



(RESEARCH ARTICLE)



IN VIVO ANTI-INFLAMMATORY AND ANTIOXIDANT EFFECTS OF *PILIOSTIGMA RETICULATUM* LEAF EXTRACT ON CARRAGEENAN-INDUCED PAW EDEMA IN WISTAR RATS

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World Journal of Advanced Research and Reviews, 2026, 32(01), 1522–1529

Publication history: Received on 15 September 2026; revised on 20 October 2026; accepted on 23 October 2026

Article DOI: <https://doi.org/10.30574/wjarr.2026.32.1.2716>

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ABSTRACT

Inflammation underlies many chronic diseases, and conventional anti-inflammatory drugs are limited by adverse effects and cost. *Piliostigma reticulatum* is used in African traditional medicine for inflammatory conditions, yet its leaves have not been pharmacologically evaluated. This study investigated the anti-inflammatory and antioxidant effects of *P. reticulatum* leaf extract in carrageenan-induced paw edema in Wistar rats. Thirty rats were allocated to five groups: distilled water control, piroxicam (10 mg/kg), and extract at 800, 400, or 200 mg/kg. Paw thickness was measured for 4 hr after carrageenan injection, and serum interleukin-1 (IL-1), interleukin-10 (IL-10), C-reactive protein (CRP), malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH), and catalase (CAT) were assayed. Control paw swelling increased to 3.10 ± 0.06 mm by 4 hr. All extract doses reduced edema relative to control at every time point ($p < .01$ to $p < .001$); the 400 mg/kg dose was most effective (-0.05 ± 0.02 mm at 4 hr), numerically exceeding piroxicam (0.59 ± 0.09 mm), whereas the effect of 200 mg/kg waned after 2 hr. IL-10 was significantly lower in all treated groups than in controls, IL-1 was lower but not significantly so, and CRP was higher in treated groups. MDA was significantly reduced by the 800 mg/kg extract ($p < .05$) and by piroxicam ($p < .01$), whereas changes in SOD, GSH, and CAT were not significant. These findings provide preliminary evidence that *P. reticulatum* leaf extract has anti-edematous and lipid peroxidation-lowering activity, accompanied by an atypical cytokine profile that warrants mechanistic investigation.

Keywords: *P. reticulatum*, Anti-Inflammatory, Antioxidant, Carrageenan, Cytokines, Wistar rats

1 INTRODUCTION

Medicinal plants remain central to health care in Nigeria. Despite advances in modern Western therapies, a large share of the population continues to rely on traditional, plant-based remedies (Offiah et al., 2011; Ojewale et al., 2013), and harnessing local ethnobotanical resources has been proposed as a route to an independent pharmaceutical industry and long-term medical security (Adefolaju, 2014; Mackintosh et al., 2018). Northern Nigeria, in particular, has a large and understudied flora that offers considerable scope for drug discovery.

The management of inflammation is a primary target for drug discovery. Inflammation is a homeostatic immune response triggered by mechanical, chemical, or pathogen-induced injury (Chen et al., 2018; Dibal et al., 2018; Furst &

Zubdorf, 2014; Toumi & Best, 2003). Acute inflammation helps eliminate foreign material through pro-inflammatory cytokines and phagocytic cells, but excessive cellular activation can cause further tissue damage through reactive oxygen species and can progress to chronic inflammation (Baizabal-Aguirre et al., 2014; Dugue et al., 2017; Ouaguia et al., 2014; Tidball, 1995). Its cardinal signs of pain, swelling, heat, and redness arise from mechanisms ranging from oxidative stress to systemic cytokine release (Ansar & Ghosh, 2016; Frangogiannis, 2018). In the laboratory, carrageenan, a sulfated galactan from red seaweed, is widely used to induce experimental inflammation, although its long-term consumption is toxic and has been linked to gastric lesions and carcinomas in animal models (Kitano et al., 1996; Kitsukawa et al., 1992; McKim et al., 2018; Shukla et al., 2016; Tobacman, 2001; Tobacman & Walters, 1999).

Chronic inflammation imposes a substantial global health burden, driving noncommunicable diseases that account for roughly 74% of all annual deaths (Furman et al., 2019; Roh & Sohn, 2018; World Health Organization [WHO], 2022). Low- and middle-income regions in Africa are disproportionately affected because of weak health infrastructure and fragmented drug supply chains. Standard treatments such as nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids carry a high risk of gastrointestinal, renal, and cardiovascular adverse effects (Bindu et al., 2020). Advanced biologic alternatives remain financially out of reach for many patients in sub-Saharan Africa, and the development of new synthetic molecules faces steep toxicological and pharmacokinetic hurdles (Munir & Badshah, 2020). Safe, affordable, plant-derived alternatives are therefore an important research priority, and researchers have increasingly focused on plants that produce bioactive compounds (Altemimi et al., 2017).

Piliostigma reticulatum is a widely distributed African shrub used across the continent for fiber and traditional medicine, including the treatment of headache, toothache, and rheumatism (Brink & Achigan-Dako, 2012; Chahad et al., 2015; Kafi et al., 2018). Its stem bark has demonstrated anti-inflammatory activity by preventing carrageenan-induced paw edema in rats (Aderogba et al., 2006). Moreover, the independent use of *P. reticulatum* for inflammatory conditions by distinct cultural groups in Nigeria, Sudan, Gabon, and Chad provides cross-cultural ethnopharmacological support for its biological activity (Sofowora et al., 2013). In contrast, the leaves have not undergone a full pharmacological evaluation, and their effects on *in vivo* inflammatory and oxidative biomarkers remain unknown.

This study is therefore justified by the clinical need for affordable, low-toxicity anti-inflammatory agents for chronic and rheumatic conditions, the ethnopharmacological plausibility of *P. reticulatum*, and a clear gap in the scientific literature. The aim was to evaluate the anti-inflammatory activity of *P. reticulatum* leaf extract in the carrageenan-induced paw edema model in Wistar rats and to assess its effects on serum IL-1, IL-10, CRP, and markers of oxidative stress (MDA, SOD, GSH, and CAT).

2 METHOD

2.1 Plant Material and Extract Preparation

Fresh, mature, and undamaged leaves of *Piliostigma reticulatum* were harvested from wild populations in Konduga LGA, Borno State, Nigeria (approx. 11.69586 °N, 13.27555°E) between February and March 2024. Collection was restricted to morning hours (07:00–10:00 h) to prevent the thermal degradation of heat-labile phytoconstituents. A total of approximately 3 kg of healthy leaf material was obtained. Botanical identification and authentication were completed at the University of Maiduguri Herbarium, where a voucher specimen (UMM/FPH/FAA/010) was deposited. The Methanol fraction (MeOHF6) extracted from this plant as described by Mshelia *et al.* 2026 was used for this experiment.

2.2 Animals and Housing

Thirty adult albino wistar rats of both sexes weighing between 220–260g were obtained from the Animal Farm of the Department of Pharmacology, Bayero University, Kano, Nigeria. The animals were housed in plastic cages in the animal house and allowed to acclimatize for 2 weeks before the experiment. They were fed grower mash (Vital Feed, Grand Cereal, Jos, Nigeria) and given water *ad libitum*. The wistar rats were kept in solid bottom plastic cages which had wood shavings as flooring at an ambient temperature having a naturally regulated 12-hour light / 12-hour dark cycle

The study was conducted in accordance with the ARRIVE guidelines (Percie du Sert et al., 2020), and blood sampling followed the procedures described by Parasuraman et al. (2010). Ethical clearance was obtained from the Animal Care and Use Research Ethics Committee (ACUREC) of the Bayero University Kano where an animal use approval certificate with number BUK/ACUREC/CAP/PG174 was issued for this experiment.

2.3 Experimental Design

The rats were randomly assigned to five groups ($n = 6$ per group). Edema was induced in all groups by injecting 0.2 ml of 1% (w/v) carrageenan suspension into the right hind paw (Abdullahi et al., 2020). Group 1 served as the control and received distilled water (DW; 1 mL/kg). Group 2 received the reference drug piroxicam (10 mg/kg). Groups 3, 4, and 5

received *P. reticulatum* leaf extract at 800, 400, and 200 mg/kg, respectively. The time of carrageenan injection was noted as 0 hr while measurements were taken at 0.5 hour, 1 hour, 2 hour, 3 hour and 4 hours post carrageenan injection. All treatment was administered via oral gavage.

2.4 Measurement of Paw Edema

Paw thickness was measured with a Vernier caliper immediately before carrageenan injection (0 hr) and at 0.5, 1, 2, 3, and 4 hr afterward. The Results were presented as absolute change in paw size (mm).

2.5 Blood Collection

Following the fourth-hour paw measurement, we anesthetized the animals via isoflurane inhalation and ketamine lotion to collect blood from the retro-orbital sinus. The blood samples were then centrifuged to isolate serum for subsequent biochemical analyses.

2.6 Biochemical Assays

2.6.1 Catalase Activity

CAT activity was measured spectrophotometrically with a commercial assay kit (Cat. No. YX-C-A501; Shanghai Coon Koon Biotech Co., Ltd., Shanghai, China) according to the manufacturer's instructions. One unit of CAT activity was defined as the amount of enzyme that catalyzes the degradation of 1 nmol H_2O_2 per minute. Serum was used without extraction. The spectrophotometer was preheated for more than 30 min and zeroed with distilled water at 240 nm. The CAT working solution (20 mL of Reagent One added to each bottle of Reagent Two) was incubated at 37 °C for 10 min. Working solution (1 mL) was added to a 1-mL quartz cuvette, followed by 35 μL of sample and mixed for 5 s. Absorbance was recorded immediately (A1) and after 1 min at room temperature (A2). CAT activity (U/mL) was calculated as $678 \times \Delta A$, where $\Delta A = A1 - A2$.

2.6.2 Reduced Glutathione

GSH was determined with a commercial kit (Cat. No. YX-C-A200; Shanghai Coon Koon Biotech Co., Ltd.). Anticoagulated blood was centrifuged at $600 \times g$ for 10 min at 4 °C; the plasma was mixed with an equal volume of Reagent One and centrifuged at $8,000 \times g$ for 10 min at 4 °C, and the supernatant was used. The spectrophotometer was zeroed with distilled water at 412 nm. The blank (100 μL distilled water), standards (GSH at 12.5–200 $\mu\text{g/mL}$), and samples (100 μL each) were mixed with 700 μL Reagent Two and 200 μL Reagent Three and incubated for 2 min, and absorbance was read at 412 nm. Sample concentration was obtained from the standard curve using ΔA ($A2 - A1$) and expressed as GSH content ($\mu\text{g/mL}$).

2.6.3 Superoxide Dismutase Activity

SOD activity was measured with a commercial kit (Cat. No. YX-C-A500; Shanghai Coon Koon Biotech Co., Ltd.) using undiluted serum samples. One unit of SOD activity was defined as the amount of enzyme producing 50% inhibition of the reaction. Reagents One, Two, and Four were preincubated at 37 °C for more than 5 min. The measuring tube contained 240 μL Reagent One, 510 μL Reagent Two, 6 μL Reagent Three, 90 μL sample, and 180 μL Reagent Four; the control tube contained 90 μL distilled water instead of sample. After incubation at room temperature for 30 min, absorbance was read at 560 nm. Percentage inhibition was calculated as $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$, and SOD activity in serum (U/mL) as $11.4 \times [\text{inhibition} / (1 - \text{inhibition})] \times \text{sample dilution factor}$, with inhibition expressed as a proportion.

2.6.4 Malondialdehyde

MDA, an end product of lipid peroxidation, was measured with a commercial kit (Cat. No. YX-C-A401; Shanghai Coon Koon Biotech Co., Ltd.) based on its condensation with thiobarbituric acid to form a red product with maximum absorbance at 532 nm; absorbance at 600 nm was used to correct for background. Reagent One (0.6 mL) was mixed with 0.2 mL serum in a 1.5-mL tube, incubated in a 95 °C water bath for 30 min, cooled on ice, and centrifuged at $10,000 \times g$ for 10 min at 25 °C. Absorbance of the supernatant was read at 532 and 600 nm, and serum MDA (nmol/mL) was calculated as $25.8 \times \Delta A$, where $\Delta A = A_{532} - A_{600}$.

2.6.5 C-Reactive Protein

Serum high-sensitivity CRP was measured with a rat hs-CRP sandwich enzyme-linked immunosorbent assay (ELISA) kit (Cat. No. ER0001; FineTest, Wuhan, China; detection range = 0.156–10 ng/mL, sensitivity = 0.094 ng/mL) according to the manufacturer's protocol. Standards, samples, and blanks were assayed in duplicate (100 μL per well). Plates were incubated for 90 min at 37 °C and washed twice; biotin-labeled detection antibody was added for 60 min at 37 °C, followed by three washes; HRP–streptavidin conjugate was added for 30 min at 37 °C, followed by five washes; and 90

μL of 3,3',5,5'-tetramethylbenzidine (TMB) substrate was added for 10–20 min at 37 °C in the dark. The reaction was stopped with 50 μL stop solution, and optical density was read at 450 nm with 570 nm correction. Concentrations were calculated from the standard curve using CurveExpert 1.4. The manufacturer-reported intra- and inter-assay coefficients of variation were <6% and 6.2%, respectively.

2.6.6 Interleukin-1 and Interleukin-10

Serum IL-1 and IL-10 were quantified using rat sandwich ELISA kits (FineTest, Wuhan, China) according to the manufacturer's instructions. For IL-1, blood was allowed to clot for 2 hr at room temperature or overnight at 2–8 °C and centrifuged at $1,000 \times g$ for 20 min, and total protein was determined by bicinchoninic acid (BCA) assay. The detection range and sensitivity were 15.625–1,000 pg/mL and <9.375 pg/mL for IL-1 β , and 31.25–2,000 pg/mL and 18.75 pg/mL for IL-10. Kit components were equilibrated to room temperature, and standards (IL-10: 2,000 to 31.25 pg/mL and a zero standard) were prepared by serial dilution. Standards, samples, and blanks were run in duplicate (100 μL per well) on precoated plates and incubated for 90 min at 37 °C. After washing, biotin-labeled antibody was added for 60 min, followed by washing and HRP–streptavidin conjugate for 30 min at 37 °C; TMB substrate was then added and incubated in the dark at 37 °C for 10–20 min. The reaction was stopped, and optical density was read immediately at 450 nm. Mean optical density of duplicates was corrected by subtracting the blank, and concentrations were derived from a four-parameter logistic standard curve and multiplied by the dilution factor where applicable.

2.7 Statistical Analysis

Data are presented as $M \pm$ standard error of the mean (SEM). Paw edema data were analyzed by repeated-measures analysis of variance (ANOVA) followed by Bonferroni post hoc tests, comparing each treatment group with the DW control at each time point and each time point with the 30-min reading within groups. Biochemical data were analyzed by one-way ANOVA followed by Bonferroni post hoc tests. Differences were considered significant at $p < .05$. All data analyses were performed using the SPSS statistical package, Version 22 (SPSS Inc., Chicago, IL).

3 RESULTS

3.1 Carrageenan-Induced Paw Edema

Table 1 summarizes the effect of the extract on carrageenan-induced paw edema over the 4-hr observation period. Paw size in the DW control group increased steadily from 1.07 ± 0.11 mm at 30 min to 3.10 ± 0.06 mm at 4 hr ($p < .001$ relative to the 30-min reading). Piroxicam (10 mg/kg) significantly suppressed edema at all time points ($p < .05$ at 30 min and $p < .001$ thereafter), with paw size decreasing slightly from 0.70 ± 0.06 mm at 30 min to 0.59 ± 0.09 mm at 4 hr.

Table 1: Effect of *Piliostigma reticulatum* Leaf Extract on Carrageenan-Induced Paw Edema in Rats

Treatment (mg/kg)	30 min	1 hr	2 hr	3 hr	4 hr
DW (1 mL/kg)	1.07 ± 0.11	1.42 ± 0.10^a	1.89 ± 0.20^c	2.22 ± 0.19^c	3.10 ± 0.06^c
Piroxicam (10)	$0.70 \pm 0.06^*$	$0.76 \pm 0.07^{***}$	$0.62 \pm 0.05^{***}$	$0.58 \pm 0.09^{***}$	$0.59 \pm 0.09^{***}$
Extract (800)	$0.62 \pm 0.08^{**}$	$0.73 \pm 0.04^{***}$	$0.76 \pm 0.10^{***}$	$0.94 \pm 0.29^{***}$	$0.90 \pm 0.32^{***}$
Extract (400)	$0.16 \pm 0.04^{***}$	$0.19 \pm 0.05^{***}$	$0.07 \pm 0.06^{***}$	$-0.08 \pm 0.07^{***}$	$-0.05 \pm 0.02^{***}$
Extract (200)	$0.45 \pm 0.11^{***}$	$0.60 \pm 0.12^{***}$	$0.57 \pm 0.12^{***}$	$1.15 \pm 0.06^{***,b}$	$1.19 \pm 0.06^{***,b}$

Note. Values are change in paw size (mm), expressed as $M \pm$ SEM ($n = 6$ per group). Asterisks denote comparison with the DW control: $*p < .05$, $**p < .01$, $***p < .001$. Superscript letters denote comparison with the same group's 30-min value: $^ap < .05$, $^bp < .01$, $^cp < .001$. Repeated-measures ANOVA with Bonferroni post hoc test. DW = distilled water.

The extract significantly reduced edema relative to the control at every dose and time point ($p < .01$ to $p < .001$). The 400 mg/kg dose produced the strongest effect of the three doses, reducing paw size to near zero at 2 hr (0.07 ± 0.06 mm) and to slightly negative values at 3 hr (-0.08 ± 0.07 mm) and 4 hr (-0.05 ± 0.02 mm), values numerically lower than those of the piroxicam group at the corresponding times. The 800 mg/kg dose produced an intermediate, sustained reduction (0.62 ± 0.08 mm at 30 min; 0.90 ± 0.32 mm at 4 hr). At 200 mg/kg, early suppression diminished over time: paw size rose to 1.15 ± 0.06 mm at 3 hr and 1.19 ± 0.06 mm at 4 hr, which differed significantly from the group's own 30-min reading ($p < .01$). Together, these results suggest a non-monotonic dose–response relationship across the range tested.

3.2 Serum Inflammatory Markers

Table 2 presents the effect of the extract on serum IL-10, IL-1, and CRP. IL-10 was markedly higher in the DW control group (165.20 ± 14.65 pg/mL) than in all treated groups, with the lowest value in the 800 mg/kg extract group (70.35 ± 8.32 pg/mL, $p < .01$), followed by piroxicam (83.86 ± 4.04 pg/mL, $p < .01$), the 400 mg/kg extract (90.75 ± 7.91 pg/mL, $p < .01$), and the 200 mg/kg extract (103.58 ± 12.79 pg/mL, $p < .05$). IL-1 followed a similar downward trend in all treated groups relative to the control (11.21 ± 2.46 pg/mL), but the reductions were not statistically significant; the lowest value was recorded in the piroxicam group (5.37 ± 1.26 pg/mL). CRP showed the opposite pattern: values in the piroxicam group (112.65 ± 5.99 mg/L) and all extract groups exceeded the control value (78.28 ± 12.78 mg/L), although none of these differences was statistically significant. Among the extract groups, CRP was lowest at 200 mg/kg (89.69 ± 6.41 mg/L).

Table 2: Effect of *Piliostigma reticulatum* Leaf Extract on Serum Inflammatory Markers Following Carrageenan-Induced Paw Edema in Rats

Treatment (mg/kg)	IL-10 (pg/mL)	IL-1 (pg/mL)	CRP (mg/L)
DW (1 mL/kg)	165.20 ± 14.65	11.21 ± 2.46	78.28 ± 12.78
Piroxicam (10)	$83.86 \pm 4.04^{**}$	5.37 ± 1.26	112.65 ± 5.99
Extract (800)	$70.35 \pm 8.32^{**}$	5.73 ± 0.48	97.61 ± 10.56
Extract (400)	$90.75 \pm 7.91^{**}$	8.46 ± 1.90	107.59 ± 8.09
Extract (200)	$103.58 \pm 12.79^{*}$	7.01 ± 1.19	89.69 ± 6.41

Note. Values are $M \pm SEM$ ($n = 4$ per group). Asterisks denote comparison with the DW control: $*p < .05$, $**p < .01$. One-way ANOVA with Bonferroni post hoc test. DW = distilled water; IL = interleukin; CRP = C-reactive protein.

3.3 Oxidative Stress Markers

Table 3 presents the effect of the extract on oxidative stress markers. MDA was markedly elevated in the DW control group (210.20 ± 28.35) and was significantly reduced by piroxicam (44.50 ± 7.44 , $p < .01$) and by the 800 mg/kg extract (88.10 ± 5.72 , $p < .05$). MDA values in the 400 and 200 mg/kg extract groups (166.50 ± 8.30 and 180.50 ± 11.10 , respectively) were lower than the control but did not differ significantly, giving a dose-related decrease across the extract groups (800 > 400 > 200 mg/kg in effect). SOD activity showed an inconsistent pattern: it was highest in the 200 mg/kg group (18.63 ± 5.87 U/mL), exceeding the piroxicam group (16.73 ± 1.48 U/mL), and lowest in the 800 mg/kg group (10.13 ± 3.27 U/mL), which was below the control (13.93 ± 1.43 U/mL). GSH rose progressively with decreasing extract dose, from 8.80 ± 3.33 at 800 mg/kg to 15.63 ± 3.33 at 200 mg/kg, the highest value of any group. CAT activity was lower than the control (8.60 ± 1.57) in the 800 and 400 mg/kg groups (4.07 ± 1.16 and 5.90 ± 1.67 , respectively) and essentially unchanged at 200 mg/kg (8.43 ± 1.55), without a consistent dose-response relationship. None of the differences in SOD, GSH, or CAT was statistically significant.

Table 3: Effect of *Piliostigma reticulatum* Leaf Extract on Oxidative Stress Markers Following Carrageenan-Induced Paw Edema in Rats

Treatment (mg/kg)	SOD (U/mL)	GSH (μ g/mL)	MDA (nmol/mL)	CAT (U/mL)
DW (1 mL/kg)	13.93 ± 1.43	7.87 ± 1.11	210.20 ± 28.35	8.60 ± 1.57
Piroxicam (10)	16.73 ± 1.48	9.97 ± 2.12	$44.50 \pm 7.44^{**}$	9.57 ± 0.48
Extract (800)	10.13 ± 3.27	8.80 ± 3.33	$88.10 \pm 5.72^{*}$	4.07 ± 1.16
Extract (400)	15.47 ± 3.45	10.40 ± 2.51	166.50 ± 8.30	5.90 ± 1.67
Extract (200)	18.63 ± 5.87	15.63 ± 3.33	180.50 ± 11.10	8.43 ± 1.55

Note. Values are $M \pm SEM$ ($n = 4$ per group). Asterisks denote comparison with the DW control: $*p < .05$, $**p < .01$. One-way ANOVA with Bonferroni post hoc test. DW = distilled water; SOD = superoxide dismutase; GSH = reduced glutathione; MDA = malondialdehyde; CAT = catalase.

4 DISCUSSION

This study evaluated the anti-inflammatory and antioxidant effects of *P. reticulatum* leaf extract in carrageenan-induced paw edema in wistar rats. All three doses significantly attenuated edema, with the 400 mg/kg dose producing the strongest effect, and the 800 mg/kg dose significantly lowered serum MDA. In contrast, the cytokine and CRP findings

did not follow the pattern typically expected of an anti-inflammatory agent, and changes in the enzymatic antioxidants were not statistically significant.

The suppression of paw edema (Table 1) is consistent with the biphasic pathophysiology of the carrageenan model, in which an early phase mediated by histamine, serotonin, and bradykinin during the first hour is followed by a later, more prolonged phase driven principally by prostaglandins and pro-inflammatory cytokines (Crunkhorn & Meacock, 1971; Vinegar et al., 1969). The persistence of the anti-edematous effect of the 400 mg/kg dose into the third and fourth hours suggests that the extract acted on mediators operating in both phases rather than on the early histaminergic phase alone. This is broadly comparable with reports for other medicinal plant extracts, in which maximal suppression has likewise been observed in the later phase of edema development (Fatima et al., 2021). The slightly negative paw-size values at 3 and 4 hr at 400 mg/kg are physiologically unusual and are best interpreted as near-complete inhibition combined with caliper measurement variability rather than true shrinkage below baseline. The response was not linear with dose: 400 mg/kg was more effective than 800 mg/kg, and at 200 mg/kg early suppression gave way to a renewed increase in paw size by the third and fourth hours. The latter may reflect a ceiling effect below which the extract's anti-inflammatory constituents are insufficient to sustain inhibition through the prostaglandin-driven phase, although biological variability in a modest sample cannot be excluded.

The cytokine results (Table 2) present a more complex picture than is typically reported for plant extracts in this model. IL-1, a pro-inflammatory cytokine central to the later phase of carrageenan inflammation, was lower in all treated groups than in controls, consistent with the expected pharmacology of an anti-inflammatory agent, but these reductions were not statistically significant and should be interpreted cautiously given the small group size ($n = 4$). IL-10 was significantly lower in all treated groups rather than higher, as is more commonly reported when effective anti-inflammatory treatment is accompanied by a rise in this regulatory cytokine. One plausible explanation is that the elevated IL-10 in the untreated control reflects a compensatory regulatory response to unchecked inflammation at the 4-hr sampling point, a response attenuated once the inflammatory drive is suppressed by the extract or by piroxicam. This interpretation would need to be tested with time-course sampling rather than asserted from a single time point. The higher CRP in the piroxicam and extract-treated groups, rather than the reduction more frequently reported for effective anti-inflammatory agents, is similarly unexpected. Although these differences were not significant, they may reflect an acute-phase response to the treatments at the single time point sampled. CRP is also only a minor acute-phase protein in rodents, which limits its value as an inflammatory readout in this species. The finding therefore warrants further investigation rather than being taken as confirmation of the extract's mechanism.

The oxidative stress findings (Table 3) are more consistent with the existing literature. The marked elevation of MDA in the untreated control is in line with the established role of carrageenan in triggering lipid peroxidation through neutrophil-derived reactive oxygen species, and the significant reduction by the 800 mg/kg extract agrees with reports that effective anti-inflammatory plant extracts characteristically lower MDA while exerting more variable effects on enzymatic antioxidants such as SOD and CAT (Fatima et al., 2021). Notably, the MDA reduction with piroxicam was larger than with the extract. The numerically higher SOD and GSH values at 200 mg/kg were not statistically significant, so any suggestion that *P. reticulatum* constituents interact non-linearly with the endogenous antioxidant system remains speculative. If confirmed, such a pattern would resemble reports for other polyphenol-rich extracts, in which moderate concentrations preserve enzymatic antioxidant activity better than higher concentrations, possibly reflecting a pro-oxidant effect of some flavonoids and tannins at higher local concentrations (Fatima et al., 2021).

5 CONCLUSION

Piliostigma reticulatum leaf extract significantly reduced carrageenan-induced paw edema in Wistar rats, with maximal activity at 400 mg/kg that numerically exceeded that of piroxicam, and the 800 mg/kg dose significantly reduced serum lipid peroxidation. The unexpected cytokine and CRP profiles and the non-significant changes in SOD, GSH, and CAT indicate that the underlying mechanisms remain unresolved. These findings provide preliminary support for the traditional anti-inflammatory use of *P. reticulatum* and justify further phytochemical, mechanistic, and toxicological investigation of the leaf extract.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Acknowledgments

The authors acknowledge the Dept. of Pharmacology and Therapeutics, where the animals were housed. The authors also acknowledge the Department of Biochemistry, Bayero University for offering the platform on which this study was conducted.

Disclosure of conflict of interest

The authors have no known conflicts of interest to disclose.

Authors' Contribution

Hamman Mshelia Stephen designed, conducted the study, performed statistical analysis, and drafted the manuscript. Muhammad Sani Sule and Nasiru Abdullahi supervised the research and reviewed the manuscript.

Statement of ethical approval

Ethical approval was obtained from the Animal Care and Use Research Ethics Committee (ACUREC) of the Bayero University Kano where an animal use approval certificate was issued.

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