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Protective Effects of *Justicia carnea* Lindl. Aqueous-Ethanol Leaf Extract and Silymarin on Thioacetamide-Induced Hepatorenal Injury in Rats

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ABSTRACT

Background and Purpose: Liver and kidney injuries pose significant global health risks and are often linked to oxidative stress and inflammation. Thioacetamide (TAA) is a hepatorenal toxin widely used in experimental models. *Justicia carnea*, has been used traditionally for treating liver disorders and silymarin, a flavonoid complex derived from *Silybum marianum*, is renowned for its hepatoprotective effects. This study investigated the potential protective effects of aqueous-ethanol leaf extract of *J. carnea* and silymarin on TAA-induced hepatorenal injury in rats.

Methods: Six groups (n=5) of male Wistar rats were used in the experiments. Group A (control) received 10 mL/kg of distilled water; group B was administered TAA at a dosage of 300 mg/kg; group C received TAA (300 mg/kg) along with 50 mg/kg of silymarin; groups D, E, and F were administered 300 mg/kg of TAA and subsequently treated with 200, 400, and 600 mg/kg of the extract, respectively. TAA was administered intraperitoneally while silymarin and the extract were administered orally. Treatment was daily for 14 days after which sera and livers from the rats were assayed for enzymes, albumin, bilirubin, urea, creatinine, electrolytes, and antioxidants.

Results: Exposure to TAA significantly elevated liver enzyme markers: alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and bilirubin but reduced albumin levels. TAA exposure also led to increased urea, creatinine, sodium, potassium, and chloride levels and caused a significant decrease in antioxidant enzymes: catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) but increased malondialdehyde (MDA) levels in liver homogenates. However, treatment with silymarin and doses of extract significantly ameliorated hepatorenal function parameters and improved the antioxidant status of the liver in the experimental animals.

Conclusion: These findings suggest that both silymarin and *J. carnea* aqueous-ethanol extract possess hepatoprotective and renoprotective properties, highlighting their potential as therapeutic agents in the management of hepatorenal injuries.

INTRODUCTION

Thioacetamide (TAA) is an organo-sulfur compound identified as a hepatotoxic agent (Stankova *et al.,* 2010). A single administration of this toxin to animals can result in centrilobular necrosis followed by a regenerative response (Chen *et al.,* 2008). Prolonged exposure can induce liver cirrhosis and hepatocarcinoma (Loh *et al.,* 2019). Thioacetamide is favored as a model hepatotoxin due to its high liver specificity, regiospecificity for the perivenous area, and a substantial time gap between its necrogenic effect and liver failure (Mgbemena *et al.,* 2015). The associated injury is characterized by significant elevations in liver function indices (Sukalingam *et al.,* 2018; Jabbar *et al.,* 2023) and the prognosis is often worse when hepatocellular necrosis is accompanied by jaundice (Chilakapati *et al.,* 2005).

The toxicity of TAA arises from bioactivation by a mixed-function oxidase system, particularly involving CYP2EI and FAD monooxygenases (Zaragoza *et al.,* 2000). The metabolic activation of TAA leads to the creation of reactive metabolites, including radicals derived from thioacetamide-S-oxide (TASO) and the reactive oxygen species (ROS) generated as intermediates. TASO is responsible for alterations in cell permeability, increase in intracellular Ca^{2+} concentration, enlargement of nuclear volume, and inhibition of mitochondrial activity, resulting in cell death. These effects severely impact cells located in the perivenous acinus region (Amad *et al.,* 2002).

For centuries, medicines derived from plants have played significant roles in promoting human health. Studies indicate that in recent times, medicinal plants have been extensively utilized in maintaining health and treating various human diseases and disorders (Salmerón-Manzano *et al.,* 2020). Several countries, including Nigeria, continue to rely on medicinal plants for their therapeutic potential. The consumption of plant-based foods regularly is linked to numerous health benefits, rooted in their diverse physiological effects that are due to their phytochemical and nutritional constituents (Altemimi *et al.,* 2017).

Silybum marianum, commonly known as milk thistle and belonging to the Asteraceae family, stands as one of the most ancient and extensively studied plants in historical herbal practices (Soleimani *et al.,* 2019). It has been used traditionally for treating liver and gall bladder disorders that encompass conditions like jaundice, cirrhosis, and hepatitis (Gillessen and Schmidt, 2020). The primary constituent of milk thistle extract is silymarin, which is a combination of flavonolignans, with silybin being the most active among them. Its most recognized attribute is its hepatoprotective effect (Mihailović *et al.,* 2023). Results from both preclinical and clinical studies have shown that silymarin and other flavonolignans possess notable antioxidant, anti-inflammatory, and pro-apoptotic characteristics (Abdel-Moneim *et al.,* 2015; Kim *et al.,* 2019; Adelina, 2022; Iqbal *et al.,* 2022; Shavandi *et al.,* 2022). These attributes contribute to their various biological and pharmacological effects, such as hepatoprotection, neuroprotection, anti-diabetic, anti-cancer, cardio-protection, photoprotection, and immunomodulation (Wadhwa *et al.,* 2022).

Justicia carnea is a plant known for its medicinal properties. According to the United States Department of Agriculture (USDA) classification, the plant belongs to the Acanthaceae family. It is a flowering plant commonly known as the Brazilian plume flower, Brazilian-plume, flamingo flower, or jacobinia. Indigenous to the Atlantic Forest eco-regions in eastern Brazil, it is also found in Nigeria (Corrêa *et al.,* 2012). The nutritional composition of the aqueous leaf extract of *J. carnea* was evaluated and the extract was found to be a potential source of nutrients being rich in vitamins A, B_2, B_{12}, B_9, B_1, C, and E, along with minerals like copper, zinc, magnesium, iron, and calcium (Orjiakor *et al.,* 2019). Vitamins play a vital role in maintaining the health of the nervous system, aid in the formation of red blood cells and help to build tissues (Calderón-Ospina and Nava-Mesa, 2020). Vitamins such as vitamins A, C, and E are antioxidants that scavenge free radicals suggesting their possible role in preventing and repairing damages caused by reactive oxygen species (Didier *et al.,* 2023). Phytochemical analysis has also revealed the presence of alkaloids, flavonoids, glycosides, carbohydrates, saponins, tannins, reducing sugars, terpenoids, and phenols in the aqueous leaf extract of *J. carnea* (Orjiakor, 2019; Ebhohon *et al.,* 2023). The extract also contains 54% carbohydrates, 11.62% ash, and 21.33% protein (Orjiakor *et al.,* 2019).

There is limited knowledge on the potential protective effects of natural compounds, such as aqueous-ethanol leaf extract of *J. carnea* on TAA-induced hepatorenal injury in rats. This study aimed to assess the protective effects of aqueous-ethanol leaf extract of *J. carnea* and silymarin on TAA-induced hepatorenal injury in rats.

MATERIALS AND METHODS

Chemicals and Reagents

Thioacetamide salt, silymarin, absolute ethanol, and chloroform were procured from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

Plant Material and Extraction

Fresh leaves of *Justicia carnea* were obtained from Umuariaga, Umudike, in May 2018 and authenticated by Mr. Nwoko Magnus, a Botanist of the Department of Plant Science and Biotechnology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike,

Umuahia, Abia State. A voucher specimen number MOUAU- 0623 was assigned, and the plant was also cross-checked at https://www.ipni.org. The leaves were washed with distilled water and air-dried at room temperature. The dried leaves were pulverized into coarse powder using a blender. A quantity of the powdered leaves (500 g) was macerated in an extraction glass jar containing 300 mL of distilled water and 700 mL of absolute ethanol (to obtain an aqueous-ethanol extraction solvent system) for 48 h, with periodic agitation. The macerated leaves were filtered using muslin cloth and Whatman filter paper #1. The filtrate was collected, stored in a glass beaker, and then subjected to freeze-drying at ≤ -40℃ for 24 h. The freeze-dried extract was preserved at 4℃ in an airtight glass container until needed for biochemical assays.

Preparation and Administration of the Aqueous-Ethanol Leaf Extract of *J. carnea*

The dry extract was reconstituted in distilled water to obtain the needed concentration for administration by gavage of the intended dosages of 200, 400, and 600 mg/kg of body weight.

Thioacetamide (TAA) and Silymarin Preparation and Administration

Thioacetamide (TAA) was prepared by dissolving it in normal saline and administered intraperitoneally (i.p.) to the rats at a dose of 300 mg/kg body weight. The standard drug (silymarin) was dissolved in distilled water and administered by gavage at a dose of 50 mg/kg body weight.

Animal Care

Thirty (30) healthy male Wistar rats were acquired from the Department of Zoology and Environmental Sciences, University of Nigeria, Nsukka. The rats were acclimatized for two weeks in the animal housing facilities of the Department of Biochemistry at Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State. The animals were housed in well-ventilated cages (stainless steel bottom and wire mesh top), under controlled environmental conditions with a twelve-hour light-dark cycle. All experimental procedures involving animal handling were carried out in strict compliance with protocols approved by the Animal Care and Ethical Committee of Michael Okpara University of Agriculture, Umudike, with approval number COLNAS18098.

Determination of oral LD_{50}

The oral median lethal dose (LD_{50}) of the aqueous-ethanol leaf extract of *J. carnea* was estimated according to the method described by Lorke (1983).

Experimental Design

Male rats were randomly allotted to six groups: group A (control) which receive 10 mL/kg of distilled water, group B (300 mg/kg of TAA only), group C (TAA and 50 mg/kg of silymarin), group D (TAA and 200 mg/kg of extract), group E (TAA and 400 mg/kg of extract), and Group F (TAA and 600 mg/kg of extract). All groups were treated for 14 days. TAA was used to induce liver and kidney damage, and the effects of silymarin and *J. carnea* leaf extract on some biochemical markers were evaluated.

Blood Sample and Tissue Collection

At the end of the experiment, the animals were fasted overnight and anaesthetized by chloroform inhalation in a closed chamber. Blood samples were drawn using a 5 mL syringe via cardiac puncture (Arunachalam and Sasidharan, 2021) into plain sample tubes and centrifuged at 4000 rpm for 10 min to obtain sera used for biochemical assays in this study. The livers were excised and transferred into containers filled with chilled saline to maintain tissue integrity and prevent degradation.

Preparation of Liver Homogenate

A piece (1 g) of liver from each rat was homogenized 9 mL of cold phosphate buffer (0.05 M, pH 7.0) with a Teflon homogenizer. The homogenate was centrifuged at 4000 rpm for 10 min. The supernatant obtained was stored frozen at -20℃ until required for the analyses of CAT, SOD, GSH and MDA levels.

Liver Function Tests

The serum activities of aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP), as well as the concentrations of albumin and total bilirubin were determined using their respective Randox® kits.

Renal Function Tests

Serum urea nitrogen and creatinine were determined using the Fawcet and Scott (1960) and Bartels and Bohmer (1972) methods respectively. Serum chloride, sodium and potassium levels were determined using the methods of Tietz (1976) as outlined in the Teco® diagnostic kit leaflet.

Antioxidant Assays

Catalase activity in serum was determined using the modified method described by Cohen *et al.* (1970). Superoxide dismutase (SOD) activity in serum was determined using the method described by Misra and Fridovich (1972). Reduced glutathione (GSH) levels in the sera were determined using the method described by Tietze (1969). The concentration of malondialdehyde (MDA) in serum was determined using the method described by Ohkawa *et al.* (1979).

Statistical Analysis

The results are presented as mean ± standard deviation (SD) and analyzed using SPSS 20 (IBM, USA). Differences between groups were statistically analyzed using one-way analysis of variance (ANOVA), and mean values that were significantly different from each other were identified by

Duncan's multiple range test. $P<0.05$ was considered significant.

RESULTS

Acute Toxicity

The oral median lethal dose (LD_{50}) of aqueous-ethanol leaf extract of *J. carnea* was greater than 5000 mg/kg. No death was recorded at the maximum dose.

Effects of Aqueous-ethanol Leaf Extract of *Justicia carnea* on Serum ALT, AST, ALP, Albumin and Total Bilirubin of Rats Treated with Thioacetamide (TAA)

The results in Table 1 indicate that treatment with TAA led to a significant increase in serum levels of ALT, AST, and ALP in the rats compared to the control group. However, when the rats were treated with 50 mg/kg of silymarin and the varying doses of the extract, there were significant decreases in the levels of these liver enzymes when compared to the rats administered TAA only. The results also show that the higher the dose of the extract, the more the reductions in the serum levels of these enzymes, suggesting a potential dose-response relationship.

There were alterations in the serum concentrations of albumin and total bilirubin in response to TAA treatment. When compared to the control group, TAA-treated rats had significant decreases in albumin and significant increases in total bilirubin concentrations. The administration of both silymarin and the extract had positive effects on these serum parameters when compared to the TAA-only rats. Both interventions led to a significant increase in albumin concentrations and a significant decrease in total bilirubin concentrations. The results also indicate that at the dose of 400 mg/kg of the extract, there was a more substantial increase in albumin concentration compared to control, silymarin, 600 mg/kg, and 200 mg/kg of the extract. This suggests that the 400 mg/kg dose of the extract was particularly effective at enhancing albumin levels, surpassing the impact of silymarin and other doses.

Similarly, at a dose of 600 mg/kg of the extract, a more pronounced decrease in total bilirubin concentration was observed compared to control, silymarin, 400 mg/kg, and 200 mg/kg of the extract. This implies that the 600 mg/kg dose of the extract exhibited greater efficacy in reducing the concentration of total bilirubin in serum, surpassing the effects of silymarin and other doses of the extract. The study suggests that specific doses of the extract, particularly 400 mg/kg for albumin and 600 mg/kg for total bilirubin, were more effective than other doses and even silymarin in ameliorating the hepatic injury induced by thioacetamide.

Effects of aqueous-ethanol leaf extract of *Justicia carnea* on serum concentrations of urea, creatinine, sodium ion, potassium ion and chloride ion of rats treated with thioacetamide (TAA)

Table 2 shows that the serum concentrations of creatinine, urea, sodium, potassium, and chloride ions were significantly elevated in the TAA-treated group compared to control, silymarin and extract-treated groups. However, the administration of 50 mg/kg of silymarin and the various doses of the extract demonstrated a significant decrease in the concentrations of urea, creatinine, and serum electrolytes of the treated animals compared to the rats treated with only TAA. The extract showed dose-dependent effects on serum urea, creatinine, and electrolyte concentrations. The doses of the extract were more effective than silymarin in mitigating the harmful effects of TAA on urea, while the dose of 600 mg/kg of the extract was particularly effective in ameliorating the detrimental effects of TAA on urea, creatinine, potassium ions, and chloride ions when compared to silymarin, and doses of 400 mg/kg, and 200 mg/kg of the extract. The protective effects of silymarin against the harmful effects of TAA on renal indices were comparable to those of the extract. Interestingly, while the doses of the extract were effective in restoring sodium ion levels to near normal, silymarin demonstrated better efficacy in restoring sodium ion levels to near normal when compared to control and the extract-treated groups.

Effects of aqueous-ethanol leaf extract of *Justicia carnea* on levels of catalase, superoxide dismutase, reduced glutathione and malondialdehyde of rats treated with thioacetamide (TAA)

Table 3 highlight noteworthy changes in serum levels of CAT, SOD, reduced glutathione (GSH), and MDA among rats treated with TAA compared to the control group. Specifically, there were significant reductions in CAT, SOD, and GSH levels, accompanied by a significant increase in MDA levels in the TAA-treated group. However, administration of 50 mg/kg of silymarin and the various doses of the extract resulted in significant increases in CAT, SOD, and GSH levels, along with a significant decrease in MDA levels compared to the group treated with TAA alone. Moreover, the extract demonstrated superior efficacy in alleviating the adverse effects of TAA on CAT, SOD, and GSH levels when compared to the silymarin-treated group. The protective effects of both the extract and silymarin against the negative impact of thioacetamide on MDA were comparable. Notably, the administration of 600 mg/kg of the extract was more effective at reducing the elevated MDA levels compared to other doses of the extract and silymarin.

Table 1: Effects of aqueous-ethanol leaf extract of *Justicia carnea* on serum ALT, AST, ALP, Albumin and total bilirubin of rats treated with thioacetamide (TAA)

GROUPS	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	ALBUMIN (mg/dL)	TOTAL BILIRUBIN (mg/dL)
A Control)	56.50 ± 0.71^{a}	56.00 ± 0.01^{a}	50.50 ± 0.71^{a}	3.70 ± 0.01^{a}	0.94 ± 0.07^{a}
B (TAA only)	69.50 ± 0.71^{b}	64.50 ± 0.71^{b}	65.50 ± 0.71^{b}	2.60 ± 0.01^{b}	1.35 ± 0.07^{b}
C (TAA + silymarin 50 mg/kg)	53.00 ± 2.83^{c}	51.00 ± 1.41^{c}	54.50 ± 0.71^{c}	3.65 ± 0.07^{c}	0.90 ± 0.01^{a}
D (TAA + *J. carnea* 200 mg/kg)	59.00 ± 1.41^{d}	58.00 ± 1.41^{d}	57.50 ± 0.71^{d}	3.30 ± 0.28^{d}	1.15 ± 0.07^{c}
E (TAA + *J. carnea* 400 mg/kg)	54.50 ± 0.71^{e}	54.00 ± 1.41^{e}	55.00 ± 1.41^{c}	3.70 ± 0.28^{a}	0.97 ± 0.03^{a}
F (TAA + *J. carnea* 600 mg/kg)	50.50 ± 0.71^{f}	50.50 ± 0.71^{c}	50.50 ± 0.71^{e}	3.45 ± 0.07^{e}	0.83 ± 0.19^{d}

Alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP). Values are represented as mean ± S.D (n=5). Means with different superscripts are significantly (P<0.05) different while those with the same superscripts are not significantly different

Table 2: Effects of aqueous-ethanol leaf extract of *Justicia carnea* on serum concentrations of urea, creatinine, sodium ion, potassium ion and chloride ion of rats treated with thioacetamide (TAA)

GROUPS	Urea (mg/dL)	Creatinine (mg/dL)	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)
A (Normal Control)	0.55 ± 0.05^{a}	52.50 ± 2.50^{a}	121.50 ± 1.50^{a}	4.15 ± 0.15^{a}	81.50 ± 1.50^{a}
B (TAA only)	0.80 ± 0.00^{b}	109.00 ± 9.00^{b}	147.50 ± 3.50^{b}	11.50 ± 0.50^{b}	121.00 ± 2.00^{b}
C (TAA + silymarin 50 mg/kg)	0.73 ± 0.04^{c}	65.50 ± 0.50^{c}	126.50 ± 3.50^{c}	6.50 ± 0.50^{c}	90.00 ± 1.00^{c}
D (TAA + *J. carnea* 200 mg/kg)	0.62 ± 0.02^{d}	71.50 ± 1.50^{d}	137.00 ± 4.00^{d}	7.50 ± 0.50^{d}	99.50 ± 0.50^{d}
E (TAA + *J. carnea* 400 mg/kg)	0.61 ± 0.01^{d}	68.50 ± 0.50^{e}	136.50 ± 0.50^{d}	6.85 ± 0.25^{e}	98.50 ± 0.50^{d}
F (TAA + *J. carnea* 600 mg/kg)	0.58 ± 0.03^{e}	60.00 ± 0.00^{f}	133.25 ± 2.65^{e}	6.40 ± 0.40^{c}	88.50 ± 0.50^{e}

Sodium ion (Na^+), potassium ion (K^+), and chloride ion (Cl^-). Values are represented as mean ± S.D (n=5). Means with different superscripts are significantly (P<0.05) different while those with the same superscripts are not significantly different.

Table 3: Effects of aqueous-ethanol leaf extract of *Justicia carnea* on levels of catalase, superoxide dismutase, reduced glutathione and malondialdehyde of rats treated with thioacetamide (TAA)

GROUPS	CAT (U/mL)	SOD (U/mL)	GSH (U/mL)	MDA $x10^{-3}$ (mmol/mL)
A (Normal Control)	1.46 ± 0.01^{a}	1.77 ± 0.02^{a}	1.66 ± 0.02^{a}	0.85 ± 0.13^{a}
B (TAA only)	1.38 ± 0.02^{b}	1.55 ± 0.38^{b}	1.55 ± 0.25^{b}	2.47 ± 0.15^{b}
C (TAA + silymarin 50 mg/kg)	1.87 ± 0.14^{c}	1.91 ± 0.69^{c}	1.83 ± 0.24^{c}	1.30 ± 0.20^{c}
D (TAA + *J. carnea* 200 mg/kg)	2.22 ± 0.59^{d}	2.13 ± 0.15^{d}	2.06 ± 0.12^{d}	1.57 ± 0.12^{d}
E (TAA + *J. carnea* 400 mg/kg)	2.15 ± 0.21^{e}	2.22 ± 0.30^{e}	2.15 ± 0.23^{e}	1.20 ± 0.14^{e}
F (TAA + *J. carnea* 600 mg/kg)	2.24 ± 0.12^{d}	2.04 ± 0.16^{f}	1.99 ± 0.12^{f}	1.03 ± 0.15^{f}

Catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) and malondialdehyde (MDA). Values are represented as mean ± S.D (n=5). Means with different superscripts are significantly (P<0.05) different while those with the same superscripts are not significantly different.

DISCUSSION

The result of the oral LD_{50} estimation suggests that the aqueous-ethanol leaf extract of *J. carnea* has a low level of acute toxicity, as evidenced by the absence of deaths in the experimental animals at the dose of 5000 mg/kg. This finding aligns with the opinions of Peters *et al.* (2022) and Akintimehin *et al.* (2021) on the extract.

TAA administration induced liver damage in experimental rats, as evidenced by elevated levels of liver enzymes, albumin and bilirubin. The enzymes are released into the bloodstream when liver cells are injured, often due to toxic exposure or liver diseases (Zargar *et al.,* 2017). ALT and AST are enzymes found in liver cells, and their elevated levels in the serum indicate liver damage or inflammation (Giannini *et al.,* 2005). The decrease in ALT and AST levels with the extract and silymarin treatment indicates a potential protective effect on the liver, as lower enzyme levels suggest reduced liver damage. ALP is an enzyme that is elevated in conditions affecting the biliary system, such as cholestasis (Pollock and Minuk, 2017). The increase in ALP after TAA administration may suggest damage or dysfunction in the biliary system. The decrease in ALP levels following treatment with the extract and silymarin suggests a protective effect, potentially mitigating the damage to the biliary system. Albumin is a protein produced by the liver, and its levels are often used as an indicator of liver function and overall health (Carvalho and Machado, 2018). A decrease in albumin after TAA administration might be a compensatory response to liver damage (Sun *et al.,* 2019). The increase in albumin levels with extract and silymarin treatment could indicate a normalization of liver function, as the levels are brought back to baseline. Bilirubin is a yellow pigment formed during the breakdown of red blood cells, and elevated levels can indicate liver dysfunction or impaired bilirubin metabolism (Guerra *et al.,* 2021). The increase in total bilirubin after TAA administration suggests compromised liver function. The decrease in total bilirubin levels with extract and silymarin treatment indicates a potential improvement in liver function and bilirubin metabolism. The results of this study suggest that the extract and silymarin have hepatoprotective properties, potentially shielding the liver from the harmful effects of TAA. These findings align with studies conducted by Sukalingam *et al.* (2018) and Jabbar *et al.* (2023).

In this study, the results also suggest that TAA treatment in rats led to kidney dysfunction, as evidenced by elevated levels of urea and creatinine, along with disturbances in serum electrolyte levels (Mohammed, 2020). Urea and creatinine are waste products excreted by the kidneys. Elevated levels in serum indicate impaired kidney function (Kellum *et al.,* 2021). The significant increase in urea and creatinine in the serum of TAA-treated rats suggests kidney damage or dysfunction. The subsequent significant decrease in urea and creatinine levels with the administration of the extract and silymarin indicates a potential protective effect on the kidneys, suggesting a restoration of normal renal function. Sodium, potassium, and chloride ions are essential electrolytes that play a crucial role in maintaining various physiological functions, including fluid balance and nerve/muscle function (Jomova *et al.,* 2022). An increase in sodium and chloride levels might indicate impaired reabsorption, while elevated potassium levels could signify decreased excretion (Ellison, 2017; Rodan, 2017). The significant increase in serum levels of sodium, potassium, and chloride ions in TAA-treated rats might indicate disturbances in electrolyte balance, possibly due to kidney dysfunction or other factors affecting ion regulation (Thompson and Joy, 2022). The subsequent significant decrease in these electrolyte levels with the administration of the extract and silymarin suggests a restoration of normal electrolyte balance. This could be indicative of improved kidney function or a direct effect of the extract and silymarin on serum ion regulation. The administration of the extract and silymarin appears to have a protective effect on the kidneys, as indicated by the significant reduction in urea, creatinine, and electrolyte levels. This implies that the extract and silymarin may have nephroprotective properties, potentially mitigating the harmful effects of TAA on renal function and electrolyte balance in the experimental rats. These findings also align with the research conducted by Ebaid *et al.* (2023).

The results of this study suggest that TAA treatment induced oxidative stress in the rats, as evidenced by reduced CAT, SOD, GSH and increased MDA levels (Jena *et al.,* 2023). CAT and SOD are enzymes that play crucial roles in antioxidant defense by neutralizing reactive oxygen species (ROS). The significant reductions in the serum levels of CAT and SOD in the rats treated with TAA suggest a compromised antioxidant defense system. The subsequent significant rise in serum levels of CAT and SOD following treatment with silymarin and the extract suggests a restoration of antioxidant capacity.

This suggests that silymarin and the extract may have antioxidant properties, enhancing the activities of these enzymes and potentially mitigating oxidative stress. GSH is an important cellular antioxidant that plays a key role in protecting cells from oxidative damage. The significant reduction in GSH levels in the serum of TAA-treated rats suggests a depletion of this antioxidant, further supporting the idea of increased oxidative stress (Kwon *et al.,* 2019). The significant increase in GSH levels with silymarin and extract treatment suggests a replenishment of GSH. This indicates that silymarin and the extract may enhance the cellular antioxidant defense system, promoting GSH synthesis or preventing its depletion. MDA is a marker of lipid peroxidation and oxidative damage. Increased MDA

levels indicate oxidative stress-induced damage to lipids. The significant increase in MDA levels in the serum of TAA-treated rats suggests enhanced lipid peroxidation and oxidative damage (Ito *et al.*, 2019). The subsequent significant decrease in MDA levels with silymarin and extract treatment indicates a reduction in lipid peroxidation. This suggests that silymarin and the extract may have antioxidant properties, protecting against oxidative damage to lipids. These results are consistent with studies carried out by Sukalingam *et al.* (2018) and Jabbar *et al.* (2023).

CONCLUSION

This study has demonstrated the potential therapeutic properties of the aqueous-ethanol leaf extract *Justicia carnea* in mitigating TAA-induced hepatorenal damage. The extract seemed to have mitigated the physiological changes induced by TAA by enhancing antioxidant defenses, as indicated by elevated CAT, SOD, GSH and reduced MDA levels. This antioxidant property may be responsible at least in part for maintaining the functionality of the liver and kidneys. Further investigations are needed to explore the underlying mechanisms and clinical applications of these protective agents in liver and kidney disorders.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR'S CONTRIBUTION

The study was conceptualized by SOE. Experiments were conducted by MK, UOL, and IAJ under the supervision of SOE. EVA was responsible for analyzing the data as well as revising the final draft of the article. SOE wrote the manuscript and all authors thoroughly reviewed and approved the final draft, taking collective responsibility for the contents.

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AUTHORS' DECLARATION

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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