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PHYTOCHEMICAL CHARACTERIZATION AND TOXICITY ASSESSMENT OF ACUTE AND SUB-ACUTE ORAL ADMINISTRATION OF AQUEOUS EXTRACT OF *Jatropha tanjorensis* LEAF

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ABSTRACT

The phytochemical evaluation of the aqueous extract of *Jatropha tanjorensis* leaf revealed the presence of alkaloids, tannins, flavonoids, phenols, terpenoids, saponins, cardiac glycosides, and steroids. Toxicity studies indicated no mortality up to 6000 mg/kg in mice, with 33.33% mortality at 7000 mg/kg and 100% mortality at 10,000 mg/kg, yielding an LD_{50} of 7745.97 mg/kg. Biochemical analyses in Wistar rats showed no significant ($p<0.05$) changes in serum ALT (Alanine Aminotransferase) and AST (Aspartate Aminotransferase) activities but a significant ($p<0.05$) reduction in ALP (Alkaline Phosphatase) activity, particularly at 400 mg/kg. Serum total protein and albumin concentrations increased significantly ($p<0.05$), while bilirubin and globulin levels remained unchanged ($p<0.05$). Urea levels decreased significantly ($p<0.05$) at 200 mg/kg, with no significant ($p<0.05$) changes in creatinine, sodium, chloride, and potassium levels. Bicarbonate levels were significantly ($p<0.05$) reduced at 200 mg/kg. Antioxidant biomarkers- SOD (Superoxide Dismutase), CAT (Catalase), GSH (Reduced glutathione), and GPx (Glutathione peroxidase) increased ($p<0.05$) dose-dependently, while MDA (Malondialdehyde) levels decreased ($p<0.05$). Histological analyses of liver and kidney sections revealed normal ultrastructures, indicating no histopathological damage. These findings suggest that *Jatropha tanjorensis* extract has a favourable safety profile at lower doses, with potential antioxidant benefits and no adverse effects on liver and kidney structures.

Keywords: Antioxidant enzymes, *Jatropha tanjorensis*, Liver enzymes, Phytochemicals, Toxicity

INTRODUCTION

Traditional herbal medicines have long been cherished for their natural origins and perceived minimal side effects, serving as integral components of local and regional healing practices worldwide (WHO, 2019; Rizvi et al., 2022). The World Health Organization defines these medicines as plant-derived substances with minimal industrial processing, used for the prevention and treatment of illnesses. Over millennia, they have found extensive application in both developed and developing countries, evolving from crude formulations like tinctures and teas to contributing directly to the synthesis of modern pharmaceuticals such as aspirin (from willow bark), morphine (from opium poppy), and quinine (from cinchona bark) (Chaachouay & Zidane, 2024). While the historical efficacy of herbal remedies is undeniable, concerns about their safety persist, prompting rigorous toxicity evaluations. Despite sparse reports of adverse reactions leading to injury or death, the presence of potentially harmful compounds like alkaloids and anthraquinone glycosides necessitates comprehensive safety assessments (Zhang et al., 2015). This underscores the critical importance of evaluating the toxicity of medicinal plants to ensure their therapeutic benefits outweigh any potential risks (Neergheen-Bhujun, 2013). The assessment of acute, sub-acute, and sub-chronic toxicity in medicinal plants plays a crucial role in ensuring their safety and efficacy for human consumption. Acute toxicity assessments provide valuable insights into the immediate adverse effects of a medicinal plant or its extracts. This helps in determining safe dosage levels and potential risks associated with high doses. Sub-acute toxicity studies, conducted over a longer duration, further elucidate the cumulative effects and potential toxicity of repeated doses, mimicking more closely the conditions of chronic use. Sub-chronic toxicity studies, on the other hand, evaluate the effects of repeated exposure over a longer duration, mimicking more closely the typical usage patterns of medicinal products. Both assessments contribute significantly to ensuring the safety of herbal medicines (Li et al., 2020; Njinga et al., 2020).

Jatropha tanjorensis (J. L. Ellis & Saroja), a member of the Euphorbiaceae family, stands as a versatile botanical wonder with a plethora of medicinal virtues (Ellis & Saroja, 1961). This gregarious shrub, reaching heights of about 1.8 meters, thrives as a common weed among field crops, primarily flourishing in the rainforest zones of West Africa (Idu et al., 2014). The multifaceted applications of *Jatropha tanjorensis* in traditional medicine and beyond underscore its significance. Abundant scientific evidence supports the medicinal properties of this plant. Every part of the *Jatropha tanjorensis* plant, from its seeds to its leaves and bark, is rich in therapeutic potential. Exploring the medicinal properties of *Jatropha tanjorensis* uncovers a wealth of benefits, including anti-diabetic, cardiovascular, antioxidant, and haematological improvements (Adebajo et al., 2001; Oyewole & Akingbala, 2011; Omoregie & Osagie, 2011; Idu et al., 2014). The antioxidant and

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hypoglycaemic properties of *Jatropha tanjorensis* leaf extracts have made them a popular treatment for conditions such as diabetes, malaria, and hypertension in Southern Nigerian regions like Edo State. Here, the leaves are consumed not only as a nutritious vegetable but also as part of daily meals, due to their reputed effectiveness in managing diabetes mellitus, thanks to their anti-hyperglycaemic effects (Olayiwola et al., 2004). These antioxidants enhance the body's defence mechanisms and are crucial in fighting oxidative stress-related conditions like diabetes, malaria, and hypertension, which are especially common in areas where this plant is cultivated. Studies administering *Jatropha tanjorensis* leaf powder to animal models have demonstrated improvements in haematological indices, indicating a potential enhancement in bone marrow function (Orhue et al., 2008). Additionally, the plant's antibacterial attributes have been delineated, affirming its role in infectious disease management (Viswanathan & Jeyaananthi, 2009). The nutritional profile of *Jatropha tanjorensis* is equally noteworthy, boasting a rich concentration of antioxidant nutrients such as phosphorus, selenium, zinc, vitamin C, and E (Omobuwajo et al., 2011).

Phytochemical investigations revealed the existence of alkaloids, tannins, saponins, flavonoids, terpenoids, cardiac glycosides, and anthraquinones in the aqueous leaf extract of *Jatropha tanjorensis* (Ebenyi et al., 2021). Similarly, alkaloids, tannins, flavonoids, cardiac glycosides, and anthraquinones were detected in the methanol leaf extract of *Jatropha tanjorensis* (Igbinaduwa et al., 2011). Studies have reported that *Jatropha tanjorensis* leaf extract contains phytochemical constituents capable of reducing blood cholesterol levels, which can be beneficial in treating cardiovascular diseases resulting from hyperlipidaemia (Oyewole & Akingbala, 2011). *Jatropha tanjorensis* leaf extract also has antimicrobial properties, inhibiting the growth of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Ewa-Udu et al., 2022). As scientific exploration continues, this botanical gem promises to unveil even more of its healing secrets, potentially revolutionising the landscape of natural medicine. An earlier study examining the toxicity characteristics of *Jatropha tanjorensis* demonstrated the absence of toxicity when administering (2000 and 5000 mg/kg) of aqueous leaf extract to mice and rats within 24 hours after oral ingestion (Ebenyi et al., 2021). Similarly, no observed toxicity was reported at 8000 mg/kg of methanol extracts in the same animal models within the same timeframe (Igbinaduwa et al., 2011).

Insufficient exploration into the toxicity evaluation of the aqueous extract derived from *Jatropha tanjorensis* leaf, especially regarding acute and sub-acute oral administration, limits a thorough understanding of the safety attributes linked to this extract. Assessing toxicity is crucial for identifying potential adverse reactions, setting safe dosage thresholds, and balancing the risks and benefits associated with utilising the extract in therapeutic or medicinal scenarios. The absence of comprehensive toxicity analyses may lead to uncertainties regarding the extract's safety, potentially prompting cautious or restricted usage within clinical settings. The study seeks to identify and quantify the key phytochemical constituents present in the aqueous extract of *Jatropha tanjorensis* leaf. This entails gauging the levels of alkaloids, flavonoids, tannins, saponins, terpenoids, cardiac glycosides, anthraquinones, and other bioactive compounds that contribute to the plant's medicinal attributes. Although *Jatropha tanjorensis* offers potential medicinal benefits, the presence of alkaloids and anthraquinones requires caution due to their potential toxic effects such as hepatotoxicity, nephrotoxicity, cardiotoxicity, gastrointestinal distress, and neurotoxicity. Proper dosing, a thorough understanding of the plant's pharmacology, are essential to mitigate these risks. Furthermore, it aims to evaluate the toxicity of the aqueous extract of *Jatropha tanjorensis* leaf through both acute and sub-acute oral administration, with the goal of elucidating its safety profile and potential therapeutic applications. In conclusion, acute and sub-acute toxicity assessment of medicinal plants is indispensable for safety evaluation, risk identification, quality control, optimizing treatment regimens, public health protection, scientific validation, and building consumer confidence. These assessments are essential steps in ensuring the safe and effective use of herbal medicines in healthcare practice.

MATERIALS AND METHODS

Collection of Plant Materials and Preparation of Aqueous Extract of *Jatropha tanjorensis* Leaves

Fresh leaves of *Jatropha tanjorensis* were collected from the National Root Crops Research Institute (NRCRI) in Umudike, Abia State, Nigeria, and identified by Mr. Pipi Okey from the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike. The specimen was assigned voucher number MOUAU/PSB/18/103592 and deposited in the Department's Herbarium. The leaves were rinsed with distilled water to remove dirt and air-dried at room temperature until completely dry. The dried leaves were ground into a fine powder using an electric blender. A known quantity (188.54 g) of the powdered sample was macerated in 2.5 L of distilled water for 24 hours in a sterile extraction jar. The filtrate was first filtered using muslin cloth and then filtered again using Whatman filter paper No. 1 into a clean, calibrated flask. The filtrate was lyophilized, using a freeze dryer set to a temperature of ≤ -40°C. A dark-brown paste with a yield of 50.85% was obtained, transferred into a sterile clean bottle and stored in a refrigerator at 4°C until needed for biochemical assays.

Qualitative Phytochemical Analysis

Preliminary analysis of the aqueous extract of *Jatropha tanjorensis* leaf was carried out to identify the presence of various phytoconstituents such as alkaloids, tannins, flavonoids, phenols, terpenoids, saponin, cardiac glycoside, and steroids.

Test for alkaloid: For qualitative alkaloid determination, a few drops of Meyer's reagent were added to 1 mL of extract. The formation of a creamy white precipitate was considered positive for the alkaloid (Archana et al., 2012).

Test for tannin: For qualitative tannin analysis, a few drops of lead acetate were added to 1 mL of extract. A large white-brown precipitate formation was considered a positive test for tannin (Archana et al., 2012).

Test for flavonoids: For the confirmation of flavonoid, 0.5 g of each extract were added in a test tube and 10 mL of distilled water, 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of 1 mL concentrated H_2S0_4. Indication of yellow colour shows the presence of flavonoid in each extract (Sofowora, 1993).

Test for phenols: To detect phenols, 1 mL of extract solution was added to 2 mL distilled water and a few drops of 10% ferric chloride ($FeCl_3$). The appearance of a green or blue colour is the indication of phenols presence. Next, 0.50 g of plant extract was added and allowed to dissolve in distilled water and then 3 mL of

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10% lead acetate was added. The presence of phenolic compounds was confirmed by the appearance of white precipitation (Trease et al., 2003).

Test for saponin: To detect the presence of saponin, 5 mL of distilled water was added to 1 mL of extract and vortexed for 10 mins. The formation of a foam column that did not disappear with the addition of HCl was evaluated as positive for saponin (Sati and Kumar, 2015; Savithramma et al., 2011).

Test for cardiac glycoside: Qualitative analysis of cardiac glycosides in the extracts was performed with the Keller Killiani test. 1 mL of acetic acid and 2 drops of ferric chloride were added to 2 mL of extract, then 2 mL of sulfuric acid (concentrated) was added and the colour change was observed. Reddish-brown colour formation was deemed to be a positive test for cardiac glycosides (Archana et al., 2012; Sati & Kumar, 2015).

Test for terpenoids and steroids: The presence of terpenoids and steroids was determined by a previously described method (Kantamreddi & Lakshmi, 2010; Siddiqui & Ali, 1997) with slight modifications. Briefly, 0.5 g of solvent-free extract was added in 2 mL chloroform and then filtered. The filtrate was placed on ice; addition of 2 mL of acetic acid and then a few drops of concentrated sulfuric acid (H_2SO_4) was carefully applied to the inner sides of the test tubes. The emergence of a pink or pinkish brown colour/ring indicates the terpenoids existence, while a blue or bluish green colour for steroids presence and the combination of pink and blue/bluish green colours confirmed the presence of both terpenoids and steroids.

Quantitative Phytochemical Analysis

Quantitative analysis of the phytochemical components, whose presence were tested by qualitative analysis, were also carried out. All the analysis were performed in triplicate.

Estimation of Total Alkaloids

Alkaloid determination was made according to the method reported by Selvakumar et al. (2019). To 1 mL of the test extract 5 mL of phosphate buffer (pH 4.7) and 5 mL of bromocresol green (BCG) solution was added to the solution. The mixture was shaken and the complex extracted with 1-, 2-, 3- and 4-mL of chloroform by vigorous shaking. The extracts were then collected in a 10 mL volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the chloroform complex was measured at 470 nm using a UV spectrophotometer against a blank prepared similarly, but without extract. To quantify the total alkaloid content in the sample, a standard curve was prepared using atropine. Standard solution of atropine was prepared at concentrations of (0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL). To generate the standard curve, the average absorbance values were plotted against the corresponding atropine concentrations. A linear regression analysis was performed to obtain the equation of the line: $y = mx + b$. This linear equation was then used to determine the total alkaloid content in the extract. The alkaloid content was expressed as mg atropine equivalent (AE)/g of sample. All tests were repeated in triplicate.

Estimation of Total Tannins

Tannin content was determined according to the method of Van-Burden & Robinson,1981. A known weight of sample (500 mg) was weighed into a 100 mL plastic bottle. 50 mL of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filtered was pipette out into a test tube and mixed with 2 mL of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferro-cyanide. The absorbance of each reaction mixture was thereafter measured at 120 nm against a blank using a spectrophotometer. To quantify the total tannin content in the sample, a standard curve was prepared using gallic acid. Standard solution of gallic acid were prepared at concentrations of (0.1, 0.2, 0.3, 0.4, and 0.5 mg/L). The average absorbance values were plotted against the corresponding concentrations of gallic acid to generate the standard curve. A linear regression analysis was performed to obtain the equation of the line: $y = mx + b$. This linear equation was then used to determine the total tannin content in the extract. Results were quantified in the form of GAE (mg of Gallic Acid Equivalents) per gram of dry plant extract/fraction. Samples were analysed in triplicates.

Estimation of Total Flavonoids

The total flavonoid content of the extract was determined using a slightly modified method reported by Meda et al. (2005). 0.5mL of the extract samples were mixed with 0.5mL methanol, 50µl of 10% $AlCl_3$, 50µl of 1mol L^{-1} potassium acetate and 1.4mL water, and allowed to incubate at room temperature for 30 mins. Thereafter, the absorbance of each reaction mixture was subsequently measured at 415 nm against blank. To quantify the total flavonoids content of the extract, a standard curve was prepared using quercetin. Standard solutions of quercetin were prepared at concentrations of 40, 80, 120, 160, 200, and 240 µg/mL. The average absorbance values were plotted against the corresponding concentrations of quercetin to generate the standard curve. A linear regression analysis was performed to obtain the equation of the line: $y = mx + b$. This linear equation was then used to determine the total flavonoids content in the plant extract samples. The flavonoids content of the extract was determined in triplicates and the results were expressed as milligram quercetin equivalent (QE) per gram of the extract (mg QE/g extract).

Estimation of Total Phenolics

Total phenolic content was determined using the method of Hageman et al. (2000). 100 mg of the extract was weighed accurately and dissolved in 100 mL of distilled water. 1.5 mL of this solution was transferred to a test tube, then 1 mL 2N of the Folin-Ciocalteu reagent and 2 mL 20% of Na_2CO_3 solution was added and ultimately the volume was made up to 8 mL with distilled water followed by vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765 nm. The blank was prepared using the reagent and solvent without the sample. Total phenol was estimated in the extract using gallic acid as standard. The calibration curve was plotted using standard gallic acid (10, 20, 40, 60, 80, 100 µg). The average absorbance values were plotted against the corresponding concentrations of gallic acid to generate the standard curve. A linear regression analysis was performed to obtain the equation of the line: $y = mx + b$. This linear equation was then used to determine the total phenolic content in the extract. The total phenolic content was expressed as mg of gallic acid equivalent (GAE) per 100 g of dry mass. The total phenolic content of the extracts was performed in triplicates.

Estimation of Terpenoids

Total terpenoid content of the extract was determined by the method as described by Ferguson, (1956). About 1 g of plant powder was weighed, placed into a conical flask, and soaked in ethyl alcohol for 24 hours. Then it was filtered and the filtrate was extracted with petroleum ether. The ether extract was taken as the

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measure of total terpenoid. The samples were analysed in triplicates.

$$\text{Total terpenoid content} = \frac{\text{(Final weight of the sample - Initial weight of the extract)}}{\text{Weight of the Sample}} \times 100$$

Estimation of Total Saponins

Estimation of total saponins content was determined by the method described by Makkar et al. based on vanillin- sulphuric acid colorimetric reaction with some modifications (Makkar et al., 2007). About 50 µL of the extract was added with 250 µL of vanillin reagent (800 mg of vanillin in 10 mL of 99.5 % ethanol) was added. Then 2.5 mL of 72% sulphuric acid was added and it was mixed well. This solution was kept in a water bath at 60 ºC for10 mins. After 10 mins, it was cooled in ice cold water and the absorbance was read at 544 nm. All measurements were performed in triplicate for each analysis. To quantify the total saponins content, a standard curve was prepared using diosgenin. Diosgenin standard solutions at concentrations of 0, 10, 20, 40, 60, 80, and 100 µg/mL were prepared. The average absorbance values were plotted against the corresponding concentrations of diosgenin to generate a standard curve. A linear regression was performed to obtain the equation of the line, which was used to determine the total saponins content in the samples. The results were expressed as mg/g diosgenin equivalent (DE) of dry extract.

Estimation of Cardiac Glycosides

Cardiac glycoside analysis was determined according to the method reported by Tofighi et al. (2016). 10 g of the extracts were mixed with 10 mL of Baljet's reagent. After 1 h of incubation, 20 mL of distilled water was added and the absorbance was measured at 495 nm. To quantify the cardiac glycoside content, a standard curve was prepared using securidaside as a standard. Securidaside standard solutions at concentrations of 0, 10, 20, 40, 60, 80, and 100 µg/mL were prepared. The average absorbance values were plotted against the corresponding concentrations of securidaside to generate a standard curve. A linear regression was performed to obtain the equation of the line. This equation was then used to determine the cardiac glycoside content in the samples, expressed as mg securidaside equivalent (SE) per gram of the sample.

Estimation of steroids

The steroid content was determined by Ejikeme et al., 2014. Firstly, 1 mL of test extract was transferred into 10 mL volumetric flasks. Sulfuric acid (4 N, 2 mL) and iron (III) chloride (0.5% *w*/*v*, 2 mL) were added, followed by potassium hexacyanoferrate (III) solution (0.5% *w*/*v*, 0.5 mL). The mixture was heated via a water bath that was maintained at 70 ± 20 °C for 30 mins, along with shaking, and afterwards diluted to the mark with distilled water. The absorbance of the plant extract solution was measured in triplicate at 780 nm against the reagent blank. To quantify the steroid content in plant extracts, cholesterol was used as a standard to prepare solutions with concentrations of 0, 10, 20, 40, 60, 80, and 100 µg/mL. A standard curve was plotted, and the linear regression equation obtained. The steroid content in the plant extract was determined and expressed as mg cholesterol equivalent per gram of dry plant extract.

Chemical and Reagent Test Kits

All chemicals used in this study were of analytical grade and procured from Sigma Chemical Company (St. Louis, MO, USA). Assay kits for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, and total protein were obtained from Randox (Antrim, U.K). Teco Diagnostics (California, USA) assay kits were used for urea, creatinine, sodium ion, potassium ion, and bicarbonate measurements.

Experimental Animals

Male healthy Wistar rats (n=24) weighing approximately 160 ± 10 g were procured from the animal house of the Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. The rats were housed in standard cages with a controlled temperature of 25 ± 2ºC and maintained under a 12:12 hours light and dark cycle. They were provided with *ad libitum* access to rat pellets as their standard diet and clean drinking water throughout the experimental period. The care and handling of the animals strictly adhered to the guidelines for the care and use of Laboratory Animals by the National Institute of Health, as well as the approved protocols of the Animal Care and Ethics Committee of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State.

Acute Toxicity Study (*in vivo*)

Acute toxicity evaluation was conducted using a slightly modified version of the methods previously reported by Lorke (1983) to determine the LD_{50}. In Phase I, nine male Wistar rats were used after a one-week acclimatization period. The rats were divided into three groups (n = 3), each receiving 10, 100, and 1000 mg/kg body weight of *Jatropha tanjorensis* aqueous leaf extract, respectively, and were observed for 24 hours for signs of behavioural changes or death. In the subsequent phases (II and III), eighteen male rats were used and divided into three groups (n = 3). Each group received doses of 1600, 2900, 5000, 6000, 7000, and 10,000 mg/kg of the extract, respectively, and were observed for another 24 hours for signs of toxicity-related behavioural changes or mortality (Tables 2, 3, and 4). The lethal dose (LD_{50}) of the extract was calculated using the formula: $LD_{50} = \sqrt{(D_0 + D_{100})} / 2$. Where D_0 is the highest dose that did not cause death and D_{100} is the lowest dose that resulted in death. The chosen doses were guided by safety data from prior studies. Some researchers employed doses like 100, 200, and 400 mg/kg body weight in one experiment (Ebenyi et al., 2021), while others used doses such as 250, 500, and 700 mg/kg body weight in another (Tarfa et al., 2021). These doses were also selected to establish a dose-response relationship, enabling the observation of diverse extract effects at varying concentrations. This approach aids in pinpointing the optimal therapeutic dose while prioritizing safety to prevent adverse effects.

Sub-acute Toxicity Study (*in vivo*)

The experimental animals were weighed, divided into four groups of five each, and fasted overnight. The aqueous extract at doses of 200, 400, and 800 mg/kg body weight was administered orally for 28 days using a gavage. The control group received 0.5 mL of distilled water orally once a day for 28 days (OECD, 2002). The animals were also monitored for signs of toxicity and mortality (Pillai et al., 2011).

Biochemical Assay

Blood sample collection

At the conclusion of the experiment, the rats were anesthetized using 25% urethane in saline intraperitoneally. The abdominal and thoracic cavities were opened and blood samples were collected into plain sample tubes using a 5 mL syringe (Arunachalam and

Sasidharan, 2021). The blood samples were centrifuged at 4000 rpm for 10 minutes to obtain serum, which was used for biochemical assays in this study.

Liver Function Tests

The activities of AST, ALT, and ALP, as well as the concentrations of total bilirubin and total protein (TP) were determined in serum using their respective Randox kits.

Renal Function Tests

Serum urea nitrogen and creatinine were determined using Fawcet and Scott, 1960 and Bartels and Bohmer, 1972 methods respectively. Serum chloride and potassium levels were determined by the methods of Tietz, 1976 as outlined in the Teco diagnostic kit leaflet. Bicarbonate determination was based on the method of Tietz *et al.*, 1986 as described in the Teco diagnostic kit leaflet.

Antioxidant Assays

Estimation of Catalase Activity

Catalase activity in serum was determined using the modified method described by Cohen et al. (1970).

Estimation of SOD Activity

Superoxide dismutase (SOD) activity in serum was determined using the method described by Misra & Fridovich (1972).

Estimation of GSH Levels

GSH levels in serum were determined using the method described by Tietze (1969).

Estimation of Glutathione Peroxidase (GPx) Activity

Glutathione Peroxidase (GPx) activity in serum was determined using the method described by Flohe & Guùzler (1984).

Assessment of Lipid Peroxidation

The concentration of MDA in serum was determined using the method described by Okhawa et al. (1979).

Histopathological Investigations of Sectioned Liver and Kidney Tissues

At the end of the experimental period, the rats were euthanized, and liver and kidney tissues were carefully excised. The tissues were immediately fixed in 10% neutral buffered formalin to preserve cellular architecture and prevent autolysis. The liver and kidney tissues were then dehydrated in a graded series of ethanol (70%, 95% and 99% absolute ethanol) and cleared using xylene. Following this, the tissues were embedded in paraffin wax to facilitate sectioning. The paraffin-embedded tissues were sectioned at a thickness of approximately 4-5 µm using a microtome. The tissue sections were mounted onto glass slides and subjected to routine histological staining procedures. Haematoxylin and eosin (H&E) staining was used to visualize general tissue morphology and assess cellular integrity and the stained sections were examined under a light microscope (Leica DM 500, Leica Biosystems, Germany) according to the method described by Windsor,1994. The liver and kidney sections were photographed at x200 magnification and multiple microscopic fields were assessed to obtain representative images of each tissue sample.

STATISTICAL ANALYSIS

Data are expressed as mean ± SEM. Significant differences were determined using one-way analysis of variance (ANOVA). Differences between means were analysed for significance using Dunnett's multiple-range tests. ANOVA and Dunnett's multiple range tests were based on the computer software, graph pad prism, version 7. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Phytochemical Analysis of Aqueous Extract of *Jatropha tanjorensis* Leaf

The result of the photochemical evaluation of Aqueous Extract of *Jatropha tanjorensis* Leaf is shown in Table1. It revealed the presence of alkaloids, tannins, flavonoids, phenols, terpenoids, saponins, cardiac glycoside, and steroids.

Table 1: Phytochemical Constituents of Aqueous Extract of *Jatropha tanjorensis* Leaf

Phytochemical parameter	Qualitative	Quantitative (mg/100g)
Alkaloids	++	27.15±0.59
Tannins	+	7.63±0.18
Flavonoids	+++	39.18±1.07
Phenols	+++	46.07±1.14
Terpenoids	+	5.41±0.15
Saponins	+++	33.10±1.25
Cardiac glycosides	+	4.83±0.19
Steroids	+	3.80±0.10

+++ = high amount, ++ = moderate amount, + = low amount

Results of acute toxicity study of Aqueous Extract of *Jatropha tanjorensis* leaf in Wistar rats

Graded doses up to 6000 mg/kg of the extract produced no mortality in rats as the treated rats remained active and physically stable throughout the 24-hour period of observation. However, 33.33 percent mortality was observed in the third phase and group administered 7000 mg/kg body weight while all the rats in the 10,000 mg/kg treated group died, representing 100 percent mortality. The application of Lorke's formular yielded an LD_{50} value of 7745.97 mg/kg body weight for the aqueous extract. The results are presented in Tables 2, 3, and 4 below.

Table 2: Stage 1 Acute toxicity (LD50) evaluation of the extract

Group	Dose (mg/kg)	No. of Deaths	Percentage of mortality	Observations
1	10	0/3	0.00	No mortality observed, instead animals remained active and physically stable.
2	100	0/3	0.00	No mortality observed, instead animals remained active and physically stable
3	1000	0/3	0.00	No mortality observed, instead animals remained active and physically stable

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Table 3: Stage 2: Acute toxicity (LD_{50}) evaluation of the extract

Group	Dose (mg/kg)	No. of Deaths	Percentage of mortality	Observations
1	1600	0/3	0.00	No mortality observed, instead animals remained active and physically stable.
2	2900	0/3	0.00	No mortality observed, instead animals remained active and physically stable.
3	5000	0/3	0.00	No mortality observed. Animals were initially calm but regained physical activity within one hour of administration.

Table 4: Stage 3: Acute toxicity (LD_{50}) evaluation of the extract

Group	Dose (mg/kg)	No. of Deaths	Percentage of mortality	Observations
1	6000	0/3	0.00	No mortality observed. Animals were initially calm but regained physical activity 24 hours of administration.
2	7000	1/3	33.33	33.33% mortality was observed. Surviving animals were weak, depressed and calm. They also did not completely regain physical activity within 24 hours of administration.
3	10000	3/3	100.00	Rats were initially calm and depressed and 100% mortality was observed by the end of 24 hours of administration.

$LD_{50} = (D_0 \times D_{100})^{1/2}$
Where:
D_0: Highest dose that gave no mortality. D_{100}: Lowest dose that produced mortality $LD_{50} = (6000 \times 10000)^{1/2}$

LD_{50} = 7745.97 mg/kg body weight

Effect of Varying Doses of Aqueous Extract of *Jatropha tanjorensis* leaf on the Activities of Liver Enzymes and Total Protein Levels in Sera Male Wistar Rats

The experimental results showed no significant differences ($P < 0.05$) in serum levels of ALT and AST between Wistar rats administered the extract at various doses and the control group (Figures 1 and 2). However, serum ALP levels were significantly reduced ($P < 0.05$) in all experimental groups given the extract compared to the control group, with the lowest ALP level observed in the group administered 400 mg/kg (Figure 3). Additionally, serum total protein and albumin concentrations were significantly increased ($P < 0.05$) in rats given the extract compared to their respective controls (Figures 4 and 5). There were no significant differences ($P < 0.05$) in serum total bilirubin levels between the experimental groups and the control group after 30 days (Figure 6). Similarly, serum globulin concentrations did not show a significant increase ($P < 0.05$) in the experimental groups compared to the control (Figure 7).

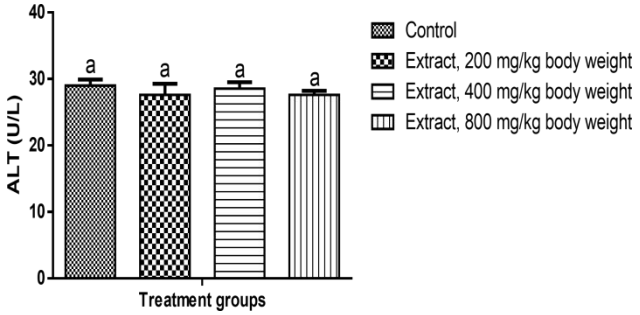
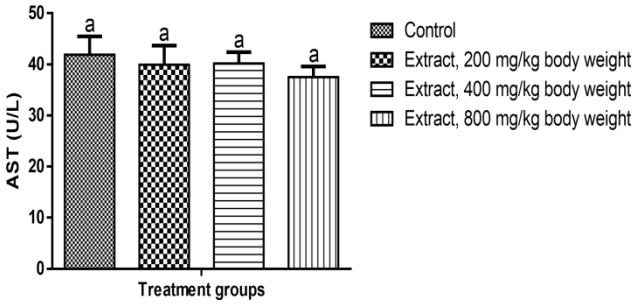


Fig. 1. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on serum levels of ALT in male Wistar rats. Data sharing the same lower-case letters are not significantly different ($P < 0.05$), whereas data with different lower-case letters are significantly different ($P < 0.05$). Values are presented as Mean ± SEM.



tanjorensis leaf on serum levels of AST in male Wistar rats. Data sharing the same lower-case letters are not significantly different ($P < 0.05$), whereas data with different lower-case letters are significantly different ($P < 0.05$). Values are presented as Mean ± SEM.

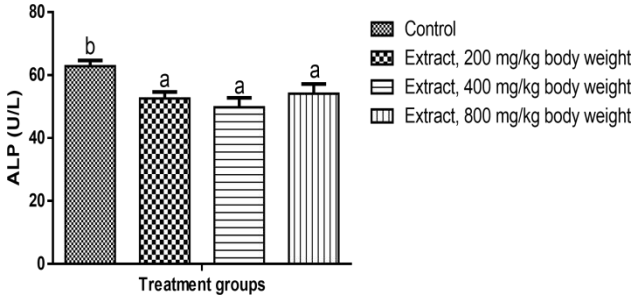


Fig. 3. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on serum levels of ALP in male Wistar rats. Data sharing the same lower-case letters are not significantly different ($P < 0.05$), whereas data with different lower-case letters are significantly different ($P < 0.05$). Values are presented as Mean ± SEM.

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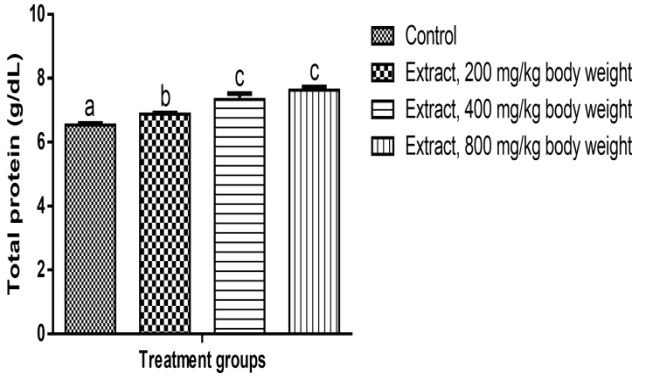

Fig. 4. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on serum total protein concentrations in male Wistar rats. Data sharing the same lower-case letters are not significantly different ($P < 0.05$), whereas data with different lower-case letters are significantly different ($P < 0.05$). Values are presented as Mean ± SEM.

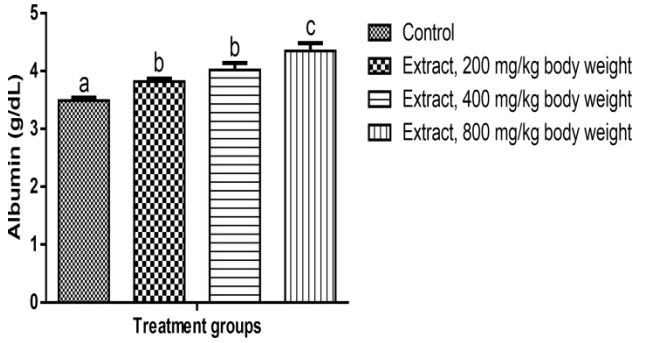

Fig. 5. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on serum albumin concentrations in male Wistar rats. Data sharing the same lower-case letters are not significantly different ($P < 0.05$), whereas data with different lower-case letters are significantly different ($P < 0.05$). Values are presented as Mean ± SEM.

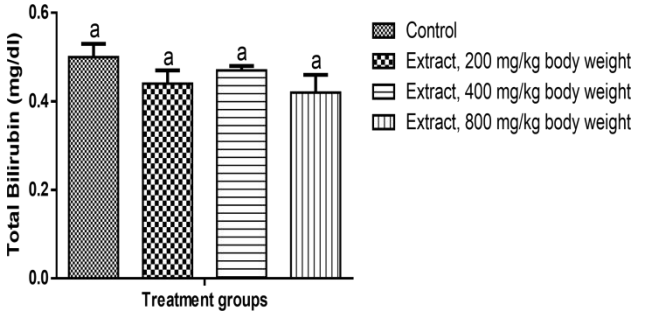

Fig. 6. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on serum total bilirubin concentrations in male Wistar rats. Data sharing the same lower-case letters are not significantly different ($P < 0.05$), whereas data with different lower-case letters are significantly different ($P < 0.05$). Values are presented as Mean ± SEM.

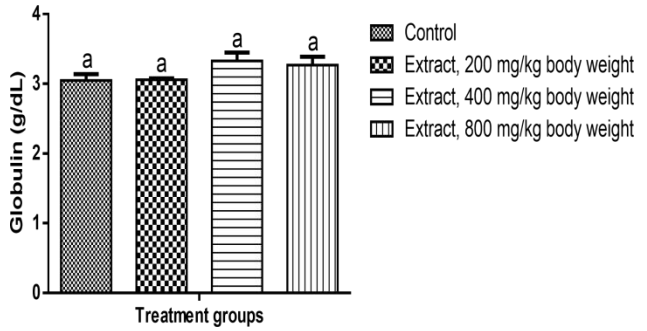

Fig. 7. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on serum globulin concentrations in male Wistar rats. Data sharing the same lower-case letters are not significantly different ($P < 0.05$), whereas data with different lower-case letters are significantly different ($P < 0.05$). Values are presented as Mean ± SEM.

Effect of Varying Doses of Aqueous Extract of *Jatropha tanjorensis* leaf on Kidney Function Indices in Sera of Male Wistar Rats

The serum urea levels in rats given 400 and 800 mg/kg of the extract did not show significant differences ($P < 0.05$) compared to the control group. However, administering 200 mg/kg of the extract resulted in a significant ($P < 0.05$) reduction in urea concentration compared to the control (Figure 8). Similarly, serum creatinine levels in rats given 400 and 800 mg/kg of the extract were not significantly different ($P < 0.05$) from the control (Figure 9). There were no significant reductions ($P < 0.05$) in serum Na^+ concentration in rats administered 200, 400, and 800 mg/kg of the extract (Figure 10). The serum chloride and potassium ion concentrations in rats given the extract were also not significantly different ($P < 0.05$) from the control (Figures 11 and 12). However, rats administered 200 mg/kg of the extract showed a significant reduction ($P < 0.05$) in serum bicarbonate ion concentration compared to the control and those given 400 mg/kg of the extract. In contrast, rats given 800 mg/kg of the extract did not show significant differences ($P < 0.05$) in serum bicarbonate levels compared to the control (Figure 13).

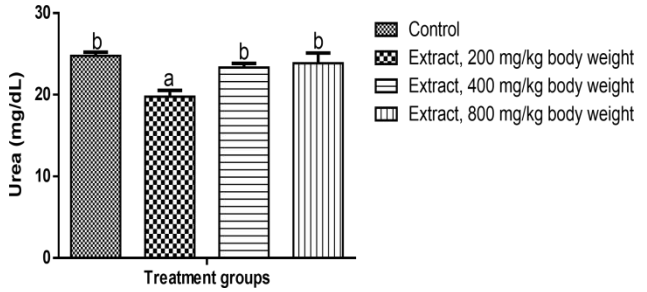

Fig. 8. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on serum urea concentrations in male Wistar rats. Data sharing the same lower-case letters are not significantly different ($P < 0.05$), whereas data with different lower-case letters are significantly different ($P < 0.05$). Values are presented as Mean ± SEM.

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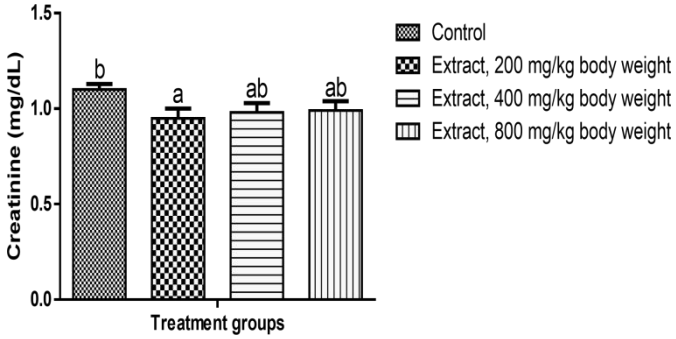

Fig. 9. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on serum creatinine concentrations in male Wistar rats. Data sharing the same lower-case letters are not significantly different ($P < 0.05$), whereas data with different lower-case letters are significantly different ($P < 0.05$). Values are presented as Mean ± SEM.

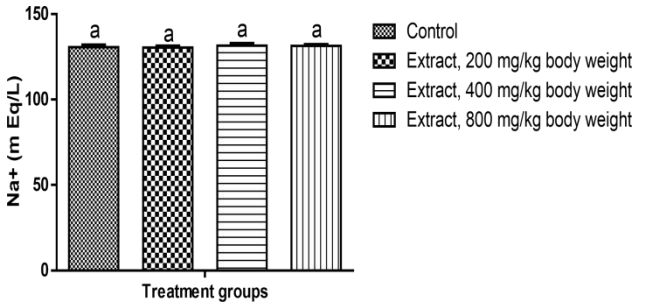

Fig. 10. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on sodium ion concentrations in male Wistar rats. Data sharing the same lower-case letters are not significantly different ($P < 0.05$), whereas data with different lower-case letters are significantly different ($P < 0.05$). Values are presented as Mean ± SEM.

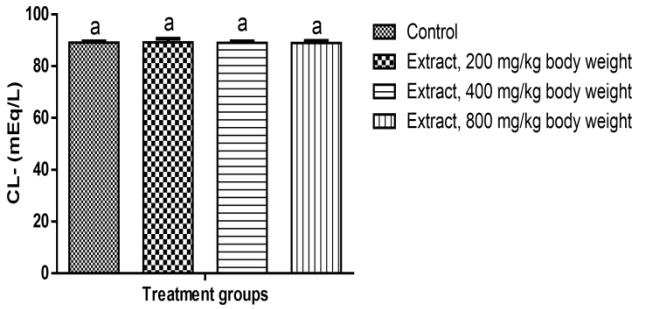

Fig. 11. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on chloride ion concentrations in male Wistar rats. Data sharing the same lower-case letters are not significantly different ($P < 0.05$), whereas data with different lower-case letters are significantly different ($P < 0.05$). Values are presented as Mean ± SEM.

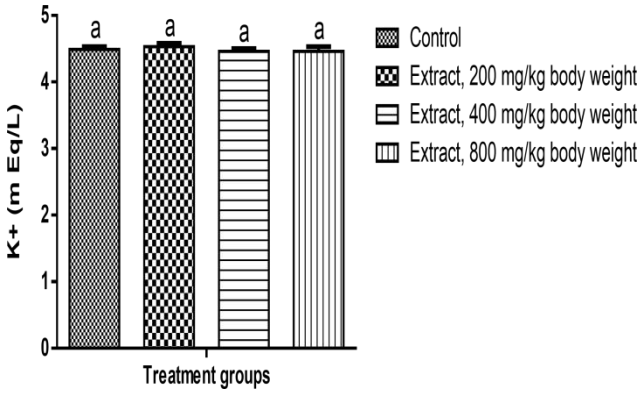

Fig. 12. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on potassium ion concentrations in male Wistar rats. Data sharing the same lower-case letters are not significantly different ($P < 0.05$), whereas data with different lower-case letters are significantly different ($P < 0.05$). Values are presented as Mean ± SEM.

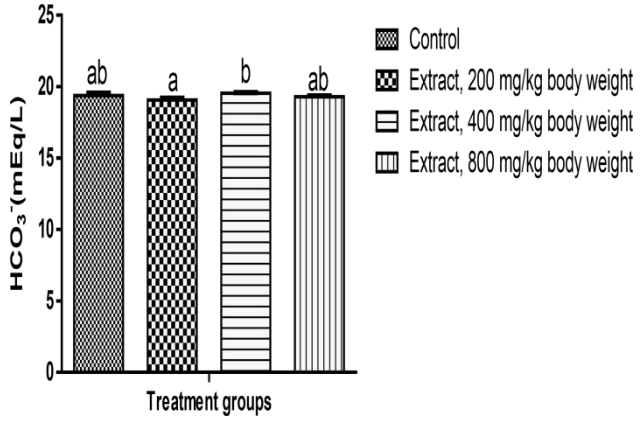

Fig. 13. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on serum bicarbonate ion concentrations in male Wistar rats. Data sharing the same lower-case letters are not significantly different ($P < 0.05$), whereas data with different lower-case letters are significantly different ($P < 0.05$). Values are presented as Mean ± SEM.

Effect of varying doses of Aqueous Extract of *Jatropha tanjorensis* Leaf on Sera Antioxidant Status in Male Wistar Rats

In this study, the rats administered varying doses of the extract exhibited significant ($P < 0.05$) increases in serum levels of SOD, CAT, GSH, and GPx, in a dose dependent manner when compared to the control groups. However, at the 800 mg/kg dose, serum levels of SOD and GSH were decreased but not statistically ($P < 0.05$) significant when compared to the 400 mg/kg group (Figures 14,15,16,17). Additionally, serum levels of MDA were significantly ($P < 0.05$) reduced in a dose dependent manner in all treatment groups when compared to the control (Figure 18).

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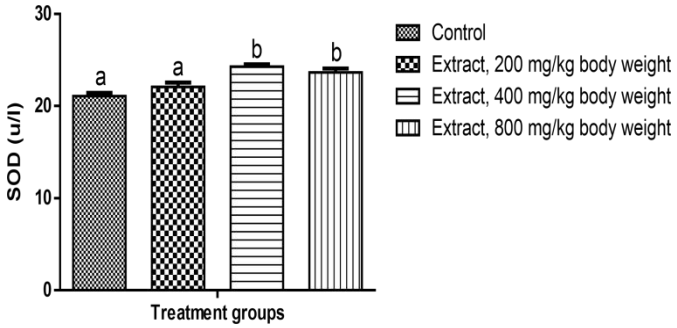

Fig. 14. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on serum SOD levels in male Wistar rats. Data sharing the same lower-case letters are not significantly different (P < 0.05), whereas data with different lower-case letters are significantly different (P < 0.05). Values are presented as Mean ± SEM.

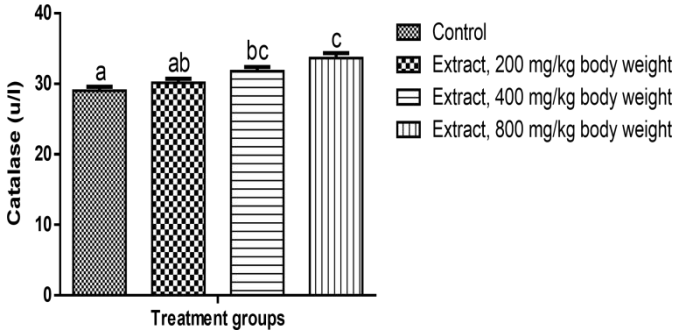

Fig. 15. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on serum CAT levels in male Wistar rats. Data sharing the same lower-case letters are not significantly different (P < 0.05), whereas data with different lower-case letters are significantly different (P < 0.05). Values are presented as Mean ± SEM.

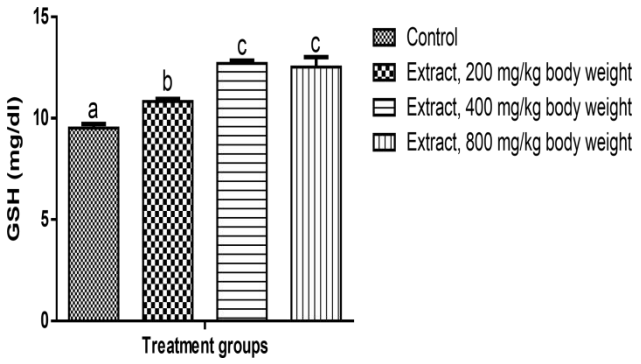

Fig. 16. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on serum GSH levels in male Wistar rats. Data sharing the same lower-case letters are not significantly different (P < 0.05), whereas data with different lower-case letters are significantly different (P < 0.05). Values are presented as Mean ± SEM.

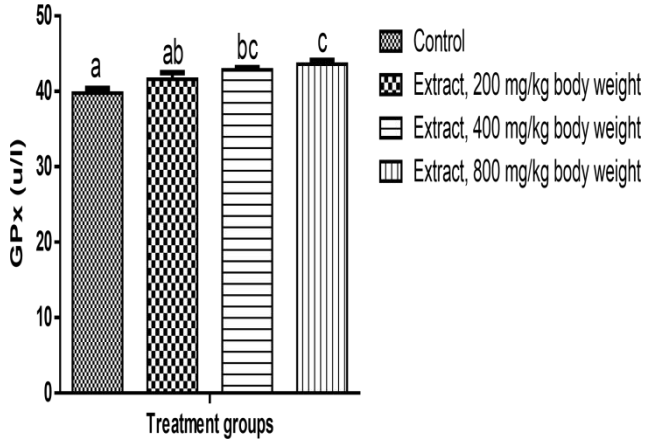

Fig. 17. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on serum GPx levels in male Wistar rats. Data sharing the same lower-case letters are not significantly different (P < 0.05), whereas data with different lower-case letters are significantly different (P < 0.05). Values are presented as Mean ± SEM.

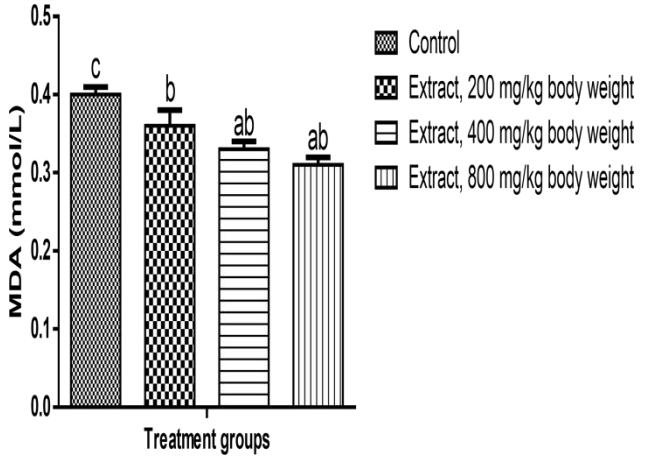

Fig. 18. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on serum MDA levels in male Wistar rats. Data sharing the same lower-case letters are not significantly different (P < 0.05), whereas data with different lower-case letters are significantly different (P < 0.05). Values are presented as Mean ± SEM.

Effect of varying doses of Aqueous Extract of *Jatropha tanjorensis* Leaf on Kidney Homogenate Supernatant and Antioxidant Status in Male Wistar Rats

In this study, rats administered varying doses of the extract showed significant increases (P < 0.05) in the antioxidant status of kidney homogenates, as evidenced by elevated levels of SOD, CAT, GSH, and GPx, in a dose-dependent manner compared to the control groups (Figures 19, 20, 21.and 22). Additionally, the levels of malondialdehyde (MDA) in the kidney homogenates were significantly reduced (P < 0.05) in a dose-dependent manner across all treatment groups compared to the control (Figure 23).

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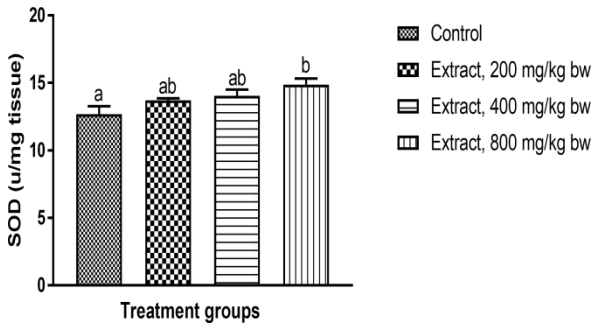

Fig. 19. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on SOD of Kidney homogenate supernatant in male Wistar rats. Data sharing the same lower-case letters are not significantly different ($P < 0.05$), whereas data with different lower-case letters are significantly different ($P < 0.05$). Values are presented as Mean ± SEM.

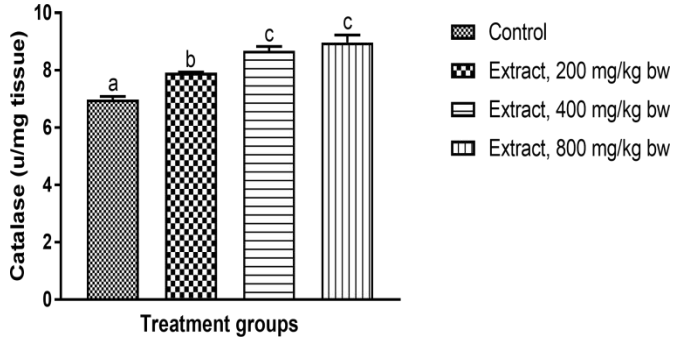

Fig. 20. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on CAT of Kidney homogenate supernatant in male Wistar rats. Data sharing the same lower-case letters are not significantly different ($P < 0.05$), whereas data with different lower-case letters are significantly different ($P < 0.05$). Values are presented as Mean ± SEM.

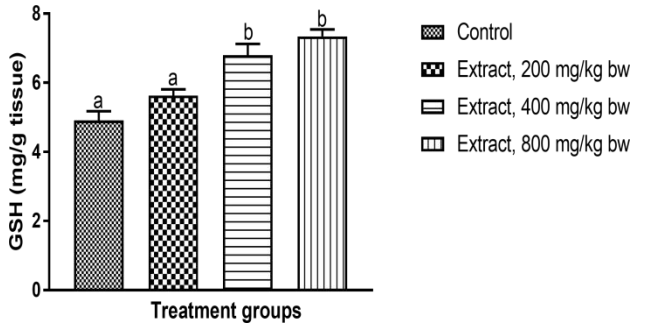

Fig. 21. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on GSH of Kidney homogenate supernatant in male Wistar rats. Data sharing the same lower-case letters are not significantly different ($P < 0.05$), whereas data with different lower-case letters are significantly different ($P < 0.05$). Values are presented as Mean ± SEM.

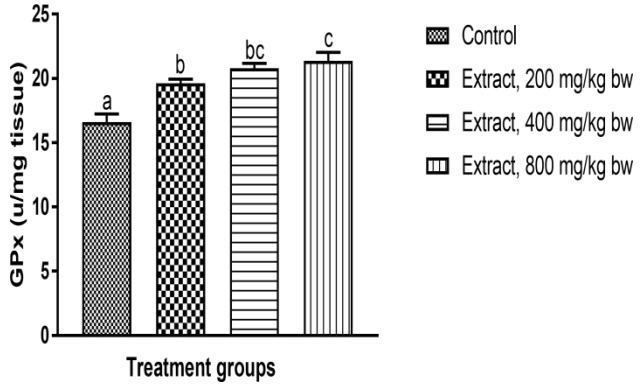

Fig. 22. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on GPx of Kidney homogenate supernatant in male Wistar rats. Data sharing the same lower-case letters are not significantly different ($P < 0.05$), whereas data with different lower-case letters are significantly different ($P < 0.05$). Values are presented as Mean ± SEM.

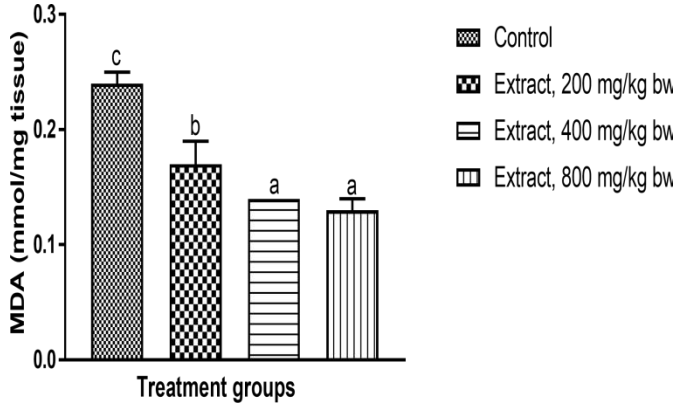

Fig. 23. Effect of different doses of aqueous extract of *Jatropha tanjorensis* leaf on MDA of Kidney homogenate supernatant in male Wistar rats. Data sharing the same lower-case letters are not significantly different ($P < 0.05$), whereas data with different lower-case letters are significantly different ($P < 0.05$). Values are presented as Mean ± SEM.

Histopathological Assessment of Liver and Kidney Ultrastructures Following Administration of Aqueous Extract of *Jatropha tanjorensis* Leaf

A. Liver

Histological analysis of liver sections from rats administered 200, 400, and 800 mg/kg of the extract revealed essentially normal histology, similar to the control group. Sections of the liver presented in all the groups showed the normal hepatic histomorphology, lacking any signs of acute or chronic damage, and portal tracts without significant inflammation (Plates 1 a, b, c and d). Normal hepatic lobules consisting of normal hepatocytes arranged in interconnecting cords, around the central veins were observed. Likewise, normal structures of the portal triads were observed.

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Group 1 (control)

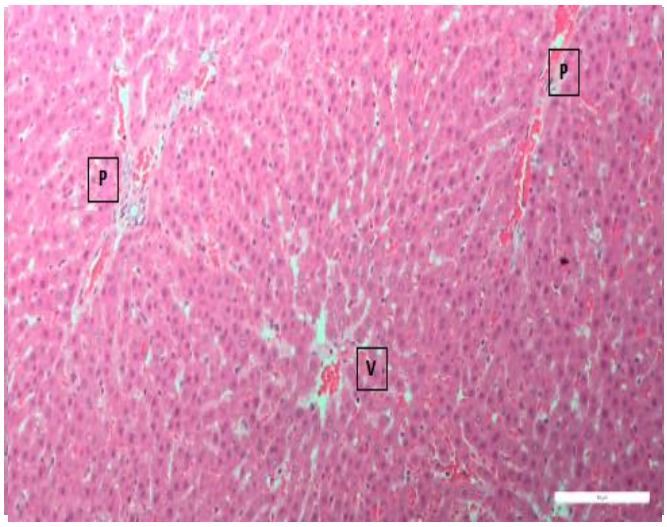

Plate 1a: The photomicrograph of the sectioned liver from control revealed normal hepatic histomorphology. The hepatic lobules appeared normal, with hepatocytes arranged in interconnecting cords around the central veins. Additionally, the structures of the portal triads were observed to be normal. H.E x200magnification/ Bar: 50µm

Central veins (V); Portal area (P) consisting of hepatic artery (HA), hepatic vein (HV) and bile duct (BD).

Group 2 (200mg/kg)

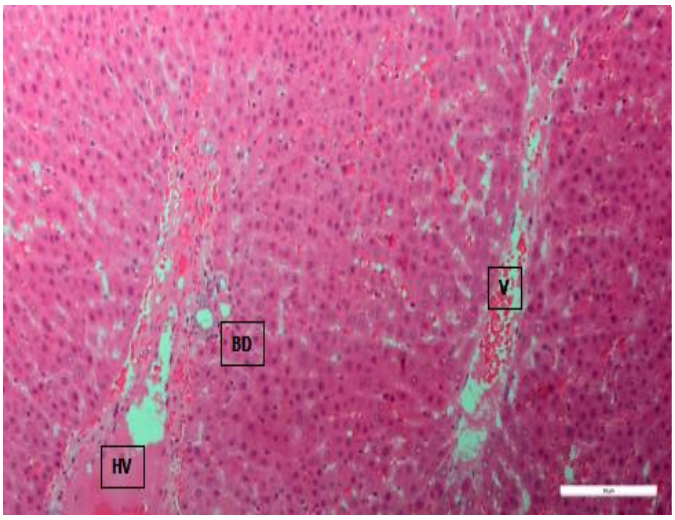

Plate 1b: The photomicrograph of the sectioned liver from 200 mg/kg treated group revealed normal hepatic histomorphology. The hepatic lobules appeared normal, with hepatocytes arranged in interconnecting cords around the central veins. Additionally, the structures of the portal triads were observed to be normal. H.E x200magnification/ Bar: 50µm

Central veins (V); Portal area (P) consisting of hepatic artery (HA), hepatic vein (HV) and bile duct (BD).

Group 3 (400mg/kg)

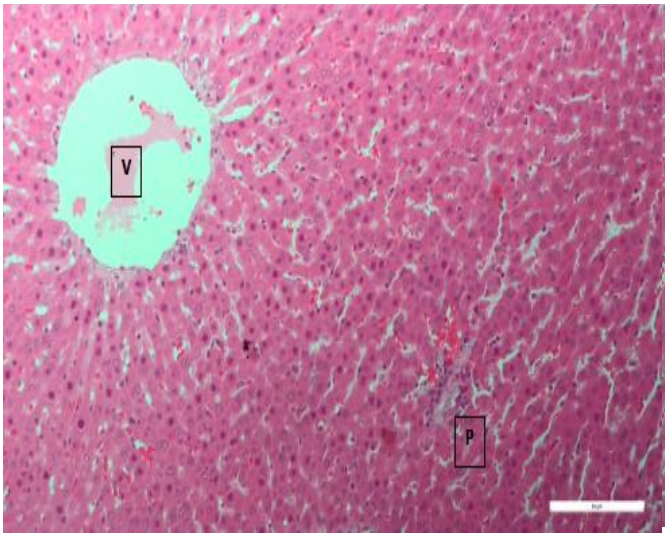

Plate 1c: The photomicrograph of the sectioned liver from 400 mg/kg treated group revealed normal hepatic histomorphology. The hepatic lobules appeared normal, with hepatocytes arranged in interconnecting cords around the central veins. Additionally, the structures of the portal triads were observed to be normal. H.E x200magnification/ Bar: 50µm

Central veins (V); Portal area (P) consisting of hepatic artery (HA), hepatic vein (HV) and bile duct (BD).

Group 4 (800mg/kg)

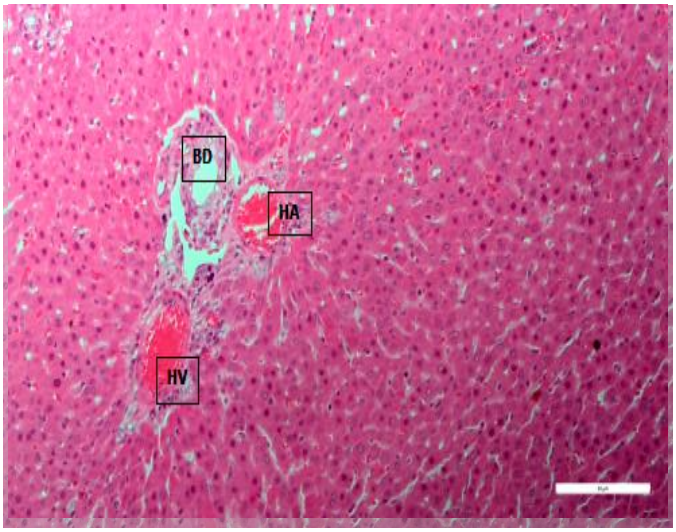

Plate 1d: The photomicrograph of the sectioned liver from 800 mg/kg treated group revealed normal hepatic histomorphology. The hepatic lobules appeared normal, with hepatocytes arranged in interconnecting cords around the central veins. Additionally, the structures of the portal triads were observed to be normal. H.E x200magnification/ Bar: 50µm

Central veins (V); Portal area (P) consisting of hepatic artery (HA), hepatic vein (HV) and bile duct (BD).

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B. KIDNEY

Histological analysis of the kidney sections from rats administered the varying doses of the extract revealed essentially normal histology, similar to the control group. The sections of the kidney presented in the various group showed normal renal histomorphology, with no indications of glomerular damage or acute or chronic tubular damage (Plate 2 a, b, c, and d). Normal glomeruli in their respective Bowmans capsules were observed, surrounded by numerous normal renal tubules (proximal convoluted tubules, pars recta, distal convoluted tubules and collecting ducts) in a highly vascularized connective tissue matrix.

Group 1 (control)

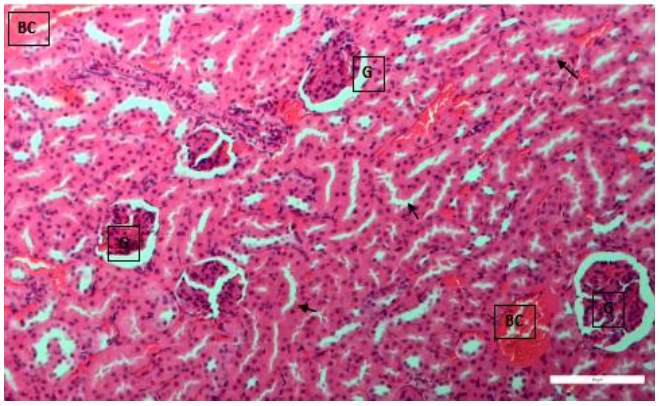

Plate 2a: The photomicrograph of the sectioned kidney from the control group displayed normal renal histomorphology. Normal glomeruli within their respective Bowman's capsules were seen, surrounded by numerous normal renal tubules in a highly vascularized connective tissue matrix. H.E x200 magnification/ Bar: 50µm

Glomeruli (G); Renal tubules (arrow); Blood capillary (BC).

Group 2 (200mg/kg)

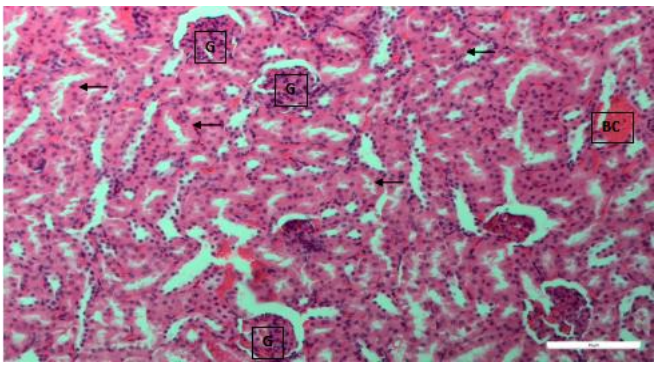

Plate 2b: The photomicrograph of the sectioned kidney from the 200 mg/kg treated group displayed normal renal histomorphology. Normal glomeruli within their respective Bowman's capsules were seen, surrounded by numerous normal renal tubules in a highly vascularized connective tissue matrix. H.E x200 magnification/ Bar: 50µm

Glomeruli (G); Renal tubules (arrow); Blood capillary (BC).

Group 3 (400mg/kg)

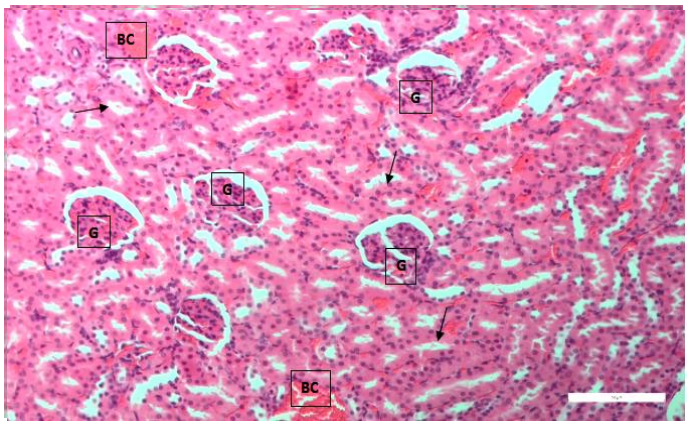

Plate 2c: The photomicrograph of the sectioned kidney from the 400 mg/kg treated group displayed normal renal histomorphology. Normal glomeruli within their respective Bowman's capsules were seen, surrounded by numerous normal renal tubules in a highly vascularized connective tissue matrix. H.E x200 magnification/ Bar: 50µm

Glomeruli (G); Renal tubules (arrow); Blood capillary (BC).

Group 4 (800mg/kg)

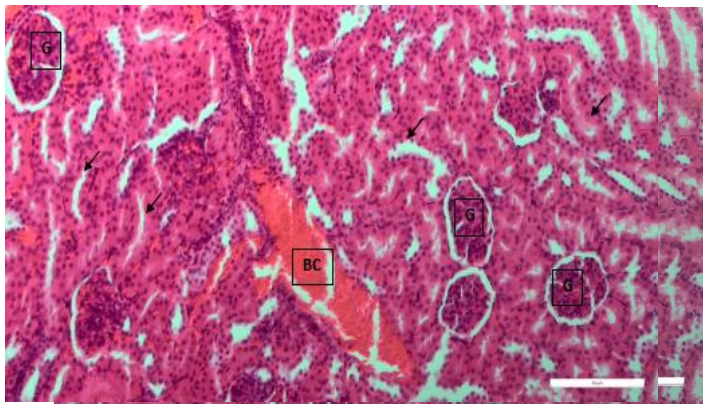

Plate 2d: The photomicrograph of the sectioned kidney from the 200 mg/kg treated group displayed normal renal histomorphology. Normal glomeruli within their respective Bowman's capsules were seen, surrounded by numerous normal renal tubules in a highly vascularized connective tissue matrix. H.E x200 magnification/ Bar: 50µm

Glomeruli (G); Renal tubules (arrow); Blood capillary (BC).

DISCUSSION

The phytochemical evaluation of *Jatropha tanjorensis* extract reveals a rich presence of bioactive compounds with diverse pharmacological properties. The extract contains a diverse array of phytochemicals, including: alkaloids, tannins, flavonoids, phenols, terpenoids, saponins, cardiac glycosides and steroids. This finding aligns with those reported by Ebenyi et al. (2021) regarding the phytochemical analysis of aqueous extract of *Jatropha tanjorensis* leaf, as well as by Igbinaduwa et al. (2011) and Oyewole et al.

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(2011) concerning the phytochemical analysis of the methanol extract of *Jatropha tanjorensis* leaf. In this study, alkaloids are present in moderate amounts. Medicinal plants generally contain alkaloids in the range of 10 to 50 mg per 100 grams. This extract, with an alkaloid content of 27.15 mg per 100 grams, falls within the typical range, suggesting a moderate level that could effectively provide therapeutic benefits like pain relief and anticancer activity without being excessively toxic. Alkaloids are recognized for their analgesic, antimalarial, antimicrobial, anticancer, and anti-inflammatory properties (Heinrich et al., 2021). Plants known for their astringent and antimicrobial qualities typically contain tannin levels ranging from 5 to 20 mg/100g. The 7.63 mg/100g tannin content in this extract falls towards the lower limit of this range, indicating its potential to deliver advantageous antimicrobial and antioxidant benefits while reducing the risk of excessive astringency. Tannins exhibit astringent, antioxidant, anti-inflammatory, and antimicrobial characteristics (Kováč et al., 2022). Flavonoids are powerful antioxidants known for their anti-inflammatory, antiviral, and anticancer properties (Hasnat et al., 2024). In medicinal plants, flavonoid content generally ranges from 20 to 50 mg/100g. With a flavonoid content of 39.18 mg/100g, this extract is on the higher end of the spectrum, suggesting it has significant antioxidant and anti-inflammatory potential. This makes the extract beneficial for preventing diseases related to oxidative stress and for managing inflammation. Plants recognized for their potent antioxidant properties usually have phenol content ranging from 30 to 70 mg/100g. This extract, with a phenol content of 46.07 mg/100g, falls within this typical range, indicating substantial antioxidant properties that can aid in the prevention and management of diseases linked to oxidative stress, such as cardiovascular diseases and cancer. Phenols are powerful antioxidants that protect against oxidative damage and help reduce the risk of chronic diseases (Kostić et al., 2023). Typically, medicinal plants have terpenoid levels ranging from 1 to 10 mg/100g. The terpenoid content of 5.41 mg/100g in this extract falls within this anticipated range, suggesting potential benefits in reducing inflammation, fighting infections, and possibly inhibiting cancer cell growth. Terpenoids exhibit anti-inflammatory, anticancer, and antimicrobial properties (Masyita et al., 2022). Plants known for their health benefits typically contain saponin levels ranging from 20 to 60 mg/100g. With a saponin content of 33.10 mg/100g falling within this usual range, the extract shows promise in managing cholesterol levels, boosting immune function, and potentially preventing cancer. Saponins are recognized for their ability to lower cholesterol, enhance immune response, and exhibit anticancer properties (Juang & Liang 2020). Cardiotonic plants typically have cardiac glycoside levels between 1 and 5 mg/100g. This extract contains 4.83 mg/100g of cardiac glycosides, which falls towards the higher end of the usual range. This suggests a robust potential for cardiotonic effects that can be beneficial in the management of heart failure and arrhythmias (Škubník et al., 2021). Cardiac glycosides are employed in treating heart conditions by fortifying cardiac contractions and regulating heart rhythm. Plants with anti-inflammatory and immunomodulatory effects typically have steroid levels ranging from 1 to 10 mg/100g. Steroids are known for their anti-inflammatory and immunomodulatory properties (Dembitsky, 2023). The extract's steroid content of 3.80 mg/100g falls within this anticipated range, indicating potential advantages in reducing inflammation and modulating immune responses. This could be valuable in treating inflammatory and autoimmune conditions like rheumatoid arthritis and type 1 diabetes.

The acute toxicity study of *Jatropha tanjorensis* in Wistar rats provided valuable insights into the safety profile of the plant extract. The absence of mortality and maintained physical stability at doses up to 6000 mg/kg indicates that *Jatropha tanjorensis* extract is relatively non-toxic at lower doses. This suggests a high margin of safety for potential therapeutic use within this dose range. At 7000 mg/kg, a significant increase in mortality rate (33.33%) indicates that toxicity begins to manifest at this dose. While the majority of mice survived, the onset of mortality at this level signals a threshold for adverse effects, highlighting the importance of dose regulation. Complete mortality at 10,000 mg/kg demonstrates that this dose is highly toxic and lethal. This drastic increase in mortality confirms a critical toxic threshold and emphasizes that doses approaching this level are dangerous and unsafe for use. The LD_{50} value (the dose at which 50% of the test population is expected to die) of 7745.97 mg/kg indicates the median lethal dose. This relatively high LD_{50} value suggests that the extract has a broad therapeutic index and is relatively safe at lower doses. The LD_{50} value is crucial for determining the safety margin and guiding dosage levels for further studies and potential therapeutic applications. This finding is consistent with the report by Ebenyi et al. (2021), which observed no behavioural changes or mortality at oral doses of 2,000 and 5,000 mg/kg within the first 24 hours and during a 14-day study period following administration of an aqueous extract of *Jatropha tanjorensis* leaf. Similarly, Igbinaduwa et al. (2011) reported no mortality or signs of behavioural changes or toxicity in mice and rats after administering methanol extract of *Jatropha tanjorensis* leaf at doses up to 8,000 mg/kg body weight.

ALT (Alanine aminotransferase) and AST (Aspartate aminotransferase) are enzymes that are commonly used as biomarkers for liver function (McGill, 2016; Chinnappan et al., 2023). The lack of significant ($p<0.05$) differences in serum ALT and AST levels suggests that the extract, at the administered doses, does not cause hepatocellular damage or stress. This outcome aligns with Ezendiokwere et al.'s 2023 study, which examined the Acute and Subacute Toxicity Profile of Diethyl ether Extract from *Jatropha tanjorensis* leaf in Male Wistar Rats. This indicates that the extract is not hepatotoxic. This implies that the extract's hepatoprotective properties may stem from its tannin content. Tannins are recognized for their antioxidant abilities, capable of scavenging free radicals and mitigating oxidative stress in cells, including those in the liver. By shielding liver cells from oxidative harm, tannins may play a role in preserving the typical levels of ALT and AST (Sobeh et al., 2017; Abu et al., 2022). While the precise mechanisms remain somewhat unclear, it's theorized that tannins might influence enzyme activity in liver cells, including ALT and AST, contributing to the maintenance of serum levels within normal ranges. ALP (Alkaline phosphatase) is another enzyme associated with liver function (McGill, 2016; Chinnappan et al., 2023), but it can also indicate bone and other tissue-related activity (Fernandez, 2007; Haarhaus et al., 2022). The significant ($p<0.05$) reduction in ALP levels suggests that the extract might have an inhibitory effect on ALP activity. This result is consistent with the findings of Chibuogwu et al. (2021) and Ezendiokwere et

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al. (2023). The presence of the phytochemicals (flavonoids, tannins, saponins, and terpenoids), through their combined antioxidant, anti-inflammatory, and enzyme-modulating properties, could be responsible for the significant reduction in ALP levels observed in the study. This effect can be beneficial in managing conditions where elevated ALP levels are a concern, such as liver diseases or bone disorders (Singab et al., 2005; Sobeh et al., 2017; Abu et al., 2022; Wang et al., 2019). The dose-dependent response, with the most significant reduction at 400 mg/kg, indicates that the extract's effect is potent and potentially dose-related. Total protein and albumin are indicators of nutritional status and liver function (Keller, 2019). An increase in these parameters suggests that the extract might have a positive effect on protein synthesis or overall nutritional status. This could indicate an anabolic effect or improved liver function in terms of protein production. This result also aligns with the findings of Ezendiokwere et al. (2023). Bilirubin is a byproduct of the breakdown of red blood cells and is processed by the liver (Guerra Ruiz et al., 2021). Normal levels of bilirubin suggest that the extract does not interfere with the liver's ability to process and excrete bilirubin, indicating that it does not induce cholestasis or impair liver function in terms of bilirubin metabolism. This result is consistent with the study conducted by Chibuogwu et al., 2021. The presence of flavonoids, tannins, saponins, and terpenoids in the extract, through their synergistic effects, may have enhanced liver function, promote protein synthesis, and ensure proper bilirubin processing, resulting in the observed improvements in total protein, albumin, and bilirubin levels (Sannigrahi et al., 2009; Wang et al., 2019; Abu et al., 2022). Globulins are a group of proteins in the blood that include antibodies (Busher, 1990). The absence of significant ($p<0.05$) changes in globulin levels suggests that the extract does not affect the immune protein synthesis or cause an immune response that would elevate these proteins. The presence of saponins and tannins in the extract, with their immunomodulatory and anti-inflammatory properties, may have helped maintain immune homeostasis without significantly impacting globulin levels (Shen et al., 2024). The extract appears to have a safe profile regarding liver enzymes (ALT, AST, bilirubin) while positively influencing ALP activity and protein levels (total protein and albumin). The reduction in ALP activity could be particularly noteworthy, indicating potential therapeutic benefits in conditions associated with elevated ALP. The increased total protein and albumin suggest potential benefits for protein synthesis or nutritional status. However, the absence of changes in globulin levels indicates no significant impact on the immune protein production, and the stable bilirubin levels suggest no negative effects on bilirubin metabolism. Further research could explore the underlying mechanisms of these effects and assess long-term safety and efficacy.

Urea is a waste product filtered by the kidneys, and its levels can indicate renal function (Kellum et al., 2021). The significant ($p<0.05$) reduction in urea levels at 200 mg/kg suggests an enhancement in renal function or increased excretion efficiency at this dose. However, this effect is not observed at higher doses (400 and 800 mg/kg), which may indicate a dose-dependent response that is optimal at lower concentrations of the extract. Creatinine is another key marker for kidney function (Zuo et al., 2008). The lack of significant ($p<0.05$) changes in creatinine levels suggests that the extract does not adversely affect renal function at these doses, maintaining normal creatinine clearance. These results are similar to the study conducted by Chibuogwu et al., 2021 on the toxicity assessment of the methanol extract of *Jatropha tanjorensis* leaf. The presence of flavonoids, tannins, saponins, and terpenoids in the extract, with their collective antioxidant, anti-inflammatory, and renal-protective properties, contributes to the observed effects on urea and creatinine levels. This indicates enhanced renal function and efficient waste excretion without negatively impacting creatinine clearance (Ullah et al., 2020; Alechinsky et al., 2020; Ma et al., 2017; Wang et al., 2019). Electrolyte balance is crucial for various physiological processes (Shrimanker & Bhattarai, 2023). The absence of significant ($p<0.05$) changes in sodium, chloride, and potassium levels indicates that the extract does not disrupt electrolyte homeostasis, which is important for maintaining overall metabolic stability. The presence of saponins and terpenoids in the extract may have supported electrolyte homeostasis and prevented disturbances in sodium, chloride, and potassium levels, ensuring overall metabolic stability (Ma et al., 2017; Wang et al., 2019). Specifically, certain terpenoids, particularly triterpenoids, possess renal-protective properties that help maintain electrolyte balance. Additionally, saponins exhibit diuretic effects, aiding in the regulation of electrolyte balance by promoting the excretion of excess sodium, chloride, and potassium while maintaining overall equilibrium. Bicarbonate is essential for maintaining acid-base balance (Hopkins et al., 2022). The significant ($p<0.05$) reduction in bicarbonate levels at 200 mg/kg could suggest a mild metabolic acidosis or an increase in bicarbonate utilization at this dose. The absence of significant changes at higher doses (400 and 800 mg/kg) suggests that this effect is specific to the 200 mg/kg dose and may not persist or might be compensated for at higher doses. The extract does not appear to impair renal function at the doses tested, as evidenced by stable creatinine levels and reduced urea levels at the 200 mg/kg dose. The reduction in urea levels at the lowest dose (200 mg/kg) suggests potential renal benefits or increased nitrogen waste excretion efficiency at this specific dose. The extract maintains normal serum concentrations of sodium, chloride, and potassium, indicating that it does not disturb electrolyte homeostasis. The reduction in serum bicarbonate at 200 mg/kg may indicate a dose-specific effect on acid-base balance, which does not extend to higher doses. The significant ($p<0.05$) reduction in bicarbonate levels could be attributed to the presence of saponins and terpenoids in the extract. However, these findings differ from the study conducted by Ezendiokwere et al., 2023, where serum potassium ion levels were not significant, but serum levels of sodium, chloride, and bicarbonate ions were significantly increased. Saponins are known for their diuretic and metabolic effects, which can influence acid-base balance. Terpenoids may impact bicarbonate levels through their effects on renal excretion and metabolic pathways, potentially causing mild metabolic acidosis at specific doses. Additionally, flavonoids have been shown to affect metabolic pathways involved in acid-base balance, and at certain doses, they may enhance metabolic activity or increase bicarbonate utilization, resulting in reduced bicarbonate levels. Further research is warranted to explore the mechanisms underlying these effects and to determine the long-term safety and efficacy of the extract at various doses.

In this study, the impact of *Jatropha tanjorensis* leaf extract on

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various antioxidant biomarkers was evaluated in rats, with the extract administered in varying doses. The biomarkers assessed included superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx), and malondialdehyde (MDA). SOD is an essential enzyme that converts superoxide radicals into oxygen and hydrogen peroxide, offering protection against oxidative stress (Wang et al., 2018). CAT, another key antioxidant enzyme, catalyses the transformation of hydrogen peroxide into water and oxygen, thereby mitigating potential damage from reactive oxygen species (Nandi et al., 2019). GSH is a crucial antioxidant that neutralizes free radicals and reactive oxygen compounds, reducing oxidative stress (Pizzorno, 2014; Basil, 2023). GPx plays a significant role in converting lipid hydroperoxides to their corresponding alcohols and reducing free hydrogen peroxide to water (Pei et al., 2023). MDA serves as a marker of lipid peroxidation and indicates oxidative stress levels (Cordiano et al., 2023).

The dose-dependent increases ($p<0.05$) in serum SOD, CAT, GSH, and GPx levels highlight the extract's potential to bolster the body's antioxidant defence mechanisms, which is crucial for mitigating oxidative stress-related damage. This result is consistent with Ezendiokwere et al., 2023, which assessed the acute and subacute toxicity of diethyl ether extract of *Jatropha tanjorensis* leaf in male Wistar rats. However, the observation that SOD and GSH levels did not significantly increase at the highest dose (800 mg/kg) compared to the 400 mg/kg dose suggests a potential ceiling effect. This indicates that beyond a certain dosage, additional extract may not provide further antioxidant benefits, and the optimal effective dose might lie around the 400 mg/kg mark. The combined presence of flavonoids, terpenoids, and saponins in the extract likely synergized to enhance the body's antioxidant defense mechanisms, crucial for mitigating oxidative stress-induced damage and preserving overall health. The significant ($p<0.05$) reduction in MDA levels across all doses further supports the extract's role in reducing oxidative stress and protecting against lipid peroxidation. These results are consistent with the findings of Ezendiokwere et al., 2023. The combination of flavonoids, saponins, and terpenoids in the extract may have acted synergistically to lower oxidative stress and thwart lipid peroxidation, leading to a notable decrease in MDA levels across all extract doses. Flavonoids act as potent antioxidants, neutralizing free radicals and hindering lipid peroxidation, thereby reducing oxidative stress and preserving cell integrity. Saponins contribute to this antioxidant effect by scavenging free radicals and regulating oxidative processes, thus shielding against lipid peroxidation and diminishing MDA levels. Additionally, certain terpenoids like carotenoids and tocopherols demonstrate robust antioxidant properties, counteracting free radicals and safeguarding cell membranes against oxidative harm, ultimately culminating in reduced MDA levels. This reduction is critical, as oxidative stress and lipid peroxidation are implicated in the pathogenesis of various diseases, including cardiovascular diseases, neurodegenerative disorders, and cancer. The dose-dependent effects observed for most antioxidants suggest that careful dosing is crucial to maximize the therapeutic benefits while avoiding potential plateaus in efficacy. This extract could be a valuable natural remedy for conditions associated with oxidative stress, although further studies are warranted to fully understand its pharmacokinetics, optimal dosing, and long-term safety.

This study also examined the impact of varying doses of the extract homogenates of Wistar rats. The findings suggest that the extract significantly enhances the antioxidant defence system in the kidneys, with each enzyme playing a crucial role in mitigating oxidative stress. The significant, dose-dependent increases in SOD, CAT, GSH, and GPx levels indicate a robust enhancement of the kidney's capacity to neutralize reactive oxygen species and protect against oxidative damage, with higher doses progressively boosting antioxidant capacity. The dose-dependent reduction in MDA levels reflects decreased lipid peroxidation and oxidative damage, confirming the increased activity of antioxidant enzymes. These results underscore the extract's potential for protecting renal tissue from oxidative damage and improving overall kidney health. Further research is needed to explore the long-term effects, optimal dosing, and mechanisms underlying these benefits to fully understand the extract's therapeutic potential for kidney health.

The observation of normal hepatic histomorphology across all groups indicates that the extract does not cause any histopathological changes or damage to liver tissue, demonstrating its non-hepatotoxic nature even at varying doses. Hepatic lobules, the functional units of the liver, with hepatocytes arranged in cords around the central veins, remained intact. This suggests that the extract does not disrupt the liver's architecture or function. The portal triad, comprising the hepatic artery, hepatic vein, and bile duct, appeared normal, indicating that the extract does not negatively impact the liver's blood supply, venous drainage, or bile production and flow. The clear identification of central veins and portal areas further confirms the intact and normal histological features of the liver, suggesting that the extract does not cause any pathological alterations that would compromise the liver's vascular or biliary systems. These findings support the potential use of the extract without the risk of liver damage. Further studies should investigate the long-term effects and the mechanisms by which the extract maintains liver health to fully establish its safety profile. The histological analysis of kidney sections from rats administered varying doses of the extract reveals normal renal histomorphology in all the treated groups. This suggests that the extract does not induce any adverse structural changes in kidney tissue, indicating its non-nephrotoxic nature. The absence of damage to glomeruli and renal tubules implies that the extract does not disrupt the filtration and reabsorption processes critical to kidney function, further supporting its safety for renal health. The presence of normal glomeruli and renal tubules signifies that the fundamental functional units of the kidney are intact and functioning properly. The highly vascularized connective tissue matrix indicates that the blood supply to the kidneys remains unaffected by the extract, ensuring proper renal function and health. Overall, the normal appearance of glomeruli, renal tubules, and blood capillaries in the treated groups suggests that the extract preserves kidney function and structural integrity. These results are consistent with the study conducted by Chibuogwu et al., 2021 on the toxicity assessment of the methanol extract of *Jatropha tanjorensis* leaves. However, the highest dose of 400 mg/kg of the methanol extract caused histopathological lesions in the livers of rats. The liver sections showed mild to moderate vacuolar degeneration and necrosis of the centrilobular hepatocytes within the hepatic lobules, indicative of toxicity. These findings support the safe use of the extract concerning kidney health. Further studies should explore the long-term renal effects and the mechanisms by which the extract maintains normal kidney histology.

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Conclusion

The aqueous extract of *Jatropha tanjorensis* leaf demonstrates a rich phytochemical profile with diverse therapeutic potentials. It is relatively safe at lower doses, enhancing antioxidant defences and showing no adverse effects on liver and kidney histology. These findings support its potential use for health benefits, particularly concerning antioxidant protection and renal health, warranting further research into long-term effects, optimal dosing, and detailed mechanisms of action to fully elucidate the extract's therapeutic potential and safety profile.

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