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Effect *of* Ethanol extract of *Justicia carnea* on Body Weight and HeamatologyAanalysis of CadmiumIinduced Hepatotoxicity in Wista Albino Rats

Okolue[1],Chinwendu J.,Nwaka[1],Andrew C., Okafor[1],Lilian A., Obi[1] , Udochukwu B., Uzor[2],Nkemjika T.

[1]Department of Biochemistry, Faculty of Natural Science, Chukwuemeka Odumegwu Ojukwu University, Anambra State.

[2]Department of Community Medicine, Nnamdi Azikiwe University, Nnewi Campus, Nigeria

***Address for correspondence:** E-mail: drjessy26@gmail.com ; Phone No: +2348066474080

ABSTRACT

The study explored the therapeutic potential of ethanol leaf extract of *Justicia carnea* in male Wistar rats subjected to cadmium-induced hepatotoxicity, focusing on changes in body weight and hematological parameters. Twenty-five rats were divided into five groups: a normal control group, a negative control group exposed to cadmium only, a positive control group treated with silymarin, and two treatment groups administered 100 mg/kg and 300 mg/kg of *J. carnea* extract, respectively.Rats exposed to cadmium without treatment experienced a reduction in body weight gain over time, suggesting systemic toxicity and overall physiological decline. In contrast, those treated with the ethanol extract of *J. carnea* showed a significant recovery in body weight, with the higher dose group exhibiting the most notable improvement. This indicates that the extract may counteract the weight loss effects of cadmium toxicity and aid in restoring metabolic balance.In addition, cadmium exposure led to marked declines in packed cell volume, hemoglobin concentration, and red blood cell counts, reflecting hematological impairment and potential anemia. Administration of *J. carnea* extract significantly reversed these effects, with both treatment groups showing improvements, and the higher dose group demonstrating the greatest enhancement in hematological values. These findings suggest a dose-dependent ability of the extract to stimulate blood formation, which supports its traditional use as a blood tonic.Overall, the ethanol extract of *Justicia carnea* contributed to weight restoration and improved hematological health in cadmium-exposed rats, reinforcing its potential as a natural remedy for heavy metal-induced toxicity and as a supportive agent for blood and liver function.

Keywords: Hepatotoxicity, Wistar rats, Hematological analysis, *Justicia carnea, C*admium

INRODUCTION

Traditional medicine, particularly the use of medicinal plants like *Justicia carnea*, has long played a critical role in health management in Africa, especially in Nigeria (Okonkwo, 2012). This is increasingly relevant as antimicrobial resistance rises and natural alternatives are explored (Kumari *et al.,* 2010; Shyamapada and Manisha, 2011). *Justicia carnea*, known locally as "ogwuobara" (Igbo) or "èwe eje" (Yoruba), belongs to the Acanthaceae family and is widely used for its hematinic (blood-enriching) and therapeutic effects (Corrêa and Alcântara, 2012). Its leaves are rich in phytochemicals such as flavonoids, alkaloids, tannins, and vitamins (Harborne, 1998; Orjiakor *et al.,* 2019), especially vitamin C, iron, and calcium, which contribute to its hematopoietic and antioxidant properties (Orjiakor, 2012; Al-Juaid and Abdel-Mojib, 2004).

Cadmium (Cd), a heavy metal and Class I carcinogen (IARC, 1993), poses significant hepatotoxic risk through oxidative stress, mitochondrial dysfunction, and interference with antioxidant enzymes like SOD, CAT, GSH, and GST (Gafar *et al.,* 2022; Noor, 2022; Branca *et al*., 2020). It also damages hematopoietic tissues, leading to anemia by binding to red blood cells and disrupting erythropoiesis (Khannazer et al., 2020; Hambach *et al.,* 2013). The liver, as the primary detoxification organ, suffers functional and structural damage, with increased levels of ALT, AST, and bilirubin, and decreased total proteins (Jacob, 2020; Andjelkovic, 2019). Inflammatory mediators like TNF-α and apoptotic proteins like caspase-3 also rise, exacerbating liver damage (Chen *et al.,* 2022; Abdelraze *et al.,* 2016).

In this context, ethanol extract of *Justicia carnea* was tested in Wistar albino rats exposed to cadmium. Cadmium caused significant reductions in body weight and hematological parameters such as RBC, hemoglobin, and packed cell volume (PCV), consistent with its known systemic toxicity. However, treatment with *J. carnea* extract, particularly at higher doses, reversed these effects. Rats showed a dose-dependent improvement in body weight and normalization of hematological indices. This suggests that *J. carnea* has a protective and restorative effect on cadmium-induced hematological and systemic toxicity,

likely due to its antioxidant and erythropoietic bioactive compounds.

However, rats treated with *Justicia carnea* extract—particularly at a higher dose of 300 mg/kg—demonstrated significant improvements in both body weight and hematological profiles. The extract restored PCV, Hb, and RBC levels in a dose-dependent manner, with the higher dose yielding the most notable effects. These findings suggest that the plant's rich content of phytochemicals, including flavonoids, alkaloids, and antioxidant vitamins (notably vitamin C), contributes to its hematopoietic and protective effects.

The study examined the effects of ethanol leaf extract of *Justicia carnea* on body weight and hematological parameters in male Wistar albino rats subjected to cadmium-induced hepatotoxicity—a model of toxic liver injury akin to drug-induced liver injury (DILI). DILI, which can result from prescription medications, over-the-counter drugs, or herbal and dietary supplements (HDS), manifests as liver dysfunction without other identifiable causes (Suk and Kim, 2012). While intrinsic DILI is predictable and dose-dependent (e.g., acetaminophen), idiosyncratic DILI is unpredictable, dose-independent, and more difficult to detect in preclinical trials (Chalasani et al., 2014). The global incidence of DILI is increasing, partly due to the rise in HDS usage (Bell and Chalasani, 2009; Larson, 2016).

In this study, cadmium exposure significantly reduced body weight and impaired hematological indices, including packed cell volume (PCV), hemoglobin (Hb), and red blood cell (RBC) counts—hallmarks of systemic toxicity and impaired erythropoiesis, similar to effects observed in DILI (Pandit, Sachdeva, and Bafna, 2014). However, treatment with *Justicia carnea* extract, particularly at 300 mg/kg, reversed these toxic effects. Treated rats showed dose-dependent increases in body weight and restoration of PCV, Hb, and RBC levels, indicating enhanced erythropoietic activity and systemic recovery.

These effects are attributed to the phytochemical composition of *J. carnea*, which includes flavonoids, alkaloids, and antioxidant-rich vitamins such as vitamin C, all of which contribute to its hematopoietic and detoxifying properties. Thus, the study supports the plant’s ethnomedicinal use as a blood tonic and highlights its potential in

mitigating hepatotoxic and hematotoxic effects of toxicants like cadmium.

In conclusion, ethanol extract of *Justicia carnea* counteracted cadmium-induced weight loss and anemia in Wistar rats, supporting its traditional use as a blood tonic and indicating its potential as a natural therapeutic agent for heavy metal-induced hematological and systemic damage.

MATERIALS AND METHODS

Materials / Equipment

- **Blood collection tools**: Syringes, needles, and collection tubes for obtaining blood samples, often from the tail vein or via cardiac puncture.
- **Centrifuge:** Used for blood sample separation to obtain serum or plasma for biochemical assays.
- **Weighing scales:** To monitor and record the weight of the rats throughout the experiment.
- **Thermometer:** For monitoring and maintaining proper environmental conditions in the animal facility.
- **Computer or lab notebooks**: for recording experimental data, observations, and results.
- **Statistical software:** For data analysis to evaluate the effects of the ethanol extract on cadmium-induced hepatotoxicity.

Experimental Design

In this study, twenty-five Wistar albino rats were randomly divided into five groups of five animals each to investigate the effect of ethanol extract of *Justicia carnea* on body weight and hematological parameters in cadmium-induced hepatotoxicity. The experiment lasted for one month. Group A served as the normal control and received only food and water without cadmium induction. Group B, the negative control, was administered cadmium at 5 mg/kg daily but received no treatment. Group C, the positive control, received the same cadmium dose and was treated with the standard hepatoprotective drug silymarin at 100 mg/kg. Groups D and E were also induced with cadmium but were treated with 100 mg/kg and 300 mg/kg of ethanol extract of *Justicia carnea*, respectively.

Cadmium was administered daily at a dose of 5 mg/kg to induce hepatotoxicity

in all groups except the normal control. At the end of the treatment period, following overnight fasting, the rats were anesthetized using chloroform. Blood samples were collected via cardiac puncture with a 10 ml syringe and placed in plain bottles. After centrifugation, the sera were separated and used for hematological and biochemical analyses to evaluate the protective effects of *Justicia carnea* extract.

Experimental Animals:

A total of 38 male Wistar rats (aged 3 weeks, 120–150g) were used in the study. Thirteen rats were used to determine the mean lethal dose (LD_{50}) of the ethanol extract of *Justicia carnea*, while the remaining 25 were assigned to the main experimental groups. The rats were obtained from Chris Experimental and Research Farm, Awka, Anambra State. The 25 test rats were divided into five groups and housed in well-ventilated stainless steel cages. They underwent a 7-day acclimatization period before the experiment began. Throughout the study, the animals were kept under ambient conditions, fed standard pelleted grower feed (Vital Feed® by Grand Cereals and Oil Mills, Jos), and given unrestricted access to water. All procedures followed ethical guidelines for animal research.

Collection, Identification and Preparation of the Plant Materials

Justicia carnea leaves were sourced from Eke Igwe Orizu, a local market in Nnewi North Local Government Area, Anambra State. The plant was authenticated by Dr. B. N. Uwalaka of the University of Agriculture and Environmental Sciences, Umuagwo, Imo State, and was assigned the voucher number UAES/HB/0037. The leaves were carefully hand-picked, thoroughly washed, and initially sun-dried, followed by further drying in a hot-air oven at 60°C. Once fully dried, they were ground into a fine powder and stored in airtight containers for subsequent extraction and analysis.

Evaluating the Hematological and Body Weight Effects of *Justicia carnea* Ethanol Extract in Cadmium-Induced Hepatotoxicity in Wistar Rats

The study evaluated the effects of ethanol extract of *Justicia carnea* on body weight and hematological parameters in cadmium-induced hepatotoxicity using Wistar albino rats. The plant material was sourced from a local market in Nnewi North LGA,

authenticated, and processed by washing, sun-drying, oven-drying at 60°C, and pulverization into powder for storage and subsequent extraction. Acute toxicity testing was conducted using Lorke's method (1983), involving two phases of dosing to determine the median lethal dose (LD_{50}), with no mortality observed up to 5000 mg/kg, indicating a favorable safety profile.

Thirty rats were randomly distributed into five groups (n=5 per group). Group A served as the normal control, receiving only food and water. Groups B to E were induced with cadmium (5 mg/kg daily), with Group B receiving no treatment (negative control), Group C treated with silymarin (100 mg/kg, positive control), Group D treated with 100 mg/kg ethanol extract of *Justicia carnea*, and Group E treated with 300 mg/kg of the extract. The treatment period lasted one month.

At the end of the study, blood samples were collected post-fasting via cardiac puncture under chloroform anesthesia. Samples were analyzed for hematological parameters using an automated hematology analyzer (Mindray BC-5300). Parameters measured included packed cell volume (PCV) via microhematocrit centrifugation, hemoglobin concentration using the cyanmethemoglobin method, white blood cell (WBC) count and red blood cell (RBC) count via hemocytometry using glacial acetic acid and Gower's solution respectively, and differential WBC analysis through stained blood smears with Leishman stain. Each method followed standard protocols, ensuring accuracy through proper sample handling, equipment calibration, and adherence to safety precautions. These analyses provided comprehensive data on the hematological impact of the ethanol extract in mitigating cadmium-induced hematotoxicity and hepatotoxicity.

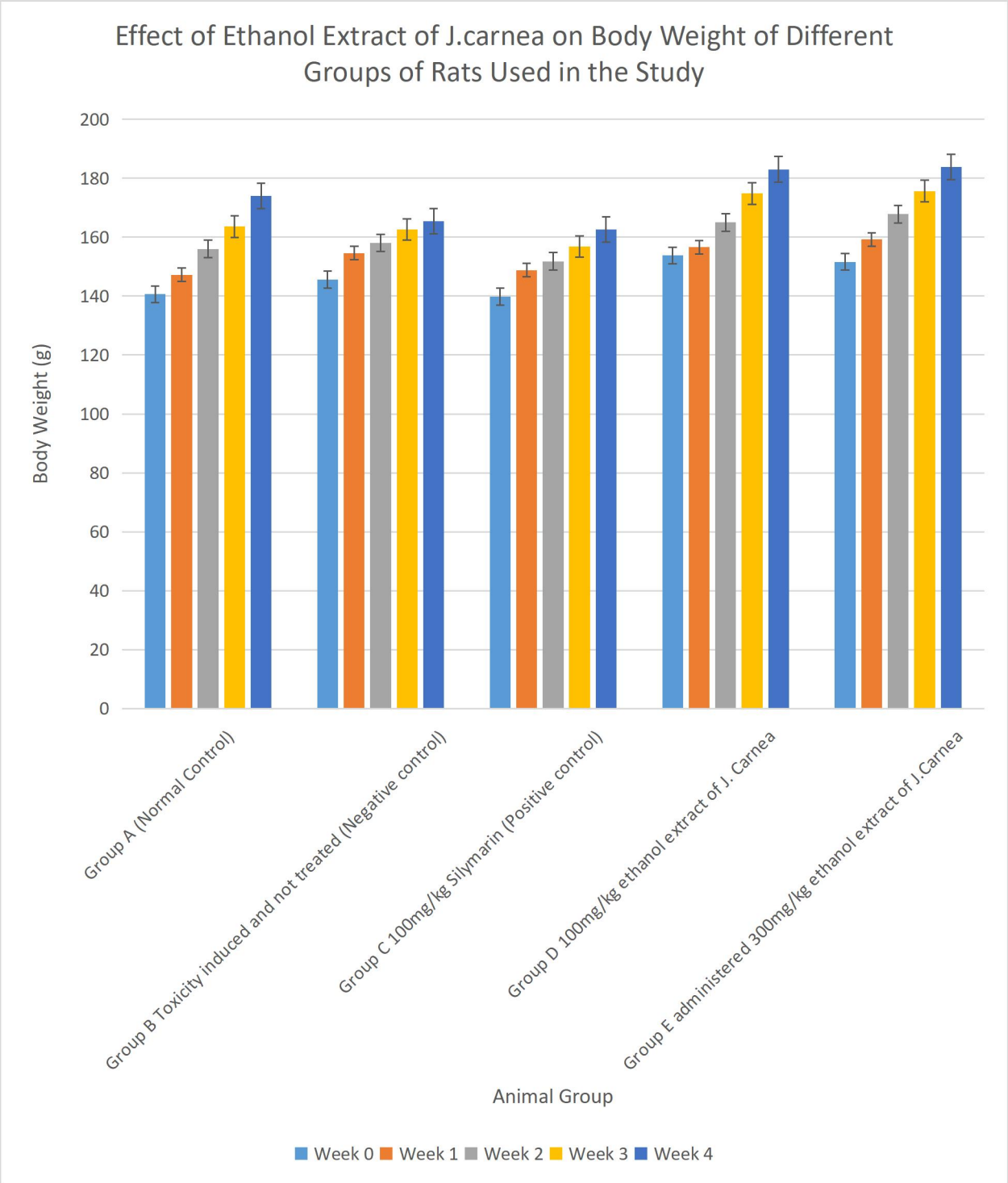
Data Analysis

The data were presented as mean ± standard deviation (SD) from three replicates. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 23. One-way Analysis of Variance (ANOVA) was employed to compare the groups, followed by a post hoc test using Least Significant Difference (LSD). A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS

Effect of Ethanol Extract of *Justicia carnea* on Body Weight of Experimental Rats

The weekly body weight measurements across the five groups revealed notable trends. Group A (normal control) maintained consistent weight gain, while Group B (cadmium-induced negative control) showed minimal or no significant increase. In contrast, Groups C, D, and E, which received treatments of 100 mg/kg silymarin, 100 mg/kg, and 300 mg/kg ethanol extract of *J. carnea* respectively, exhibited significant weight gain over the study period. Notably, Group E (300 mg/kg *J. carnea*) demonstrated the greatest improvement, followed by Group D. These results suggest that *J. carnea* extract supports recovery from cadmium-induced toxicity, with the higher dose yielding more pronounced effects.



Effect of Ethanol extract of *J. carnea* on Full blood count of different groups of rats used.

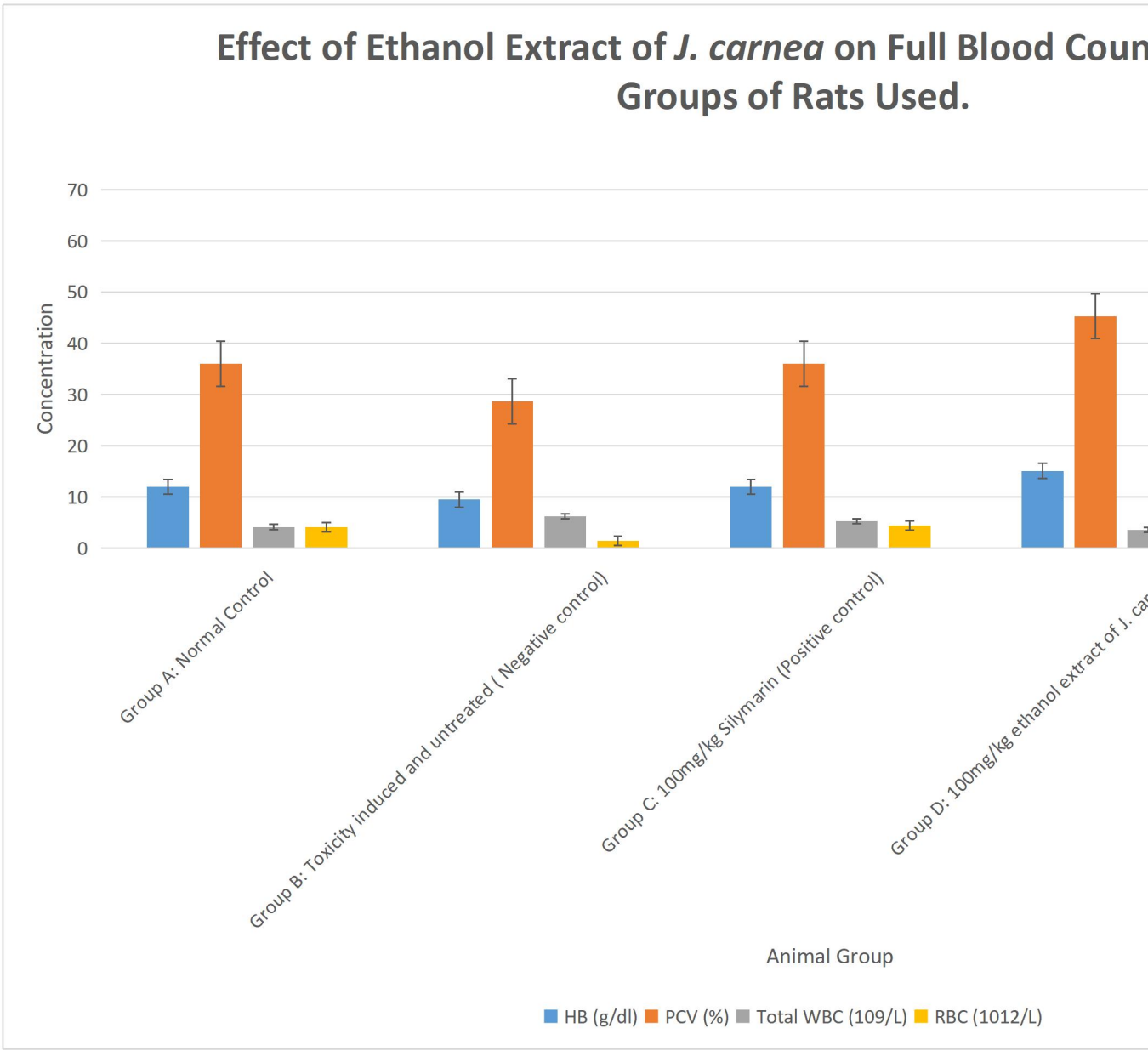
Groups	HB (g/dl)	PCV (%)	Total WBC (109/L)	RBC (1012/L)
Group A: Normal Control	12.00± 0.00	36.00± 0.00	4.17 ± 0.07	4.10 ± 0.00
GroupB: Toxicity induced and untreated (Negative control)	9.50± 0.17 b	28.67 ± 0.33 b	6.23± 0.09 a	1.50 ± 0.10 b
Group C: 100mg/kg Silymarin (Positive control)	12.00± 0.00	36.00 ± 0.02	5.27 ± 0.03 a	4.43 ± 0.17 c
Group D: 100mg/kg ethanol extract of *J. carnea*	15.10 ± 0.10a	45.33 ± 0.33 ac	3.60 ± 0.00 d	5.17 ± 0.07 c
Group E: 300mg/kg ethanol extract of *J. carnea*	18.00 ± 0.01a	54.00 ± 0.00 ac	3.70 ± 0.00 d	7.10 ± 0.00 c

- a; signifies increase W.R.T normal control group;
- b; signifies decrease W.R.T normal control group;
- c; signifies increase W.R.T cadmium chloride toxicity- induced untreated;
- d; signifies decrease W.R.T cadmium chloride toxicity - induced untreated.

Effect of Ethanol extract of *J. carnea* on Full Blood Count of Different Groups of Rats used.

The study evaluated the effect of ethanol extract of *Justicia carnea* on body weight and hematological parameters in Wistar albino rats subjected to cadmium-induced hepatotoxicity. The experimental design comprised five groups: a normal control (Group A), a cadmium toxicity-induced negative control (Group B), a positive control treated with 100 mg/kg Silymarin (Group C), and two treatment groups receiving 100

mg/kg (Group D) and 300 mg/kg (Group E) of *J. carnea* extract, respectively. Cadmium exposure led to significant reductions in hemoglobin (HB), packed cell volume (PCV), and red blood cell (RBC) counts, particularly in Group B. Treatment with *J. carnea* extract resulted in dose-dependent improvements in these parameters, with Group E showing the highest increases. White blood cell (WBC) counts were reduced in the extract-treated groups, suggesting a potential anti-inflammatory effect. Overall, the ethanol extract of *J. carnea*, especially at 300 mg/kg, demonstrated a protective and restorative impact on hematological indices affected by cadmium toxicity.



Effect of Ethanol extract of *J. carnea* on Full blood count of different groups of rats used.

Groups	HB (g/dl)	PCV (%)	Total WBC (109/L)	RBC (1012/L)
Group A: Normal Control	12.00± 0.00	36.00± 0.00	4.17 ± 0.07	4.10 ± 0.00
GroupB: Toxicity induced and untreated (Negative control)	9.50± 0.17 b	28.67 ± 0.33 b	6.23± 0.09 a	1.50 ± 0.10 b

Group C: 100mg/kg Silymarin (Positive control)	12.00± 0.00	36.00 ± 0.02	5.27 ± 0.03 a	4.43 ± 0.17 c
Group D: 100mg/kg ethanol extract of *J. carnea*	15.10 ± 0.10a	45.33 ± 0.33 ac	3.60 ± 0.00 d	5.17 ± 0.07 c
Group E: 300mg/kg ethanol extract of *J. carnea*	18.00 ± 0.01a	54.00 ± 0.00 ac	3.70 ± 0.00 d	7.10 ± 0.00 c

- a; signifies increase W.R.T normal control group;
- b; signifies decrease W.R.T normal control group;
- c; signifies increase W.R.T cadmium chloride toxicity- induced untreated;
- d; signifies decrease W.R.T cadmium chloride toxicity - induced untreated.

DISCUSSION

This study explored the effects of ethanol extract of *Justicia carnea* on body weight and hematological parameters in Wistar albino rats with cadmium-induced hepatotoxicity. The primary aim was to assess the extract's ability to improve lipid profiles and hematological indices, particularly in anaemic conditions. *J. carnea* leaves were found to be rich in antioxidant phytochemicals, notably flavonoids and polyphenols, known for their protective roles against oxidative stress and cellular damage. Nutritional analysis revealed high moisture and carbohydrate content, along with moderate levels of protein, fibre, and essential vitamins (A, C, and E), all of which support antioxidant activity, immune function, and energy production.

Toxicological assessment confirmed the safety of the extract, showing zero mortality at doses up to 5000 mg/kg. Hematologically, cadmium exposure led to significant reductions in RBC, Hb, and PCV, which were substantially restored upon administration of *J. carnea* extract, particularly at higher doses. The extract also contributed to significant reductions in LDL cholesterol, triacylglycerol, and total cholesterol levels in treated groups, reflecting an improvement in lipid metabolism. Overall, the extract of *Justicia carnea* exhibited hepatoprotective, hematopoietic, and potential lipid-lowering effects, suggesting its therapeutic promise in managing cadmium-induced oxidative damage and anaemia.

CONCLUSION

It was concluded that ethanol extract of *Justicia carnea* offers significant protection against cadmium-induced hepatotoxicity in male Wistar rats. Cadmium exposure caused marked liver and kidney damage, indicated by elevated liver enzymes, bilirubin, and signs of renal impairment. Treatment with *J. carnea* extract notably improved these parameters, suggesting strong hepatoprotective and nephroprotective effects, likely due to its antioxidant and anti-inflammatory properties. These findings support the potential of *J. carnea* as a natural therapeutic agent for mitigating cadmium-induced organ toxicity and improving hematological health

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CONFLICT OF INTEREST

We declare no conflict of interest.

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