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Original Research Article

Hepatorenal protection of *Justicia carnea* leaf aqueous extract on sheep red blood cell-induced immunotoxicity in mice

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Abstract

Purpose: *To evaluate the hepatorenal protective properties of Justicia carnea (Acanthaceae) aqueous leaf extract against Sheep Red Blood Cells (SRBC)-induced immunotoxicity in mice.*
Method: *Thirty-six (36) male mice were randomly selected into six groups of six mice each. Group I (VEH control) was administered distilled water only, while Groups II – VI mice were immunized intraperitoneally with SRBC (0.2 mL/kg) on days 1 and 5. In addition, Groups III – V were given 125, 250 and 500 mg/kg of JC aqueous extract, respectively and Levamisole (LEV; 7.5 mg/kg) was administered to mice in Group VI. From days 3 to 9, the mice received daily oral doses of their assigned treatment agents. On day 10, the animals were sacrificed, and blood, liver, and kidney tissues were collected for biochemical analysis.*
Result: *There were significant changes in Aspartate Transferase (AST), Alanine Transferase (ALT), Alkaline Phosphatase (ALP) and Albumin (ALB) levels of SRBC-induced mice ($p < 0.05$), which were reversed almost to the baseline value of VEH group. However, at JC 250 and 500 mg/kg, there was a significant increase in total protein (TP) level compared to VEH, LEV and SRBC groups ($p < 0.05$). Aqueous JC extract significantly reduced the increased urea concentration induced by SRBC, though not to baseline, compared to LEV ($p < 0.05$). Also, the extract significantly altered activities of oxidative enzymes such as aldehyde oxidase (AO), monoamine oxidase (MAO) and peroxidase (PO) compared to control ($p < 0.05$).*
Conclusion: *Justicia carnea significantly restores liver and kidney functions in mice treated with SRBCs, comparable to levamisole. These findings unveil the potential of J. carnea leaf extract as an adjuvant in vaccine therapy.*

Keywords: *Immunized, Immunostimulant, Immunotoxicity, Immunostabilizing, Nephroprotective, Antigenic and hepatoprotective effects.*

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INTRODUCTION

Sheep Red Blood Cells (SRBC) are widely recognized as T-cell dependent antigens capable of inducing a strong humoral immune response, necessitating the cooperation of both B and T lymphocytes. In rodents, SRBCs are identified as foreign elements, making them ideal candidates for use in immunotoxicological studies. Their ability to provoke T-dependent antibody production has established SRBC as a standard model in evaluating the immunotoxic effects of xenobiotics [1].

Liver function assessment relies heavily on specific biomarkers, particularly those related to enzyme activity and deviations from physiological norms. Key indicators include Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), bilirubin, total protein (TP) and AST/ALT ratio [2]. Hepatic damage may result from direct toxic effects of xenobiotics or immune-mediated injury affecting the liver's biliary structures and vasculature [3]. While an AST/ALT ratio below 1 typically suggests hepatic dysfunction, higher values may indicate alcoholic hepatitis or cirrhosis. Elevated AST and ALT levels are classic signs of liver injury and ALP elevation often correlates with hepatobiliary diseases or systemic conditions such as malignancy or renal failure [2]. Conversely, abnormally low ALP may be associated with disorders like Wilson's disease and hypothyroidism.

Kidney function, essential for metabolic waste removal and fluid balance, is influenced by and interdependent with other major organs. Traditional renal diagnostics include assessments of creatinine and urea levels, urinary albumin and albumin/creatinine ratio. More refined methods, including mRNA expression and permeability assays, are now employed for precise functional evaluation [3].

Enzymes and biochemical parameters such as ALT, AST, ALP, TP, bilirubin, monoamine oxidase (MAO), aldehyde oxidase (AO), peroxidases, urea and creatinine play critical roles in monitoring both hepatic and renal health. Antigenic stimulation modulates biogenic amines such as catecholamines, which are degraded by MAO with concurrent generation of reactive oxygen species (ROS), potentially compromising immune integrity. Aldehyde oxidase contributes to drug metabolism and oxidative stress through ROS production, while peroxidases help regulate ROS to prevent cellular damage [4]. Immune challenges induced by SRBC affect antioxidant systems, including enzymes such as catalase, superoxide dismutase and glutathione peroxidase [5].

Levamisole, a synthetic compound with anthelmintic and antineoplastic properties, has been widely utilized as an immunostimulant and immunomodulator in clinical and experimental settings. Its efficacy in managing autoimmune conditions and as an adjunct in cancer therapies further supports its role as a benchmark drug in immune studies [6,7].

Justicia carnea (Acanthaceae), also known as the Blood of Jesus plant or Brazilian plume, is an evergreen shrub native to South America and cultivated in West and Central Africa. Known locally for its medicinal applications, including anemia management, immune enhancement and postpartum care, the plant contains high levels of iron and bioactive compounds like flavonoids, phenols and carotenoids. These phytochemicals have demonstrated strong antioxidant properties, which could be valuable in mitigating oxidative stress [5].

This study investigates the protective potential of aqueous *J. carnea* leaf extract against SRBC-induced immunotoxicity in mice. By exploring the plant's role in modulating immune-related oxidative stress and preserving hepatic and renal integrity, the study proposes *J. carnea* as a candidate for inclusion in vaccine formulations as a natural adjuvant, potentially minimizing organ dysfunction commonly observed following immunization.

EXPERIMENTAL

Collection of plants

Fresh *J. carnae* leaves were collected from a garden in Abraka, Ethiope East LGA, Delta State, Nigeria (Latitude: 5° 47' 21.9552" N, Longitude: 6° 6' 8.4492" E). The leaf specimens were identified by Dr. Joseph Erabor of the Department of Plant Biology and Biotechnology at the University of Benin, Benin City and authenticated with a voucher number allocated (UBH-J386) and deposited in the Herbarium.

Preparation of extracts

The leaves were washed with distilled water to remove debris and were air-dried for two weeks, until a constant weight was obtained. The dried leaves were pulverized using a mill and 100 g of coarsely powdered leaves was extracted with 1 L of distilled water using cold maceration for 72 h. The extract was filtered using a cheese-cloth with fine pores and the filtrate obtained was filtered

for the second time using Whatman No. 1 filter paper to ensure purity and consistency of the extract. The resulting extract was concentrated using a rotary evaporator RII –HB (Buchi Labortechnik AG, Switzerland CH-9230) at 50 °C and then stored in a laboratory refrigerator (-4 ^{o}C) for biochemical analysis [5].

Preparation of aqueous J. carnea extract stock

A stock solution of 20 mg/mL was prepared by weighing 200 mg of paste-like aqueous *J. carnea* extract and dissolving it in 10 mL of distilled water. The solution was stirred continuously until dissolution was achieved. Doses of administration per mouse were calculated using Eq 1.

VE = {(Ds x Bwt)/EC}1000 …… (1)

Where VE = Volume of extract administered, Ds = Dose (mg/kg), Bwt = Body weight of mice (g), EC = Extract concentration (mg/mL) x1000.

Preliminary phytochemical analysis

Phytochemical analysis of the plant was carried out in accordance with the methods described by Iwetan and colleagues [5].

Preparation of antigen

Sheep Red Blood Cells (SRBCs) were obtained aseptically with a syringe from the left ear lobe of a male sheep and dispensed into an anticoagulant tube. The cells were washed with normal saline in a 1:3 ratio (SRBCs: Normal Saline) by centrifugation at 3000 rpm for 10 min. The process was repeated three times and the washed cells were subsequently suspended in normal saline adjusted to a concentration of 1 x 10^9 cells/mL. Experimental mice were immunized with the suspended SRBC solution [5].

Preparation of standard drug

Levamisole hydrochloride, a standard drug in tablet dosage (40 mg), was obtained from the Pharmacy Division, Delta State University Health Centre, Abraka, Nigeria. A stock solution of the standard drug (4 mg/mL) was prepared by dissolving a tablet of Levamisole hydrochloride in 10 mL of distilled water and stirred continuously until the dissolution was achieved.

Design

Thirty-six (36) male mice, aged 12 – 16 weeks and weighing 22 – 32 g, were used in this study. The mice were bred, fed with grower mash and water, and housed at ambient temperature in a well-ventilated Animal House of the Department of Basic Medical Sciences, Delta State University, Abraka. They were allowed to acclimatize in their respective groups in line with the experimental design. Ethical approval was obtained from the University of Port Harcourt's Study Research Ethics Committee (UPH/CEREMAD/REC/MM74/003). The experimental protocol for this study is stated in Table 1 below [5].

All groups were immunized at days 1 to 5 with SRBC to induce an immune response, except for VEH (control) group. Treatment with standard drug and JC extract for respective groups commenced on day 3 until day 9. All animals were sacrificed on day 10.

Collection of blood samples

On day ten (10), mice were sacrificed via cervical dislocation. Blood samples were collected via retro-orbital puncture and 3 mL was transferred into non-anticoagulant containers. Serum was obtained from the supernatant of the coagulated blood after centrifugation at a speed of 3000 rpm for five (5) min.

Table 1: Experimental protocol for administration of *J. carnea* extract

Group	Vehicle (10 mL/kg distilled H_2O; oral)	SRBC (0.2 mg/kg; i.p)	*J. carnea* Aqueous Extracts (oral)			Levamisole (7.5 mg/kg; orally)
			125 mg/kg	*250 mg/kg*	*500 mg/kg*	
I (VEH)	+ (1-9) days	-	-	-	-	-
II (SRBC)		+(day 1 & 5)		-	-	-
III (JC 125)	-	+(day 1 & 5)	+ (day 3-9)		-	-
IV (JC 250)	-	+(day 1 & 5)	-	+ (day 3-9)	-	-
IV (JC 500)	-	+(day 1 & 5)	-	-	+ (day 3-9)	-
VI (LEV)		+(day 1 & 5)	-	-	-	+ (day 3-9)

Collection of tissue samples and preparation of homogenates

Also, liver and kidney tissues were carefully harvested into well-labeled, sterilized sample containers from which 0.3 g of each harvested tissue was weighed into a homogenizing tube, submerged in an ice-packed condition. Thereafter, 1.7 mL of 0.1 M phosphate buffer (pH 7.4) was added to the respective tissues to form 10 % tissue homogenates. The homogenates were centrifuged at 3000 rpm for 10 minutes; supernatants were filtered with Whatman (90 mm) filter paper into labeled plain tubes and kept in the Laboratory refrigerator for subsequent analyses.

Determination of ALT, AST and ALP activities

The method described by Reitman and Frankel [7] was adopted to quantify alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in the mice serum in this research. Also, alkaline phosphatase activity was determined in accordance with the principle of Englehardt using commercially available kit (Randox) [8].

Determination of albumin and total protein concentrations

Albumin was determined according to a previously reported protocol [9] while total protein was assayed using the method described by Tietz [10].

Assessment of serum urea and creatinine levels

Serum urea concentration was determined using the modified method of Berthelot [11] while creatinine levels in the serum were estimated using a previously described method [12].

Assay for aldehyde oxidase (AO), peroxidase (PO) and monoamine oxidase (MAO)

The method described by Omarov and colleagues was adopted in the determination of AO activities in the serum, liver and kidney samples with slight modification [13] while PO and MAO levels were determined by the methods of Rad *et al* [14] and McEwen [13], respectively.

Data analysis

GraphPad Prism version 7 software was used to analyze the data, applying statistical tools like One-way analysis of variance and Two-way analysis of variance (ANOVA). Tukey's multiple comparison test (*post hoc*) was used for statistical differences, while statistical significance was observed at $p < 0.05$. Data are expressed as mean ± standard error of the mean (SEM) with sample size (n = 5).

RESULTS

Serum urea and creatinine concentrations

Serum urea and creatinine concentrations of mice immunized with SRBCs and treated with aqueous extract of JC leaves are presented in Table 1. Results obtained show significant increase in serum urea level of mice in SRBCs, 125, 250, and 500 mg/kg of JC and LEV groups when compared to VEH (control) group ($p < 0.05$). However, changes in urea concentration were not significant in mice treated with JC extract when compared to treatment and SRBCs groups ($p > 0.05$). Serum creatinine concentration was significantly reduced in mice treated with 125, 250, and 500 mg/kg of JC, compared to mice in VEH, SRBCs and LEV groups ($p < 0.05$).

Liver and kidney peroxidase activities

Analysis of peroxidase activities, shown in Table 2, indicates that liver peroxidase activities significantly increased in SRBC and LEV groups when compared to VEH group and JC treatment groups ($p < 0.05$). However, a significant decrease in PO levels was observed in mice administered JC at 125, 250, and 500 mg/kg, when compared to SRBC and LEV groups ($p < 0.05$). Meanwhile, in the kidney, SRBC, JC 125, 250 mg/kg, and LEV groups were increased significantly in comparison to VEH group ($p < 0.05$), but mice in JC 500 mg/kg group showed statistically not significant PO activities compared to VEH group ($p > 0.05$).

AST, ALT and ALP levels

Serum AST, ALT and ALP levels are presented in Figure 1. Results obtained revealed that AST significantly decreased in SRBC, JC; 125, 250, and 500 mg/kg and LEV 7.5 mg/kg groups compared to VEH group ($p < 0.05$). Also, AST levels in JC 125 and 500 mg/kg groups were significantly decreased and increased, respectively, when compared to mice of LEV group ($p < 0.05$). Furthermore, ALT levels were significantly decreased in all treatment groups compared to VEH, except in JC 500 mg/kg group, which was not significant ($p > 0.05$).

Table 1: Urea and creatinine concentrations following treatment with *J. carnea* in mice induced with SRBCs

Treatment (dose)	Urea (mg/dL)	Creatinine (mg/dL)
VEH (10 mL/kg)	5.5±0.26	1.10±0.05
SRBCS (0.2 mL/kg)	12.0±1.2*#	1.20±0.04#
JC (125 mg/kg)	11.0±0.23*#	0.74±0.09*&
JC (250 mg/kg)	15.0±0.82*#	0.47±0.05*&
JC (500 mg/kg)	10.0±0.65*#	0.29±0.04*&#
LEV (7.5 mg/kg)	4.6±0.22	0.57±0.05*

Significant differences between the mice groups with respect to VEH, SRBCS and LEV are indicated by *, &, and #, respectively ($p < 0.05$). VEH: Vehicle (10 mL/kg); SRBCS: Sheep Red Blood Cell (0.2 mL/kg); JC: *J. carnea* (125 - 500 mg/kg); and LEV: Levamisole (7.5 mg/kg)

Table 2: Liver and kidney peroxidase activities following treatment with *J. carnea* in mice induced with SRBCs

Treatment group	Liver PO (Unit/mg tissue)	Kidney PO (Unit/mg tissue)
VEH (10 mL/kg)	31.0±0.59	39.0±0.47
SRBCS (0.2 mL/kg)	37.0±0.63*	45.0±0.34*#
JC (125 mg/kg)	29.0±0.34&#	48.0±0.41*&#
JC (250 mg/kg)	29.0±0.42&#	46.0±0.31*#
JC (500 mg/kg)	23.0±0.37*&#	39.9±0.59&
LEV (7.5 mg/kg)	36.0±0.63*	41.0±0.24*

VEH: Vehicle, JC: *J. carnea*, LEV: Levamisole, SRBCs: Sheep Red Blood Cell; PO: Peroxidase. *, &, and # indicate significance ($p < 0.05$) compared to VEH, SRBCs and LEV, respectively

Also, ALT levels of SRBC and JC 125 mg/kg groups were significantly decreased compared to LEV group, while that of JC 500 mg/kg group was significantly increased compared to SRBCs and LEV groups ($p < 0.05$). In addition, ALP levels in SRBC group were significantly increased compared to all groups ($p < 0.05$), except LEV group, where no significant difference was observed ($p > 0.05$). There was also a significant decrease in ALP levels in all extract treatment groups compared to VEH group ($p < 0.05$).

AST/ALT ratio

The AST/ALT ratios of SRBC and JC 125 mg/kg treatment groups were decreased significantly ($p < 0.05$) as well as the LEV group compared to VEH group, while JC 125, 500 mg/kg and LEV groups were statistically not significant ($p > 0.05$; Figure 2). However, the ratios for JC 125, 250, and 500 mg/kg were significantly decreased compared to SRBC group ($p < 0.05$).

Serum total protein level

Results of the serum total protein analysis (Figure 3) indicate that serum total protein levels of mice in Groups IV (JC 250 mg/kg) and V (JV 500 mg/kg) significantly increased in comparison with Group I (VEH), II (SRBCs), III (JC 125 mg/kg) and VI (LEV; $p < 0.05$).

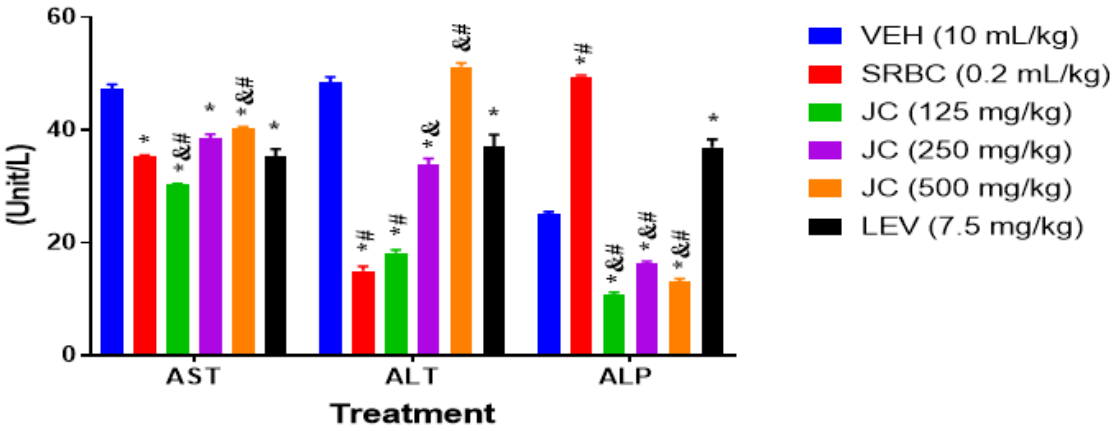

Figure 1: AST, ALT and ALP levels following treatment with *J. carnea* in mice induced with sheep red blood cells. VEH: Vehicle (Control), JC: *J. carnea*, LEV: Levamisole, SRBCs: Sheep Red Blood Cell. Bar with different symbols is significantly different ($p < 0.05$)

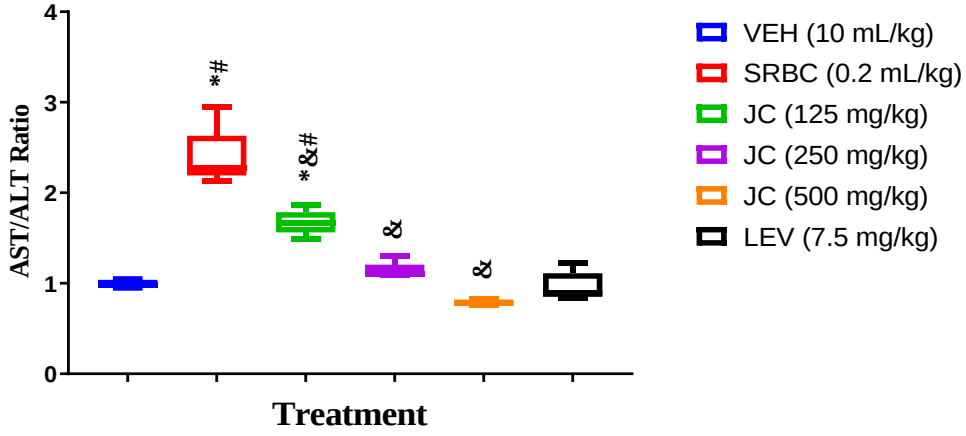

Figure 2: Serum AST/ALT ratio following treatment with *J. carnea* in mice induced with sheep red blood cells. VEH: Vehicle (Control); JC: *J. carnea*; LEV: Levamisole; SRBCs: Sheep Red Blood Cells. Bars are Mean ± SEM (n = 5). Bars with different characters are significantly different ($p < 0.05$)

Mice in Group VI (LEV) had significantly decreased total protein levels when compared to control (VEH) group, SRBC and JC 125 mg/kg groups.

Albumin levels of mice treated with *J. carnea*

Figure 4 presents the serum albumin levels of mice induced with sheep red blood cells and treated with aqueous extract of *J. carnea*. Results showed a non-significant increase in serum albumin levels in all Groups ($p > 0.05$) except for Group III (JC 125 mg/kg), where a significant decrease ($p < 0.05$) in the albumin levels in comparison with control (VEH), SRBC and LEV groups was observed.

Kidney, liver and serum monoamine oxidase (MAO) activities

The kidney and liver MAO activities are presented in Figure 5 A. Kidney MAO activities in SRBC and JC 125 mg/kg groups showed significant increase in comparison with VEH group ($p < 0.05$), whereas JC 250 and 500 mg/kg groups significant decrease in MAO activities compared to VEH and SRBC groups ($p < 0.05$). Liver MAO activity showed significant increase in mice treated with JC 125 mg/kg and JC 500 mg/kg when compared to VEH, SRBC, JC 250 mg/kg and LEV groups of mice ($p < 0.05$). In addition, results obtained for serum MAO activity indicate that JC 125 and 250 mg/kg groups significantly had reduced MAO activities compared to VEH group ($p < 0.05$).

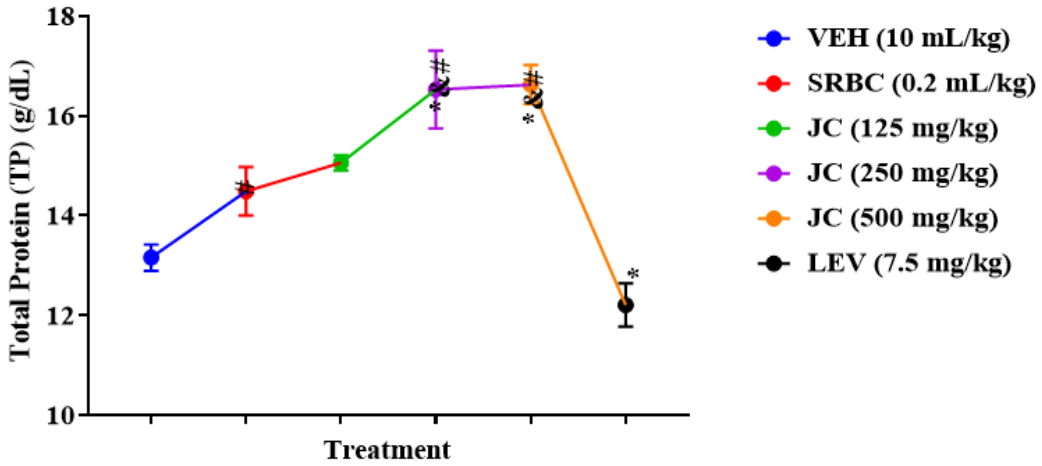

Figure 3: Total protein concentration following treatment with *J. carnea* in mice induced with sheep red blood cells. VEH: Vehicle (Control); JC: *J. carnea*; LEV: Levamisole; SRBCs: Sheep Red Blood Cells. Values represented in graph as dot lines are Mean ± SEM (n = 5). Dot lines with different characters are significantly different ($p < 0.05$)

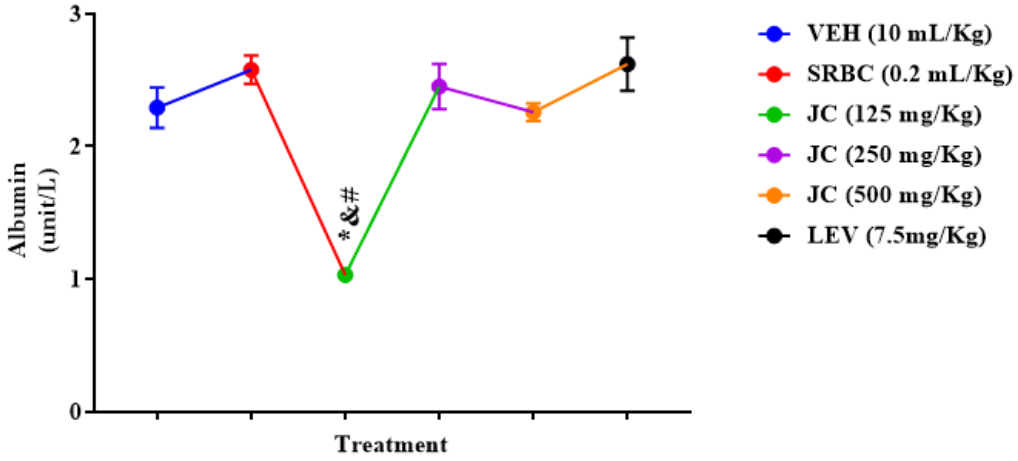

Figure 4: Albumin of all treatment groups in mice induced with sheep red blood cells. VEH: Vehicle (Control); JC: *J. carnea*; LEV: Levamisole; SRBCs: Sheep Red Blood Cells. The dotted line represents Mean ± SEM (n = 5). Dotted lines with different symbols are significantly different ($p < 0.05$)

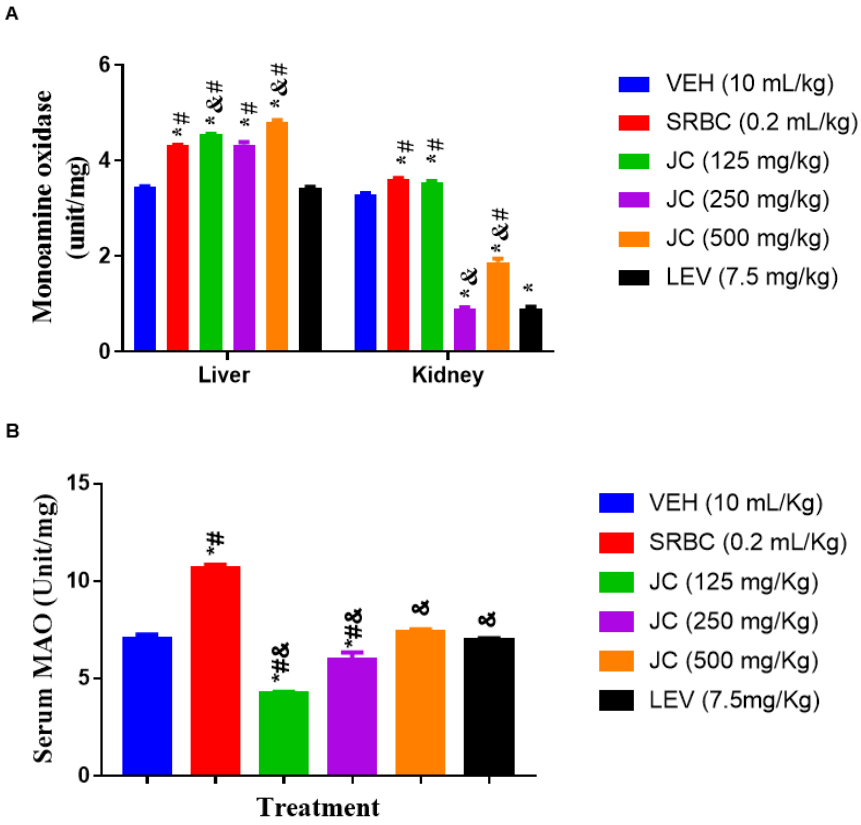

Figure 5: Monoamine oxidase activities of (A) liver and kidney, (B) serum of all treatment groups in mice induced with sheep red blood cells. VEH: Vehicle (Control); JC: *J. carnea*; LEV: Levamisole; SRBCs: Sheep Red Blood Cells. Bar with different symbols is significantly different ($p < 0.05$)

On the other hand, values in SRBC group showed significantly increased activities when compared to all the other treatment groups ($p < 0.05$). However, JC 500 mg/kg and LEV groups were statistically non-significant when compared to VEH group with respect to the MAO activities (Figure 5 B; ($p > 0.05$).

Aldehyde oxidase (AO) activities of mice treated with *J. carnea* extract

Serum AO activities of SRBC-induced mice and treated with aqueous extract of JC are shown in Figure 6. Aldehyde oxidase (AO) activity of mice in SRBC, JC 125 mg/kg and LEV 7.5 mg/kg groups were significantly ($p < 0.05$) reduced compared to VEH group, while JC 250 and 500

mg/kg groups were increased non-significantly ($p < 0.05$) compared to VEH group, but were significantly ($p < 0.05$) increased when compared to both SRBC and LEV groups.

DISCUSSION

The presence of phenolic compounds and flavonoids in *J. carnea* has been well-documented, particularly for their antioxidant capabilities as previously reported [5,14]. In addition, the detection of saponins and other bioactive compounds known for their wide-ranging pharmacological properties further supports the plant's therapeutic potential, particularly as a candidate for vaccine adjuvants in both antiviral and antibacterial formulations [5].

Findings from this study demonstrate that administration of aqueous *J. carnea* leaf extract at 500 mg/kg effectively restored AST activity to baseline levels, comparable to VEH group. This result is noteworthy given that, under disease conditions, AST activity typically increases [2]. Interestingly, in this study, SRBC administration led to a significant reduction in AST, suggesting that the immunization did not promote hepatic inflammation typically associated with elevated transaminase levels. Similarly, ALT activity, which was significantly decreased by SRBC, was normalized in a dose-dependent manner by both the JC leave extracts and the reference drug, LEV groups. In addition, alkaline phosphatase, often elevated during tissue injury or muscle wasting [2], was significantly increased following SRBC treatment. However, this increase was attenuated by *J. carnea* leaf extract and levamisole, with the extract demonstrating a modest but consistent effect. The AST/ALT ratio, also a major diagnostic marker for hepatic conditions such as alcoholic hepatitis and cirrhosis [2], was elevated in SRBC group. Notably, treatment with *J. carnea* extract reversed this trend, restoring the ratio toward normal levels, and indicating hepatoprotective activity. This observation aligns with findings from earlier studies that highlighted the liver-protective effects of *J. carnea* [13]. Furthermore, elevated total protein levels in the JC 250 and 500 mg/kg groups may reflect increased globulin production, possibly due to enhanced antibody synthesis [16]. In contrast, the JC 125 mg/kg group exhibited significantly ($p < 0.05$) lower albumin levels, which could indicate hepatocellular dysfunction or toxicity, consistent with previous assessments of liver health markers [2,16].

Renal function, assessed through urea and creatinine levels, are surrogate markers of glomerular filtration rate (GFR) that offer additional insights. Although the JC 500 mg/kg group showed a non-significant reduction in urea, the trend suggested improved renal function. Notably, the levamisole-treated group exhibited a significant decrease in urea, implying a protective effect against SRBC-induced renal damage. This is important, as elevated creatinine and urea are often associated with impaired GFR and early renal dysfunction [3,13]. Creatinine levels in SRBC group were not significantly different from VEH control but bordered on early toxicity thresholds [13].

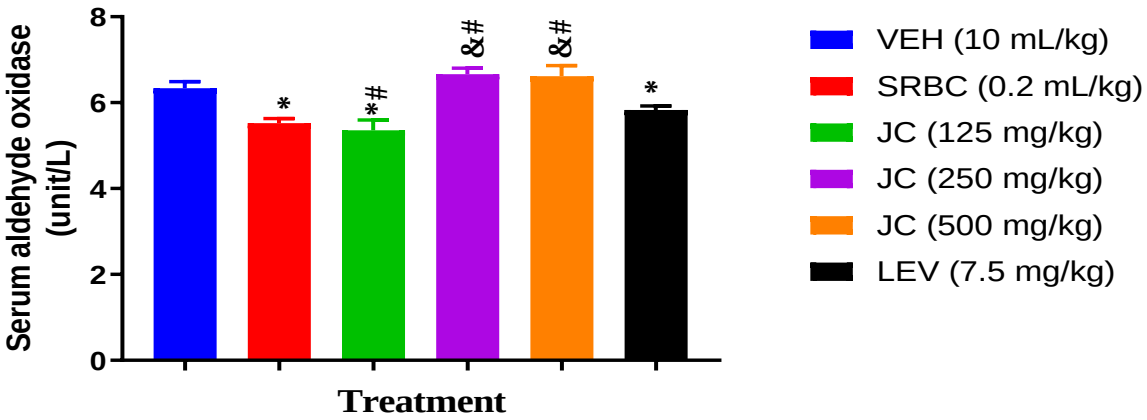

Figure 7: Serum AO activities of all treatment groups in mice induced with sheep red blood cells. VEH: Vehicle (Control); JC: *J. carnea*; LEV: Levamisole; SRBCs: Sheep Red Blood Cells. Bars with different symbols differ significantly ($p < 0.05$)

However, mice treated with *J. carnea* at all doses showed significantly lower creatinine levels, suggesting the extract may mitigate renal toxicity. These results are in agreement with prior findings that support the nephroprotective potential of plant-based therapeutics [16].

Monoamine oxidase (MAO) activity was significantly increased in all *J. carnea*-treated groups compared to Vehicle, except for the levamisole group. This enzyme is essential for the metabolism of biogenic amines and its elevation may indicate increased oxidative metabolism. While enhanced MAO activity could raise ROS levels contributing to oxidative stress, it also supports immune function through the breakdown of neurotransmitters [17]. However, excessive MAO activity has been linked to pathologies such as pheochromocytoma, a condition characterized by overproduction of catecholamines, leading to hypertension and organ damage [17]. Interestingly, in renal tissues, *J. carnea* extract appeared to reduce MAO activity, potentially preventing excessive catecholamine accumulation and preserving kidney function. This dual modulation – upregulating MAO in serum while downregulating it in renal tissue – suggests that *J. carnea* may exhibit organ-specific biochemical actions, possibly via interaction with different substrate receptors [17,5]. Such selective behavior may also indicate the extract's immunostabilizing potential, a trait shared with levamisole.

Aldehyde oxidase (AO), primarily active in the liver, plays a crucial role in the oxidation of aldehydes, including those derived from alcohol metabolism. Administration of SRBC suppressed AO activity, potentially impairing the breakdown of toxic aliphatic compounds and affecting innate immune responses via reduced ROS generation [13]. Levamisole also showed reduced AO activity, confirming SRBC's suppressive effect. Conversely, *J. carnea* extract successfully reversed this suppression, restoring AO to baseline levels, which further supports its hepatoprotective and immunomodulatory properties. Aldehyde oxidase activity, while beneficial in detoxification, can also contribute to oxidative stress through ROS generation in combination with xanthine dehydrogenase. Therefore, modulation of AO may offer a balance between detoxification and the prevention of ROS-induced tissue damage. Additionally, AO is implicated in alcohol metabolism and may be a target for managing alcohol-related disorders [13]. Peroxidase enzymes, which utilize cysteine and haem cofactors, serve as key defenses against elevated ROS. While moderate ROS levels support immune function, excess accumulation can cause cellular damage. Sheep red blood cell immunization increased peroxidase activity, potentially suppressing ROS-driven immune mechanisms. These findings suggest impaired oxidative balance. Interestingly, the aqueous extract of *J. carnea* normalized peroxidase activity in a dose-dependent manner, aligning with previous findings on its antioxidant potential [5,18]. This ability to regulate ROS and support antioxidant enzyme function underlies its therapeutic promise in managing oxidative stress-related immune dysfunctions.

CONCLUSION

Sheep red blood cell-induced immune responses disrupt liver and kidney functions, as shown by altered biomarkers. *Justicia carnea* aqueous extract significantly restores these functions, comparable to levamisole. Improvements in AST/ALT ratio, ALP, total protein, albumin and renal markers observed in tissues and serum further support the potential of the plant extract in this regard. The extract's effect on enzymes like peroxidase and aldehyde oxidase suggests antioxidant involvement in immune regulation. These findings unveil the potential of *J. carnea leaf extract* as an adjuvant in vaccine therapy, with its yet-to-be-identified active compounds, possibly by moderating immune-related side effects in vaccine recipients.

DECLARATIONS

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Ethics approval

Ethical approval was obtained from the University of Port Harcourt's Study Research Ethics Committee (UPH/CEREMAD/REC/MM74/003). We confirm that all study processes were carried out in accordance with the relevant guidelines and regulations and under the supervision of the Ethics Committee and other Regulatory Authorities.

Authors' contributions

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this

article will be borne by the authors. All authors read and approved the final draft of the manuscript for publication.

Conflict of interest

No conflict of interest is associated with this work.

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