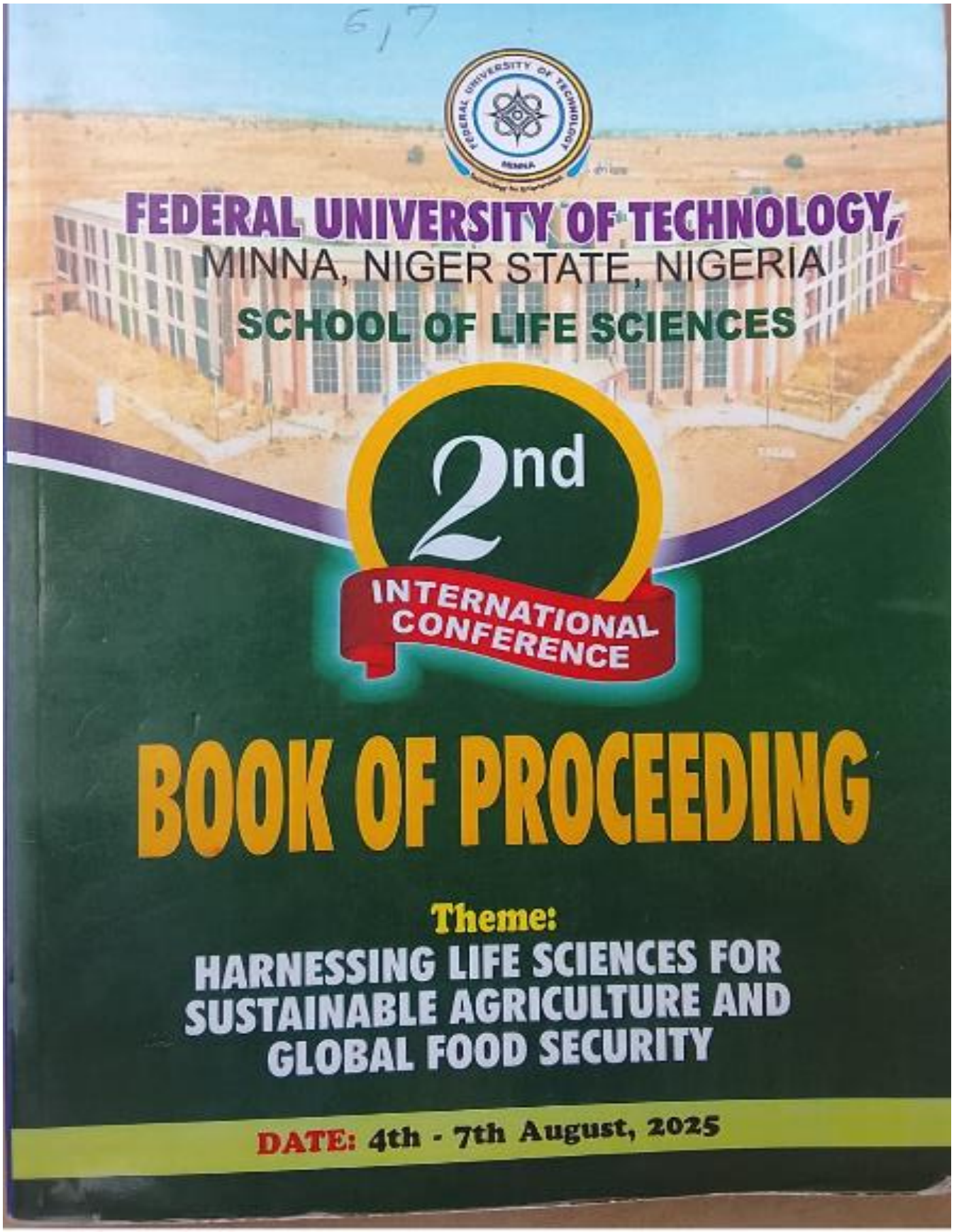
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Nephroprotective Effect of Phenol-Rich Extract of *Tridax Procumbens* Plant on Cisplatin-Induced Renal Damage in Rats

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ABSTRACT

Cisplatin (cis-diamminedichloroplatinum II) is one of the most effective cancer chemotherapies, but its usage is limited due to the severity of adverse effects especially its dose-related nephrotoxicity. *Tridax procumbens is* rich in phenols with immense medicinal properties. Thirty Wister rats were divided into six groups of five rats each. Group I (normal group) was not administered Cisplatin, while rats in groups II-VI were intraperitoneally administered 5mg/kg bodyweight of Cisplatin to induce renal damage. Group II (negative control) received no treatment with the plant extracts. The positive control group (III) was orally administered 100 mg/kg bodyweight of silymarin. Rats in groups IV-VI were orally administered 100, 200 and 400 mg/kg bodyweight tannin-rich extract of *T. procumbens* plant respectively once daily for fourteen days. The rats were euthanized on the 15th day, blood and tissue samples were collected for biochemical and histologic investigations. Significant decreases ($p<0.05$) in serum urea, uric acid, creatinine, Na^+, Cl^- and $H_2CO_3^{2-}$, and an elevation in serum K^+ were observed in all phenol-rich extract treated groups compared with the negative control group. The activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidise (GSH-Px) in kidney tissues were dose-dependent (400 mg/kg bodyweight has highest activity), while that of MDA decreased in all treated groups compared to the negative control group. Nephrocytes repair was dose dependent with 400 mg/kg bodyweight phenol-rich extract being able to restore normal kidney architecture. The haemorrhage and necrosis that resulted from nephrotoxicity induced by Cisplatin administration were ameliorated by phenol-rich extract of *T. procumbens*.

Keywords: Cisplatin, nephrotoxicity, tannin-rich, *Tridax procumbens*, cancer

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INTRODUCTION

The kidney is the primary eliminator of xenobiotics and their metabolites. This important organ has a high metabolic activity and transporter capacity facilitating the elimination of toxins via the urine (Pardeshi and Bhiungade, 2016). Due to the large volume of blood supply (20-25% of the cardiac output), high level of toxicant is delivered over a period of time thereby making this organ vulnerable to toxicity and various forms of injury (Pardeshi and Bhiungade, 2016). The epithelial cells of the renal proximal convoluted tubules (PCT) are a crucial target for nephrotoxicants due to their functions in glomerular concentrations, drug delivery, and metabolism (Iqbal *et al.*, 2022). Nephrotoxic agents damage the renal tubular epithelial cells due to their

interaction with membrane components and cellular macromolecules (Iqbal et al, 2022).

About 60% of cases of acute kidney injury (AKI) in hospitalized patients are believed to result from drug induced nephrotoxicity. The prevalence of kidney failure is 10-15% in men and 10% in women worldwide. In sub Saharan Africa, the prevalence is 13.9% and 16% in West Africa (Okonkwo et al, 2020). In nephrotoxicity the glomerular filtration rate is reduced while creatinine and blood urea nitrogen in the serum is increased, ultimately increasing the blood pressure and fluid retention in the body (over-hydration) (Yadav et al, 2017; Saylor et al, 2021). Humans are frequently exposed to a wide variety of potential nephrotoxins, many of which are either prescribed or available to the general population as over the-counter medications. Antimicrobial agents, chemotherapeutic agents, analgesics, and immunosuppressive agents are important therapeutic agents with known nephrotoxic potential (Shi et al, 2018).

Cisplatin (Cis) is the most commonly used chemotherapeutic agent against different solid tumors, however Cis induces several side effects including neurotoxicity, gastrotoxicity, myelosuppression, and allergic reactions (Dasseneux et al, 2020). The main toxic effect is the dose-related nephrotoxicity that is responsible for mortality and morbidity (Yang et al, 2020). Previous studies have indicated the nephrotoxicity of Cis at a single dose (50–100 mg/m²) (Pinzi et al, 2020). The Cis disturbs the equilibrium between antioxidants and peroxides, while renal fibrosis is closely related to a rise in oxidative damage (Iqbal et al, 2022). Cisplatin acts as a DNA crosslinking agent and interferes with mitosis and breakdown of DNA damage repair tissue triggering apoptosis (Nian et al, 2014).

For centuries, herbal products have been used for medicinal purposes and many plants are readily available, providing a rich source of bioactive chemicals with minimal side effects thereby possessing powerful pharmacological actions. The extracts of *Tridax procumbens* have shown significant medicinal properties and pharmacological activities. It is estimated that about 80% of the world population relies on botanical preparations as medicines to meet their health needs. This may be attributable to the downturn in the economy, as herbal medicine is perceived to be a cheaper means of treatment (Opara et al, 2018).

Tridax procumbens Linn. (Family: Asteraceae) is a common plant which belongs to a species of flowering plant in the daisy family. It is commonly known as coat buttons or tridax daisy and best known as a widespread weed and pest plant. It is native to the tropical Americas but has been introduced to tropical, subtropical, and mild temperate regions worldwide. It grows primarily during rainy season in tropical areas (Paredes-Escamilla, 2016).

Significant medicinal properties have been attributed to the extract of this plant. The plant has also demonstrated various pharmacological activities including immuno-modulatory, anti-hepatotoxic, anti-diabetic, antioxidant, anti-inflammatory, analgesic, and nitrous depressant action on respiration (Opara et al, 2018).

Most organ function impairment is a direct consequence of changes in the histological structures as well as biochemical

parameters of the organ. *T. procumbens* has been reported to have a protective effect against toxicity of the liver (Sampathkumar *et al*, 2018); however, there is limited information on its effect on toxicity of the kidney. This research was designed to investigate the effects of phenol-rich extracts of *T. procumbens* on cisplatin-induced nephrotoxicity in Wister rats.

MATERIALS AND METHODS

Plant collection and identification

Tridax procumbens plant was collected from the Bosso Area (latitude 9.6522 and longitude 6.5261) Minna, Niger State, Nigeria. The plant was authenticated at the herbarium section of the Plant Biology Department, Federal University of Technology Minna and voucher number (FUT/PLB/AST/033) was allocated.

Experimental animals

Wister rats of either sex (weighing between 150-200 g) were purchased from the Animal house of the Department of Biochemistry Federal University of Technology, Minna. The rats were housed in plastic cages and allowed to acclimatize for two weeks to standard laboratory conditions in the Departmental Animal house (12:12 light dark cycle, room temperature 24±2°C, relative humidity 33-70 %). They had access to feed and water *ad libitum* according to the guidelines for animal care (National Research Council, 2010).

Preparation of plant samples

Tridax procumbens plant was washed under running tap water, rinsed with distilled water and air dried under shade at room temperature for one week. The dried leaves were milled into coarse powder with an electric blender (Silver Crest blender model SC 2130 made in Germany) and stored in airtight containers.

Extraction of phenol

To obtain phenol-rich extract of *T. procumbens* plant, 420ml of methanol/acetone (60:40) was added to 50g of *T. procumbens* plant powder and was subjected to Soxhlet extraction for 3 hours to obtain phenol-rich mixture. The mixture was filtered using Whatman (Number-1) filter paper, concentrated under reduced pressure in a rotary vacuum evaporator and dried to obtain the dried with an oven to obtain the phenol-rich extract (Sethi and Sujeeta, 2017).

Quantitative phytochemical screening

Determination of total phenol

The method described by Singleton *et al.* (1999) was used to determine the total phenol content of the extracts. Exactly 0.01 g of the extract was dissolved in 10 mL of distilled water and 0.5 mL was pipetted by 2.5 mL of 10 % Folin Ciocalteu's reagent which was then neutralized by 2 mL of 7.5 % sodium carbonate. The reaction mixture was incubated at 45 °C for 40 minutes. Absorbance was read at 765 nm using double beam Shimadzu UV spectrophotometer, UV-1800. Standard gallic acid was used to prepare the calibration curve.

Determination of the Antioxidant Activity of Phenolic-rich Extracts

Determination of 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of DPPH radicals was monitored using the method of Marrocco et al (1998). Different concentrations of the extracts and ascorbic acid (125, 250, 500 and 1000 µg/ml) were prepared by weighing and dissolving 0.01 g of the extract and ascorbic acid respectively in 10 ml of methanol. Then, 2 ml of 0.004 % DPPH in methanol was added to 2 mL of the various concentrations of extracts and ascorbic acid. The reaction mixture was incubated at 25 °C for 30 minutes, the absorbance was measured at 517 nm using spectrophotometer (double beam Shimadzu UV-1800 series, made in India). The percentage of the DPPH radical scavenging was then calculated using the following equation:

$$\% \text{ inhibition of DPPH radical} = \frac{Abc - Aat}{Abc} \times 100$$

Where Abc is the absorbance before reaction and Aat is the absorbance after reaction has taken place.

Determination of inhibition of lipid peroxidation

The method described by Khalig et al (2015) was used to study the anti-lipid peroxidative properties of the extracts. To prepare the stock solutions (1000 µg/mL) of the extracts as well as the ascorbic acid respectively, 0.01 g was weighed and dissolved in 10 mL of methanol. Various concentrations (125 µg/mL, 250 µg/mL, 500 µg/mL and 1000 µg/mL) of the extracts and ascorbic acid were then prepared from the stock solutions. One gram of egg yolk was weighed and diluted to 100 mL with 100 mM Tris-HCl pH7.4 and used as homogenate. The homogenate was incubated with Fe (II) and the extract was added, color reaction was carried out by adding 500 µL of Thiobarbituric acid (TBA) and 500 µL of acetic acid (pH 3.4) for 1 h. The tubes were then cooled and 2 ml of n-butanol was finally added and centrifuged at 1600 x g for 10 minutes. The absorbance was read with a spectrophotometer at 532 nm.

Ferric reducing antioxidant power (FRAP) assay

This assay was carried out using the method developed by Benzie and Strain (1996) which measures the ability of antioxidants to reduce ferric ion. It is based on the reduction of the complex of ferric ion and 2,4,6-tripyridyl-S-triazine-2-sodium-acetate-1,4-dienyl chloride (TPTZ) to the ferrous form at low pH. This reduction was monitored by using a diode-array spectrophotometer to measure the change in absorption at 595 nm. Three milliliters of FRAP reagent was mixed with 100 ml of diluted sample, the absorbance at 595 nm was recorded after a 30 minute incubation at 37 °C. FRAP values were obtained by comparing the absorption change in the test mixture with those obtained from increasing concentrations of Fe^{2+} and expressed as mM of Fe^{2+} equivalents per L of sample.

Acute Oral Toxicity Studies

The acute toxicity study of 70% *Procumbens* leaves rich extract was carried out using the method of Lorke (1983). The method involved two stages (phases I and II). In phase I, nine (9) rats

were distributed into three groups of three rats each (for each of the extracts) and administered doses of 10, 100 and 1000 mg/kg bodyweight of the extracts respectively. The animals were then observed for 24 hours for signs of toxicity and mortality. In the absence of mortality in phase I, another set of rats were grouped for phase II of the method. In phase II again, a total of nine (9) rats were distributed into three groups of three rats each (for each of the extracts) and administered 2900, 3600, and 5000 mg/kg body weight. The animals were also observed for 24 hours for signs of toxicity and mortality after which the LD_{50} was calculated using formula below:

$$LD_{50} = \sqrt{(D0 \times Dm)}$$

Where D0= Maximum tolerated dose, Dm= minimum lethal dose.

Experimental Design

In this study, 30 *Wistar* rats weighing (150-200) g were used. 5 rats in each cage. Rats were acclimatized to standard laboratory conditions (12:12 light dark cycle, environmental temperature 24±2 °C) for 15 days with access to water and food *ad libitum* before the start of the experiment. The weights of the animals were taken at the beginning and end of the study, and every day for two weeks. The rats were divided into six groups of five rats each and 5 mg/kg bodyweight of cisplatin was intraperitoneally administered to rats in groups II-VI.

Group I (Normal group) was the normal rats and not administered cisplatin.

Group II (Negative control) was administered a single dose of 5 mg/kg bodyweight of cisplatin on the 7th day of the experiment.

Group III (Positive control): The rats were administered a dose of 100 mg/kg bodyweight of Silymarin by oral gavage daily for 14 days of the experimental period and were then given a single intraperitoneal dose of 5 mg/kg bodyweight of Cis on the day 7 of the experiment.

Group IV: The rats administered a dose of 100 mg/kg bodyweight of *T. procumbens* phenol-rich extract by oral gavage daily for 14 days of the experimental period including administration of a single intraperitoneal dose of 5 mg/kg bodyweight of Cis on the 7th day the experiment.

Group V: The rats were administered a dose of 200 mg/kg bodyweight of *T. procumbens* phenol-rich extract by oral gavage daily for 14 days of the experimental period including administration of a single intraperitoneal dose of 5 mg/kg bodyweight of Cis on day 7 of the experiment.

Group VI: The rats were administered a dose of 400 mg/kg bodyweight of *T. procumbens* phenol-rich extract by oral gavage daily for 14 days of the experimental period including administration of a single intraperitoneal dose of 5 mg/kg bodyweight of Cis on the day 7 of the experiment.

Blood and Tissue Sampling

On the 15th day of the experiment, the rats in all groups were anesthesized under chloroform vapour and were euthenised by jugular vein puncture. Blood samples were collected into sample bottles for biochemical parameters while kidneys were harvested for histopathological

studies. Blood samples were left for 20 min at room temperature (27°C) and centrifuged at 3000 rpm for 10 min to separate the serum. The resultant serum samples were kept in a deep freezer (-20°C) until ready for biochemical analyses.

The harvested kidney tissue samples from all animals were immediately immersed in ice-cold saline solution and kept in the refrigerator prior to histopathological analyses. Kidney tissues were homogenized in phosphate buffer (pH 7.4) in test tubes.

Biochemical Analyses

Serum biochemical parameters such as electrolytes (sodium, potassium, chloride and bicarbonate), urea, creatinine and antioxidant enzymes such as Catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GSH-Px) were assayed with the aid of a UV-visible spectrophotometer as specified manufacturer.

Determination of serum sodium concentration

Serum sodium concentration was determined using commercial kit (AGAPPE, Switzerland). An aliquot of 1000 µL of reagent was added to Blank, Standard and Sample (test) tubes. Thereafter, 10 µL of standard reagent was added to the standard test tube, while 25 µL of each serum sample (test) was added to each of the sample test tube. The content of the test tube was mixed and incubated for 5 min at 37 °C. Thereafter, 250 µL of R2 reagent was added to all tubes. The mixtures were mixed for 1 minute at 37 °C and the absorbance (A1) was read against blank at 405 nm. The mixtures were further incubated for 5 min at 37 °C and the absorbance (A2) was read against blank at 405 nm.

Sodium concentration (mEq/L) = $\frac{\text{test reading}}{\text{standard reading}} \times 150$

Determination of serum potassium concentration

An aliquot of 1000µL of R1 reagent was added to blank, standard and sample (test) tubes. Thereafter, 25 µL of standard reagent was added to the standard test tube, while 25 µL of each serum sample (test) was added to each of the sample test tube. The content of the test tube was mixed and incubated for 5 min at 37 °C. Thereafter, 250 µL of R2 reagent was added to all tubes. The mixtures were mixed for 1 minute at 37 °C and the absorbance (A1) was read against blank at 405 nm. The mixtures were further incubated for 5 min at 37 °C and the absorbance (A2) was read against blank at 405 nm.

Potassium concentration (mEq/L) = $\frac{\text{test reading}}{\text{standard reading}} \times 150$

Determination of serum chloride concentration

Serum chloride concentration was determined using commercial kit (AGAPPE, Switzerland). Test tubes were arranged corresponding to the number of serum samples. Two other tubes were set for the standard and blank. 10 µL of each sample was pipetted into the appropriate test tube. Correspondingly, 10µL of chloride standard (100 mmol) was added to the standard tube while the blank contained 10 µL of distilled water. Finally, 1000 µL of the working reagent (thiocyanate reagent containing a blend of ferric III nitrate, mercury nitrate, ammonium chloride, ammonium thiocyanate and nitric acid) was added to each tube. The contents of each tube were well mixed and incubated for 5 min at 37 °C in a water bath. The absorbance of the sample and standard were measured against reagent blank at 480 nm.

Chloride concentration = $\frac{\text{Absorbance of Sample}}{\text{Absorbance of standard}} X$ standard concentration (mg/dl)

Determination of serum bicarbonate concentration

Serum carbonate concentration was determined using commercial kit (SPECTRUM, Egypt). Test tubes were labeled according to each sample identity and two tubes were labeled standard and blank respectively. 1000µL of reagent k was added to all the tubes. 10µL of calibrator was added to the calibrator tube while 10µl of each sample was added to the corresponding tube. The reaction mixtures were mixed and incubated at room temperature for 2minutes. Absorbance A1 was read immediately after incubation period while absorbance A2 was read exactly after 1minute for reagent blank, calibrator and samples.

Co2 (mmol/L) = $\frac{\Delta AS - \Delta AB}{\Delta AC - \Delta AB} X$ concentration of c

al

Where ΔA is change in absorbance $\Delta A = A1 - A2$

B, C and S are the blank, calibrator and sample respectively

Estimation of serum urea concentration

Urea concentration was determined by using a randox commercial kit. Ten microlitres (10 µL) of each sample, distilled water and standard were added to the sample, blank and standard tubes followed by the addition of 100 µL of R1 reagent to each of the test tubes. The content of the tubes was properly mixed and incubated at 37 °C for 10mins after which 2.5 mL of both reagents R2 and R3 was added. The mixture mixtures were incubated at 37°C for 1minute. The absorbance of the samples (Asample) and standard (Astandard) was read against the blank at 340 nm.

Urea $= \frac{A_{sample}}{A_{standard}} X$ Concentration (mg/dl) X Standard Concentration

Estimation of serum creatinine concentration

Creatinine concentration in samples were determined using Randox commercial kit (Ireland). Test tubes were labeled according to the serum samples identity and two tubes were labeled standard and blank respectively. 1200 µL of working reagent was added to all tubes. 120 µL of standard reagent was added to the tube labeled standard and 100 µL of each serum sample was added to the corresponding tube. The reaction mixtures were properly mixed and absorbances A1 and A2 of both standard and serum sample against blank was read at 510nm.

Concentration of Creatinine = $\frac{\Delta S \times K}{\Delta Standard}$ X Standard Concentration (130)

Where $\Delta A = \Delta Sample = A_2 - A_1$

Determination of serum uric acid concentration

Serum uric acid concentration was determined using commercial kit (URAP/E, Switzerland). Test tubes were labeled according to each sample identity, and two tubes were labeled standard and blank respectively. 1000 µL of uric acid reagent was added to all tubes. 25 µL of standard solution was added to the tube labeled standard while 25 µL of each sample was added to the corresponding tube. The content of the tubes was mixed

and incubated at 37°C for 5minutes. Absorbance of standard and each sample against reagent blank was measured at 546nm

Catalase (CAT) assay

The method of Sikiru (2019) was used to measure CAT activities. The decomposition rate of hydrogen peroxide (H_2O_2) into O_2 and H_2O by the samples in 3 minutes was tested at 240 nm wavelength using a UV spectrophotometer (Shimadzu UV Visible- 1800 series spectrophotometer). The reaction was initiated by the addition of 20 µl of serum to the tube 1 ml reaction mixture containing 100 µl of 100 mM H_2O_2 and 880 µl of 50 mM potassium phosphate buffer (pH 7.0). The change in the absorbance at 240 nm was measured at 20 seconds interval until 3 minutes. The enzyme activity was calculated by 0.0436mM extinction coefficient of H_2O_2 at 240 nm. The catalase activities units/ml of serum/plasma was calculated based on the conversion ability of catalase of µmol/min H_2O_2 at 25 °C.

$$\text{Activity} = \frac{\Delta A \text{ sample} - \Delta A \text{ control}}{\text{minutes} \times \text{millimolar extinction coefficient of } H_2O_2}$$

ΔA = change in absorbance, 0.0436= millimolar extinction coefficient of H_2O_2 at 240 nm

Superoxide dismutase (SOD) assay

Superoxide dismutase (SOD) activity was determined using the method of Sikiru *et al* (2019). One milliliter (1 ml) of each of the samples was diluted in 9 ml of distilled water to make 1:10 dilutions. An aliquot of the dilute samples was then added to 2.5 ml of 0.05M carbonic acid buffer, pH 10.2 to equilibrate in the spectrophotometer and the reaction was initiated by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 0.3 ml substrate (adrenaline) and 0.2 ml distilled water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds.

$$\text{Increase in absorbance per minute} = \frac{A_{150} - A_{30}}{120}$$

Where A_{30} absorbance at 30 second A_{150} absorbance at 150 seconds

$$\%\text{Inhibition} = \frac{\text{Increase in absorbance for sample}}{\text{Increase in absorbance for blank}} X100$$

Glutathione peroxidase (GSH-Px) assay

The method of Sikiru *et al*, (2019) was used to determine GSH-Px activity as described below.

Standard solution: first, 1 mM NADPH standard solution was prepared from NADPH salt in diluent buffer (phosphate buffer 50 mM, pH 7.2 containing 5 Mr ethylene diamine tetraacetic acid). Then from this stock solution 0, 0.78, 1.56, 3.12, 6.25, 12.5, 25, 50 and 100 µl volumes were pipetted into respective microplate wells and all wells were brought to 100 µl volume by diluent solution. Thus, the standard solution with concentrations of 0, 0.78, 1.56, 3.12, 6.25, 12.5, 25, 50 and 100 mM were prepared in the wells. Absorbance readings at 340 nm became the base to plot standard curve.

Positive control solution: 10 µl positive control solution of Abnorm kit which contains glutathione peroxidase, plus 90 µl of diluent buffer were used.

Blank solution: Diluent buffer was used as a blank solution and 100 µl of it was poured in the respective well.

were consisted of 10 µL glutathione (5
0, 10 µL glutathione reductase solution
U/ml), 10 µL NADPH (40 mM), 10 µL
menhydroperoxide solution (0.5 mM),
10 µL diluent buffer.

ocedure: According to the
rementioned method, 100 µl of the
ndard solution was added to the wells
ated to standards, 10 µl of positive
ntrol, 10 µl of blank solution and 10 µl of
d test samples were poured into
spective microplate wells. Then, 50 µl of
action mixture was added to all wells,
cept for the standard wells. In order to
ve the same volume as the final one of
e standard wells, each well was made up
00 µl by volume with diluent buffer.
bsorbance of wells at 340 nm was
mediately read (A1) by microplate
reader (Sun Rise, TECAN, Austria).
terwards, microplate was incubated for
10 minutes at 37°C. At the end of this time,
bsorbance of wells at 340 nm was
asured again (A2). GPX activity related
o test samples was calculated by ELISA
program based on the standard curve and
the difference of absorption of test sample
at two different times (A1-A2)

Malondialdehyde (MDA) assay

Lipid peroxidation assay was carried out using the method of Sikiru *et al.* (2019). Exactly 200µl of each sample was combined with 0.2 ml of 8.1 % SDS; 1.5 ml acetic acid and 1.5 ml TBA and the solution made up to 4 ml with distilled water. This was boiled for 60 minutes in a boiling water bath (95 °C). The reaction product (TBA–MDA complex) was extracted by adding 1 ml of n-butanol-pyrimidine (15:1 v/v) after cooling. The flocculent precipitate was removed by centrifugation at 3500 rpm for 15 minutes. The supernatant was obtained and absorbance

reading of the supernatant at 532 nm against a blank that contains the reagents minus the samples was carried out. The malondialdehyde concentration of the sample was calculated using the molar extinction coefficient of 1.56 x 105 M-1 cm-1 for MDA.

Malondialdehyde concentration (N) = $\frac{\text{Abs/cm}}{\text{ME}}$

MDA conc (µM): N x 1000 = MDA conc (µM/ml) of the sample

Histological Analysis

Kidney tissue specimens were fixed in formalin for light microscopic analyses. After fixation the kidney tissues were processed routinely for embedding in paraffin. Tissue sections of 4 µm were stained with Hematoxylin eosin (H&E) and Masson Trichrome to evaluate the parenchymal and stromal structure of the kidney tissues. The sections were examined under a light microscope and a modified scoring system was used to evaluate the severity of hepatic injury according to the method reported by Jamshidzadeh *et al.* (2008). The parameters include the extent of inflammatory cell infiltration, sinusoidal dilation, congestion and hydropic degeneration, (cytoplasmic vacuolization and swelling of hepatocytes). Slide scores were obtained after examination of the complete microscopic fields for each sample section.

Data Analysis

The results obtained were expressed as mean ± SEM. The data were analysed statistically by using One-way ANOVA of spss software version 20. The means were compared by Duncan's multiple range test. Significant difference was accepted at 95 % confidence level. ($p < 0.05$).

RESULTS

Quantitative phytochemical compositions of phenol-rich extract of *Tridax procumbens* plant

The result of quantitative analysis of phenol-rich extract of *Tridax procumbens* plant revealed the presence of [illegible] mg/100g of tannins.

Acute oral toxicity test of phenol-rich extract of *Tridax procumbens* plant

The result of acute oral toxicity of phenol-rich extract of *Tridax procumbens* plant which was carried out using Lorke's method showed that the extract was safe at the highest dose of 5000mg/kg because no death was recorded.

***In vitro* antioxidant activity of phenol-rich extract of *Tridax procumbens* plant**

The result of DPPH, FRAP and [illegible] activities of phenol-rich extract of *Tridax procumbens* is presented in Table 1. There was no significant difference in the DPPH inhibition percentage, FRAP and [illegible] of ascorbic acid across all concentrations. The DPPH inhibition percentages, [illegible] and LP of ascorbic acid were significantly higher than that of the extract across all concentrations. However, the DPPH inhibition percentages, FRAP and LP of the extract were significantly higher at the dose of 1000 µg/ml.

There was a dose dependent increase in the antioxidant activities of phenol-rich extract. However, there was a significant difference in the scavenging activity of the standard ascorbic acid compared to the extract across all concentrations and assays.

Table 1: *In vitro* Antioxidant Activity of Phenol-rich Extract of *Tridax procumbens* Plant

Sample (µg/ml)	DPPH (%)	FRAP (%)	LP (%)
Tp 1000	92.32±[illegible]	[illegible]	[illegible]
Ascorbic acid 1000	[illegible]	[illegible]	[illegible]
Tp 500	[illegible]	[illegible]	[illegible]
Ascorbic acid 500	[illegible]	[illegible]	[illegible]
Tp 250	[illegible]	[illegible]	[illegible]
Ascorbic acid 250	[illegible]	[illegible]	52.22±[illegible]
Tp 125	[illegible]	[illegible]	[illegible]
Ascorbic acid 125	[illegible]	[illegible]	[illegible]

Tp: *Tridax procumbens* phenol-rich extract. Values are [illegible]

Effect of phenol-rich extract of *Tridax procumbens* plant on serum urea, uric acid and creatinine of cisplatin-induced rats

Table 2 shows the effect of phenol-rich extract of *Tridax procumbens* plant on serum urea, uric acid and creatinine of cisplatin-induced rats.

Serum level of urea, creatinine and uric acid significantly increased in the negative control group when compared to the normal and Tp-treated groups. There was no significant difference in the serum [illegible]

acid level of normal and 100mg Tp-treated groups.

Administration of phenol-rich extract of *Tridax procumbens* prevented the effect of cisplatin on serum urea, creatinine and uric acid in a dose-dependent pattern.

Table 2: Effect of Tannin-rich Extract of *Tridax procumbens* Plant on Serum Urea, Uric acid and Creatinine of Cisplatin-induced rats

Sample	Urea	Creatinine	Uric acid
Tp 100mg	33.55 ± 0.45	7.80 ± 0.23	3.98 ± 0.26
Tp 200mg	31.51 ± 1.13	6.59 ± 0.43	4.72 ± 0.75
Tp 400mg	27.83 ± [illegible]	4.61 ± 0.34	3.50 ± 0.50
Positive control	25.96 ± 1.55	8.18 ± 0.32	3.93 ± 0.59
Negative control	35.96 ± 1.18	[illegible]	5.54 ± 0.62
Normal	22.82 ± 0.14	[illegible]	3.74 ± 0.87

Tp (cisplatin + *Tridax procumbens* phenol-rich [illegible]), [illegible] (cisplatin), normal (Normal rats). Values are expressed as mean [illegible] different superscripts in the same column are significantly different at p<0.05.

Effect of phenol-rich extract of *Tridax procumbens* plant on serum electrolytes of cisplatin-induced rats

Table 3 shows the effect of phenol-rich extract of *Tridax procumbens* plant on serum electrolytes of cisplatin-induced rats.

Serum levels of Na, Cl and HCO_3^- significantly increased while serum level of K significantly decreased in the negative control group when compared to the Tp-treated, positive control and normal groups. There was no significant difference in the serum level of Na in the positive control and Tp-treated groups except for the 400mg Tp-treated group which showed no significant difference with the normal.

Also there was no significant difference in the serum levels of Cl in normal and Tp-treated groups. There was no significant difference in serum K level between the normal and 200mg Tp-treated groups. There was no significant difference in the serum HCO_3^- levels of normal and 100mg Tp-treated groups.

Table 3: Effect of Phenol-rich Extract of *Tridax procumbens* Plant on Serum Electrolytes of Cisplatin-Induced Rats

Sample	Na	K	Cl	HCO3
Tp 100mg	126.23±2.51	4.16±3.15	78.37±2.04	38.78±1.17
Tp 200mg	120.41±1.70	5.05±3.35	76.41±1.82	33.45±1.83
Tp 400mg	120.45±1.87	7.08±1.60	72.17±1.49	25.91±1.48
Positive control	120.59±2.51	7.23±0.40	68.77±1.50	21.57±2.56
Negative control	142.59±1.69	1.85±0.21	61.43±2.26	27.25±2.26
Normal	113.94±1.72	3.49±0.26	73.21±2.24	18.53±1.46

[illegible]

Effect of phenol-rich extract of *Tridax procumbens* plant on kidney-based enzymes of cisplatin-induced rats

Tissue CAT, SOD, MDA and GSH-Px results are summarized in Table 4. CAT, SOD and GSH-Px activity decreased significantly in the negative control group compared with the Tp-treated, positive control and normal groups. However, administration of tannin-rich extract of *Tridax procumbens* lowered the effect of cisplatin in a dose-dependent pattern. There was no significant difference in the positive control, normal and 400mg Tp treated groups for CAT. Also no significant difference was recorded in the normal and 200mg Tp treated for SOD. There was no significant difference in the positive control, 400mg Tp-treated groups for GSH-Px.

Cisplatin treatment significantly increased the MDA level as evident in the negative control group.

For MDA, there was no significant difference in the normal and 400mg Tp-treated groups.

Table 4: Effect of Phenol-rich Extract of *Tridax procumbens* Plant on Kidney-based Enzymes of Cisplatin-Induced Rats

Sample	CAT (U/mg)	SOD (U/ml)	MDA (nM)	GSH-Px (U/mg)
Tp 100mg	[illegible]	73.52±0.76	44.42±1.52	29.31±2.60
Tp 200mg	3.42±1.20	145±3.40	45.24±1.22	27.14±1.36
Tp 400mg	5.06±0.40	108±3.40	40.20±1.35	43.27±1.41
Positive control	5.22±0.51	85.26±0.51	44.26±1.76	25.13±1.29
Negative control	1.2±0.17	37±2.41	57.74±1.39	32.18±2.15
Normal	5.70±3.4	75±2.00	34.26±2.4	45.93±2.87

Histological analysis of renal tissue of rats administered *Tridax procumbens* plant phenol-rich extracts

The histological analysis results of renal tissues are presented in Figure 1. Examination with hematoxylin and eosin dye under the light microscope showed that the renal tissues of the positive control, normal and 400mg/kg bw-treated groups were normal. This indicates renal tissue with preserved architecture that is composed of normal glomeruli, tubules and capsular space. However, some degree of hemorrhage/congestion and capsular spaces dilation of the nephrocytes were observed in the negative control group when compared with the normal groups. Also, 200 mg/kg bw treated showed mild haemorrhage of the nephrocytes compared to the negative control group

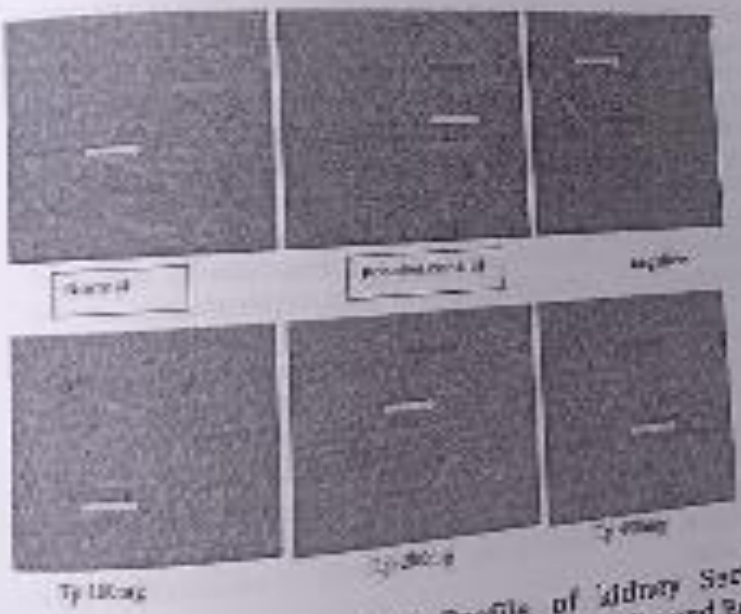

Figure 1: Histopathological Profile of Kidney Section of Rats Administered *Tridax Procumbens* Phenol-rich Extracts (Hematoxylin and Eosin Stained H&E, X 400)

Blue arrow= Capsular space, red arrow= Distal convoluted tubules, Yellow arrow= Glomerulus

Negative control: Section shows renal tissue with severely haemorrhagic glomeruli and capsular space. Necrosis of the nephrocytes was observed as well

Normal: Section shows renal tissue with preserved architecture composed of normal glomeruli, tubules and capsular space.

Positive control: Section shows renal tissue with preserved architecture composed of normal glomeruli, tubules and capsular space.

Tp 100mg: Section shows renal tissue with distorted glomeruli and capsular space with mild haemorrhage of the nephrocytes.

Tp 200mg: Section shows renal tissue with distorted glomeruli and capsular space with mild hemorrhage of the nephrocytes.

Tp 400mg: Section shows renal tissue with preserved architecture composed of normal glomeruli, tubules and capsular space

DISCUSSION

Despite being one of the most potent and effective cancer chemotherapies (Wang *et al*, 2018), cisplatin usage is limited due to the severity of adverse effects especially its dose-related nephrotoxicity. Different drugs used clinically in response to cisplatin-induced acute kidney injury (CIAKI) have shown to be inadequate (Duffy *et al*, 2018).

Tridax procumbens is rich in phytochemicals responsible for its physiological effects and hence medicinal properties. Of great importance are the phenols that are present in substantial amount (Himanshu and Rohit, 2022). The presence of phenols in plants is linked to the antioxidant properties of the plant. The presence of promising phytochemicals in *T. procumbens* may partly explain why it is largely used in folkloric medicine for the treatment of ailments. Unraveling the mechanism by which *T. procumbens* plant elicits its therapeutic effects is very important in understanding its pharmacogenicity. In this study, phenol rich extracts of *T. procumbens* were evaluated with a view of getting an insight into its nephroprotective potential.

This study revealed the presence of phenols in *T. procumbens* plant extract, which agrees with the results of Umedine *et al* (2015).

Similar to the observation made by Burgos pino *et al* (2022), the *Tridax procumbens* plant extract showed no acute toxicity as no death was recorded up to 5000 mg/kg bodyweight.

In this study, the DPPH assay showed that phenol rich extract has a high radical scavenging activity at a dose of 1000 µg/ml. This result is in line with the findings of Syed *et al*. (2020) that extracts of *T. procumbens* exhibited high radical scavenging activity. Various studies have reported the role of secondary metabolites such as phenols, tannins, catechins and flavonoids as antioxidants in various diseases conditions (Andrians *et al*, 2019).

It has been established that there are some connections between reducing power and the antioxidant activity of antioxidant, the reducing power increases with increasing antioxidant activity. Therefore, reducing power can be used as an indication of antioxidant activity (Zhang *et al*, 2012). In this present study, the ability of phenols to chelate transition metals, specifically Fe (II), forming a complex that prevents initiation of lipid peroxidation is demonstrated (Obuh and Ikeda, 2008).

may be due to the high phenols
ot which have been reported as a
chelator of iron (Omololu et al,
).

tion and reabsorption of cisplatin
its metabolites take place in the
l tubules, resulting in their
mulation in the kidney (4–5 times
er in proximal tubular epithelial
s (PTECs) than in the blood). This
umulation is mainly due to the
olvement of various transport-
diated uptake processes in renal
ECs. CIAKI occurs possibly due to
cumulation of cisplatin leading to
llular oxidative damage, inflammation,
scular injury, apoptosis and proximal
bular cell damage. In this context of
idney dysfunction, creatinine, urea and
ric acid are not filtered properly,
ndicating a decrease in glomerular
iltration rate (GFR), this results in high
evels of these markers of renal functions
(Anwer *et al.* 2023). In this study,
isplatin administration is responsible
for the decrease in glomerular filtration
rate (GFR) along with an elevation in
serum creatinine, urea and uric acid.
These findings support previous reports
(Abdelrahman *et al.* 2019; Anwer *et al.*
2023). However, the no significant
difference in uric acid was reported by
Faouzi *et al.* (2012) after cisplatin
administration.

This study also recorded electrolytes (Na^+, K^+, Cl^- and HCO_3^-) disturbances in cisplatin administration, which may result from cisplatin-induced atrophy. This is because electrolytes are mostly reabsorbed in the tubular system and cisplatin administration affects the tubulointerstitial components also the glomeruli is relatively spared (Airfael et al. 2023). This also supports the study of
et al (2012).
of cisplatin in the
epithelial cells
lowers the activity of antioxidant enzymes, depletes intracellular concentration of GSH and also causes peroxidation of lipids thereby inducing reactive oxygen species (Orbek, 2012). LPO is an important marker of cellular oxidative stress that damages cell membrane lipids via the generation of ROS, which subsequently increases malondialdehyde (MDA) levels a metabolic product of lipid peroxidation (Anwer et al. 2021). In this study, elevated MDA levels from cisplatin administration indicated its initiation of lipid peroxidation.

The cell has enzymatic and non-enzymatic antioxidant systems to protect itself against oxidative damage. SOD is present in ample amounts in all cells and is a very important enzyme that protects them against the renal oxidative injury resulting from ROS. It converts highly toxic superoxide anions (O_2^-) to the less toxic hydrogen peroxide (H_2O_2), which is then detoxified by CAT and GSH-Px. CAT and GSH-Px protect cells from oxidative damage by utilizing protons generated from the oxidation of GSH to convert free H_2O_2 to water. GSH is oxidized to GSSG so as to provide more protons to protect cells from oxidative damage, hence, the GSH level starts depleting in cells that undergo oxidative damage. The GSH level is maintained in a cell by GR, which converts GSSG back to GSH. Depleted antioxidant enzymes reduce the clearance of H_2O_2 and facilitate the production of toxic hydroxyl radicals, leading to renal cell oxidative damage (Anwer et al. 2023). This study demonstrated antioxidant properties of *T. procumbens* plant as evidenced by the activities of the kidney's paired enzymes (Table 4). These results are consistent with previously published reports that pretreatment with *Portumbens* attenuated doxorubicin-induced oxidative stress (Nwokwuchi et al. 2021). It also agrees with the ear

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