Protective Effects of *Citrullus Lanatus* Seed Extract on Diclofenac-Induced Splenic Oxidative Stress and Histopathological Alterations in Adult Male Wistar Rats

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ABSTRACT

Diclofenac, a widely used non-steroidal anti-inflammatory drug (NSAID), has been associated with oxidative stress and tissue injury following prolonged administration. *Citrullus lanatus* (watermelon) seeds contain bioactive phytochemicals with antioxidant properties that may protect against oxidative damage. This study investigated the effect of ethanolic seed extract of *Citrullus lanatus* on oxidative stress markers and splenic histology in diclofenac-induced splenic injury in male Wistar rats. Twenty-five adult male Wistar rats were randomly assigned into five groups (A–E; n = 5). Group A served as the control; Group B received 100 mg/kg body weight of diclofenac; Group C received 300 mg/kg body weight of *C. lanatus* extract; while Groups D and E received diclofenac concurrently with 150 mg/kg and 300 mg/kg body weight of the extract, respectively. Treatments were administered orally for 28 days. Body weight, relative spleen weight, serum malondialdehyde (MDA), and superoxide dismutase (SOD) levels were assessed, while splenic histology was evaluated using hematoxylin and eosin staining. Diclofenac administration resulted in reduced body weight gain, elevated MDA levels, decreased SOD activity, and severe splenic alterations characterized by hemorrhage and fibrosis. Treatment with *C. lanatus* extract reduced MDA levels and significantly improved SOD activity compared with the diclofenac-only group. Histological examination revealed preservation of normal splenic architecture in rats treated with the extract alone and attenuation of splenic lesions in co-treated groups. The findings suggest that ethanolic seed extract of *Citrullus lanatus* exerts protective effects against diclofenac-induced splenic injury, possibly through its antioxidant activity and enhancement of endogenous antioxidant defenses.

Keywords: Diclofenac; *Citrullus lanatus*; spleen; oxidative stress; malondialdehyde; superoxide dismutase.

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INTRODUCTION

Background of The Study

Watermelon (*Citrullus lanatus*) is a widely cultivated fruit, renowned for its high water content and refreshing taste (Bamidele et al., 2021). While the fruit itself is well known for its hydrating properties and nutritional value (Nissar et al., 2025), the seeds of watermelon have recently attracted significant interest in the field of pharmacology due to their rich composition of bioactive compounds (Eke et al., 2021; Sorokina et al., 2021). Watermelon seeds are a source of protein, B vitamins, minerals (such as magnesium, potassium, phosphorus, sodium, iron, zinc, manganese, and copper), and fat, among others (Bamidele et al., 2021; Collins et al., 2007). Additionally, they are rich in polyphenolic compounds, which are well-documented for their antioxidant, anti-inflammatory, and hepatoprotective activities (Bamidele et al., 2021; Nissar et al., 2025)

These bioactive compounds found in watermelon seeds have been shown to exert potent antioxidative effects, primarily by scavenging reactive oxygen species (ROS) and inhibiting the activities of oxidative stress markers such as malondialdehyde (MDA) (Bamidele et al., 2021; Eke et al., 2021). This makes watermelon seeds a valuable natural source of protection against oxidative damage, which is a key contributor to a range of pathologies, including inflammation, tissue damage, and degenerative diseases (Nikolakakis et al., (2026). The antioxidant properties of watermelon seed extract have been shown to enhance the endogenous antioxidant defense system by modulating enzyme activities such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, thereby reducing cellular damage (Eke et al., 2021). Furthermore, watermelon seeds are known to possess anti-inflammatory properties that help in reducing the levels of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), in response to oxidative stress (Nikolakakis et al., (2026); Hirsa et al., (2025). This capacity to modulate the inflammatory cascade is particularly important in preventing or mitigating damage to vital organs (Messaoudi et al., 2019).

Diclofenac is a widely used non-steroidal anti-inflammatory drug (NSAID), primarily employed for the management of pain and inflammatory conditions such as arthritis, musculoskeletal disorders, and post-surgical pain (Alfaro & Davis, 2023). It exerts its pharmacological effects through inhibition of cyclooxygenase (COX-1 and COX-2) enzymes, thereby reducing prostaglandin synthesis, which mediates pain and inflammation (Gan, 2010; Alfaro & Davis, 2023).

Although diclofenac is clinically effective, prolonged or excessive use has been associated with adverse effects across multiple organs, including the liver, kidneys, gastrointestinal tract, and spleen (Elnashar et al., 2024; Alfaro & Davis, 2023). These toxic effects are largely mediated through the induction of oxidative stress and amplification of inflammatory responses, ultimately leading to cellular injury and organ dysfunction (Thai et al., 2023; Elnashar et al., 2024). The spleen has been identified as a particularly sensitive organ to diclofenac-induced toxicity (Elnashar et al., 2024). Experimental studies have demonstrated that prolonged exposure to diclofenac may result in cellular degeneration, necrosis, and inflammatory changes within splenic tissue, thereby impairing its immunological and hematopoietic functions (Yoo et al., 2025; Gabr et al., 2025).

The spleen plays a central role in immune defense and blood filtration, and its vulnerability to oxidative injury is linked to its continuous involvement in hematopoiesis and immune surveillance, including removal of aged or damaged erythrocytes (Bronte & Pittet, 2013; Lewis et al., 2019). Excessive oxidative stress induced by pharmacological agents such as diclofenac can disrupt the normal splenic architecture, leading to alterations in both white pulp (lymphoid regions) and red pulp (vascular regions), thereby compromising immune competence (Gabr et al., 2025; Elnashar et al., 2024).

Structurally, the spleen is a secondary lymphoid organ composed of white pulp, responsible for adaptive immune responses through lymphocyte activation, and red pulp, which filters blood and recycles iron from senescent erythrocytes (Bronte & Pittet, 2013; Lewis et al., 2019). Despite extensive literature on splenic physiology and drug-induced toxicity, there remains a gap in knowledge regarding the combined effect of watermelon seed extract and diclofenac on the spleen of adult male Wistar rats. This study therefore seeks to address this gap.

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Statement of the Problem

The use of non-steroidal anti-inflammatory drugs (NSAIDs), particularly diclofenac, is widespread for the management of pain and inflammation (Alfaro & Davis, 2023). Despite its therapeutic benefits, prolonged use of diclofenac has been associated with several adverse effects, particularly organ toxicity (Elnashar et al., 2024). Among the organs affected, the spleen is highly susceptible to oxidative stress and inflammatory damage induced by diclofenac (Yoo et al., 2025; Elnashar et al., 2024). Histopathological evidence shows that chronic exposure to diclofenac can lead to cellular degeneration, inflammation, and dysfunction in the spleen, which plays a critical role in immune response and blood filtration (Yoo et al., 2025; Elnashar et al., 2024). The adverse effects of diclofenac on the spleen necessitates the exploration of potential protective agents that can mitigate such damage. Watermelon seed extract, known for its potent antioxidant and anti-inflammatory properties, has shown promise in protecting against oxidative stress and organ damage in various tissues (Bamidele et al., 2021; Eke et al., 2021). However, there is limited research on the protective effects of watermelon seed extract specifically in relation to diclofenac-induced damage to the spleen.

Given the antioxidant and anti-inflammatory properties of watermelon seeds, it is plausible that co-administration of watermelon seed extract with diclofenac could offer a protective effect against diclofenac-induced spleen toxicity. However, the specific effects of this co-administration on the spleen of adult male Wistar rats remain unclear.

METHODOLOGY

Ethical Approval

Ethical approval for this study was obtained from the Ethical Committee of the Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus (NAU/CHS/NC/FBMS/923). All procedures involving the use of experimental animals were conducted in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals* (2011).

Area of Study

This study was carried out at the Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Nnewi, Anambra State, Nigeria.

Materials Used in The Study

Fifty-one (51) Male Wistar Rats, Diclofenac, Watermelon (*Citrullus lanatus*) seed, Standard plastic cages and water can, Beddings, electronic weighing balance (M-Metlar M311L; China), Oral cannula, Slide, Microscope (Olympus XSZ-107BN), and Centrifuge 90(1) (Alpin Medical, England). Plain Blood tube (Fantastik, China), Filter paper (Whatman Qualitative Filter Paper No. 1, Sigma Aldrich WHA1001042), distilled water, Cotton wool, Latex Medical Hand gloves, and Chloroform (Guondghuo, China), Vital feed grower (JOS, Nigeria), and Dissecting kits. Randox Reagent Kits (Sigma Aldrich, Germany), UV-VIS 752N Spectrophotometer, and Rotary evaporator (Digital) TT-52 (Techmel and Techmel, USA).

Procurement and Housing of Experimental Animals

Fifty-one (51) male Wistar rats weighing 120 to 130 grams were purchased from the Animal House Unit, Department of Zoology, University of Ilorin, Nigeria and was conveyed to the Animal House Unit, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. The experimental male rats were kept in standard cages at a room temperature of 27±2°C and were maintained with normal laboratory chow (Grower feed) and distilled water *ad libitum*. They were acclimatized for two weeks before administration of diclofenac and the ethanolic seed extract of *C. lanatus*, and were observed under a 12-hour light-dark cycle.

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Of the 51 male Wistar rats used in this research, 26 were allocated to the acute toxicity study of diclofenac and the ethanolic seed extract of *Citrullus lanatus*, while the remaining 25 rats were used for the main experimental study.

Procurement of Diclofenac

Diclofenac Sodium produced by Aarti Drugs, India, was purchased from a licensed drug dealer at Onitsha Drug Market, Onitsha, Anambra State, Nigeria.

Procurement of *Citrullus lanatus* seed and Ethanoic Extraction

Fresh seeds of *Citrullus lanatus* were purchased from Eke-Amobi market, Nnewi, Anambra State, washed under running water and shed-dried. The dried seeds of *Citrullus lanatus* were ground into a fine powdered form using a local grinder. Three hundred (300) grams of the powdered form of *Citrullus lanatus* was dissolved in 1000mls of 95% ethanol for 48-hours with intermittent shaking. Thereafter, the mixture was first filtered through a clean muslin cloth and subsequently through Whatman No. 1 filter paper. The filtrate obtained was concentrated using a rotatory evaporator and further dried using a Laboratory oven at 45ºC into a gel-like form, preserved in an air-tight container and kept in a refrigerator at -4ºC for further usage (Azwanida, 2015).

Toxicity Test for *Citrullus lanatus* seed and Diclofenac

The acute oral toxicity (LD_{50}) of ethanolic seed extract of *Citrullus lanatus* and diclofenac was evaluated using the method described by Lorke (1983). Each study was conducted in two phases using a total of 13 adult male Wistar rats per test substance. In Phase I, animals were divided into groups and administered graded doses of 10, 100, and 1000 mg/kg body weight of the test substances via oral gavage and observed for signs of toxicity and mortality for 24 hours. In Phase II, additional animals received higher doses selected based on the outcomes of Phase I to further refine the LD_{50} estimation.

All animals were observed for clinical signs of toxicity, behavioural changes, and mortality following administration.

The median lethal dose (LD_{50}) of the ethanolic seed extract of *Citrullus lanatus* was found to be greater than 5000 mg/kg body weight, indicating a high margin of safety. The LD_{50} of diclofenac was estimated to be approximately 3800 mg/kg body weight following oral administration in adult male Wistar rats.

Treatment Protocol

The experimental animals were grouped according to their body weights into five groups of five animals each and received the following:

Group A received feed and distilled water *ad libitum* only.

Group B received 100 mg/kg/bw of Diclofenac only.

Group C received 300 mg/kg/bw of ethanolic seed extracts of *Citrullus lanatus* only.

Group D received a co-administration of 100 mg/kg/bw of Diclofenac and 150 mg/kg/bw of ethanolic seed extracts of *Citrullus lanatus,* while

Group E received a co-administration of 100 mg/kg/bw of Diclofenac and 300 mg/kg/bw of ethanolic seed extracts of *Citrullus lanatus*.

Both doses of diclofenac and ethanolic seed extracts of *Citrullus lanatus* were reconstituted freshly in the laboratory daily prior to administration, which was done via oral gavage. Also, during this period signs of toxicity and behavioral changes were checked for. Administration of both diclofenac and ethanolic seed extracts of *C. lanatus* lasted for 28-days.

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Termination of Experiment and Sample Collection

Twenty-four hours after the end of the experimental period, the animals were anaesthetized using ketamine hydrochloride (80 mg/kg body weight, intraperitoneally). Blood samples were collected from the retro-orbital plexus using a heparinized capillary tube as described by Parasuraman et al., (2010). The collected blood was transferred into labelled plain tubes and allowed to clot for 5 minutes at room temperature, after which it was centrifuged at 3000 revolutions per minute (rpm) for 20 minutes. The serum obtained was carefully separated using a micropipette and stored for biochemical analysis of oxidative stress markers, including malondialdehyde (MDA) and superoxide dismutase (SOD).

Following blood collection, the animals were sacrificed, and a midline abdominal incision was made to expose the spleen. The spleen was carefully excised, rinsed in normal saline to remove blood contaminants, and blotted dry using filter paper. The absolute spleen weight was then determined using a digital weighing balance. Tissue samples for histological examination were immediately fixed in 10% neutral buffered formalin and stored in properly labelled containers for subsequent processing and microscopic evaluation.

Biochemical Analysis

Oxidative Stress Biomarkers

Malondialdehyde (MDA) Estimation

Malondialdehyde (MDA) levels were determined using the thiobarbituric acid reactive substances (TBARS) assay as described by Ohkawa et al., (1979).

Principle:

MDA, a secondary product of lipid peroxidation, reacts with thiobarbituric acid (TBA) under acidic conditions and high temperature to form a pink MDA–TBA adduct. The intensity of the color produced is directly proportional to the concentration of MDA and is measured spectrophotometrically at 532 nm.

Reagent Preparation:

A 4.0 mM solution of thiobarbituric acid (TBA) was prepared by dissolving 57.66 mg of TBA in 100 mL of glacial acetic acid.

Procedure:

One millilitre (1 mL) of standard or sample was mixed with 1 mL of TBA reagent in a test tube. The mixture was incubated in a boiling water bath at 95°C for 60 minutes. Thereafter, the tubes were cooled to room temperature, and absorbance was measured at 532 nm using a UV-visible spectrophotometer (PharmaSpec 1700, Shimadzu, Japan). Calibration standards were run in triplicate (n = 3), while blanks were prepared by replacing the sample with distilled water or acetic acid (n = 5). Each sample was analysed in five replicates (n = 5).

MDA concentration was calculated from the calibration curve and expressed as TBARS using the formula:

TBARS (µmol/g) = (Ac x V) / W

Where ‘Ac’ is the amount determined from the calibration curve, and ‘W’ is the weight of the sample taken while ‘V’ is the volume in mL or dilution factor of the total extract prepared.

Superoxide Dismutase (SOD) Activity

SOD activity was determined using the method described by Misra & Fridovich, (1972).

Principle:

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The method is based on the ability of superoxide dismutase to inhibit the auto-oxidation of epinephrine to adrenochrome under alkaline conditions. The rate of adrenochrome formation is measured spectrophotometrically at 480 nm, and SOD activity is proportional to the degree of inhibition of this reaction.

Procedure:

A total of 80 µL of serum sample was added into a test tube labelled "Test," followed by 1000 µL of carbonate buffer (pH 10.2). The mixture was gently mixed and incubated at 37°C for 5 minutes. Subsequently, 600 µL of freshly prepared epinephrine solution was added to initiate the reaction. Absorbance was recorded at 30-second intervals for 150 seconds at 480 nm. A blank was prepared similarly, except that distilled water was used in place of serum.

The percentage inhibition of epinephrine auto-oxidation was calculated as:

% Inhibition = [(OD blank − OD test) / OD blank] × 100

SOD activity was expressed in U/mL, where one unit represents the amount of enzyme required to inhibit 50% of epinephrine auto-oxidation.

OD= Optical density

RESULTS

Table 1.0: Effect of ethanolic seed extract of *Citrulus lanatus* on body weight in Diclofenac-induced Splenic toxicity

	Initial body weight (g)	Final body weight (g)	Weight change	t-value	p-value
	Mean±SEM	Mean±SEM			
Group A	170.83±6.31	230.50±14.85	59.67	4.217	0.003*
Group B	275.50±10.09	266.00±8.02	-9.50	0.694	0.519
Group C	277.00±17.35	289.33±21.11	12.33	0.451	0.675
Group D	265.67±13.57	234.67±9.94	-31.00	1.843	0.139
Group E	236.50±11.72	228.67±6.44	-7.83	0.526	0.621

Data was analysed using t-test and values were considered significant at $p \leq 0.05$.

*: significant

Table 1.0 result reveals an increase in the mean body weight in groups A ($p < 0.05$) and C, while groups B, D, and E had a decrease in body weight when the initial weight was compared to the final weight. The weight changes showed a decrease in groups B, C, D, and E when compared to group A. this is represented in fig. 1.0.

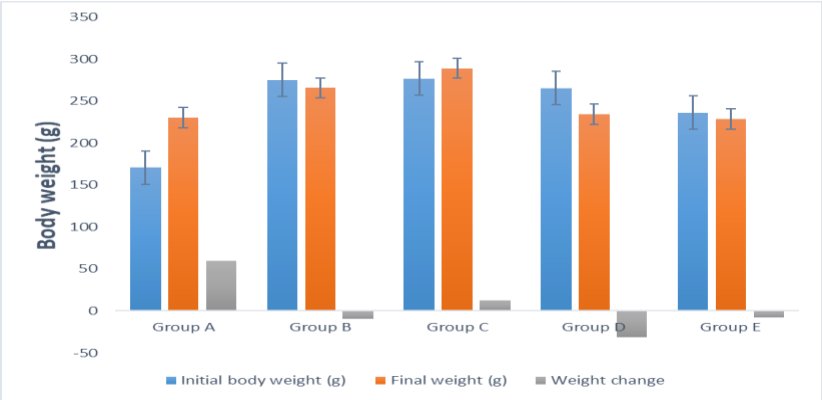

Fig 1.0: Effect of ethanolic seed extract of *Citrulus lanatus* on body weight in Diclofenac induced toxicity

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Table 2.0: Effect of ethanolic seed extract of *Citrulus lanatus* on relative spleen weight in Diclofenac-induced Splenic toxicity

	Relative spleen weight (g)
	MEAN±SEM
Group A	0.27±0.04
Group B	0.35±0.05
Group C	0.34±0.04
Group D	0.65±13.57*#
Group E	0.48±0.09
F-ratio	3.883

Data was analysed using ANOVA followed by post Hoc LSD comparison. *: significant when compared to group A, #: significant when compared to group B.

Table 2.0 result reveals higher values in the relative spleen weight in groups B, C, D, and E (0.441, p=0.465, p=0.005, p=0.070 respectively) when compared to group A. Groups D also showed higher relative spleen weight (p< 0.05), when compared to Group B (fig. 2.0).

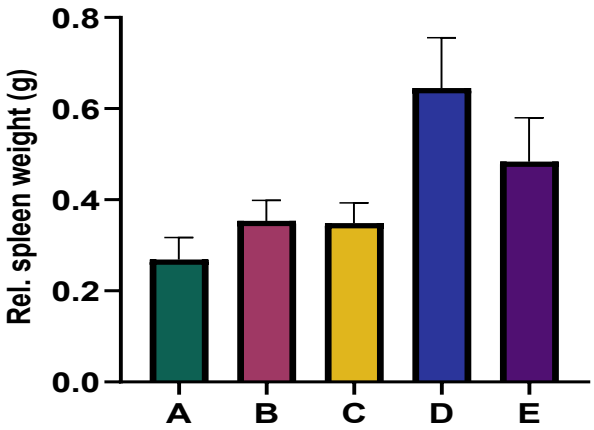

Fig 2.0: Effect of ethanolic seed extract of *Citrulus lanatus* on the relative spleen weight in Diclofenac-induced splenic toxicity.

Table 3.0: Effect of ethanolic seed extract of *Citrulus lanatus* on serum MDA and SOD level in Diclofenac-induced Splenic toxicity

	Serum MDA level (nmol/ml)	Serum SOD level (U/l)
	MEAN±SEM	MEAN±SEM
Group A	0.95±0.00	16.84±0.13
Group B	1.63±0.14*	9.22±0.64*
Group C	1.42±0.08*	13.38±0.61*#
Group D	1.36±0.08*	15.14±1.16#
Group E	1.40±0.07*	14.00±0.52*#
F-ratio	7.503	16.556

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Data was analysed using ANOVA followed by post Hoc LSD comparison. *: significant when compared to group A, #: significant when compared to group B.

Table 3.0 shows that the mean serum MDA level was higher ($p< 0.05$) in groups B, C, D, and E when compared to group A. Although the mean serum MDA level were lower in groups C, D, and E when compared to group B, these differences were however not statistically significant (fig. 3.0).

There were statistically significant lower values in the mean serum SOD level in groups B, C, and E when compared to compared to group A. when test groups were compared against Group B (exposed to Diclofenac alone), they all showed higher ($p< 0.05$) values ($p=0.002$, $p=0.001$, $p=0.001$ respectively) (fig.4.0).

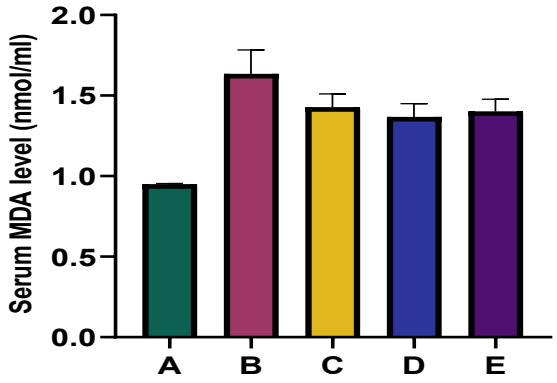


Fig 3.0 Effect of ethanolic seed extract of *Citrulus lanatus* on serum MDA level in Diclofenac-induced toxicity

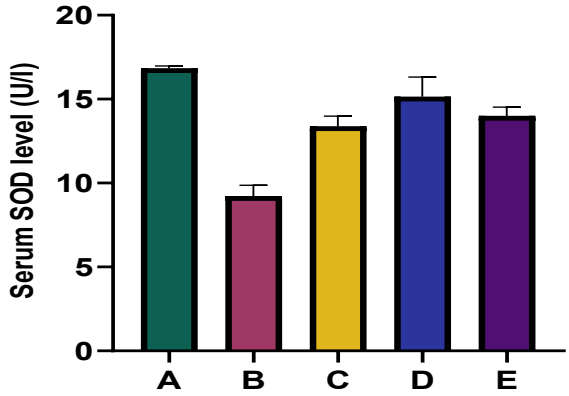
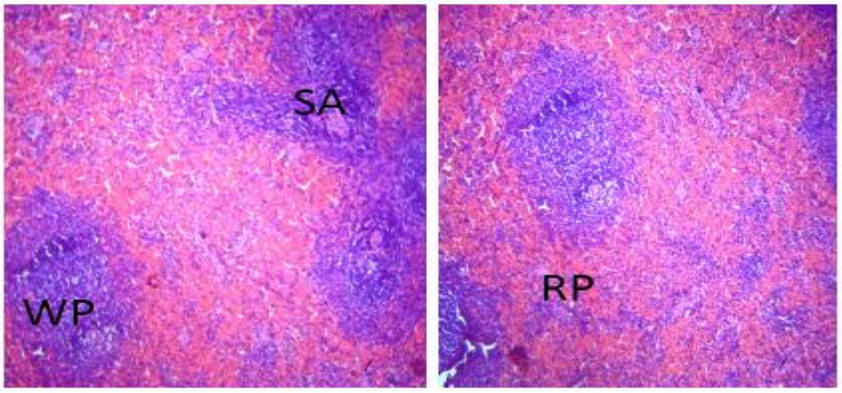


Fig 4.0 Effect of ethanolic seed extract of *Citrulus lanatus* on serum SOD level in Diclofenac-induced toxicity

Histological findings



Plates 1A & 1B: Photomicrographs of spleen of Group A Wistar rats that received feed and distilled water *ad*

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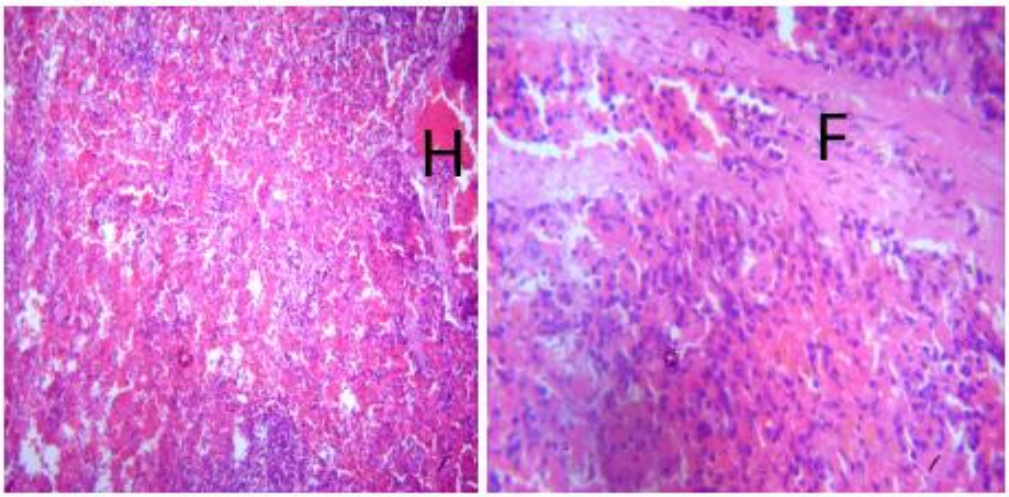
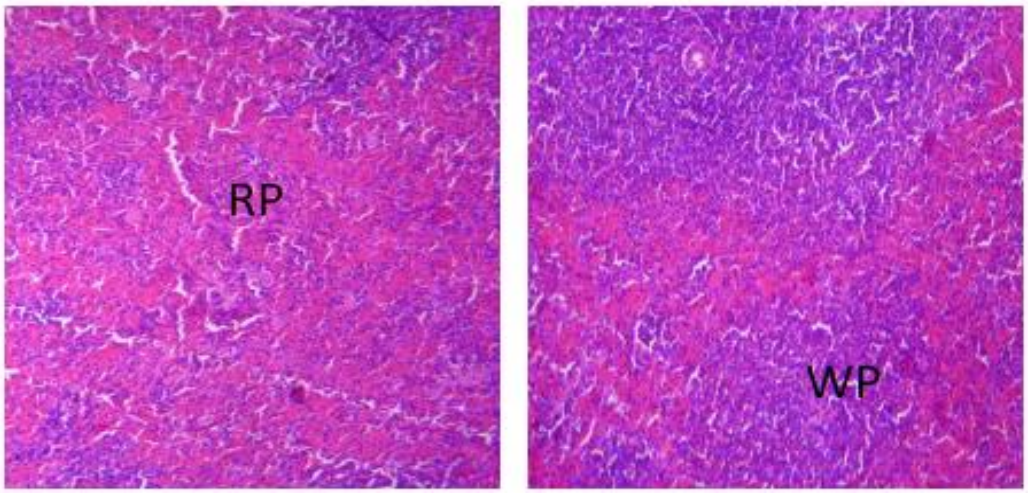
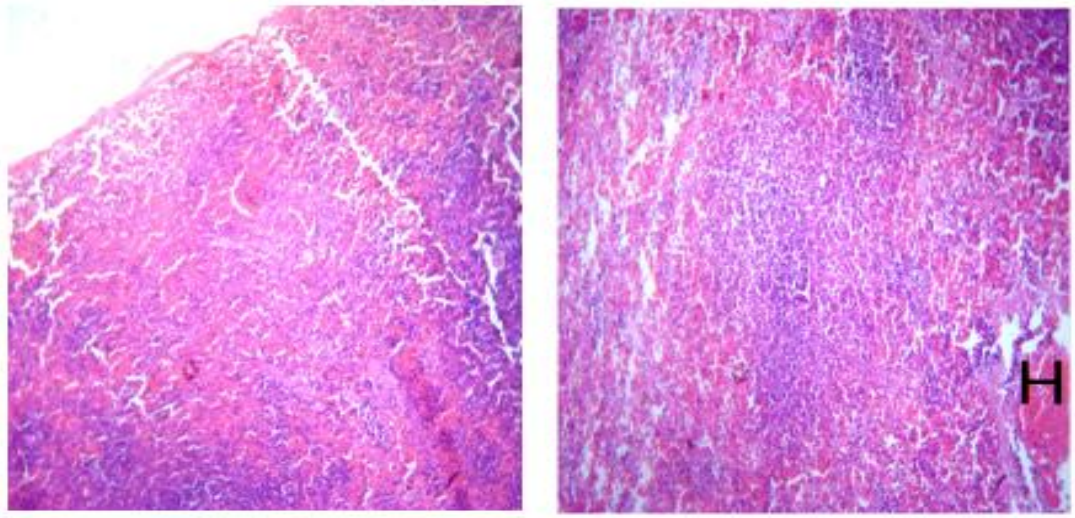
libitum only (X150) (H/E), shows normal splenic tissue with red pulp (RP) and white pulp (WP) with central splenic artery (SA).



Plates 2A &2B: Photomicrographs of spleen of Group B Wistar rats that received 100 mg/kg/bw of Diclofenac only (X150) (H/E), shows severe effect on the splenic tissue with hemorrhage (H) and severe fibrosis (F).



Plates 3A & 3B: Photomicrographs of spleen of Group C Wistar rats that received 300 mg/kg/bw of ethanolic seed extract of *Citrullus lanatus* only (X150) (H/E), shows normal splenic tissue with well outlined white pulp (WP) and red pulp (RP).



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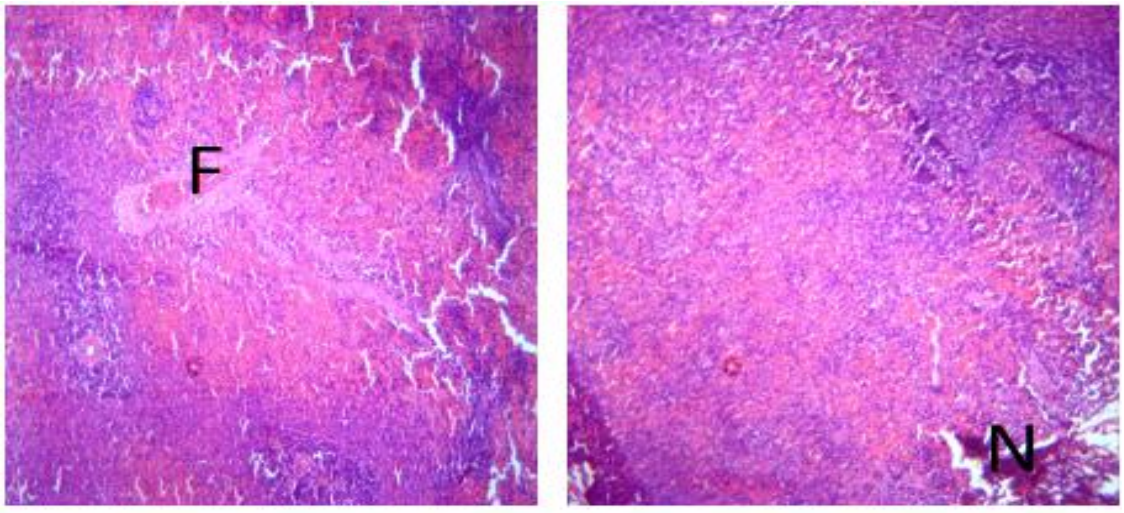
Plates 4A & 4B: Photomicrographs of spleen of Group D Wistar rats that received a co-administration of 100 mg/kg/bw of Diclofenac and 150 mg/kg/bw of ethanolic seed extract of *Citrullus lanatus* (X150) (H/E), shows mild effect on the splenic tissue with mild area of hemorrhage (H) otherwise normal splenic histoarchitecture.



Plates 5A & 5B: Photomicrographs of spleen of Group E Wistar rats that received a co-administration of 100 mg/kg/bw of Diclofenac and 300 mg/kg/bw of ethanolic seed extract of *Citrullus lanatus* (X150) (H/E), shows mild effect on the splenic tissue characterized by mild fibrosis (F) and focal areas of necrosis (N).

DISCUSSION

The present study investigated the effects of ethanolic seed extract of *Citrullus lanatus* on body weight, relative spleen weight, oxidative stress markers, and splenic histology in diclofenac-induced splenic injury in male Wistar rats. The findings demonstrated that diclofenac administration was associated with reduced body weight gain, increased relative spleen weight, elevated serum malondialdehyde (MDA) levels, reduced superoxide dismutase (SOD) activity, and marked histological alterations in the spleen. Administration of *C. lanatus* seed extract ameliorated some of these changes, suggesting a protective role against diclofenac-induced oxidative and structural damage.

The body weight findings showed that rats administered diclofenac alone exhibited a reduction in body weight relative to their initial body weight. Although the reduction was not statistically significant, it may reflect the systemic effects of diclofenac-induced oxidative stress and tissue injury. Oxidative stress has been associated with metabolic disturbances, impaired nutrient utilization, and reduced growth performance in experimental animals as well as CNS depressant activities suppressing hunger centers (Burke et al., 2006). Similar reductions in body weight following exposure to toxic agents that induce oxidative stress have been reported in experimental studies (Owumi & Dim, 2019; Abbas et al., 2014; Burke et al., 2006). In contrast, rats treated with *C. lanatus* extract alone showed a slight increase in body weight. This observation may be attributable to the nutritional composition of watermelon seeds, which are rich in proteins, essential fatty acids, minerals, and bioactive compounds (Bamidele et al., 2021; Eke et al., 2021). The absence of marked weight loss in the extract-treated group further suggests that the extract was well tolerated at the administered dose.

The relative spleen weight results revealed a tendency toward increased spleen weight in diclofenac-treated animals compared with the control group. Alterations in organ weight are frequently associated with inflammatory responses, vascular changes, and cellular adaptations following tissue injury. Diclofenac has been reported to induce oxidative stress and inflammatory responses in experimental animals, which may contribute to changes in organ morphology and weight (Moradi et al., 2021; Owumi & Dim, 2019). The increase observed in the present study may therefore reflect adaptive or pathological responses to diclofenac-induced splenic injury. Interestingly, the co-treatment groups demonstrated variable spleen weight responses, suggesting that factors beyond oxidative stress alone may have contributed to the observed changes.

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Malondialdehyde is a major end product of lipid peroxidation and serves as a reliable biomarker of oxidative damage to cellular membranes (Del Rio et al., 2005; Ofoego et al., 2020). In the present study, diclofenac administration resulted in a significant elevation of serum MDA levels compared with the control group. This finding indicates increased lipid peroxidation and supports the occurrence of oxidative stress following diclofenac exposure. Similar increases in MDA levels have been reported in experimental models of diclofenac toxicity, where excessive production of reactive oxygen species (ROS) promotes membrane damage and cellular dysfunction (Moradi et al., 2021; Izak-Shirian et al., 2022; Varışlı et al., 2023). The elevated MDA levels observed in the present study therefore suggest that oxidative damage contributed substantially to the splenic alterations induced by diclofenac.

Administration of *C. lanatus* extract was associated with lower MDA levels compared with the diclofenac-only group, although the reductions did not attain statistical significance. Nevertheless, this trend suggests attenuation of lipid peroxidation by the extract. Watermelon seeds and rand contain numerous antioxidant phytochemicals, including phenolic compounds, flavonoids, tocopherols, and other bioactive constituents capable of scavenging free radicals and reducing oxidative damage (Eze & Ofoego, 2022; Zamuz et al., 2021; Olukayode & Clara, 2021). Previous studies have similarly reported antioxidant effects of *C. lanatus* seed preparations, including reductions in MDA concentrations and improvements in oxidative stress indices (Daniel et al., 2021; Eke et al., 2021). The inability of the extract to completely normalize MDA levels in the present study may indicate that the administered doses provided only partial protection against diclofenac-induced oxidative stress.

Superoxide dismutase is a critical endogenous antioxidant enzyme that protects tissues against oxidative injury through the dismutation of superoxide radicals into hydrogen peroxide and molecular oxygen. In the present study, diclofenac administration produced a significant reduction in serum SOD activity compared with the control group. This finding is consistent with oxidative stress-mediated depletion or impairment of antioxidant defense systems. Similar reductions in SOD activity following diclofenac exposure have been documented in experimental studies investigating diclofenac-induced toxicity and oxidative injury (Moradi et al., 2021; Izak-Shirian et al., 2022; Varışlı et al., 2023). The reduced SOD activity observed in the diclofenac-treated group therefore further confirms the establishment of an oxidative stress state.

Importantly, rats treated with *C. lanatus* extract alone and those co-administered the extract and diclofenac exhibited significantly higher SOD activity than the diclofenac-only group. This finding suggests that the extract enhanced antioxidant defense mechanisms and helped restore redox balance. The antioxidant activity of watermelon seeds has been attributed to their rich phytochemical profile, particularly flavonoids and phenolic compounds, which can directly neutralize reactive oxygen species or preserve endogenous antioxidant enzyme systems (Nnamani et al., 2022; Zamuz et al., 2021; Eke et al., 2021; Okafor & Elemuo, 2018). The observed increase in SOD activity therefore provides biochemical evidence supporting the antioxidant potential of *C. lanatus* seed extract.

Histological examination corroborated the biochemical findings. Rats administered diclofenac alone showed severe splenic alterations characterized by hemorrhage and fibrosis, indicating substantial disruption of normal splenic architecture. Oxidative stress is known to promote cellular injury, vascular damage, inflammatory responses, and subsequent fibrotic changes in tissues exposed to toxic insults. Consequently, the severe histological lesions observed in the diclofenac-treated group are consistent with the elevated MDA levels and reduced SOD activity recorded in the same group. Previous studies have similarly demonstrated that diclofenac-induced oxidative stress is accompanied by structural tissue damage and histopathological alterations in experimental animals (Abbas et al., 2014; Moradi et al., 2021).

In contrast, rats treated with *C. lanatus* extract alone exhibited normal splenic architecture with well-defined red and white pulp regions, suggesting that the extract did not adversely affect splenic morphology. Furthermore, co-administration of the extract with diclofenac attenuated the severity of splenic lesions. Rats in Group D showed only mild hemorrhage with preservation of the overall splenic architecture, while rats in Group E demonstrated mild fibrosis and focal areas of necrosis. These findings indicate that the extract conferred partial protection against diclofenac-induced splenic injury. The protective effect is likely related to the antioxidant properties of *C. lanatus* phytochemicals, which may have reduced oxidative damage and

limited tissue degeneration (Bamidele et al., 2021; Eke et al., 2021). Interestingly, the higher dose did not provide superior histological protection compared with the lower dose, suggesting that the protective effects of the extract may not be strictly dose-dependent under the conditions of the present study.

Overall, the findings of this study indicate that diclofenac induces oxidative stress and structural damage in the spleen, as evidenced by increased lipid peroxidation, reduced antioxidant enzyme activity, and marked histopathological alterations. Administration of ethanolic seed extract of *Citrullus lanatus* ameliorated these changes by reducing oxidative stress, enhancing antioxidant defenses, and preserving splenic histoarchitecture, thereby demonstrating a protective effect against diclofenac-induced splenic injury.

CONCLUSION

The findings of the present study indicate that diclofenac administration induced oxidative stress and histopathological alterations in the spleen of male Wistar rats, as evidenced by elevated serum malondialdehyde (MDA) levels, reduced superoxide dismutase (SOD) activity, and marked splenic lesions characterized by hemorrhage and fibrosis. Administration of ethanolic seed extract of *Citrullus lanatus* attenuated these adverse effects, as demonstrated by improved antioxidant status, lower MDA levels, higher SOD activity, and reduced severity of histological alterations in the spleen. These findings suggest that the extract possesses antioxidant properties capable of mitigating diclofenac-induced splenic injury. Although changes in body weight and relative spleen weight were observed among the experimental groups, most of these differences were not statistically significant. Overall, the study demonstrates the potential protective role of ethanolic seed extract of *Citrullus lanatus* against diclofenac-induced oxidative and histological damage in the spleen.

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Competing Interests

The authors declare that they have no known competing financial or non-financial interests that could have influenced the conduct or reporting of this research.

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