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Original Article

Ethanol Extracts of *Justicia carnea* Leaves Mitigate Pancreatic Oxidative Stress and Preserve Islet Glucagon Expression in TNBS-treated Mice

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Abstract

Background and objectives: Chronic pancreatitis is an inflammatory disease and is difficult to manage despite advancements in medical science. This study examined the effect of water/ethanol extracts of *Justicia carnea* leaves on oxidative stress and glucagon expression in a mouse model of chronic pancreatitis induced by trinitrobenzenesulfonic acid (TNBS).

Methods: Twenty-five male Swiss albino mice were randomized and treated intrarectally with vehicle (the control group) or TNBS. Some TNBS-treated mice were treated orally with 200 mg/kg or 400 mg/kg *J. carnea* extracts, or with the positive control, 500 mg/kg sulfasalazine, every other day on three occasions. Oxidative stress markers and pancreatic glucagon expression were assessed.

Results: Compared with the healthy control mice, treatment with TNBS significantly decreased the levels of pancreatic glutathione (0.89 μmol/g tissue vs. 7.16 μmol/g tissue in the control) and glutathione peroxidase activity, but significantly increased the levels of α-amylase and lipase activities, lipid peroxidation, total antioxidant capacity, and nitric oxide, as well as serum C-reactive protein ($P < 0.05$ for all), accompanied by severe inflammation and reduced glucagon expression in the pancreatic tissues. The toxic effects of TNBS were significantly mitigated by treatment with *J. carnea* extracts.

Conclusions: These findings provide evidence that treatment with *J. carnea* extracts inhibited oxidative stress and preserved glucagon expression in the pancreatic tissues of mice.

Introduction

Common gastrointestinal conditions like pancreatitis, which can arise during hospital and emergency room visits, result in significant morbidity, mortality, and higher medical bills. Its symptoms include bloating, nausea, and abdominal pain. Its pathology involves an inflammatory response triggered by multiple factors, including bleeding, edema, digestion, and even pancreatic tissue necrosis and systemic inflammation.[1,2] Due to its function in controlling blood sugar, the pancreas is an essential organ in diabetes mellitus.[3] Depending on the kind and stage of the disease, pancreatitis can have a substantial impact on the expression and secretion of glucagon (a peptide hormone produced by the alpha cells of the islets of Langerhans in the pancreas). Chronic pancreatitis can lead to dysregulation and reduced glucagon output because of islet cell destruction.[4,5]

Over six million instances of pancreatitis were reported globally in 2017.[6] Patients' lives and health are put in grave danger when acute pancreatitis (AP) advances to severe acute pancreatitis (hereinafter referred to as SAP).[7] Certain treatments, like fluid resuscitation, pain management, and nutritional assistance, can postpone the onset of AP.[8] The current treatment for AP is a phased, interdisciplinary, and gradual symptomatic approach, partly due to the absence of suitable targets for AP treatment. There are current-

Keywords: Pancreas; Glucagon; Trinitrobenzenesulfonic acid; TNBS; *Justicia carnea*; Inflammation.

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ly no specific medications for treating SAP or AP, despite recent advancements in AP treatment.[9]

A common chemical agent used in models intended to induce inflammation and ulcerative colitis is 2,4,6-trinitrobenzenesulfonic acid (TNBS).[10] This chemical is useful in researching the complex pathways that lead to inflammation due to its ability to initiate immunological reactions and inflammatory processes.[11] This enables the simulation and analysis of situations akin to those observed in inflammatory illnesses in humans.[10,12,13] This experimental approach creates room to systematically examine the underlying mechanisms, find potential therapeutic targets, and create innovative treatments meant to lessen the detrimental effects of inflammatory diseases on human health.[14,15]

According to Fousekis *et al*.,[16] ulcerative colitis and pancreatitis have similar immunologic characteristics, microbiologic alterations, genetic susceptibility, and clinical symptoms. According to some research, there is a connection between colitis and pancreatitis,[16,17] and people with Crohn's disease and ulcerative colitis are more likely to have these pancreatic conditions.[18] Although TNBS is commonly applied to induce colitis, its biological actions extend beyond the intestine and trigger systemic oxidative stress, immune activation, and cytokine release, all of which contribute to pancreatic injury.

The pathophysiology of AP is significantly influenced by oxidative stress. Malondialdehyde (MDA), a consequence of lipid peroxidation, is produced in pancreatic tissue when reactive oxygen species (ROS) damage the lipids and proteins of cell membranes.[19,20] One important pathophysiologic component that contributes to the development and course of inflammation is oxidative/nitrosative stress.[21–24] Because inflammatory cells secrete a lot of cytokines and chemokines, inflammation causes an excess of ROS to be produced, which in turn triggers oxidative stress.[25,26] From the foregoing, treatment approaches that include compounds with anti-inflammatory and antioxidant potentials are being explored.[27–30] Değer *et al*.[31] showed that glutamine had protective effects against colonic and pancreatic harm triggered by TNBS.

Kolgazi *et al*.[32] concluded that α-lipoic acid has benefits in TNBS-induced gut inflammation in animals by suppressing the accumulation of neutrophils, preserving endogenous glutathione (GSH), and inhibiting the generation of reactive oxidants. Polat *et al*.[33] examined the influence of hesperetin on inflammatory and oxidative status in an experimental colitis model induced by TNBS. Hesperetin administration significantly decreased levels of proinflammatory agents (TNF, IL-6, and NF) and lipid peroxidation (MPO, MDA).

According to Orororo *et al*.[34] and Ekakitie *et al*.,[35] medicinal plants have proven advantageous qualities that make them less hazardous and less expensive when used to treat conditions triggered by oxidative damage. Natural products provide a sustainable source with considerable efficacy to treat and overcome several disorders and fatal diseases, including cancer.[36] *Justicia carnea*, a flowering plant in the Acanthaceae family, is one of these plants that researchers have recently focused on.[37] *J. carnea*, called Pink jacobinia, and known by its native Yoruba and Igbo names, "Ewe eje" and "Ogwuobara," which means "blood tonic," is a perennial shrub grown extensively in subtropical and tropical regions.[38] *Justicia* species are deployed in treating different illnesses, including gastrointestinal issues, arthritis, diabetes, cancer, anemia, and inflammation.[39,40] The histological lesions on the pancreas, liver, and kidney of streptozotocin-induced diabetic rats were lessened by *J. carnea* methanol leaf extract.[41] Additionally, the plant has been reported to have hepato-protective, lipid-lowering, antiviral, hypocholesterolemic, antidiabetic, antioxidant, and cardioprotective qualities.[11,22,37,38,42–44] Active phytochemicals (phenols, flavonoids, alkaloids, etc.), essential micronutrients, and minerals (selenium, folic acid, iron, zinc, and copper) are associated with the health benefits of *J. carnea* leaves.[45,46]

With few effective treatment choices, pancreatitis is still a difficult condition to manage despite advancements in medical science. Most of the existing treatments concentrate on supportive care and symptom management, which emphasizes the need for new therapeutic agents that can influence the underlying inflammatory processes and encourage tissue healing. Examining *J. carnea*'s effects on TNBS-induced pancreatitis could reveal important information about its potential for use in treating pancreatitis. For this reason, this study examined how ethanol extracts of *J. carnea* leaves mitigate pancreatic oxidative stress and preserve islet glucagon expression in TNBS-treated mice.

Materials and methods

Reagents

Standard reagents were employed in carrying out this study.

Plant identification and extract preparation

A natural farm in the rural area of Abraka, Delta State, provided this plant in its freshest state. Authentication was done by a licensed botanist in Delta State University's Botany Department in Abraka with voucher number DELSU/BOT/272. Distilled water was used to wash the fresh leaves. At temperatures between 28 and 30 °C, they were then air-dried in the laboratory for a week.[47] Thereafter, they were blended smoothly using a sterilized blender. Fifty (50) grams of the powder were extracted with 500 milliliters of 70% ethanol for three days while being shaken periodically. After filtration through filter paper, the mixture was further concentrated using a water bath at 40 °C. Following that, the extracts were stored at 4 °C in the refrigerator until further use.[48]

Animal grouping and treatment

Twenty-five male mice were used in this investigation. The mice had an average weight of 22 ± 2.5 grams. The mice were given standard mice feed and unlimited access to clean water as they acclimated for seven days. Animals were handled according to the Institutional Animal Ethics Committee guidelines, and the experimental protocol was approved by the Research and Ethics Committee of the Faculty of Basic Medical Science, Delta State University, Abraka (RBC/FBMC/DELSU/24/444).

The mice were randomized into the following groups:

- Group 1 - Control (given normal saline orally);
- Group 2 - TNBS only (intrarectally);
- Group 3 - TNBS + 200 mg/kg of *J. carnea* extract orally;
- Group 4 - TNBS + 400 mg/kg of *J. carnea* extract orally;

Group 5 - TNBS + 500 mg/kg of sulfasalazine orally.Initiation of inflammation and treatments

Some mice were administered intrarectally with TNBS (150mg/kg body weight) for 72 h to induce inflammation, as described by Tanideh *et al*.[49]

This dose had been demonstrated to reliably deplete endogenous antioxidants (GSH, superoxide dismutase (SOD), catalase (CAT)) and elevate lipid peroxidation (MDA) while stimulating pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, thereby

creating a reproducible inflammatory environment for evaluating therapeutic interventions.[22,50] The mice in the therapeutic groups were treated orally with the specific dose of *J. carnea* extracts or sulfasalazine every other day for three doses.

Animal sacrifice

After one week of treatment, the mice were sacrificed, and blood samples were collected. After standing at room temperature for ten minutes, the blood samples were centrifuged at 1,200 g, and their serum samples were used for biochemical assays. The pancreatic tissues were dissected; some were homogenized in 0.2 mL of phosphate buffer at a pH of 7.4. The remaining pancreatic tissues were fixed in 10% formalin overnight for histological and immunohistochemical analyses.

Biochemical analysis

Measurement of pancreatic oxidative stress indices in tissue homogenate

A thiobarbituric acid reactive substance assessment was used to measure the amount of MDA.[51] Thiobarbituric acid and MDA, a marker of lipid peroxidation, combined to generate the pink MDA-thiobarbituric acid adduct, which was detected at 532 nm.

The compound 5,5′-dithiobis-2-nitrobenzoic acid (hereinafter referred to as DTNB) was the reagent utilized in a spectrophotometric examination to assess reduced glutathione (GSH levels.[52] In the experiment, GSH reacted with DTNB to yield 5-thio-2-nitrobenzoic acid, a yellow substance that was detected at 412 nm.

Cumene hydroperoxide was employed in a spectrophotometric technique to measure glutathione peroxidase (GPx) activity.[53]

The Misra and Fridovich method was used to measure the activity of SOD.[54] The superoxide anion (O_2^-) is dismutated into hydrogen peroxide and oxygen by SOD. In the test, superoxide radicals produced reacted with a chromogenic substance, and the ability of SOD to inhibit this reaction was quantified at 560 nm.

Using hydrogen peroxide as a substrate, a spectrophotometric technique was used to evaluate the activity of CAT.[55] The CAT-containing samples were added to a reaction mixture previously prepared with phosphate buffer (pH 7.0) and H_2O_2. The absorbance at 240 nm was observed as it decreased over time.

2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was used in a spectrophotometric assay to evaluate total antioxidant capacity (TAC).[56] The experiment assessed the sample's total capacity to scavenge free radicals. ABTS and potassium persulfate were reacted to produce the ABTS•+ radical cation. The sample was then added to the reaction mixture, and the decline in absorbance at 734 nm was used to track the decrease of ABTS•+.

The Griess reaction was employed to measure nitric oxide (NO) levels.[57] The Griess reagent measures nitrite, which is created when NO combines with oxygen. Nitrate reductase was used in the test to convert nitrate to nitrite, which was further reacted with Griess reagent. The resulting colored azo dye was detected at 540 nm.

Assay in serum

Measurement of C-reactive protein (CRP)

CRP levels were determined using a high-sensitivity enzyme-linked immunosorbent assay following the manufacturer's protocol. Serum samples were diluted 1:3 and tested in duplicate in 96-well plates. The serum samples were probed with anti-CRP, and bound antibodies were detected with horseradish peroxidase–conjugated secondary antibody, followed by the addition of chromogenic substrate solution (tetramethylbenzidine). The absorbance was measured at 450 nm in a microplate reader. Serum CRP levels were calculated using the standard curve established with known concentrations of CRP.[58]

Lipase assay

Following the guidelines provided by Nurcan,[59] lipase levels were ascertained spectrophotometrically. Triglycerides are hydrolyzed by lipase to produce glycerol and free fatty acids. The hydrolysis of p-nitrophenyl palmitate, which yields p-nitrophenol (pNP), is a typical spectrophotometric procedure. The yellow pNP product absorbs at 410 nm. P-nitrophenyl palmitate was dissolved in an appropriate solvent (e.g., isopropanol) to create a substrate solution. Additionally, bile salts and gum arabic were added to an assay solution (Tris-HCl buffer, pH 8.0) to emulsify the substrate. The enzyme sample was then introduced to the assay buffer's substrate solution. The resultant mixture was incubated at 37 °C for 15 min. The release of pNP was determined by measuring the rise in absorbance at 410 nm.

Amylase assay

According to Xiao *et al*.,[60] the spectrophotometric method was used to ascertain α-amylase activity by tracking variations in absorbance at specific wavelengths. The breakdown of starch into sugars, such as glucose and maltose, is catalyzed by amylase. The assay quantifies the increase in reducing sugars or the decrease in starch content. The iodine-starch assay was employed, in which iodine forms a blue complex with starch. Spectrophotometric measurement reveals a reduction in blue color when amylase breaks down starch. The enzyme sample was added to a reaction mixture prepared with soluble starch as the substrate and phosphate buffer (pH 6.9). Incubation was carried out for ten minutes at 37 °C. Iodine solution (iodine-potassium iodide), which combines with the remaining starch to generate a blue complex, was added to stop the reaction. The blue-starch-iodine complex's absorbance was then measured at 660 nm. The amylase activity is directly correlated with the absorbance decrease.

Immunohistochemical staining

Antibodies against glucagon (ThermoFisher Scientific, Waltham, MA) at a dilution of 1:100 were used to stain pancreatic sections. After immunohistochemical staining, the slides were digitally imaged at 600× magnification using microimaging software (Cell Sens Entry, Olympus Corp., Center Valley, PA) and a digital camera attached to a microscope (DP25, Olympus Corp., Center Valley, PA). To ascertain the degree of immunoreactivity for the hormone, the images were digitally examined using image processing software.[61]

Data analysis

All collected data were presented as mean ± SD of triplicate measurements, and differences among groups were tested by ANOVA and post hoc Tukey's test using GraphPad Prism (v7.0). A P-value <0.05 was considered statistically significant.

Results

J. carnea leaf extracts mitigate the effect of TNBS on oxidative stress in the pancreas of mice

The mitigating effect of *J. carnea* leaf extract on oxidative stress in the pancreas of mice exposed to TNBS is shown in Table 1.

Table 1. *Justicia carnea* leaf extracts mitigate the effect of TNBS on oxidative stress in the pancreas of mice

Groups	Parameters of oxidative stress						
	MDA	GSH	GPx	SOD	CAT	TAC	NO
1 - Control (normal saline)	19.24 ± 3.2^{a}	7.16 ± 3.1^{a}	4.72 ± 1.9^{a}	0.72 ± 0.5^{a}	11.20 ± 0.8^{a}	0.07 ± 0.02^{a}	16.24 ± 2.8^{a}
2–TNBS only (intrarectally)	58.68 ± 6.8^{b}	0.89 ± 0.04^{b}	0.29 ± 0.05^{b}	2.62 ± 0.6^{b}	17.02 ± 0.9^{b}	0.03 ± 0.01^{b}	78.82 ± 1.64^{b}
3 – TNBS + *J. carnea* extract (200 mg/kg)	20.84 ± 3.6^{a}	9.24 ± 3.4^{c}	4.98 ± 0.88^{c}	0.87 ± 0.2^{a}	12.46 ± 0.8^{a}	0.08 ± 0.04^{a}	20.28 ± 3.0^{c}
4- TNBS + *J. carnea* extract (400 mg/kg)	21.48 ± 2.4^{a}	8.92 ± 4.1^{c}	3.71 ± 1.5^{d}	0.82 ± 0.2^{a}	12.02 ± 0.6^{a}	0.09 ± 0.02^{c}	18.90 ± 1.9^{a}
5 - TNBS + sulfasalazine (500 mg/kg).	21.50 ± 5.4^{a}	9.88 ± 4.8^{d}	3.69 ± 1.2^{d}	0.89 ± 0.3^{a}	12.28 ± 0.7^{a}	0.08 ± 0.04^{a}	19.92 ± 6.8^{a}

Values are shown as mean ± SD of triplicate measurements. Superscripts (a, b, c, d) indicate statistical differences. Values bearing dissimilar superscripts within the same column are significantly different ($P < 0.05$). Values are presented in µmol/g tissue (GSH), nmol/g tissue (MDA), and U/g tissue (CAT, SOD, and GPx). CAT, catalase; GPx, glutathione peroxidase; GSH, reduced glutathione; MDA, malondialdehyde; NO, nitric oxide; SD, standard deviation; SOD, superoxide dismutase; TAC, total antioxidant capacity.

MDA levels in Group 1 were 19.24, which was significantly (P <0.05) elevated to 58.68 in the group exposed to TNBS alone (Group 2). Treatment of TNBS-exposed mice with the two selected doses of the extract caused a significant reduction in MDA levels compared to the untreated group. This reduction brought MDA levels to a value not significantly different from the control. A similar trend was observed in TNBS-exposed animals treated with the standard drug, sulfasalazine.

There was a statistically significant decrease in GSH levels in Group 2 (TNBS alone) compared to the control group. TNBS-exposed animals treated with the extract (Groups 3 and 4) and those treated with the standard drug (Group 5) showed significantly higher GSH levels. However, no statistically significant difference was observed between TNBS-exposed animals treated with 200 mg of the extract (Group 3) and those treated with 400 mg of the extract (Group 4).

GPx activity in the pancreas of mice exposed to TNBS alone (Group 2) was 0.29, a significant ($P < 0.05$) decrease from that observed in the control animals (Group 1). When TNBS-exposed mice were treated with the extract (Groups 3 and 4) or the standard drug (Group 5), there was a significant increase in GPx activity to 4.98, 3.71, and 3.69, respectively. Animals treated with 200 mg of the extract (Group 3) had the highest GPx activity.

As shown in Table 1, SOD activity was not significantly changed in animals given normal saline alone (Group 1) and those given TNBS and subsequently treated with *J. carnea* extracts (Groups 3 and 4) or the standard drug (Group 5). However, administration of TNBS alone (Group 2) caused a significant ($P < 0.05$) increase in SOD activity compared to the control and all groups administered *J. carnea* extract.

J. carnea's modulatory effect on CAT activity in the pancreas of mice administered TNBS is also depicted in Table 1. Group 2 displayed a statistically significant ($P < 0.05$) increase in CAT activity compared to Group 1 (Control), TNBS-exposed mice administered 200 mg of the extract (Group 3), those treated with 400 mg of the extract (Group 4), and those treated with the standard drug (Group 5).

TAC in the pancreas of mice was 0.07 in the control group, 0.03 in animals exposed to TNBS alone (Group 2), 0.08 in TNBS-exposed mice treated with 200 mg of the extract, 0.09 in TNBS-exposed mice treated with 400 mg of the extract, and 0.08 in TNBS-exposed mice treated with sulfasalazine. The results showed that exposure to TNBS caused a significant ($P < 0.05$) reduction in TAC levels, which was significantly ameliorated upon administration of the extract. Conversely, a statistically significant increase in NO levels was observed upon TNBS exposure (Group 2) compared to the control and all other experimental groups.

J. carnea's modulatory effect on CRP levels in mice administered TNBS

J. carnea's modulatory effect on CRP levels in mice administered TNBS is shown in Figure 1. A statistically significant increase in CRP levels was observed in mice exposed to TNBS alone (Group 2) compared to the control and TNBS-exposed mice subsequently treated with 200 mg and 400 mg of the extract (Groups 3 and 4) or the standard drug (Group 5). No statistically significant difference was observed between TNBS-exposed mice treated with 200 mg (Group 3) and 400 mg (Group 4) of the extract.

J. carnea's modulatory effect on lipase activity in mice given TNBS

Figure 2 shows *J. carnea*'s modulatory effect on lipase activity in mice given TNBS. A statistically significant increase in lipase

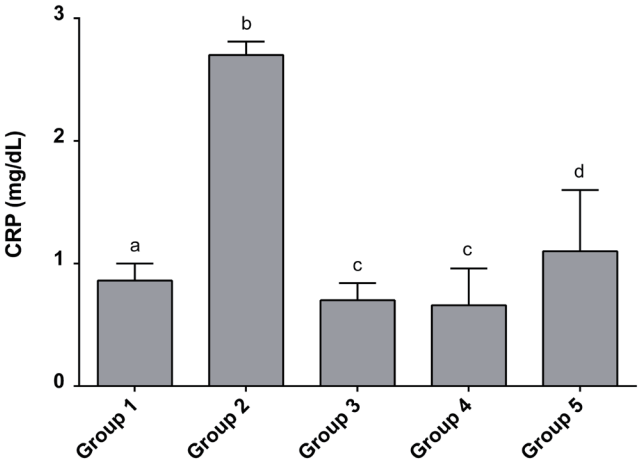

Fig. 1. *Justicia carnea* leaf extracts mitigate TNBS-elevated CRP levels in the pancreatic tissues of mice. Values are mean ± SD (n = 3). Values with different superscripts differ significantly ($P < 0.05$). Group 1 – Control (normal saline orally); Group 2 – TNBS only (intrarectally); Group 3 – TNBS + *J. carnea* extract (200mg/kg orally); Group 4 – TNBS + *J. carnea* extract (400mg/kg orally); Group 5 – TNBS + Sulfasalazine (500mg/kg orally). CRP, C-reactive protein; SD, standard deviation; TNBS, trinitrobenzenesulfonic acid.

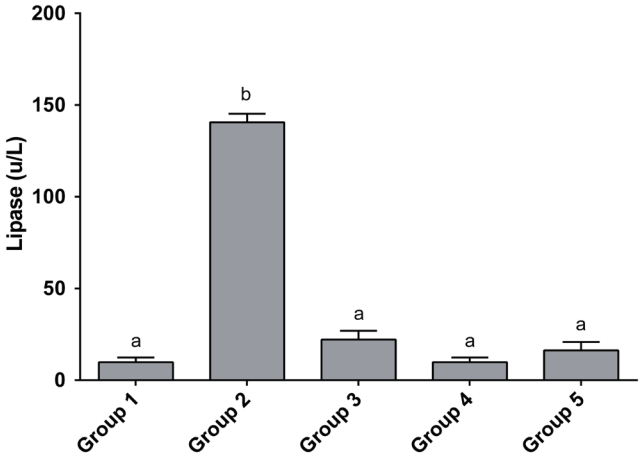

Fig. 2. *Justicia carnea* leaf extracts mitigate TNBS-elevated lipase activity in the pancreatic tissues of mice. Values are mean ± SD (n = 3). Values with different superscripts differ significantly ($P < 0.05$). Group 1 – Control (normal saline orally); Group 2 – TNBS only (intrarectally); Group 3 – TNBS + *J. carnea* extract (200mg/kg orally); Group 4 – TNBS + *J. carnea* extract (400mg/kg orally); Group 5 – TNBS + Sulfasalazine (500mg/kg orally). SD, standard deviation; TNBS, trinitrobenzenesulfonic acid.

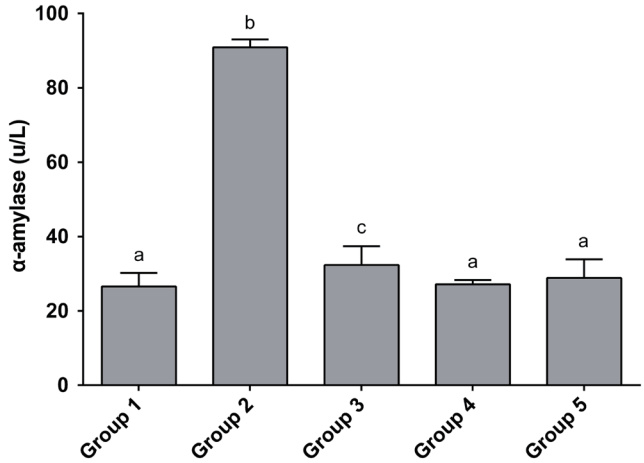

Fig. 3. *Justicia carnea* leaf extracts mitigate TNBS-elevated α-amylase activity in the pancreatic tissues of mice. Values are mean ± SD (n = 3). Values with different superscripts differ significantly ($P < 0.05$). Group 1 – Control (normal saline orally); Group 2 – TNBS only (intrarectally); Group 3 – TNBS + *J. carnea* extract (200mg/kg orally); Group 4 – TNBS + *J. carnea* extract (400mg/kg orally); Group 5 – TNBS + Sulfasalazine (500mg/kg orally). SD, standard deviation; TNBS, trinitrobenzenesulfonic acid.

activity was seen in mice exposed to TNBS alone (Group 2) compared to the control (Group 1), animals treated with the standard drug (Group 5), and all groups treated with *J. carnea* extract (Groups 3 and 4). No statistically significant difference was observed between the control and TNBS-exposed mice treated with the two doses of the extract or the standard drug.

J. carnea's modulatory effect on α-amylase activity in mice exposed to TNBS

Figure 3 shows that TNBS exposure (Group 2) significantly increased α-amylase activity compared with the control group and all groups administered *J. carnea* extract or the standard drug (Group 5).

J. carnea's modulatory effect on glucagon expression in mice administered TNBS

J. carnea's modulatory effect on glucagon expression in TNBS-administered mice is depicted in the immunohistochemical results in Figure 4, Tables 2 and 3. Exposure to TNBS (Group 2) caused reduced glucagon expression and significant histopathological damage to the pancreas, which were ameliorated upon treatment with *J. carnea* extract.

Discussion

One important pathophysiologic component that contributes to the toxicological consequences of TNBS is oxidative/nitrosative stress.[11,23] Because inflammatory cells emit a large number of cytokines and chemokines, inflammation causes an overproduction of ROS, which in turn triggers oxidative stress.[22,23,30,62] In light of this, treatment approaches that include compounds with anti-inflammatory and antioxidant potentials are being explored as ways to lessen the harmful consequences of TNBS.[63]

The present study examined *J. carnea's* modulatory effects on pancreatic oxidative harm instigated by TNBS. To investigate the inflammatory pathways and possible therapeutic medicines, it is standard practice to use TNBS to produce inflammation in experimental mice. According to Kitadi,[13] TNBS-induced models can be used to study therapeutic targets and mechanisms because they closely resemble human inflammatory situations. This strategy is buttressed by the technique used in the current study, which offers a reliable model for evaluating *J. carnea's* effectiveness. The findings indicated that *J. carnea* significantly improved a number of biomarkers, signifying that it may have therapeutic benefits.

In comparison to the group maintained on TNBS alone, the study established that mice treated with *J. carnea* had statistically significantly lower MDA levels. This result supports the findings of Oloruntola *et al*.,[45] who documented the antioxidant activity of powdered *J. carnea* leaf and showed a notable lessening in lipid peroxidation. Similarly, in a model of oxidative stress, Orjiakor *et al*.[38] discovered that *J. carnea* decreased MDA levels, demonstrating its ability to suppress lipid peroxidation. This proposes that by lowering lipid peroxidation and cell damage, *J. carnea* may be deployed as a treatment for oxidative stress-related illnesses like pancreatitis.[64]

Onyeabo *et al*.[47] reported consistent antioxidant properties of *J. carnea*, supporting the observed increases in GSH, GPx, and TAC levels. The potential of *J. carnea* as a preventive agent against diseases connected to oxidative stress is supported by the fact that improving these antioxidant enzymes helps shield cells from oxidative damage. SOD, GPx, and CAT are examples of enzymatic antioxidants that preserve a balance essential to cellular health.[65] Superoxide anions are converted into hydrogen peroxide by SOD, which is then neutralized by GPx and CAT. Catalase contributes to cellular tolerance against oxidative stress by shielding cells from damage triggered by hydrogen peroxide.[66]

In alignment with Falode *et al*.,[43] who documented comparable anti-inflammatory potentials of *J. carnea* in a diabetes model, the drop in NO, CRP, lipase, and α-amylase levels in the treated groups points to an anti-inflammatory effect. Moreover, *J. carnea* aqueous leaf extract showed strong antioxidant action and suppressed α-amylase activity, suggesting possible anti-inflammatory and anti-diabetic properties, according to studies by Anigboro *et al*.[44] and Oloruntola *et al*.[45] *J. carnea* may be used to treat inflam-

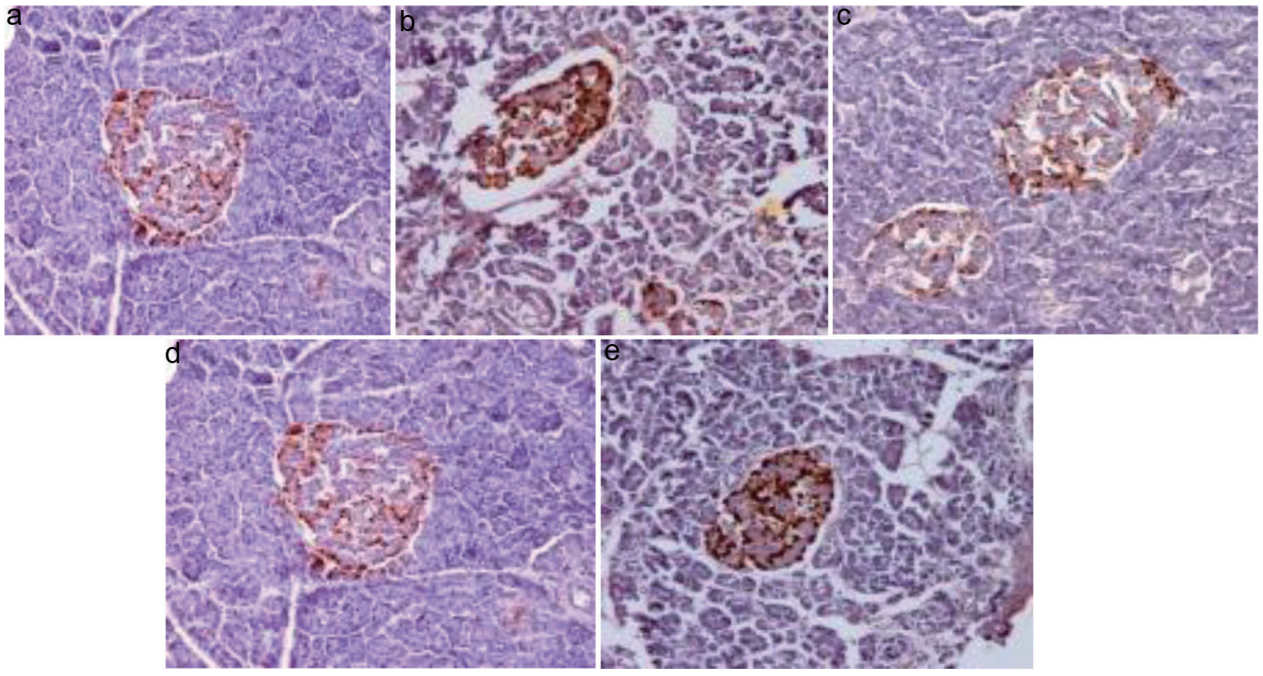

Fig. 4. Immunohistochemistry of pancreas (glucagon stain). (a) Control (normal saline orally); (b) TNBS only (intrarectally); (c) TNBS + *J. carnea* extract (200 mg/kg orally); (d) TNBS + *J. carnea* extract (400 mg/kg orally); (e) TNBS + sulfasalazine (500 mg/kg orally). TNBS, trinitrobenzenesulfonic acid.

Table 2. Histopathological grading criteria for pancreatic tissue evaluation

Histopathological criteria	0	1	2	3
Presence of BRBCs	None	<40%/HPF	41–70%/HPF	71–100%/HPF
Oedema	None	Interlobular septum	Interglandular septum, mild	Interglandular septum, Severe
Haemorrhage	None	Interlobular septum	Interglandular septum, mild	Interglandular septum, Severe
Inflammatory infiltration	None	In 1–2 lobules	In 3–4 lobules	In >4 lobules
Acinar necrosis	None	In 1–2 lobules	In 3–4 lobules	In >4 lobules
Fat necrosis	None	Mild	Moderate	Severe
Fibrosis	None	In 1–2 lobules	In 3–4 lobules	In >4 lobules
Total score 3+3+3+3+3+3+3=21				

BRBCs, blood red blood cells; HPF, high-power field.

Table 3. Histopathological grading of pancreatic tissue

Histopathological criteria	Group 1 Control	Group 2 – TNBS alone	Group 3 – TNBS + 200mg *J. carnea* extract	Group 4 – TNBS + 400mg *J. carnea* +TNBS	Group 5- TNBS + sulfasalazine
Presence of BRBCs	0	3	0	0	0
Oedema	0	3	1	1	0
Hemorrhage	0	3	1	0	1
Inflammatory infiltration	0	3	0	0	1
Acinar necrosis	0	2	1	0	0
Fat necrosis	0	2	0	1	0
Fibrosis	0	3	0	0	0
Total score	0/21	19/21	3/21	2/21	2/21

BRBCs, blood red blood cells; TNBS, trinitrobenzenesulfonic acid.

matory diseases including pancreatitis and other chronic inflammatory conditions, since lower levels of these inflammatory markers suggest less inflammation. In the early stages of AP, aberrant alterations in abdominal imaging may be seen, along with typical upper abdominal pain and abnormal increases in serum lipase or amylase.[9]

One of the most important methods used in the study to assess the expression of certain markers associated with inflammation and oxidative stress in the pancreatic tissues of mice with TNBS-induced pancreatitis was immunohistochemistry. The study evaluated the influence of *J. carnea* treatment visually and quantitatively on cellular and molecular alterations in the pancreas by employing antibodies that bind to these markers.

In this study, TNBS administration caused significant pancreatic damage and a reduction in glucagon expression. TNBS is known to induce systemic oxidative stress and inflammatory responses, resulting in the generation of ROS and depletion of antioxidants such as GSH, SOD, and CAT. This oxidative imbalance contributes to acinar and islet cell injury, as evidenced by edema, cellular degeneration, and inflammatory infiltration observed in pancreatic tissue. The reduction in glucagon likely reflects α-cell vulnerability to oxidative and inflammatory stress, as well as cytokine-mediated suppression of hormone production. Impairment of the islet microvasculature may further compromise α-cell function, impairing glucagon secretion and potentially affecting glucose homeostasis. These findings demonstrate that TNBS reliably models pancreatic oxidative injury and functional impairment, making it suitable for evaluating interventions aimed at preserving pancreatic structure and endocrine function.

The results also showed that treatment with the extract lessened TNBS-induced pancreatic damage and reduction in glucagon expression. The particular goal of examining how *J. carnea* affects pancreatic glucagon expression was accomplished, and the results showed that the extract affects glucagon levels, which are essential for controlling inflammation and glucose homeostasis. Depending on the kind and stage of the disease, pancreatitis can have a substantial impact on glucagon expression and secretion, resulting in both increases and decreases. While chronic pancreatitis can lead to dysregulation and reduced glucagon output because of islet cell destruction, AP frequently causes increased glucagon levels, particularly during the initial episode. This may contribute to post-pancreatitis diabetes. Thus, the immunohistochemistry data demonstrate the extract's capacity to alter substantial inflammatory pathways and offer molecular support for the observed biochemical and histological findings.

In TNBS-induced pancreatitis, *J. carnea* extract dramatically decreased inflammatory indicators such as oedema, bleeding, and inflammatory infiltration, according to the biochemical and histological examination of the current study. This is in line with Onyeabo *et al*.,[47] which provided evidence that *J. carnea* leaf extract had anti-inflammatory and haematological effects in rats with phenylhydrazine-induced anemia. Overall, the tissue-protective effects were in line with the present findings, even though the precise parameters varied.

This study is limited by several factors. First, the lack of a dose–response evaluation for *J. carnea* restricts accurate assessment of its optimal therapeutic range. Second, reliance on crude plant extract without isolating its bioactive constituents limits reproducibility and clarity regarding the specific agents mediating the observed effects. Third, the TNBS-induced model may not completely mimic the complex oxidative and hormonal disturbances seen in human pancreatic disorders, which affects the generalizability of the findings. Finally, a key limitation of this study is its short duration, which restricts the ability to evaluate the long-term effects, safety profile, and potential cumulative impact of *J. carnea* on oxidative stress and pancreatic glucagon expression.

Future directions

Given that *J. carnea* extract meaningfully reduced inflammation and oxidative stress markers, it may be turned into a natural anti-inflammatory treatment. For diseases like pancreatitis, where inflammation is a major factor, this could be especially helpful. In both conventional and alternative medicine, *J. carnea* may be marketed as an adjuvant management for inflammatory diseases. It is a viable option for herbal treatments and supplements due to its natural origin and proven effectiveness. The study's encouraging findings open the door for additional investigation and clinical testing. A crucial next step would be to carry out human clinical trials to verify *J. carnea*'s safety and effectiveness in treating inflammatory diseases. Its approval and broad use in clinical settings could result from successful experiments.

Conclusions

In mice with pancreatitis initiated by TNBS, extract from *J. carnea* showed strong antioxidant and anti-inflammatory properties. While increasing levels of antioxidant enzymes including GSH, GPx, SOD, CAT, and TAC, the therapy effectively decreased oxidative stress markers like MDA. Additionally, a noteworthy anti-inflammatory effect is indicated by the reduction in NO, lipase, and α-amylase levels. The study's immunohistochemical and histological analyses provide evidence of *J. carnea's* ability to effectively treat pancreatic damage instigated by TNBS.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

Study design and analysis (EMT, OOC, JJE), data collection (DCC, EKC, TOE, MJ, OOB), data analysis (OOC, DCC), writing and editing of the manuscript (OOC, EMT). All authors contributed to conception, design, analysis, and/or interpretation of the data. All authors have read and approved the manuscript.

Ethical statement

Ethical approval for this study was obtained from the Research and Ethics Committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka (Approval No.: RBC/FBMC/DELSU/24/444). All experimental procedures involving animals

were conducted in accordance with institutional guidelines of Delta State University for the care and use of laboratory animals, as well as national guidelines for animal research and welfare. Animals were handled humanely throughout the study. Anesthesia was administered prior to all invasive procedures to minimize pain and distress, and all efforts were made to reduce animal suffering and the number of animals used. Sample collection and sacrifice procedures were performed using approved humane methods to ensure minimal discomfort.

Data sharing statement

Data presented in this study are available on request from the corresponding author.

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