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Effects of *Justicia carnea* leaf extract on cerebral oxidative stress parameters and expression of cellular adhesion molecule-1 in albino mice exposed to TNBS

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Abstract

Background Although *Justicia carnea* possesses antioxidant qualities, its neuroprotective potential has not been well examined. Therefore, this study investigated the therapeutic potential of *J. carnea* extract in reducing tissue damage and oxidative stress brought on by exposure to 2,4,6-trinitrobenzenesulphuric acid (TNBS) in albino mice. A total of 35 Swiss albino adult mice were split up into seven groups ($n=5$) and given a variety of treatments, such as normal saline (neutral control), TNBS (0.2 ml) intrarectally as a negative control, TNBS (0.2 ml) + sulfasalazine (500 mg/kg orally) as a positive control, and two different doses of *J. carnea* leaf extract (200 mg/kg and 400 mg/kg) given orally and intrarectally. The leaf of *J. carnea* was dried, extracted, and then formulated into a gel using sodium carboxymethyl cellulose (NaCMC) and glycerol. The animals received treatment for seven days before being sacrificed. Samples of blood and brain were gathered for immunohistochemical and biochemical examination.

Results The findings demonstrated that TNBS significantly decreased glutathione (GSH) levels and the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) while increasing C-reactive protein (CRP) levels, platelet counts, malonaldehyde (MDA), and nitric oxide (NO). On the other hand, *Justicia carnea* therapy successfully decreased platelet counts, MDA, CRP, and NO levels, especially at 400 mg doses. Additionally, it considerably ($P > 0.05$) increased GSH levels and antioxidant enzymes activities. The extract's neuroprotective and antioxidant properties were demonstrated by the considerable reduction in cellular adhesion molecule-1 (ICAM-1) expression as well as the mitigation of TNBS-induced cortical necrosis and neuronal degeneration.

Conclusion The study showed that *J. carnea* leaf extract offers a substantial defense against tissue damage and brain oxidative stress brought on by TNBS.

Keywords *Justicia carnea*, Cerebral oxidative stress, Cellular adhesion molecule-1, TBNS, Mice

Background

The brain's biochemical integrity is essential to the central nervous system's (CNS) proper operation. Since free radicals have unpaired valence electrons, they are extremely reactive molecules, including reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Ekakitie et al., 2021; Oyem et al., 2021). Metabolism and other enzymatic functions are inextricably linked

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to the generation of free radicals. Potentially intracellular mitochondria and extracellular inflammation are the main producers of free radicals. In physiological settings, these processes are tightly controlled. These reactive chemicals can result in DNA damage, protein oxidation, and lipid peroxidation when there is an imbalance with antioxidant processes, a condition known as oxidative stress. This can lead to tissue damage and cell toxicity (Jelinek et al., 2021). The brain is particularly vulnerable to oxidative stress because of its high oxygen demand and lipid-rich composition. Consequently, there is a good chance that damage to the brain brought on by oxidative stress will impair regular central nervous system processes.

Located along the wall of the gastrointestinal (GI) tract, the enteric nervous system (ENS), which is made up of neurons and glial cells, is essential for regulating digestive processes. According to recent studies, complex relationships between the ENS, intestinal epithelium, immune system, intestinal microbiota, and central nervous system (CNS) are necessary for the GI system to function at its best (Suman, 2024). Stress reactions and mood disorders are influenced by communication between the central nervous system and the peripheral nervous system. According to a recent study by Schneider et al. (2023), psychological stress alters enteric glia and neurons, which worsens gut inflammation and dysmotility. Experiences that can upset a person's homeostasis and impact various bodily physiological functions are known as psychological stressors. Perception of threat triggers the autonomic nervous system (ANS) and the hypothalamic–pituitary–adrenal (HPA) axis, which release catecholamines and corticosteroid hormones into the bloodstream, respectively (Solano & Menard, 2023). The resolution or persistence of this reaction is modulated by self-regulating feedback loops (Kloet & Joëls, 2022).

Inflammation represents a significant physiological source of free radicals. According to Lauridsen (2019), inflammation is a component of a complicated biological reaction to pathogenic stimuli like bacteria. A vital defense mechanism, inflammation has developed in higher species to deal with damaging insults such as tissue damage, microbial infection, and other unfavorable circumstances. It is an important immunological response that makes it possible to eliminate dangerous stimuli and promotes tissue repair. The initial line of protection against harmful substances and external invaders is innate immunity, which includes acute inflammation. Redness, discomfort, swelling, and heat are the traditional signs of inflammation that have been recognized for hundreds of years (Mridha et al., 2017). Substances such as trinitrobenzene sulfonic acid (TNBS) can cause inflammation (Riaz et al., 2017).

Trinitrobenzenesulfonic acid is a nitroaryl oxidizing acid with the chemical formula $C_6H_3N_3O_9S$. Because of its strong oxidative characteristics, it can start a violent reaction that nearly instantly detonates when combined with reducing agents including nitrides, sulfides, and hydrides (Atsushi et al., 2000). In scientific study, its main purpose is to deactivate the terminal amino groups of peptides. On rare occasions, it serves as a detonator for further explosive substances. In order to simulate inflammatory bowel illness and post-infectious irritable bowel syndrome, it is also utilized to cause colitis in the colon of laboratory animals (Antoniou et al., 2016). Scientists are presently investigating this compound's toxicological characteristics.

Inflammation and tissue healing are significantly influenced by cellular adhesion molecule-1 (CAM-1). CAM-1, a member of the immunoglobulin superfamily, facilitates leukocyte–endothelial cell adhesion and has a role in leukocyte transmigration to inflammatory areas. Even while endothelial cells, some lymphocytes, and monocytes constitutively express ICAM-1 at low levels, cytokines and reactive oxygen species can dramatically boost this expression (Marinović et al., 2023).

To strengthen the body's antioxidant defense mechanism, scientists are looking for naturally occurring antioxidants from plant sources because biological systems have an unbalanced pro-oxidant/antioxidant ratio (Eduviere et al., 2022; Orororo & Asagba, 2022; Orororo & Udi, 2023; Ovuakporaye, et al. 2020). According to Awad et al. (2018), dietary fruits and vegetables have been shown to contain substantial amounts of antioxidants through assessments of their phenol content, total flavonoid content, and other antioxidant activities like reducing power and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability. In addition to plants used for food, therapeutic plants that contain bioactive components with unique antioxidant qualities are also gaining a lot of attention and being discovered (Efekemo & Orororo, 2022; Odeghe et al., 2023a). In addition to being used as food, medicinal plants are the primary source of tradomedicines, which are an essential component of both human and animal healthcare systems. Approximately 80% of the world's population still relies on medicinal plants for their fundamental medical needs (Anorado et al., 2021; Odeghe et al., 2023b).

Commonly referred to as pink jacobinia, flamingo flower, or pine-bur begonia in English, and known as "Ewe eje" in Yoruba, *Justicia carnea* (Acanthaceae family) is a delicate perennial shrub that is extensively grown in tropical and subtropical regions. According to reports, *Justicia* species can help with a variety of health issues, including cancer, diabetes, gastrointestinal issues, and arthritis (Onyeabo et al., 2017). Additionally, it has

positive effects on hematological parameters (Asakizi et al., 2020; Peters et al., 2022). Active phytochemicals, minerals, and essential micronutrients like iron, copper, zinc, folic acid, and selenium have all been connected to the health advantages of *J. carnea* leaves (Asogwa et al., 2020). Although *J. carnea* has been used for a variety of medical purposes in folklore, there is no scientific or experimental proof that it can reduce oxidative stress in the brain or affect the expression of cellular adhesion molecule-1. Based on these findings, the current study was created to assess how *Justicia carnea* leaf extract affected the expression of cellular adhesion molecule-1 and brain oxidative stress in albino mice that had been experimentally exposed to trinitrobenzene sulfonic acid (TNBS).

Methods

Chemicals and reagents

Analytical-grade chemicals were used in this study.

Collection of plant materials, extraction, and gel preparation

The leaves were obtained from their natural habitat in a farm in Abraka, Ethiopia East L.G.A. of Delta State, Nigeria. Identification and authentication by a certified botanist were done at the Delta State University, Abraka herbarium (voucher number 272). After being removed from their stems, the fresh leaves were cleaned with distilled water. They were then allowed to air dry for a week at room temperature (28–30 °C) in the lab (Achi et al., 2017). A sterilized electric blender was used to grind them into a fine powder. After that, the 50 g of powder was combined with 500 ml of distilled water and ethanol in a sterile conical flask, and it was let to stand for 72 h while being shaken occasionally. After using a filter paper to help filter the mixture, it was concentrated for three hours at 70 °C in a water bath. The extracts were put in a sterile conical flask with a clear label and kept in the refrigerator at 4 °C for the duration of the experiment (Umeh et al., 2023).

The *Justicia carnea* leaves were cleaned with tap water and put in a drying cabinet that was set at 40 °C to prepare the gel. 2% sodium carboxymethylcellulose (NACMC) and 5% glycerol were constantly mixed with a mixer. The *J. carnea* extract was then melted in deionized water, and the resulting mixes were gradually added to the Glycerol-NACMC. Finally, the produced gel was homogenized for half an hour. Gel was then put in the refrigerator in an aluminum tube.

Procurement of experimental animals and handling

Mice weighing between 20 and 30 g were acquired from Delta State University Abraka, Nigeria's Faculty of Basic

Medical Science. The animals were acclimated for seven days prior to the start of the trial. They were treated in accordance with the Institutional Animals Ethics (IAEC) guidelines, which were established by the Faculty of Basic Medical Science's ethical committee at Delta State University in Abraka, Nigeria (RBC/FBMC/DELSU/24/449). They were also provided with food and clean water at will.

Experimental design and grouping

The experimental animals were grouped into seven groups of five animals, and the experimental design was as follows:

Group A—control (normal saline orally)

Group B—trinitrobenzene sulfonic acid (TNBS) (0.2ml intrarectally)

Group C—trinitrobenzene sulfonic acid (0.2 ml) + sulfasalazine (500mg/kg orally).

Group D—trinitrobenzene sulfonic acid (0.2 ml) + *Justicia carnea* (JUC) (200mg/kg orally)

Group E—trinitrobenzene sulfonic acid (0.2 ml) + *Justicia carnea* (JUC) (400mg/kg orally)

Group F—trinitrobenzene sulfonic acid (0.2 ml) + gel of *Justicia carnea* (JUC) (200mg/kg rectally)

Group G—trinitrobenzene sulfonic acid (0.2 ml) + gel of *Justicia carnea* (JUC) (400mg/kg rectally)

TNBS was given in accordance with Zhou et al. (2006)'s earlier description. A 2-diameter polypropylene catheter was gently placed 4 cm through the rectum into the colon after the mice had been fasting for 24 h and had been mildly sedated with thiopental. To disrupt the intestinal barrier, a solution of 2,4,6-trinitrobenzenesulfonic acid (TNBS, 150 mg/kg) was mixed in 50% (V/V) ethanol and injected gradually into the colon; the control group was given only 50% ethanol. After 40 s of vertical hanging, the animals were put back in their cages (Tanideh et al., 2016). The two doses of plant extract were selected following the study of Umeh et al. (2023).

Sample collection

Following seven days of therapy and inflammatory induction, the animals were fasted overnight and sacrificed by cervical dislocation. Using a needle attached to 1 ml syringes, blood samples were drawn from the inferior vena cava and placed into a plain bottle with a clear label. Blood samples were centrifuged at 3000 rpm for 10 min. Clear supernatants were then aspirated into plain bottles with proper labels and kept cold until they were needed for biochemical analysis. Brain (hippocampus) samples were taken and homogenized using 0.2 phosphate buffer at a pH of 7.4 for biochemical examination. Additionally, sections of the brain (hippocampus) were fixed in 10%

formal saline and removed for immunohistochemistry examination.

Biochemical analysis

Assay in Blood A spectrophotometric technique was used to quantify the levels of C-reactive protein (CRP) (Pepys & Hirschfield, 2003). A spectrophotometric approach outlined by Marcus (2003) was used to count the platelets.

Assay in Brain Tissue A thiobarbituric acid reactive substance (TBARS) assay was used to measure MDA (Varshney & Kale, 1990). 5'-dithiobis-2-nitrobenzoic acid (DTNB) was used as a reagent in a spectrophotometric test to assess GSH levels (Ellman, 1959). Cumene hydroperoxide was used as the substrate in a spectrophotometric procedure to assess glutathione peroxidase (GPx) activity (Paglia and Valetine, 1967). The Misra and Fridovich (1972) approach was used to measure the activity of superoxide dismutase (SOD). Hydrogen peroxide was used as a substrate in a spectrophotometric experiment to measure catalase (CAT) activity (Aebi, 1984). A spectrophotometric assay using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as the reagent was used to assess total antioxidant capacity (TAC) (Re et al., 1999), and the Griess reaction was used to measure nitric oxide (NO) levels (Odeghe et al., 2023b).

Immunohistochemical analysis

Sections of brain (hippocampus) tissue (5–10 μ m) were taken out and placed on glass slides that were positively charged. Samples were then rehydrated using a graded ethanol series after being deparaffinized in xylene. A citrate buffer (pH 6.0) and heat treatment (microwave)

for ten to fifteen minutes were used to achieve antigen retrieval. After that, non-specific binding sites were blocked for 30 min using either 1% bovine serum albumin (BSA) or 5% normal goat serum (NGS) in PBS. The primary anti-ICAM-1 antibody (1:50–1:100 dilution) was then incubated for the entire night at 4 °C in PBS with 1% NGS or BSA. Sections were then incubated for one to two hours at room temperature with the biotinylated secondary antibody (1:200–1:500 dilution) in PBS containing 1% NGS or BSA. As directed by the manufacturer, sections were then incubated using Vector Laboratories' ABC Elite reagent. Further staining was developed using Vector Laboratories' DAB substrate kit and counterstained for one to two minutes using Sigma-Aldrich hematoxylin (Seguí et al., 2004).

Data analysis and presentation

Tukey's post hoc test and one-way analysis of variance (ANOVA) were used to analyze all gathered data, which were provided as mean $\pm$ standard error of mean (SEM). The analysis was conducted using GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA). P-values were deemed significant if they were less than 0.05.

Results

Effects of *Justicia carnea* leaf extract on C-reactive protein (CRP) levels in the blood of albino mice exposed to TNBS

The effects of *Justicia carnea* leaf extract on C-reactive protein (CRP) levels in the blood of albino mice exposed to TNBS are presented in Fig. 1. The extract significantly reduced C-reactive protein (CRP) levels in a dose-dependent manner. CRP, a biomarker for inflammation, was notably higher in the TNBS-only treatment,

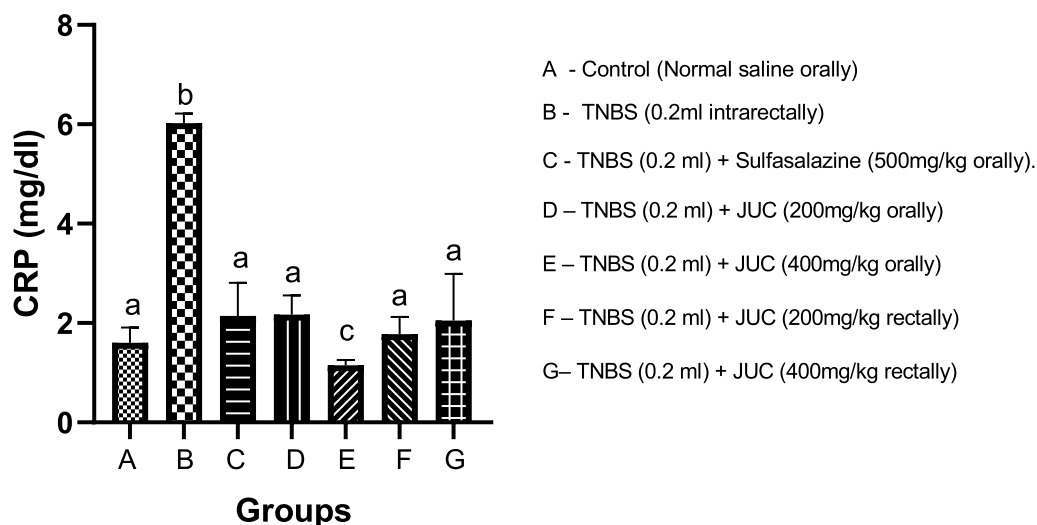


Fig. 1 Effects of *Justicia carnea* leaf extract on C-reactive protein (CRP) levels in the blood of albino mice exposed to TNBS

indicating a robust inflammatory response. However, groups treated with *J. carnea* extract, particularly at a 400 mg dose, exhibited CRP levels comparable to the TNBS + sulfasalazine group. This demonstrates the anti-inflammatory efficacy of *J. carnea* extract, which appears to attenuate the TNBS-induced inflammation effectively.

Effects of *Justicia carnea* leaf extract on platelet count in the blood of albino mice exposed to TNBS

The effects of *Justicia carnea* leaf extract on platelet count in the blood of albino mice exposed to TNBS are presented in Fig. 2. Platelet counts significantly increased in the TNBS-only group compared to the control group. Treatment with *J. carnea* extract restored platelet counts, especially at the highest dose (400 mg), which shows similar levels to the mice exposed to TNBS and treated with sulfasalazine. This suggests that *J. carnea* may help

maintain normal platelet function under inflammatory conditions.

Effects of *Justicia carnea* leaf extract on cerebral oxidative stress parameters in albino mice exposed to TNBS

Figures 3, 4, 5, 6, 7, 8, and 9 show the effects of *J. carnea* leaf extract on cerebral oxidative stress parameters in albino mice exposed to TNBS.

SOD levels are significantly reduced in the TNBS group compared to the control group. Treatment with *J. carnea* extract increased SOD levels, particularly at higher doses (400 mg), indicating its potential antioxidant properties. Similarly, GSH levels were significantly reduced in the TNBS group compared to the control group. *J. carnea* extract treatment restored GSH levels, suggesting enhanced antioxidant defenses.

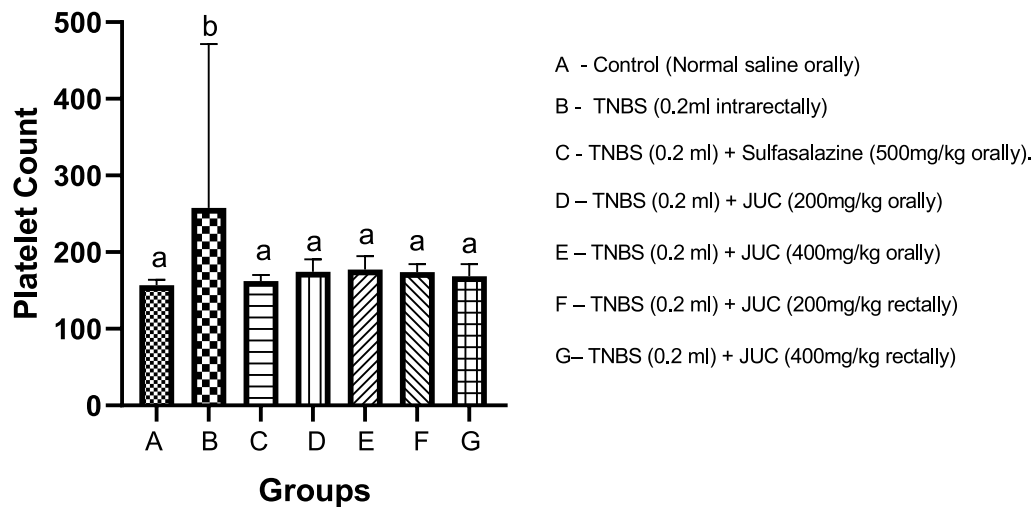


Fig. 2 Effects of *Justicia carnea* leaf extract on platelet count in the blood of albino mice exposed to TNBS

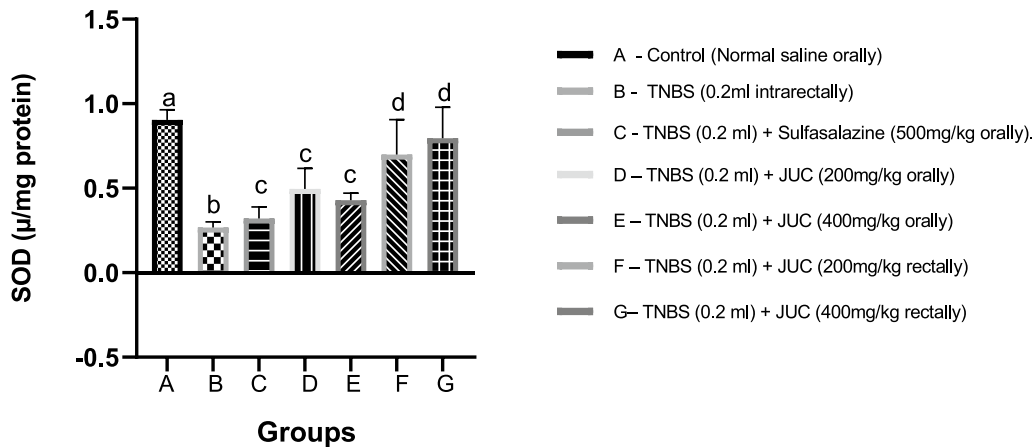


Fig. 3 Effects of *J. carnea* leaf extract on SOD activity in the brain of albino mice exposed to TNBS. Values in groups having same superscript are not significantly different

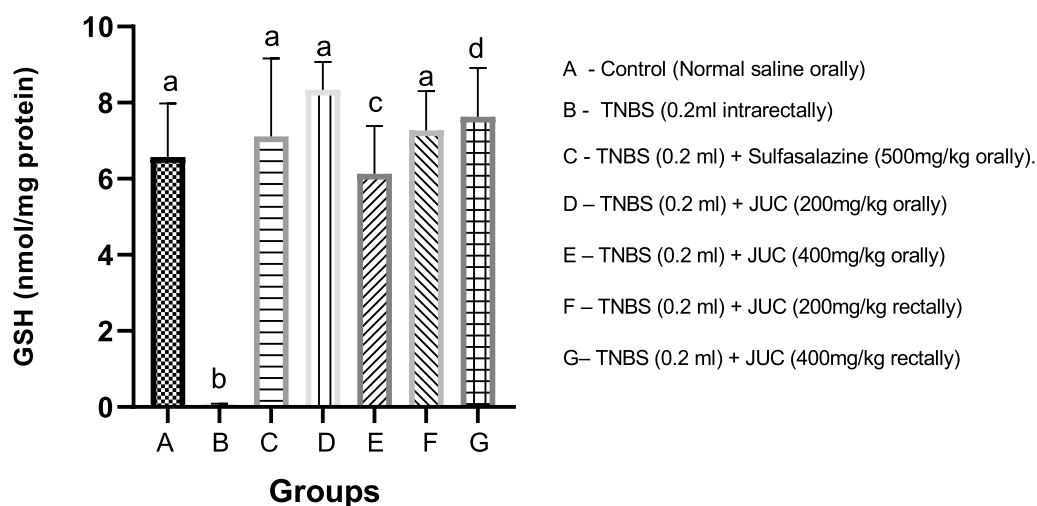


Fig. 4 Effects of *J. carnea* leaf extract on GSH activity in the brain of albino mice exposed to TNBS. Values in groups having same superscript are not significantly different

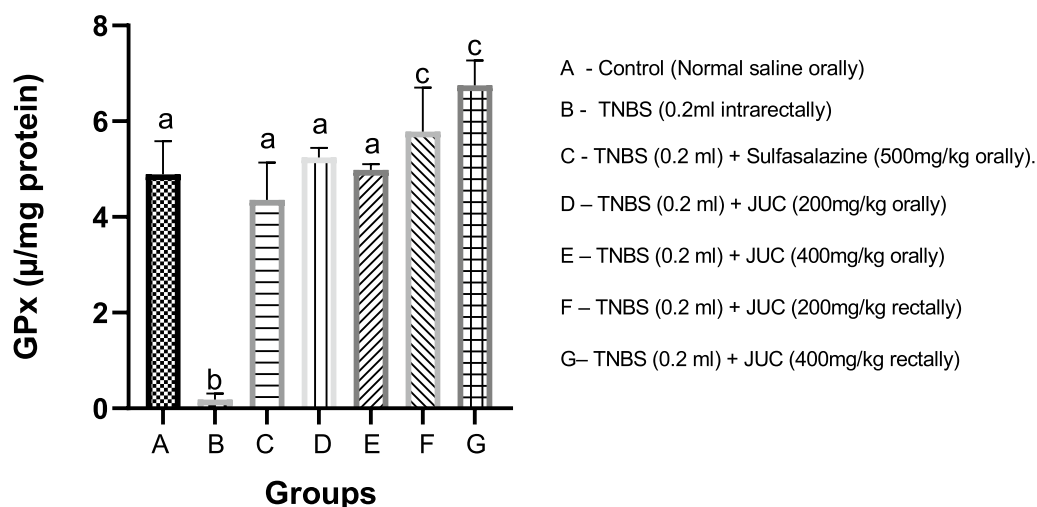


Fig. 5 Effects of *J. carnea* leaf extract on GPx activity in the brain of albino mice exposed to TNBS. Values in groups having same superscript are not significantly different

Figure 8 displays the total antioxidant capacity (TAC) levels across different treatment groups. The neutral control represents baseline TAC levels, indicating normal antioxidant capacity. In the negative control group, which received only TNBS, TAC levels are significantly higher than the neutral control, suggesting that TNBS induces oxidative stress and depletes antioxidants. The positive control group, treated with sulfasalazine and TNBS, shows TAC levels lower than the negative control but still below the neutral control, indicating that sulfasalazine partially mitigates oxidative stress but does not fully restore antioxidant capacity. The group receiving 200 mg of *Juticia carnea* extract orally alongside TNBS exhibited

TAC levels almost similar to the neutral control, suggesting that this dose effectively restores antioxidant capacity. The 400 mg dose of *Juticia carnea* extract administered orally also shows TAC levels lower than the negative control and similar to the neutral control, reflecting a more pronounced effect of the higher dose. The 200 mg dose administered rectally results in TAC levels almost similar to the neutral control, indicating effective restoration of antioxidant capacity. Similarly, the 400 mg rectal dose shows TAC levels lower than the negative control and comparable to the neutral control, suggesting effective normalization of TAC with both oral and rectal higher doses.

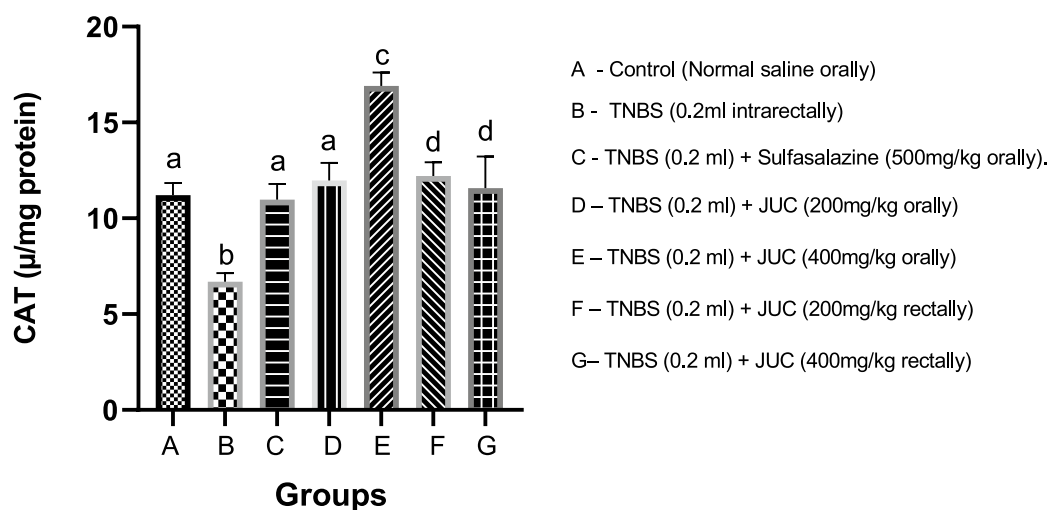


Fig. 6 Effects of *J. carnea* leaf extract on CAT activity in the brain of albino mice exposed to TNBS. Values in groups having same superscript are not significantly different

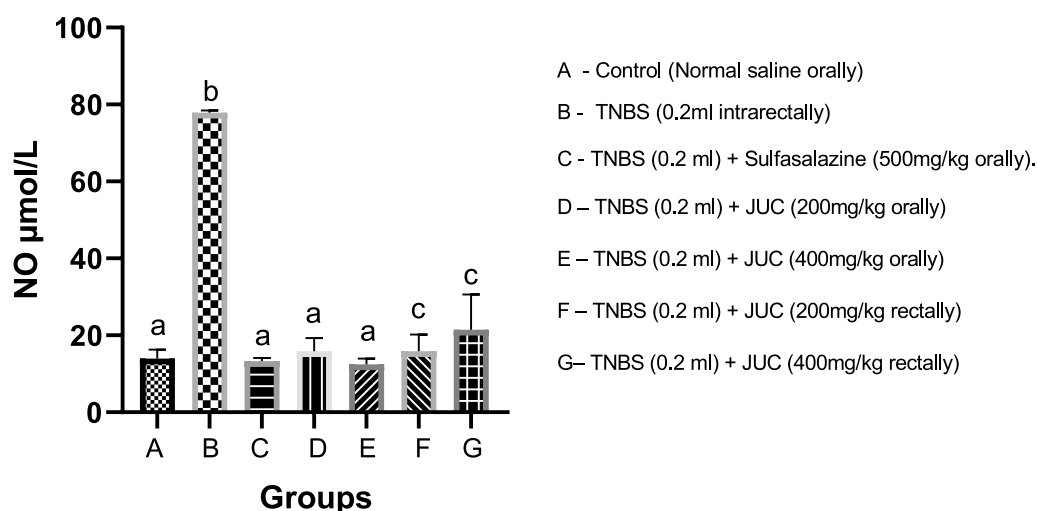


Fig. 7 Effects of *J. carnea* leaf extract on NO level in the brain of albino mice exposed to TNBS. Values in groups having same superscript are not significantly different

Figure 9 illustrates malondialdehyde (MDA) levels in various treatment groups. The control showed baseline MDA levels, reflecting normal lipid peroxidation. The group which received only TNBS demonstrated significantly higher MDA levels compared to the control, indicating increased oxidative stress and lipid peroxidation due to TNBS. In Group C, treated with sulfasalazine and TNBS, MDA levels are lower than the TNBS group but still higher than the control group, suggesting that sulfasalazine reduces lipid peroxidation but does not fully restore it to baseline levels. The group receiving 200 mg of *Justicia carnea* extract

orally showed MDA levels almost similar to the Control, indicating effective reduction in lipid peroxidation. The 400 mg dose of *Justicia carnea* extract administered orally also exhibited MDA levels lower than the TNBS group and comparable to the Control, reflecting effective reduction in lipid peroxidation with the higher dose. The 200 mg rectal dose of *Justicia carnea* showed MDA levels almost similar to the control, suggesting effective reduction in lipid peroxidation. Similarly, the 400 mg rectal dose displayed MDA levels lower than the TNBS group and similar to the control, indicating effective reduction in lipid peroxidation with both oral and rectal higher doses.

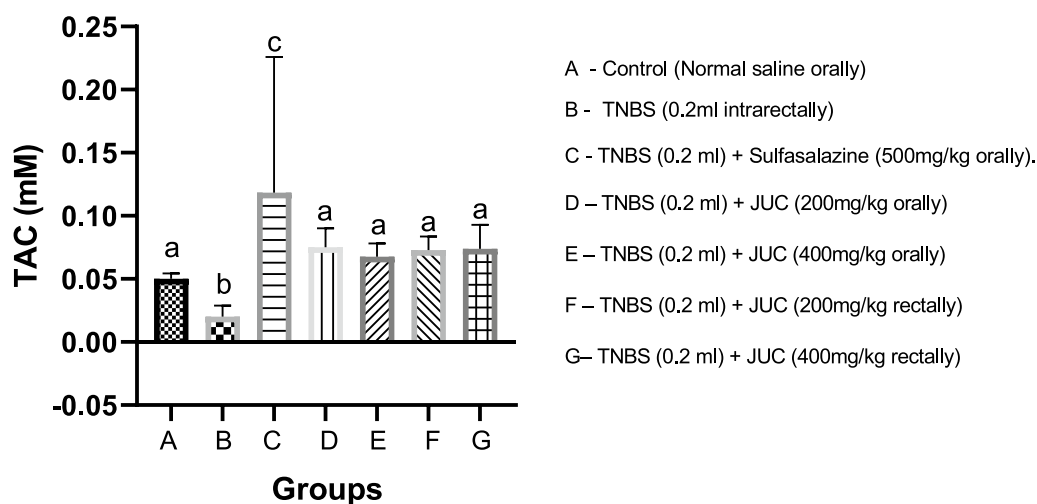


Fig. 8 Effects of *J. carnea* leaf extract on TAC in the brain of albino mice exposed to TNBS. Values in groups having same superscript are not significantly different

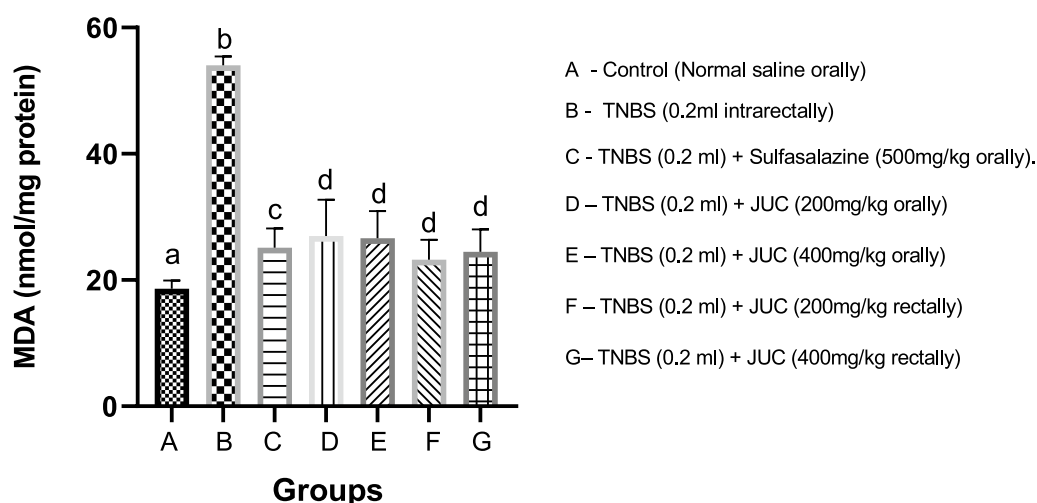


Fig. 9 Effects of *J. carnea* leaf extract on MDA levels in the brain of albino mice exposed to TNBS. Values in groups having same superscript are not significantly different

Effects of *Justicia carnea* leaf extract on the expression of cellular adhesion molecule-1 in TNBS-induced albino mice

Figure 10 shows the effects of *J. carnea* leaf extract on the expression of cellular adhesion molecule-1 in albino mice exposed to TNBS with the TNBS group showing strong positive staining indicating a pronounced inflammatory response due to TNBS-induced neuro-inflammation, while treatment groups exhibit weaker staining, supporting the anti-inflammatory effect of *J. carnea*. This is supported by the semiquantitative analysis shown in Tables 1, 2 and 3.

Discussion

This work investigated how *J. carnea* extract affected biochemical and immunohistochemical markers in a model of inflammation generated by TNBS, offering fresh perspectives on its possible medicinal uses. According to the findings, *J. carnea* exhibits strong antioxidant and anti-inflammatory qualities, lowering oxidative stress and systemic inflammation.

The inflammation brought on by TNBS is highlighted by the notable rise in C-reactive protein (CRP) levels in Group B (TNBS only) as compared to the control (Group A). Numerous studies have shown that elevated

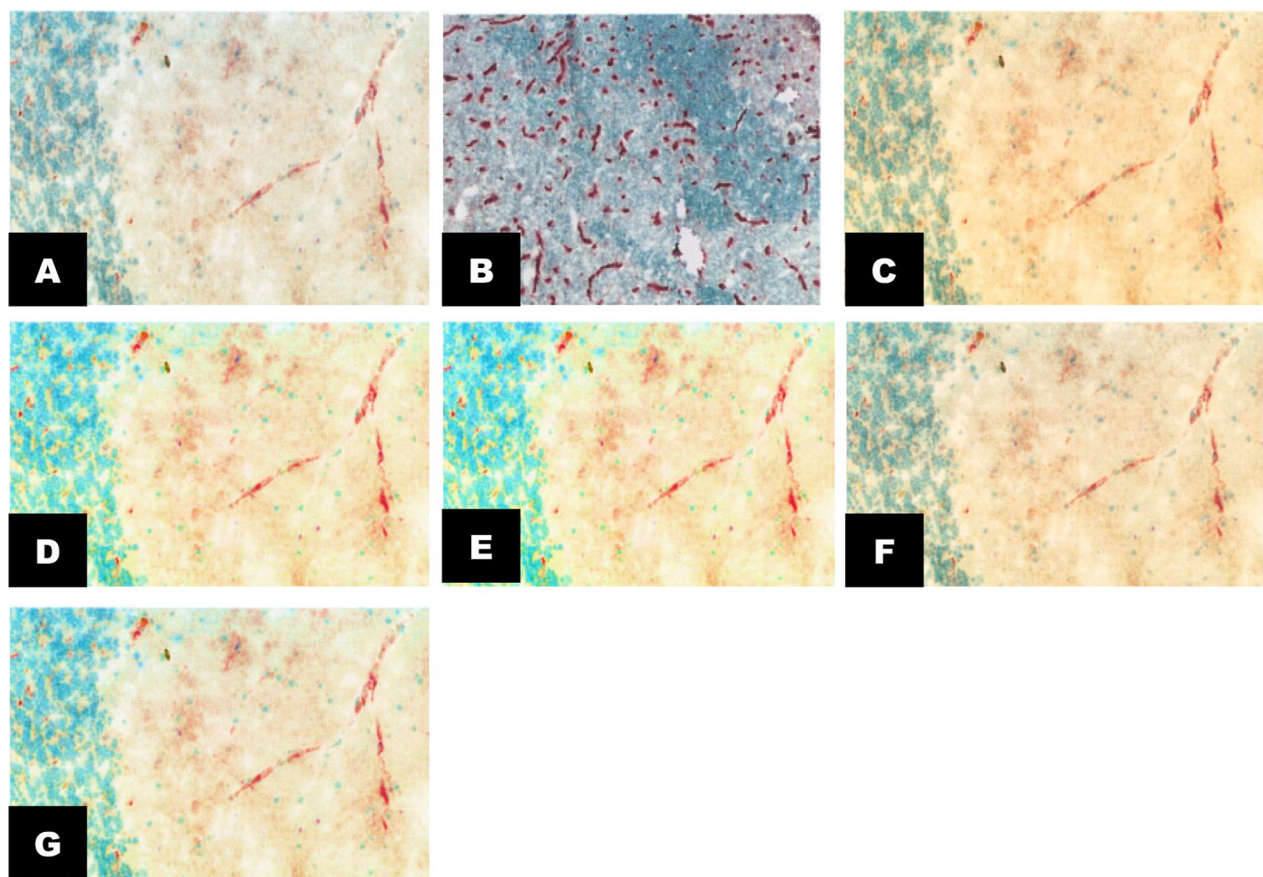


Fig. 10 Effects of *J. carnea* leaf extract on the expression of cellular adhesion molecule-1 in albino mice administered TNBS. **A.** Control (normal saline orally); **B.** TNBS (0.2 ml intrarectally); **C.** TNBS (0.2 ml) + sulfasalazine (500 mg/kg orally); **D.** TNBS + *J. carnea* (200 mg/kg orally); **E.** TNBS + *J. carnea* (400 mg/kg orally); **F.** TNBS + gel of *J. carnea* (200 mg/kg rectally); **G.** TNBS + gel of *J. carnea* (400 mg/kg rectally)

Table 1 Immunohistochemical scoring according to the intensity and extent

Score	Positive area (%)	Intensity
0	0–15	Negative
1	16–40	Weak positive
2	41–150	Moderate positive
3	> 150	Strong positive

CRP levels are a well-known indicator of inflammation (Zhou et al., 2020). A significant anti-inflammatory impact is suggested by the decrease in CRP levels following *J. carnea* treatment, particularly at the highest dosage (400 mg). This finding is consistent with earlier studies by Oguwike et al. (2021), which showed that *J. carnea* has anti-inflammatory qualities in a variety of inflammatory conditions. The fact that the CRP levels of the positive control group (sulfasalazine + TNBS) and the highest dose of *J. carnea* were comparable suggests that *J. carnea*

might be just as effective as conventional anti-inflammatory therapies.

Justicia carnea's rich phytochemical profile, which includes flavonoids, phenols, and alkaloids, has been linked to its anti-inflammatory qualities. These compounds have been demonstrated to have both antioxidant and anti-inflammatory characteristics. According to Opara and Agomuo (2022), *J. carnea* leaf powder demonstrated strong anti-inflammatory properties, most likely due to its ability to lower oxidative stress and alter pro-inflammatory pathways. Additionally, *J. carnea* extracts were shown to reduce oxidative stress and inflammation markers in diabetic rats by Falode et al. (2023), confirming the plant's potential as a treatment for inflammatory diseases.

Another important measure of inflammatory response, platelet count, was markedly elevated in the negative control group. According to the literature, thrombosis and inflammation are linked to elevated platelet counts (Andrew et al., 2024; Katsanos et al., 2016; Orororo & Asagba, 2022). Platelet counts were restored after

Table 2 Histological grading according to the intensity and extent of expression

Histological grading	Control (normal saline orally)	TNBS (0.2 ml intrarectally)	TNBS (0.2 ml) + sulfasalazine (500 mg/kg orally)	TNBS + <i>J. carnea</i> (200 mg/kg orally)	TNBS + <i>J. carnea</i> (400 mg/kg orally)	TNBS + gel of <i>J. carnea</i> (200 mg/kg rectally)	TNBS + gel of <i>J. carnea</i> (400 mg/kg rectally)
Intra-cerebral hemorrhage	0	2	0	0	0	0	0
Neuronal degeneration	0	2	1	1	0	1	0
Region of cortical necrosis	0	3	1	1	1	1	1
Total score	0/9	7/9	2/9	2/9	1/9	2/9	1/9

treatment with *J. carnea*, especially at the highest dose, indicating that it may help preserve normal platelet function in inflammatory situations. This result is consistent with that of Eze et al. (2019), who found that in inflammatory models, *J. carnea* supports the maintenance of normal platelet function. *J. carnea* can successfully offset the inflammatory effects of TNBS, as evidenced by the platelet counts returning to levels comparable to those of the positive control group (Fasuyi & Nonyelum, 2018).

Oxidative stress was also shown by the significantly greater levels of superoxide dismutase (SOD) in the animals maintained on TNBS alone (Group B) when compared to the control (Group A). This increase in levels of SOD, an essential antioxidant enzyme that shields cells from superoxide radicals, is a compensating reaction to oxidative stress (Orororo et al., 2018b). Treatment with *J. carnea* demonstrated its antioxidant potential by raising SOD levels, especially at higher doses. This result is in line with studies conducted by Dangana (2021), which demonstrated that *J. carnea* strengthens antioxidant defenses under circumstances of oxidative stress. The extract may strengthen the body's natural antioxidant defense systems, as seen by the notable rise in SOD levels following *J. carnea* treatment (Akintimehin et al., 2023; Amole & Ilori, 2010; Eduviere et al., 2022).

Particularly at higher dosages, *J. carnea* therapy restored glutathione (GSH) levels, which had been markedly decreased in the TNBS alone group. Oxidative stress frequently results in the depletion of GSH, an essential antioxidant that shields cells from oxidative damage (Orororo et al., 2018a). The results of Akubugwo et al. (2017), who noted elevated GSH levels after *J. carnea* administration in oxidative stress models, are supported by the restoration of GSH levels with *J. carnea* treatment, which supports improved antioxidant defenses. Because of its flavonoid and phenolic content, which contributes to redox equilibrium, *J. carnea* has the potential to reduce oxidative damage and preserve cellular health, as evidenced by its ability to restore GSH levels (Olowoselu,

2020). By restoring GSH stores, which are essential for eliminating reactive oxygen species (ROS) and preserving cellular antioxidant defenses, these substances fight oxidative stress (Baranov, 2023).

Similar patterns were seen in glutathione peroxidase (GPx) activity, with treatment with *Justicia carnea* increasing GPx activity, suggesting that it supports antioxidant defenses. Another important antioxidant enzyme that prevents oxidative damage is GPx, which converts hydrogen peroxide to water. The antioxidant qualities of *J. carnea* are supported by the rise in GPx activity following therapy. Due to its capacity to regulate both enzymatic and non-enzymatic antioxidants, research has demonstrated that *J. carnea* possesses hepatoprotective and anti-inflammatory qualities (Dangana, 2021). *J. carnea* reduces oxidative damage by increasing GPx activity, which aids in the breakdown of lipid hydroperoxides and hydrogen peroxide (Adegbesan et al., 2022). Anthocyanins and tannins, two of *J. carnea*'s bioactive substances, may directly scavenge ROS or increase GSH production routes. GSH's sulfhydryl groups are essential for preserving the cellular redox equilibrium, particularly in inflammatory situations like those brought on by TNBS (Pushpakiran et al., 2021). The antioxidant qualities of the extract were further supported by the identical pattern of catalase (CAT) activity. These findings align with the research conducted by Oloruntola et al., (2022), which found that *J. carnea* extract increases the activity of important antioxidant enzymes. Oxidative stress was shown by a decrease in CAT activity in the neutral control, which is consistent with research showing that TNBS-induced colitis is characterized by oxidative stress (Zhu & Li, 2020).

The TNBS group's nitric oxide (NO) levels were noticeably greater, indicating elevated oxidative stress and inflammation. Treatment with *J. carnea* decreased NO levels, particularly at higher dosages, indicating that it may help reduce inflammation and oxidative stress. This is consistent with research by Ogundele et al. (2015),

Table 3 Qualitative score used to access the strength of the stain

Receptor expression	Control (normal saline orally)	TNBS (0.2 ml intrarectally)	TNBS (0.2 ml) + sulfasalazine (500 mg/kg orally)	TNBS + <i>J. carnea</i> (200 mg/kg orally)	TNBS + <i>J. carnea</i> (400 mg/kg orally)	TNBS + gel of <i>J. carnea</i> (200 mg/kg rectally)	TNBS + gel of <i>J. carnea</i> (400 mg/kg rectally)
ICAM-1	±	±	+	±	±	±	±

The qualitative score used to access the strength of the staining of this antigen was given as —no staining section, ± scattered staining in some section, + consistent in all section, ++ consistent strong staining in all section

who showed that in inflammatory models, *J. carnea* lowers NO levels. Oxidative stress and depleted antioxidants were indicated by the negative control group's considerably higher total antioxidant capacity (TAC) values. Treatment with *J. carnea* highlighted its antioxidant potential by bringing TAC levels back to almost baseline, particularly at higher dosages. Ajiboye et al. (2018) reported similar findings, indicating elevated TAC levels after administering *J. carnea*. TAC levels were nearly returned to the control (Group A) levels in the group that received 200 mg of *Justicia carnea* extract orally in addition to TNBS. Because *J. carnea* contains bioactive chemicals such as flavonoids and phenolics, which are known for their antioxidant qualities, this implies that a lower oral dose can reduce oxidative stress. Additionally, the larger oral dose (400 mg) demonstrated dose-dependent antioxidant properties, with TAC levels comparable to the control. It is interesting to see that TAC levels were similar to the control when 200 mg and 400 mg dosages of *J. carnea* were administered rectally. Consistent with research on improved drug delivery via the rectal route, this implies that rectal distribution improves bioavailability, possibly permitting more direct and effective absorption of active substances into the systemic circulation (Shohin et al., 2011). It has been demonstrated that *Justicia carnea* contains a wealth of antioxidant components, such as flavonoids and anthocyanins, which can scavenge free radicals and restore antioxidant defenses (Ajiboye et al., 2018). *J. carnea's* promise as a treatment for illnesses linked to oxidative stress is supported by the notable TAC restoration shown in the groups that received *J. carnea*.

As a measure of lipid peroxidation, malondialdehyde (MDA) levels were noticeably higher in the TNBS group. MDA levels were lowered by *J. carnea* therapy, particularly at higher dosages, suggesting less lipid peroxidation. This result is in line with Onyema et al. (2013)'s research, which found that *J. carnea* lowers MDA levels under oxidative stress. According to Awogbindin et al. (2019), elevated MDA levels in the TNBS group validate the oxidative damage caused by TNBS. Remarkably, the effects of rectal administration of *Justicia carnea* extract on MDA levels were comparable to those of oral treatment, suggesting that both delivery methods have similar bioavailability and effectiveness. For the targeted therapy of oxidative stress in gastrointestinal inflammation, this may be essential. Inflammation was shown by the negative control group's strong positive staining in the immunohistochemical examination of ICAM-1 expression. *Justicia carnea's* anti-inflammatory qualities are supported by the fact that treatment decreased ICAM-1 expression, especially at higher dosages. Flavonoids and antioxidant chemicals found in *J. carnea* are well known for their anti-inflammatory properties. The suppression

of important inflammatory pathways, such as NF- κ B, which controls the production of adhesion molecules, may be the cause of the observed decrease in ICAM-1 expression (Olaleye et al., 2019; Saba et al., 2010).

In line with dose-dependent effects frequently documented for plant-derived therapies, higher dosages (400 mg) demonstrated superior anti-inflammatory capabilities in comparison with lower doses (Akinmoladun et al., 2020).

Conclusions

The study offered solid proof of *J. carnea's* therapeutic effectiveness in reducing oxidative stress, in the brain of mice given TNBS. Its promise as an anti-inflammatory, antioxidant, and neuroprotective pharmaceutical is highlighted by the notable decrease in CRP levels, restoration of platelet counts, improvement in antioxidant defenses, and decrease in expression of cellular adhesion molecule-1 in the brain. Future research should examine the molecular pathways that underlie *J. carnea's* medicinal properties. To confirm these results in human populations and identify the ideal dosages for medicinal uses, clinical trials are also required.

Abbreviations

SOD	Superoxide dismutase
CAT	Catalase
ROS	Reactive oxygen species
TBARS	Thiobarbituric acid reactive substance
MDA	Malondialdehyde
TNBS	2,4,6-Trinitrobenzenesulphuric acid
CRP	C-reactive protein
ICAM-1	Cellular adhesion molecule-1

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Author contributions

EMT, BIOP, and OGO conceived and designed the experiment; OGO, MJ, DCC, and EKC performed the experiment and acquired the data; OOC and JJE analyzed and interpreted the data; OOC and MJ drafted the manuscript; and all authors read and approved the manuscript.

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Availability of data and materials

Experimental data and materials employed in the development of this research report would be made available upon a reasonable request, but for the meantime, they are kept in the correspondent's repository.

Declarations

Ethics approval and consent to participate

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This study followed the principles in the Declaration of Helsinki on the humane treatment of animals used in research (<http://www.wma.net/en/30publications/10policies/a18/>) and the principles in the AVMA guidelines for the euthanasia of animals (AVMA 2013). Laboratory animals were handled in accordance with the laboratory principles of handling and care of experimental animals.

Clinical trial number

Not applicable.

Consent for publication

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Competing interests

Not applicable.

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