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Evaluation of Proximate Composition, Quantitative Phytochemical and Antioxidant Vitamin Level of *Justicia carnea*

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ABSTRACT

Cadmium-induced hepatotoxicity poses significant health risks, and conventional treatments have limitations. The traditional use of Justicia carnea as a blood and liver tonic needs scientific validation, prompting this study to investigate its hepatoprotective effects and potential therapeutic applications. This study evaluated the hepatoprotective effects of ethanolic leaves extract *Justicia carnea* against cadmium-induced liver damage in male Wistar rats. The extract's traditional use as a blood and liver tonic was validated by assessing its potential to mitigate cadmium-induced liver and blood toxicity. Phytochemical analysis revealed the presence of Naringin, Vinnillic acid, and Nobiletin, while proximate composition showed high moisture content, carbohydrates, and protein. Acute toxicity tests confirmed the extract's safety. Cadmium exposure led to significant ($p \leq 0.05$) liver damage, marked by elevated liver enzymes and bilirubin levels. However, treatment with *J. carnea* extract (100mg/kg and 300mg/kg) showed significant ($p \leq 0.05$) improvements in liver function, with the higher dose exhibiting more pronounced effects. The extract also improved hematological parameters, including PCV, RBC, and Hb levels. Renal function markers, such as urea and creatinine, were also improved. The study's findings suggest that *J. carnea* ethanol extract has hepatoprotective and hematological benefits, validating its traditional use. The extract's potential as a natural remedy for cadmium-induced hepatotoxicity and related oxidative stress disorders warrants further research to explore its mechanisms, optimal dosage, and therapeutic applications. The results of this study provide scientific evidence for the traditional use of *J. carnea* as a blood and liver tonic, and highlight its potential as a natural therapeutic agent for liver-related disorders.

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

INRODUCTION

The use of traditional medicine in Africa, particularly in Nigeria, with a focus on *Justicia carnea* (Flamingo Flower). This plant has gained attention for its medicinal properties, particularly for treating various ailments, including gastrointestinal issues, respiratory infections, pain, and diabetes. *Justicia carnea* contains several bioactive compounds, such as phenols, terpenoids, tannins, alkaloids, flavonoids, saponins, and glycosides, along with essential vitamins like A, B1, B2, B6, B12, C, and E. Among these, vitamin C is found in the highest concentration. The plant's leaves also have significant amounts of iron, calcium, and other minerals, though it contains relatively low levels of magnesium, zinc, and copper.

In addition to its medicinal properties, *Justicia carnea* has a significant role in combating oxidative stress and inflammation. It also shows potential in addressing issues related to cadmium toxicity, which is a concern for liver health and overall body function. Cadmium exposure is associated with liver damage, oxidative stress, and immunotoxicity, and *Justicia carnea* has been explored for its protective effects against such toxicity due to its antioxidant and anti-inflammatory properties.

The study of *Justicia carnea* involves evaluating its proximate composition, phytochemical content, and antioxidant capacity. Research has identified the plant's potential for promoting health and mitigating damage caused by toxins like cadmium. This includes its high antioxidant activity and various essential nutrients, making it a valuable subject for exploring natural remedies for diseases related to oxidative stress and inflammation. The plant's active compounds, such as vitamins, alkaloids, and flavonoids, contribute to its therapeutic effects, supporting its use in traditional medicine across Africa.

In summary, *Justicia carnea* (Flamingo Flower) stands out for its rich phytochemical content, high antioxidant potential, and significant levels of vitamins, particularly vitamin C. It holds promise as a natural remedy for a variety of health conditions, including those exacerbated by environmental toxins like cadmium.

MATERIALS AND METHODS

Experimental Design

In this study evaluating the proximate composition, quantitative phytochemical content, and antioxidant vitamin levels of *Justicia carnea*, an experimental animal model was used to assess the plant's protective effects against cadmium-induced hepatotoxicity. A total of 25 Wistar rats were divided into five groups (A–E), with five rats per group. Group A served as the normal control and received only food and water. Group B was the negative control, receiving 5 mg/kg of cadmium (Cd) daily to induce hepatotoxicity without treatment. Group C received the same Cd dosage but was treated with 100 mg/kg of silymarin, a standard hepatoprotective drug, serving as the positive control. Groups D and E were administered 100 mg/kg and 300 mg/kg of ethanol extract of *Justicia carnea*, respectively, following Cd induction. The induction and treatment period lasted for one month.

This design allowed for the evaluation of *Justicia carnea*'s antioxidant potential—owing to its rich phytochemical constituents such as flavonoids, alkaloids, saponins, and vitamins (notably vitamin C)—in mitigating oxidative stress and liver damage caused by cadmium exposure. The outcomes aim to support the ethnomedicinal relevance of *Justicia carnea* and its therapeutic application in liver protection and detoxification.

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

The Justicia carnea was acquired from Eke Igwe Orizu, a local market in Nnewi North Local Government Area, Anambra State , were authenticated by

Dr. B. N. Uwalaka of the University of Agriculture and Environmental Sciences, Umuagwo, Imo State, and assigned voucher number UAES/HB/0037. The leaves were hand-picked, thoroughly washed, sun-dried, and further dried in a hot-air oven at 60°C. After complete drying, they were pulverized into fine powder and stored in air-tight containers for later extraction and analysis.

Preparation of Ethanol Extracts of *Justicia carnea*:

The dried and powdered leaves of *Justicia carnea* were subjected to ethanol extraction. A measured quantity of the powdered plant material was soaked in 70% ethanol and left to macerate for 72 hours with occasional stirring to enhance extraction efficiency. After maceration, the mixture was filtered using Whatman No. 1 filter paper to separate the liquid extract from the plant residue. The filtrate was then concentrated using a rotary evaporator under reduced pressure and at a controlled temperature not exceeding 45°C. The resulting semi-solid extract was further dried to obtain a solid ethanol extract, which was stored in an air-tight container under refrigeration until use for experimental analysis.

Phytochemical and Bioactive Composition of *Justicia carnea*

The phytochemical and bioactive constituents of *Justicia carnea* were analyzed using Gas Chromatography-Flame Ionization Detection (GC-FID). The analysis was conducted with a BUCK M910 Gas Chromatograph fitted with a Flame Ionization Detector (FID) and a RESTEK 15-meter MXT-1 capillary column (15 m × 250 µm × 0.15 µm). This technique enabled precise separation and quantification of volatile and semi-volatile phytochemicals present in the ethanol extract.

The GC-FID analysis revealed the presence of several key phytochemicals, including flavonoids, alkaloids, saponins, tannins, and phenolic compounds. These bioactive components are known for their antioxidant, anti-inflammatory, hepatoprotective, and antimicrobial properties. Flavonoids and phenols, in particular, are associated with free radical scavenging and liver cell protection. The presence of these compounds supports the traditional use of *Justicia carnea* in managing oxidative stress-related conditions, including liver toxicity.

This detailed phytochemical profile underscores the therapeutic potential of

Justicia carnea, particularly in mitigating cadmium-induced hepatotoxicity, and justifies its continued investigation as a plant of pharmacological interest.

Evaluation of Proximate Composition of *Justicia carnea*

The proximate composition of *Justicia carnea* was assessed following standard AOAC (1998) procedures to determine the basic nutritional components: moisture, ash, fiber, fat, protein, and carbohydrates.

Moisture Content:
Determined using the oven-drying method at 105°C. The sample was weighed before and after drying, and the moisture percentage was calculated based on weight loss.

Ash Content:
Estimated by incinerating the sample in a muffle furnace at 550°C to eliminate organic matter. The residual inorganic mineral content (ash) was expressed as a percentage of the original sample weight.

Crude Fiber Content:
Determined gravimetrically by sequential acid (H_2SO_4) and alkaline (NaOH) digestion to remove digestible components. The undigested residue was dried, ashed, and weighed to calculate the fiber content.

Fat Content:
Extracted using a Soxhlet apparatus with petroleum ether or hexane as the solvent. The percentage fat was calculated from the weight difference before and after extraction.

Protein Content:
Estimated using the Kjeldahl method, which measures nitrogen content. The nitrogen value was multiplied by a conversion factor (6.25) to obtain the total protein content.

Carbohydrate Content:
Calculated by difference using the formula:
Carbohydrates (%) = 100 – (Moisture + Ash + Fat + Protein)

All analyses were performed in duplicate or triplicate for reliability, with proper sample preparation, calibration, and adherence to safety and quality control measures throughout the procedures. This comprehensive proximate analysis highlights the nutritional potential and phytochemical richness of *Justicia carnea*.

Antioxidant Vitamin Analysis of *Justicia carnea*

The antioxidant vitamin content of *Justicia carnea* was determined using standard analytical methods described by Kirk and Sawyer (1998) and James (1995). The vitamins analyzed included Vitamins A, C, E, and B-complex (Thiamine, Riboflavin, and Niacin), each evaluated using specific colorimetric, titrimetric, or fluorometric techniques as outlined below.

Determination of Vitamin A (Retinol)

Vitamin A was determined by the colorimetric method of Kirk and Sawyer (1998). This method is based on the ability of Vitamin A (retinol) to form a colored complex with phosphomolybdic acid in the presence of sulfuric acid. The intensity of the color, which is proportional to the concentration of Vitamin A, was measured spectrophotometrically at 328 nm.

Approximately 1 g of the sample was extracted using hexane or acetone. The resulting extract was reacted with phosphomolybdic acid and sulfuric acid and allowed to stand for 5–10 minutes for full color development. Absorbance readings were taken against a blank, and Vitamin A content was quantified using a standard calibration curve prepared from known concentrations of standard Vitamin A solution.

Determination of Vitamin E (Tocopherol)

The Vitamin E content was analyzed using the Futter-Mayer colorimetric method, also reported by Kirk and Sawyer (1998). Vitamin E (tocopherols) reacts with bathophenanthroline, ferrous chloride, and orthophosphoric acid to produce a red-colored complex. The absorbance of this complex was measured at 520 nm using a spectrophotometer.

A known weight of the sample (1–5 g) was extracted with ethanol. The clear extract was then reacted with the bathophenanthroline reagent, ferrous chloride, and orthophosphoric acid. After 10 minutes of incubation at room temperature, the absorbance was measured. Quantification was done by comparing the absorbance values to a standard curve prepared from alpha-tocopherol solutions of known concentrations.

Determination of Vitamin C (Ascorbic Acid)

Vitamin C was determined using the titrimetric method based on the redox reaction between ascorbic acid and 2,6-dichlorophenolindophenol (DCPIP), as described by Kirk and Sawyer (1998). In

this method, ascorbic acid reduces the blue-colored DCPIP to a colorless compound in acidic medium. The endpoint was observed as a faint pink color that persisted for 10–15 seconds.

A known weight (5–10 g) of the sample was homogenized in 0.4% oxalic acid and filtered. A 10 mL aliquot of the filtrate was titrated with the standardized DCPIP solution. The volume of titrant used was noted, and the Vitamin C content was calculated using the dye factor derived from standard ascorbic acid solutions. Results were expressed in mg/100 mL of extract or mg/100 g of sample.

Determination of Vitamin B-Complex (Thiamine, Riboflavin, Niacin)

Vitamin B-complex content, comprising thiamine (B1), riboflavin (B2), and niacin (B3), was determined using the method described by James (1995). This involved the following analytical procedures:

Thiamine (Vitamin B1)

Thiamine in the sample was oxidized to thiochrome using alkaline potassium ferricyanide. Thiochrome exhibits fluorescence under ultraviolet light. The fluorescence intensity, measured with a fluorometer (excitation at 365 nm and emission at 440 nm), was compared with that of standard thiamine solutions for quantification.

Riboflavin (Vitamin B2)

Riboflavin, which naturally fluoresces under UV light, was stabilized by adding hydrochloric acid and heated in a water bath at 70°C for 5 minutes. The absorbance was then measured at 450 nm using a spectrophotometer. The concentration of riboflavin was calculated by comparison to a standard calibration curve.

Niacin (Vitamin B3)

Niacin content was determined colorimetrically by reacting the sample with cyanogen bromide (or chloramine-T), forming a colored complex. The absorbance was read at 470 nm using a spectrophotometer. Quantification was based on a standard curve prepared from nicotinic acid standards.

Calculation of Vitamin Content

For Vitamins A, E, B1, B2, and B3, the concentration in the sample was calculated using the following formula:

$$\text{Vitamin Concentration (mg/100 g)} = \frac{(\text{Abs}_{\text{sample}} \times C_{\text{standard}} \times \text{Dilution Factor} \times 100)}{\text{Abs}_{\text{standard}} \times \text{sample weight (g)}}$$

For Vitamin C, titration data was used to calculate the dye factor (DF) and the Vitamin C concentration as:

$$DF = \frac{\text{Weight of standard ascorbic acid (mg)}}{\text{Volume of DCPIP used for standard (mL)}}$$

$$\text{Vitamin C (mg/100 mL)} = \left(\frac{V_{\text{sample}} \times C_{\text{DCPIP}} \times M_{\text{ascorbic acid}}}{V_{\text{standard}} \times W}\right) \times 100$$

Where:

V_{sample} : Volume of DCPIP used for the sample (mL)

C_{DCPIP} : Concentration of DCPIP solution (mg/mL), determined during standardization

$M_{ascorbic\ acid}$: Molar mass of ascorbic acid (176.12 g/mol)

$V_{standard}$: Volume of ascorbic acid used for standardization (mL)

W: Weight/volume of the sample (e.g., grams or mL).

All measurements were performed in duplicate or triplicate to ensure precision, and strict precautions were taken to prevent degradation of vitamins by light, heat, or oxidation. Glassware and reagents were verified to be contaminant-free prior to analysis.

Data Analysis

The data obtained were expressed as mean ±SD of three replicates. Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 23. One Way Analysis of Variance were adopted for comparison, and the results were subjected to post hoc test using least square deviation (LSD). $p<0.05$ was considered significantly for all the results.

RESULTS

The proximate composition of *Justicia carnea* revealed a high moisture content (51.69%), substantial carbohydrate level (37.43%), and moderate protein content (8.05%). Ash (1.60%), fat (0.75%), and fibre (0.48%) were present in lower amounts, indicating low mineral, fat, and fibre contents. Overall, the plant sample is characterized by high water content and appreciable energy-yielding nutrients.

Table 1: Proximate Compositions of *J. carnea* used in the study.

Proximate composition (%)	**Mean ± SEM**
1. Moisture content	51.69 ± 0.10
2. Ash	1.60 ± 0.00
3. Fibre	0.48 ± 0.00
4. Fat	0.75 ± 0.00
5. Protein	8.05 ± 0.02
6. Carbohydrate	37.43 ± 0.04

Phytochemical Compositions of *J. carnea* used in the Study

Table 2 presents the concentrations of 26 phytochemicals in *Justicia carnea*, measured in nanograms per gram (Ng/G), along with their mean values and standard errors. These compounds are categorized into classes such as flavonoids, polyphenols, phenols, and alkaloids. Concentrations range from non-detectable (0.00 Ng/G) to

17.17 Ng/G, with Naringin (17.17 Ng/G), Vinnillic acid (14.80 Ng/G), and Nobiletin (11.96 Ng/G)—all flavonoids—showing the highest levels. Several compounds were not detected. Overall, the data indicate a predominance of flavonoids and phenols in *J. carnea*, both known for their strong antioxidant properties.

Table 2: Phytochemical compositions of *J. carnea* used in the study are as follows:

Phytochemicals (Ng/G)	Mean ± SEM
1. Kaempferol	0.00 ± 0.00
2. Epicatechin	0.00 ± 0.00
3. Epigallocatechin	0.00 ± 0.00
4, Quercetechin (Flavonoids)	4.53± 0.02
5. Gallocatechin-3- gallate (Polyphenol)	0.0 ± 0.00
6. Robinetin	0.00 ± 0.00
1. Myricetin	0.00 ± 0.00
2. Nobiletin (Flavonoids	11.96 ± 0.05
3. Baicalin	0.00 ± 0.00
4. Tangeretin(Flavonoids)	1.23± 0.00
5. Artemetin(Flavonoids)	0.52 ± 0.00
6. Naringin (Flavonoids)	17.17 ± 0.04
7. Lunamarin (Alkaloids)	1.17 ± 0.00
8. Cinnamic acid (Flavonoids)	0.42± 0.00
9. Vinnillic acid (Flavonoids)	14.80 ± 0.02
10. Coumaric acid (Phenols)	0.74 ± 0.00
11. Ferrulic acid (Phenols)	0.78 ± 0.00

12. Piperic acid	0.00 ± 0.00
13. Ellagic acid (Polyphenol)	6.97 ± 0.00
14. Flavone (Flavonoids)	5.23±0.00
15. Flavone-3-ol (Flavonoids)	5.01 ± 0.00
16. Gentisic acid (Phenols)	2.59 ±0.00
17. Cinnamic acid (Polyphenol)	0.37± 0.00
18. Syringic acid	0.00 ± 0.00
25. Sinapic acid	0.00 ± 0.00
26. Rosmarinic acid	0.00 ± 0.00

Table 3 contains the vitamin composition table for *J. carnea* which shows the mean and standard error of the mean (SEM) for three vitamins, expressed in milligrams per kilogram (Mg/Kg). The vitamins and their concentrations are: Vitamin A (29.43 Mg/Kg), Vitamin C (65.24 Mg/Kg), and Vitamin E (15.66 Mg/Kg). Vitamin C has the highest concentration, indicating a significant amount of this antioxidant vitamin in *J. carnea*. Vitamin A is present in a moderate amount, contributing to its potential health benefits. Vitamin E, another antioxidant, is present in a relatively lower concentration. The result in Table 3 revealed that *J. carnea* has a very good antioxidant Vitamins, with Vitamin C having the highest concentration.

Table 3: Vitamins A, C and E Concentrations present in *J. carnea* Sample used in the Study

Vitamins (Mg/Kg)	**Mean ± SEM**
1. Vitamin A	29.43 ± 0.02
2. Vitamin C	65.24 ± 0.10

3. Vitamin E	15.66 ± 0.01

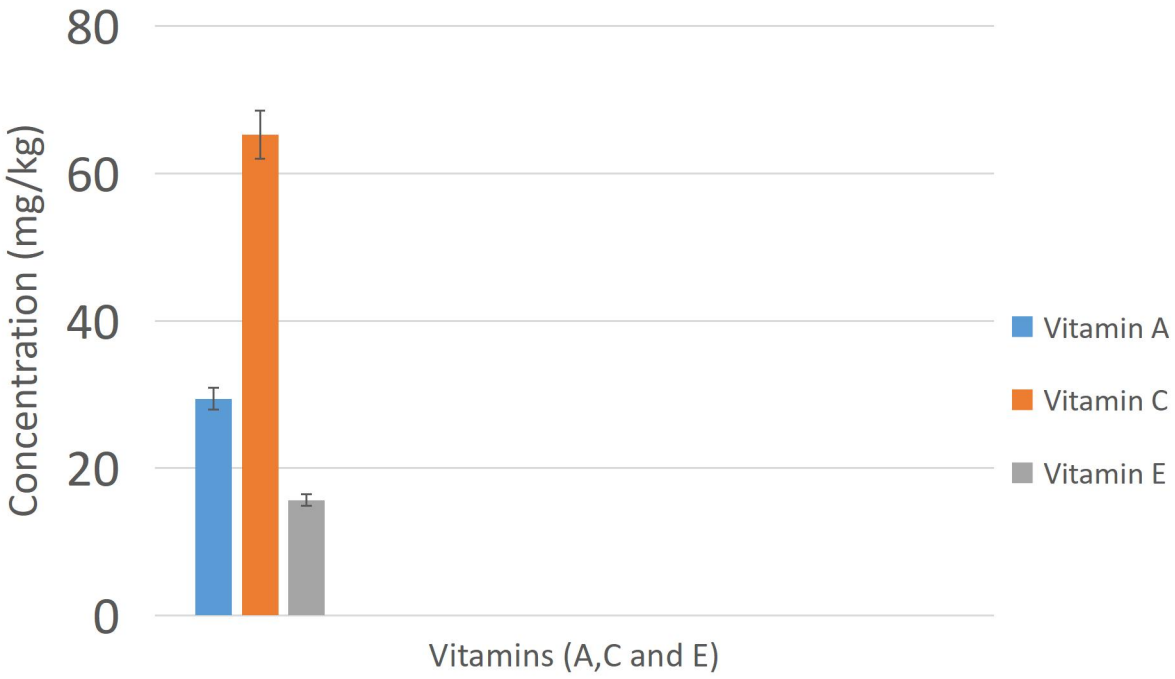

Figure 1: Antioxidant vitamins A, C and E concentrations present in *J. carnea* sample used in the study

DISCUSSION

The study explored the nutritional and medicinal potential of *Justicia carnea* leaves by evaluating their proximate composition, phytochemical content, and levels of antioxidant vitamins, particularly in relation to their impact on lipid profiles and haematological parameters. The findings revealed that the leaves contained 51.69% moisture, 1.60% ash, 0.48% fiber, 0.75% fat, 8.05% protein, and 37.43% carbohydrates. This composition suggested a significant carbohydrate presence, which implies potential energy-yielding properties. These results aligned with previous studies, such as that of Andrew *et al*. (2024), which similarly reported high carbohydrate levels and considerable amounts of ash, fiber, protein, and fat in the leaves.

In addition to their nutritional content, *Justicia carnea* leaves were rich in flavonoids and phenolic compounds, two classes of phytochemicals known for their strong antioxidant properties. Flavonoids, which are naturally occurring polyphenolic compounds, have been linked to numerous health benefits, including cancer prevention. Polyphenols, widely found in fruits, vegetables, and legumes, helped protect cells from oxidative stress and degenerative conditions by neutralizing harmful free radicals and reducing DNA damage.

The leaves also contained appreciable amounts of antioxidant vitamins—namely, vitamins A, C, and E. These vitamins are vital for maintaining physiological health. Vitamin A contributes to immune function and cellular regulation, while vitamin C supports protein metabolism, collagen formation, and iron absorption. Vitamin E, being fat-soluble, safeguards cell membranes from oxidative damage and enhances the immune system. Together, these vitamins support wound healing, reduced inflammation, and strengthened the body's defense mechanisms.

Overall, the study highlighted that *Justicia carnea* leaves possessed a rich nutritional profile and a substantial amount of antioxidant compounds, suggesting they have a beneficial role in managing oxidative stress and improving lipid and haematological health. Furthermore, toxicity tests indicated zero fatalities at all tested dosages, confirming the plant's safety for consumption and its potential application in therapeutic settings.

CONCLUSION

The study provided a comprehensive assessment of *Justicia carnea* leaves, focusing on their proximate composition, phytochemical content, and antioxidant vitamin levels in the context of their

protective effects against cadmium-induced liver toxicity in male Wistar rats.

The proximate analysis revealed that the leaves contain a high carbohydrate content (37.43%), moderate protein (8.05%), and low fat (0.75%), along with significant moisture (51.69%), suggesting their potential as an energy-yielding and nutritive plant source. Phytochemical evaluation indicated a rich presence of flavonoids and phenolic compounds—key antioxidants known to combat oxidative stress and cellular damage. These bioactive compounds play crucial roles in protecting against degenerative diseases and enhancing overall cellular resilience.

Furthermore, the leaves were found to be rich in antioxidant vitamins A, C, and E. These vitamins contribute to cellular protection, immune regulation, and tissue repair. Vitamin A supports cell growth and immune function; vitamin C aids in collagen synthesis, iron absorption, and antioxidation; while vitamin E protects cell membranes from oxidative injury and boosts immune defense.

Together, these nutritional and biochemical constituents likely underpinned the observed hepatoprotective and nephroprotective effects of *Justicia carnea* extract in the study. The ethanol extract significantly improved liver and kidney function markers following cadmium exposure, indicating that the plant's antioxidant and anti-inflammatory properties may offer a natural therapeutic strategy against toxin-induced organ damage.

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CONFLICT OF INTEREST

We declare no conflict of interest.

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