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Effect of Ethanol extract of *Justicia carnea* on Liver and Kidney function of Cadmium - induced Hepatotoxicity in Male Wistar Rats

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ABSTRACT

The traditional use of Justicia carnea as a blood and liver tonic lacks scientific validation, and cadmium-induced hepatotoxicity poses significant health risks, prompting this study to investigate the hepatoprotective and nephroprotective effects of Justicia carnea on cadmiuminduced toxicity in male Wistar rats. The extract's traditional use as a blood and liver tonic was scientifically validated through various biochemical assessments. Phytochemical analysis revealed high concentrations of naringin, vinnillic acid, and nobiletin, while proximate composition showed notable levels of moisture, carbohydrate, and protein. The extract also contained high vitamin C content, indicating strong antioxidant potential. Cadmium exposure significantly elevated liver enzymes, bilirubin, urea, and creatinine levels, indicating liver and kidney damage. However, treatment with J. carnea extract (100mg/kg and 300mg/kg) significantly (p ≤ 0.05) improved these parameters, with the higher dose showing more pronounced effects. The extract also improved hematological indices, including RBC, Hb, and PCV levels, supporting its blood-enhancing reputation. Body weight gain was observed in treated groups, indicating better health and recovery. The study's findings suggest that J. carnea ethanol extract possesses significant hepatoprotective, nephroprotective, and hematopoietic properties, effectively mitigating cadmium-induced liver and kidney damage. The extract was found to be safe at both tested doses, with no signs of acute toxicity. Overall, this study validates the traditional use of *J. carnea* as a blood and liver tonic and highlights its potential as a natural therapeutic agent for managing heavy metal-induced toxicity and oxidative stress-related disorders.

Keywords: Proximate, Phytochemical, Antioxidant, Vitamins, Justicia Carnea

INRODUCTION

Traditional medicine has long served as a cornerstone for treating various human particularly in ailments, African societies where its integration continues to grow. In Nigeria, plants like Justicia carnea (commonly referred to "ogwuobara" by the Igbo, "èwe ajeri" or "èwe eje" by the Yoruba, and known locally as "Hospital too far" or "Blood of Jesus") are extensively used for their medicinal presumed properties, especially as blood and liver tonics (Okonkwo, 2012; Corrêa and Alcântara, 2012).

The plant belongs to the Acanthaceae family and has been shown to contain phytochemicals numerous such phenols, terpenoids, flavonoids, tannins, alkaloids, saponins, glycosides, and essential vitamins (A, B-complex, C, and E), with vitamin C occurring in the highest concentration (Harborne, 1998; Orjiakor et al., 2019). Its high iron and calcium content further supports its traditional use in improving hematological health. Medicinally, J. has been used carnea to treat inflammation, respiratory infections, gastrointestinal disorders. arthritis. diabetes, liver diseases, and more (Badami et al., 2003; Al-Juaid and

Abdel-Mojib, 2004; Correa and Alcantara, 2012).

Cadmium (Cd), a non-essential and highly toxic heavy metal, poses a significant environmental and public health threat due to its prevalence in industrial processes such electroplating, cigarette production, and petrochemical refining (Poli, 2022). Classified as a Class I carcinogen by the IARC (Wan et al., 2021), cadmium exerts its toxic effects primarily by with essential competing metals, disrupting mitochondrial function, binding sulfhydryl groups in proteins, and altering gene expression related to antioxidant enzymes (Gafar et al., 2022; Okutu et al., 2022; Domouky et al., 2022; Andjelkovic, 2019). It triggers oxidative stress by generating reactive oxygen species (ROS) and reducing endogenous antioxidants such glutathione, catalase, and superoxide dismutase (Branca et al., 2020; Noor, 2022).

Cadmium's effect on the liver includes increased levels of liver enzymes (AST, ALT, ALP), total and direct bilirubin, and a decline in total protein synthesis due to impaired hepatic antioxidant systems. It also interacts with the immune system by promoting

proinflammatory cytokines like TNF-α and activating apoptotic pathways involving caspase-3 (Kumar *et al.*, 2021; Mirkov *et al.*, 2021; Chen *et al.*, 2022; Abdelraze *et al.*, 2016). Additionally, cadmium adversely affects the hematopoietic system, contributing to anemia (Khannazer *et al.*, 2020), and compromises renal function by elevating blood urea and creatinine levels.

Wistar rats are commonly used in and toxicology pharmacological research due to their predictable biology, moderate size, and lower spontaneous tumor incidence compared to other strains (McCormick, 2017). In assessing hepatotoxicity, aminotransferase levels are used as indicators. According to Tseng et al. (2014), hepatotoxicity grading is based on the elevation of AST and ALT levels above the upper limit of normal (ULN), with five classifications ranging from Grade 0 (<1.25× ULN) to Grade IV ($>10 \times$ ULN).

In this context, the ethanol extract of *Justicia carnea* has shown promising protective effects against cadmiuminduced liver and kidney toxicity in male Wistar rats. The extract demonstrated significant antioxidant and hepatoprotective properties, reversing cadmium-induced alterations in

biochemical markers such as ALT, AST, ALP, bilirubin, creatinine, and urea levels. It also improved hematological parameters, supporting its traditional use and suggesting its potential as a natural remedy for cadmium toxicity and related oxidative stress disorders.

MATERIALS AND METHODS

Experimental Design

A total of twenty-five (25) male Wistar rats were used for the main study and were randomly divided into five (5) experimental groups, each comprising five (5) animals. The study was conducted over a period of one month. Hepatotoxicity was induced in the experimental groups (Groups B–E) through daily intraperitoneal administration of cadmium chloride (Cd) at a dose of 5 mg/kg body weight. regimens Treatment commenced concurrently with cadmium induction and were administered daily. The groups and their specific treatments are as follows:

• Group A – Normal Control:

This group served as the baseline control. Animals received standard feed and water only, without cadmium induction or treatment.

• **Group B – Negative Control:**

Rats in this group were administered cadmium (5 mg/kg/day) to induce hepatotoxicity but received no therapeutic intervention. This group was used to observe the full extent of cadmium toxicity.

Group C – Positive Control (Standard Drug):

Following cadmium induction (5 mg/kg/day), rats in this group were treated with silymarin at a dosage of 100 mg/kg/day. Silymarin, a known hepatoprotective agent, served as the standard treatment reference.

Group D – Low Dose Justicia carnea Extract:

After cadmium induction (5 mg/kg/day), rats in this group received 100 mg/kg/day of ethanol leaf extract of *Justicia carnea*. This dosage was used to evaluate the extract's efficacy at a standard therapeutic dose.

Group E – High Dose Justicia carnea Extract:

Similar to Group D, rats in this group were subjected to cadmium-induced hepatotoxicity (5 mg/kg/day), but were treated with a higher dose of ethanol extract of *Justicia carnea* at 300

mg/kg/day. This group was included to assess the dose-dependent effects of the plant extract.

This experimental design allowed for the comparative assessment of *Justicia carnea*'s hepatoprotective and renoprotective potentials at two dosage levels, in relation to a known standard drug (silymarin) and untreated toxicity.

Collection, Identification and Preparation of the Plant Materials

Fresh leaves of Justicia carnea were sourced from Eke Igwe Orizu Market located in Nnewi North Local Government Area, Anambra State, Nigeria. The plant species was identified taxonomically and authenticated by Dr. B. N. Uwalaka at the University of Agriculture and Environmental Sciences, Umuagwo, Imo State, where it was deposited with herbarium voucher the number UAES/HB/0037.

Upon collection, the leaves were carefully selected, rinsed with clean water to remove surface contaminants, and initially sun-dried. To ensure complete dehydration, the leaves were further subjected to drying in a hot-air oven maintained at 60°C. The dried

leaves were then ground into a uniform fine powder using an electric grinder and stored in sealed, air-tight containers until required for extraction and subsequent experimental procedures.

Experimental Animals:

This investigation involved a total of 38 male Wistar albino rats, each weighing between 120–150g and approximately three weeks old. Thirteen of these animals were exclusively utilized for determining the median lethal dose (LD₅₀) of the ethanol leaf extract of *Justicia carnea*. The remaining twenty-five rats were designated for the primary experimental phase. All animals were procured from Chris Experimental and Research Farm, located in Awka, Anambra State.

Prior to the commencement of treatment, the test animals were housed in groups of five per cage using well-aerated stainless steel cages and were allowed a 7-day acclimatization period to adapt to the laboratory environment. The rats were maintained under standard environmental conditions and received free access to clean drinking water and a nutritionally balanced pelleted grower diet (Vital Feed®, produced by Grand Cereals and Oil Mills, Jos, Nigeria). All experimental protocols adhered strictly

to internationally accepted ethical standards for the care and use of laboratory animals.

Preparation of Ethanol Extracts of Justicia carnea:

In this study investigating the hepatoprotective and nephroprotective potential of Justicia carnea, the dried and pulverized leaves of the plant underwent ethanol-based extraction. A specific quantity of the leaf powder was immersed in 70% ethanol and left undisturbed for a 72-hour period, with intermittent agitation to enhance solute dissolution. Following the maceration phase, the mixture was passed through Whatman No. 1 filter paper to remove insoluble plant debris. The ethanol-rich solution obtained was then concentrated using evaporator rotary at temperature maintained below 45°C under reduced pressure. This process yielded a semi-solid extract, which was further air-dried into a solid form and preserved in a sealed container under refrigerated conditions until further use in experimental assays.

To characterize the chemical composition of the ethanol extract, Gas Chromatography-Flame Ionization Detection (GC-FID) was employed. The analysis was conducted using a BUCK

M910 gas chromatograph equipped with a flame ionization detector and a RESTEK MXT-1 capillary column, designed to effectively separate and detect the volatile components of the extract. The analysis revealed the of several secondary presence metabolites, including flavonoids, alkaloids, tannins, saponins, and phenolic compounds. These bioactive constituents are recognized for their antioxidant potent and antiinflammatory functions, as well as their roles in promoting hepatic and renal health.

In particular, the identified flavonoids and phenolic compounds are known to exert free radical neutralizing effects and to contribute to the stabilization of cell membranes under toxic stress. Their presence in the extract lends strong support to the ethnomedicinal reputation of Justicia carnea as a plant used for blood and liver health, especially in conditions involving oxidative damage. The rich phytochemical profile of this extract strengthens the rationale for its application in the management of cadmium-induced liver and kidney toxicity, highlighting its value as a promising natural therapeutic agent.

Aspartate Transaminase Enzyme (AST) Assay determination:

The activity of aspartate transaminase (AST), an enzyme involved in amino acid metabolism and an essential biomarker for evaluating liver function, was quantified using a colorimetric method based on the procedure originally described by Reitman and Frankel (1957), utilizing a Randox diagnostic kit. The enzymatic reaction is based on the transamination between Laspartate and α-oxoglutarate catalyzed by AST, forming L-glutamate and oxaloacetate. The oxaloacetate produced with subsequently reacts 2.4dinitrophenylhydrazine (DNPH) to form hydrazone complex, which measured spectrophotometrically at a wavelength of 505 nm.

required The assay fresh, nonhemolyzed serum samples and specific reagents including AST substrate (containing L-aspartate and ketoglutarate) and DNPH, all supplied by Randox. The procedure involved incubating serum with substrate at 37°C for 30 minutes, halting the reaction with DNPH, and allowing color development over 20 minutes. Thereafter, 0.4 N NaOH was added to stabilize the developed color before measuring the absorbance. Blank controls were prepared using distilled water in place of serum to account for baseline absorbance. The final AST activity was calculated using a calibration factor provided in the kit or by referencing a standard curve, with results expressed in units per liter (U/L).

The reliability of the method was upheld by adhering to strict quality control protocols, including the use of normal and pathological serum controls and regular spectrophotometer calibration. To prevent erroneous elevation in AST values, samples were handled cautiously hemolysis, and avoid precise incubation times and temperatures were maintained. The accuracy and reproducibility of this assay make it a standard method for routine clinical and experimental assessment ofliver integrity, particularly in studies involving hepatotoxicity such as those induced by cadmium exposure. This method remains a vital tool biochemical diagnostics, confirming its relevance in liver function analysis in both human and animal studies (Reitman and Frankel, 1957).

Determination of Alanine
Transaminase (ALT) Activity Using
Colorimetric Method

The assay for alanine transaminase (ALT), a key liver enzyme indicative of hepatic function, was performed using a colorimetric method with the Randox diagnostic kit following the procedure described by Reitman and Frankel (1957). The assay is based on the enzymatic conversion of L-alanine and α-oxoglutarate to pyruvate and Lglutamate, catalyzed by ALT (also known as GPT). The resulting pyruvate forms a hydrazone complex with 2,4dinitrophenylhydrazine (DNPH), which produces a measurable color change. This color intensity, stabilized with sodium hydroxide (NaOH), is then measured spectrophotometrically at 505 nm.

To conduct the test, reagents and serum samples were prepared according to the manufacturer's instructions. The procedure involved incubation at 37°C for 30 minutes, followed by color development with DNPH and stabilization with NaOH. Absorbance readings of test samples were taken against a blank containing distilled water. ALT enzyme activity was calculated using a calibration factor provided by the kit, or alternatively, by plotting on a standard curve. Quality control measures included the use of both normal and abnormal control sera to validate assay

reliability, and care was taken to prevent hemolysis and degradation of serum samples.

This method is a standard and reliable procedure used in clinical and research settings for diagnosing and monitoring liver function, as ALT elevation is closely associated with liver cell damage. The procedure's precision, reproducibility, and sensitivity make it valuable for detecting hepatocellular injury in toxicological studies, such as the evaluation of hepatoprotective agents like *Justicia carnea*.

Estimation of Alkaline Phosphatase (ALP) Activity Using Colorimetric Method

Alkaline phosphatase (ALP) activity, a key biomarker for liver and bone health, was assessed colorimetrically using the Randox diagnostic kit, following the guidelines set by the Deutsche Gesellschaft für Klinische Chemie (GSCC). The assay is based on the enzymatic hydrolysis of nitrophenylphosphate to p-nitrophenol and inorganic phosphate, with pnitrophenol forming a yellow-colored chromogen measurable at 405 nm.

To perform the test, 1.0 mL of ALP working reagent was added to test tubes

designated for sample and blank. Then, 0.02 mL of either the serum sample or distilled water (for the blank) was added. The tubes were mixed and incubated at 37°C for 1 minute, after which the initial absorbance (A1) was recorded. Following a further 1-minute incubation, the final absorbance (A2) was measured. ALP activity was calculated using the change in absorbance over time (ΔA), the total reaction volume, sample volume, cuvette path length, and the molar absorptivity of p-nitrophenol (18.75 L/mmol•cm). Alternatively, the calibration factor provided in the Randox kit was used for simplified calculations.

Strict quality control measures were observed using known normal and abnormal serum The controls. spectrophotometer was calibrated, and all reagents were handled according to manufacturer protocols. Only fresh, nonhemolyzed serum samples were used to avoid data interference, and precise pipetting techniques were applied. This method is a standard and reliable tool in clinical and experimental settings for evaluating liver function, particularly useful in toxicological studies such as those involving Justicia carnea for mitigating cadmium-induced hepatotoxicity.

Evaluation of Renal Biomarkers in Cadmium-Induced Nephroprotective Effects of *Justicia carnea* Ethanol Extract in Male Wistar Rats:

In assessing kidney function following cadmium-induced hepatotoxicity and the potential protective influence of Justicia carnea ethanol extract in male Wistar rats, several biochemical parameters evaluated validated were using colorimetric techniques. Urea concentration was measured following the Berthelot method, which involves enzymatic hydrolysis of urea ammonia, followed by the formation of a blue-colored indophenol complex in the presence of phenol, hypochlorite, and sodium nitroprusside under alkaline conditions. This color intensity was quantified spectrophotometrically and used to calculate urea levels by comparison to a standard.

Creatinine levels were determined through the Jaffe method, creatinine reacts with picric acid in an alkaline medium to yield a reddishorange complex. The absorbance of this complex was recorded and used to calculate the creatinine concentration, providing insight into glomerular filtration efficiency. To further examine renal function, serum bicarbonate was analyzed enzymatically by exploiting its conversion to oxaloacetate and subsequent reduction to malate, coupled with oxidation of NADH to NAD⁺. The decrease in NADH absorbance was monitored at 340 nm, and bicarbonate levels were deduced based on this change.

Chloride ion levels were determined via a colorimetric reaction in which chloride displaces thiocyanate from mercuric thiocyanate, forming free thiocyanate ions. These then reacted with ferric ions to yield a red-colored complex, with absorbance measured at 480 nm. This intensity directly reflected chloride concentration. Sodium concentration was assessed using a chromogenic reaction where sodium interacts with a selective reagent to produce a colored complex. The strength of this color, measured at 580 nm, correlated with sodium content in the sample.

Potassium determination involved using sodium tetraphenylboron, which reacts with potassium to form a turbid suspension. The degree of turbidity, indicative of potassium levels, was recorded at 580 nm. All samples were processed under strictly controlled conditions, using only fresh, non-hemolyzed serum or plasma to prevent

analytical interference. Each assay followed precise incubation periods and reagent handling protocols to ensure reproducibility and reliability.

Collectively, these methods allowed for a comprehensive evaluation of renal parameters in the experimental groups, helping to elucidate whether *Justicia* carnea exerted a nephroprotective effect in the context of cadmium-induced toxicity. The results derived from these assays contribute to understanding the therapeutic potential of this plant extract in preserving kidney function under toxic stress.

Data Analysis

The experimental data were presented as the mean values accompanied by their standard deviations (mean \pm SD), based on triplicate measurements to ensure accuracy and reliability. Statistical evaluations were performed using SPSS software, version 23. To determine significant differences among treatment groups, a one-way analysis of variance (ANOVA) was employed. Where ANOVA indicated significance, pairwise group comparisons were further assessed using the Least Significant Difference (LSD) post hoc test. Statistical significance was established at a confidence level of p <

0.05, which served as the threshold for determining meaningful differences in kidney function biomarkers across the various experimental conditions involving cadmium-induced toxicity and treatment with *Justicia carnea* ethanol extract.

RESULTS

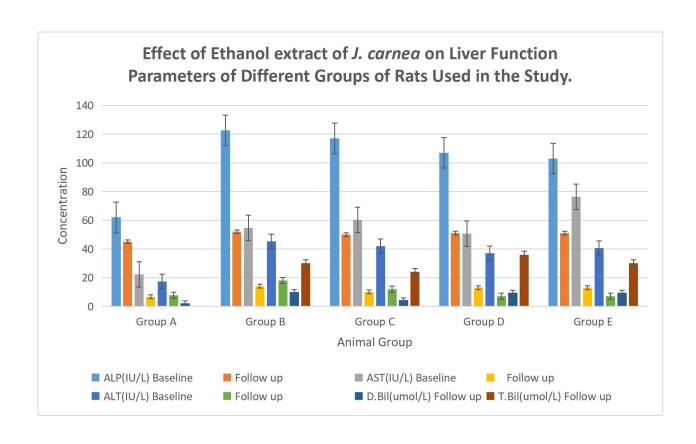
Effect of Ethanol Extract of *J. carnea*on Liver Function Parameters of Different Groups of Rats Used In the Study

The graph summarizes liver function test outcomes across five experimental groups, highlighting the impact of Justicia carnea ethanol extract on cadmium-induced hepatotoxicity in male Wistar rats. Key liver biomarkers assessed included ALP, AST, ALT, direct bilirubin (D.Bil), and total bilirubin (T.Bil). Group A served as the normal control, while Group B (negative control) received cadmium without treatment and displayed elevated liver enzyme levels, indicating hepatic injury. Group C, treated with 100 mg/kg Silymarin, moderate showed improvement.

Notably, Groups D and E, administered 100 mg/kg and 300 mg/kg of *J. carnea* extract respectively, demonstrated

marked restoration of liver function. Group D exhibited reduced levels of AST (50.67 ± 4.26), ALT (37.00 ± 3.21), ALP (107.33 ± 8.76), and D.Bil (9.50 ± 0.00), alongside a rise in T.Bil (36.00 ± 0.00). Group E, receiving the higher

dose, showed even greater normalization of liver markers, suggesting a dose-dependent hepatoprotective effect of the extract. Overall, the findings support the therapeutic potential of *Justicia carnea* in mitigating liver damage caused by cadmium toxicity.



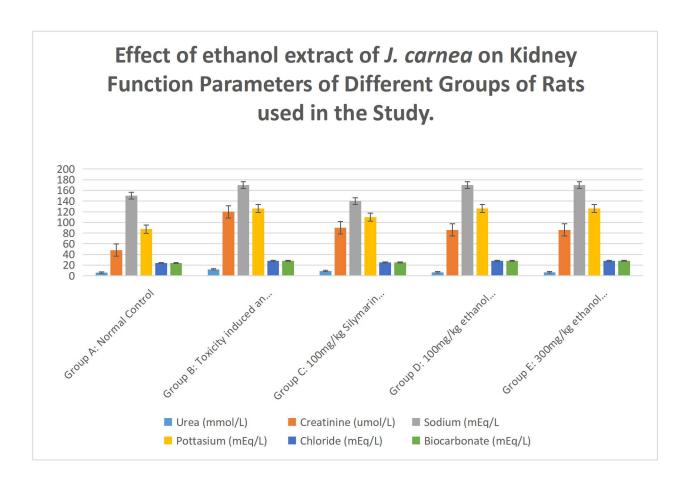
Effect of ethanol extract of *J. carnea* on Kidney Function parameters of different groups of Rats used in the study

The results of the renal function and electrolyte profiles for five experimental groups demonstrate the potential nephroprotective effect of *Justicia carnea* ethanol extract in cadmium-induced toxicity. Group A (normal control) displayed standard renal parameters, while Group B (negative control) exhibited elevated urea (12.00 ± 0.00) and creatinine (120.00 ± 0.00) levels, along with disrupted electrolyte balance, indicating significant kidney impairment due to cadmium exposure.

Group C, treated with 100 mg/kg Silymarin, showed partial improvement in renal function (urea: 9.00 ± 0.00 ; creatinine: 90.00 ± 0.00). However, Groups D and E,

which received 100 mg/kg and 300 mg/kg of J. carnea extract respectively, showed greater restoration of renal function. Group D had reduced urea (6.60 \pm 0.00) and creatinine (86.00 \pm 0.00) levels, with electrolyte values returning closer to normal. Similar outcomes were observed in Group E, indicating that both doses of the extract provided comparable renal protection.

Overall, the findings suggest that *Justicia carnea* ethanol extract can effectively mitigate cadmium-induced renal damage, supporting its nephroprotective potential.



DISCUSSION

In this investigation, the potential role of Justicia carnea ethanol leaf extract in mitigating cadmium-induced hepatic and renal dysfunction in male Wistar rats was explored. Though traditionally used in some ethnomedical practices, there is a lack of scientific validation for its impact on lipid metabolism. The current study sought to address this gap by assessing the plant's bioactivity, especially regarding haematological and biochemical parameters in an anaemia model, while drawing parallels with the outcomes observed under cadmium toxicity. Phytochemical analysis of J. carnea revealed a high presence of flavonoids and phenolic compounds two potent antioxidant classes that have demonstrated efficacy in neutralizing oxidative stress. Flavonoids, with their extensive diversity across over 8000 identified compounds, are commonly found in the outer parts of plant tissues and are recognized for their antiinflammatory, anti-carcinogenic, immune-modulating properties. Likewise, polyphenols, which abundant in fruits, vegetables, and contribute significantly legumes, cellular defense mechanisms by protecting against ultraviolet radiation pathogen invasion. and These compounds originate primarily through the shikimic acid and phenylpropanoid biosynthetic pathways and have been widely documented to reduce oxidative damage and enhance DNA protection, particularly within lymphocytes.

Nutritional profiling of *Justicia carnea* leaves also revealed significant contents of moisture, carbohydrates, and protein, implying its potential for energy support and basic nourishment. Results showed 51.69% moisture, 37.43% carbohydrates, 8.05% protein, and trace levels of fat and fiber. Comparable findings by other researchers reaffirmed its carbohydratedense nature, with implications for metabolic energy supply. In addition to

these macronutrients, J. carnea also demonstrated considerable levels of antioxidant vitamins A, C, and E-each known for their vital physiological roles. Vitamin A plays a key part in gene expression, cell differentiation, immunity, while also mitigating DNA damage caused by free radicals. Vitamin C is indispensable in collagen synthesis, neurotransmitter production, and immune modulation, and it enhances non-heme iron absorption. Vitamin E contributes significantly to cellular defense against oxidative stress and supports vascular health and immune function.

Toxicological evaluation confirmed the safety of *J. carnea* even at high doses. Animals administered up to 5000 mg/kg showed no signs of toxicity or mortality, indicating a high safety margin. These findings support its suitability for further pharmacological studies. From a haematological standpoint, administration of *J. carnea* extract led to

marked blood improvement in parameters that were previously suppressed following anaemia induction. There was a significant rebound in red blood cell haemoglobin count, concentration, and packed cell volume after treatment, aligning with earlier findings that demonstrated its efficacy in restoring haematological balance.

Biochemically, the showed extract protective effects on liver and kidney function in cadmium-exposed animals. The hepatotoxic model revealed that J. carnea mitigated enzyme elevations typically associated with liver injury. Rats exposed to cadmium exhibited increased levels of liver enzymes such as AST, ALT, and ALP, as well as elevated bilirubin concentrations. indicative of hepatic stress. However, groups administered with J. carnea especially at higher doses-exhibited normalized enzyme levels, suggesting liver stabilization improved and hepatocyte integrity. Similarly, in terms of renal function, the extract significantly reduced elevated urea and creatinine levels, suggesting preserved glomerular function and renal protection. Electrolyte levels also improved, indicating restoration of normal kidney handling of ions.

Overall, the findings of this study indicate that Justicia carnea ethanol extract offers dual protection against cadmium-induced hepatic and renal damage. These effects are likely mediated through its rich composition of antioxidant phytochemicals and essential vitamins. which together enhance cellular resilience against oxidative stress and toxic insults. The extract's safety profile further supports its potential therapeutic pending use, mechanistic and additional clinical investigations.

CONCLUSION

Ultimately, the current research examined the possible hepatoprotective benefits of Justicia carnea (flamingo

flower) ethanol extract against cadmium-induced hepatotoxicity in male Wistar rats. The findings showed that exposure to cadmium resulted in severe liver damage, as shown by increased bilirubin levels, liver enzymes, and renal However, these effects were failure. considerably reduced by treatment with Justicia carnea ethanol extract, which improved renal and liver parameters. The extract may have hepatoprotective and nephroprotective benefits because of its anti-inflammatory and antioxidant According to the study's qualities. findings, extract from Justicia carnea may be a useful natural treatment for hepatotoxicity brought on by cadmium and associated conditions.

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CONFLICT OF INTEREST

We declare no conflict of interest.

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