduction in the activity of SOD points to tissue pathology, especially SOD, which is a major factor when it comes to the enzymatic antioxidant defense system in that it removes the anion superoxide by converting it to hydrogen peroxide, thus mediating its toxic effects (41). During toxicity, GPx activity reduces due to increased formation of anions of superoxide by removing hydrogen peroxides from the system. CP caused a considerable decrease in the levels of SOD and GST in the cardiac and renal tissues. There was a significant increase ($P<0.05$) in the levels of SOD and GST in the treated group relative to the toxicant group. CP was also observed to cause a significant decrease ($P<0.05$) in the levels of SOD, GST, and GPx in hepatic tissues. The decrease level of enzymatic antioxidants observed in CP toxicity was in line with Ilyad et al (42), that studied the antioxidant and hepatoprotective effect of alcoholic extract of ginger against the CP-induced oxidative stress in rats due in large part to lipid peroxidation and disintegration of the cell membranes of the hepatic, renal and cardiac tissues with resultant leakages of the enzymes. Also, the increased level of SOD, GPx, and GST observed in some treated groups may be associated with the membrane cell repair effect and antioxidants properties of the phytochemical constituents such as flavonoids and phenols.

Reduced glutathione (GSH), a non-enzymatic component of the antioxidant body defense system, maintains cell integrity due to its reducing properties. It also can detoxify certain endogenous toxins, including CP, which causes inhibition of protein synthesis (43). CP chemotherapy could cause depletion in the tissue GSH level of rats in response to oxidative stress (44). In this study, there was a significant decrease in GSH activity as observed in the liver and kidney of the toxicant groups when compared with the control group. Also, there was a dose-dependent increase in renal and hepatic GSH levels in the treated groups when compared to the toxicant group and of the significant increase in the groups treated with a higher dosage of 200 mg/kg of *P. nitida* leaf extract. This was in accordance with Shahid et al results (35) that showed *Nigella sativa* oil could blunt the effect of CP on the antioxidant system in the rat intestine, which might be due to the inhibition of functional-SH groups of the GSH enzyme by CP or CP generated ROS (45). The increase in GSH activity of the treated groups in this study may be due to the ability of the administered extract to prevent

CP-induced suppression of the antioxidant body defense system.

The MCR is activated by aldosterone, which is produced by the adrenal glands. It plays an important role in blood pressure regulation and electrolyte and fluid homeostasis. Stimulation of aldosterone secretion could be seen in renin-angiotensin-aldosterone system (RAAS) activation in response to decreased vascular volume. Besides its role in kidney function, MCR could also be expressed through enhanced oxidative stress and inflammation (46). Its enhanced expression in the toxicant group may be due to CP-induced oxidative stress. The action of the extract in reducing its expression is an indication of the antioxidant property of this plant, as earlier pointed out, which is similar to the work of Tata et al (47), in which increased expression of MCR was observed in the toxicant group. This observation was similar to previous reports that showed that L-NAME treatment activated the renal renin-angiotensin system (48). It thus means that high blood pressure elevation observed in CP-induced toxicities is attributable to RAAS activation. CP could thus contribute to cardiorenal syndrome. The extract of *Picralima nitida* was able to reverse this.

Nuclear factor (erythroid-derived 2)-like factor 2 (Nrf2) is a transcription factor that regulates the expression of many detoxification enzymes. Hence, this signalling pathway could be a target for cancer chemoprevention (49). Nrf2 activation in this study is an indication of the anticancer potential of this plant extract, while the downregulation of its expression by CP further confirms its toxicity in tissues.

The biomarker of choice for detecting cardiac injury is troponin; hence, its significant expression in the toxicant group indicates its damage to the cardiac tissue (50). This effect was; however, reversed in the groups treated with the extract. CP is known to cause bradycardia due to cardiac injury, but administration of the extract prevents cardiac damage; hence restoration of normal blood pressure is seen in this study.

One of the steps in regulating inflammatory and immune responses is the activation of the NF-κB family of transcription factors (51). The upregulation of this protein by the toxicant in all the organs used in this study indicates that this toxicant brought about inflammatory changes. The ability of the extract to suppress its expression also indicates the anti-inflammatory ability of the plant extract.

Conclusion

The administration of this plant extract alongside CP in cancer chemotherapy may go a long way in reducing the side effects of this drug because of the anti-oxidant property of this plant, which will help to mitigate the reactive oxidative stress generated by the drug. Also, the study showed that CP has a toxic effect on the liver and the heart, but the administration of the plant extract mitigated these effects.

Authors' contributions

AAA, OOO, MAY conceived and designed the study, AAY, OOF, IOO, OAA, BSO carried out the experiments, supervised by AAO, TOO, and FBY. AAA wrote the manuscript that was read and corrected by all authors.

Conflict of interests

The authors have no conflict of interest to declare

Ethical considerations

All experimental procedures were in conformity with the University of Ibadan Ethics Committee on Research in Animals and internationally accepted principles for Laboratory animal upkeep and use. The institutional Ethical Committee, i.e., Animal Committee for Use in Research (ACUREC), approved the study, and the number is UI-ACUREC/17/0064.

Funding/Support

No funding was received for the study.

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