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Anti-inflammatory Effects of Aqueous and Ethanolic *Dactyloctenium aegyptium* Whole-plant Extracts in Carrageenan-induced Paw oedema in Rats

***Temitayo Iyanuoluwa Adesipe, and Oluwatobi Victor Olowu**

Department of Science Laboratory Technology, Federal Polytechnic, P.M.B 50, Ilaro, Ogun state, Nigeria.

*Corresponding author : ADESIPE, Temitayo Iyanuoluwa, Department of Science Laboratory Technology, Federal Polytechnic, P.M.B 50, Ilaro, Ogun state, Nigeria.

* Corresponding authors' email: iyanu.adesipe@federalpolyilaro.edu.ng
ORCID ID: https://orcid.org/0000-0002-8830-6164

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ABSTRACT
The anti-inflammatory activity of aqueous (AE) and ethanol (EE) extracts of whole-plant *Dactyloctenium aegyptium* was evaluated in carrageenan (Carr)-induced paw oedema in male albino rats, together with their effects on paw malondialdehyde (MDA) and reduced glutathione (GSH) levels. Rats (n = 3 per group) received distilled water, Carr alone, or Carr preceded by diclofenac (20 mg/kg), AE (400 mg/kg) or EE (400 mg/kg). Paw thickness was measured hourly for six hours and again at 24 hours, after which the animals were euthanised and paw tissue was collected for MDA, GSH and histological evaluation. At six hours, oedema inhibition relative to the Carr group was 53% (AE), 53% (EE) and 49% (diclofenac) ($p < 0.0001$ versus Carr). Paw thickness in the extract-treated groups was significantly lower than in the diclofenac group at 3–4 hours ($p < 0.05$), whereas no significant difference from diclofenac was indicated at 5–6 hours. AE, EE and diclofenac significantly reduced paw MDA and increased paw GSH relative to the Carr group ($p < 0.05$), and histology showed reduced inflammatory cell infiltration. These findings provide preliminary preclinical evidence of anti-inflammatory and antioxidant effects of *D. aegyptium* extracts and warrant further dose–response, mechanistic and toxicity studies.

Keywords: Convergence Insufficiency, Near Point of Convergence, Positive Fusional Vergence, Convergence Insufficiency Symptom Survey, Undergraduate Students, Near Work

INTRODUCTION

The body's natural reaction to a wide range of stimuli, including damaged cells, infectious or toxic chemicals is called inflammation. These stressors can trigger acute or long-term inflammatory responses in the kidney, reproductive system, liver, heart, pancreas, lung, brain and digestive tract which can lead to illness or tissue damage (Chen et al., 2017). Molecular and cellular interactions usually lower the risk of injury or harm during acute inflammatory reactions. This mitigation technique lessens acute inflammation and aids in the restoration of tissue homeostasis. However, untreated acute inflammation that progresses to a chronic condition can lead to a number of chronic inflammatory illnesses (Zhou et al., 2016).

Carrageenan (Carr)-induced inflammation in the rat paw is a validated acute inflammation model in which new anti-inflammatory drugs are often evaluated (Bello et al., 2020; Jeong et al., 2019). Biphasic oedema is the outcome of injecting carrageenan into the rat paw's sub-plantar region. Bradykinin, histamine, serotonin, and to a lesser extent, prostaglandins (PGs), are released during the first phase (0–1 hour). The next stage, which happens an hour later, is linked to prostaglandin synthesis by cyclooxygenase (COX) enzymes and neutrophil infiltration (Fatima et al., 2021). The therapeutic control of inflammation therefore presents a substantial health issue because of the several pathways by which it is induced. Also, negative consequences are associated with the use of immunosuppressants, steroidal agents, and non-steroidal anti-inflammatory medications (Sohail et al., 2023). An innovative approach that will help reduce inflammation in a homeostatic, modulatory, and well-tolerated way by the body is required (Huang et al., 2024). Given the critical role of reduced glutathione (GSH) depletion in inflammatory disorders, medications that raise GSH levels may be necessary for reducing the severity and negative effects of these diseases.

Medicinal or pharmaceutical formulations frequently use plants with therapeutic qualities. According to Ibrahim et al. (2018), because of the strong pharmacological effects of their active metabolites, several ailments have been treated with medicinal plants. Thus, a careful examination of traditional botanicals might create new opportunities for the discovery of ground-breaking drugs. However, grasses and related weeds have received comparatively little attention relative to other plant genera frequently employed in traditional medicine, and few pharmaceutical studies have addressed them to date (Atanasov et al., 2015; Gebashe & Van Staden, 2019). Investigating such under-studied plant biomass may broaden the range of plant materials available for medicinal research (Grosu & Ichim, 2020), provided that their botanical identity and quality are properly authenticated (Simmler et al., 2018). *Dactyloctenium aegyptium* (L.) Willd. (Egyptian crowfoot grass) possesses rhizomiferous main stems and is used in semi-arid areas as feed for ruminants as it yields notable annual pasture in addition to good hay (Al-Snafi, 2017).

In our prior investigation, we screened *Dactyloctenium aegyptium* aqueous and ethanol extracts for their bioactive constituents, employed them for silver nanoparticles' biosynthesis, and subsequently evaluated the potential medical applications of resulting nanoparticles. Phytochemical screening result revealed that both extracts of *Dactyloctenium aegyptium* contained cardiac glycosides,

tannins, alkaloids, flavonoids, phenols, and reducing sugars. The ethanol extract additionally contained steroids and terpenoids, while the aqueous extract contained saponins (Adesipe et al., 2023). Some of these constituents, notably cardiac glycosides, have been documented to possess anti-inflammatory properties (Fürst et al., 2017; Jansson et al., 2021). Previous work on *D. aegyptium* has largely addressed its phytochemical composition and its antioxidant and antimicrobial properties (Adesipe et al., 2023); to the authors' knowledge, its in vivo anti-inflammatory efficacy has not been reported.

Therefore, this study aimed to evaluate the anti-inflammatory activity of the aqueous (AE) and ethanol (EE) extracts of whole-plant *D. aegyptium* in carrageenan-induced paw oedema in rats, and to assess their effects on the oxidative-stress markers malondialdehyde (MDA) and reduced glutathione (GSH) and on paw histopathology.

MATERIALS AND METHODS

Plant Collection and Identification

The whole *Dactyloctenium aegyptium* plant was gathered at the Federal Polytechnic Ilaro school yard in Ogun State, Nigeria. Dr. George N. of the Botany Department, University of Lagos verified the plant and gave it a voucher specimen number (LUH 8694). All of the chemicals used in this study were of research quality.

Plant Extract Preparation

Following botanical identification, the grasses were separated, thoroughly washed with distilled water, and air-dried at room temperature for three weeks. The dried plant materials were pulverized using an electric grinder and sieved through a 40 µm mesh. Powdered whole plant material (140 g each) was separately macerated in distilled water (2,000 mL) and 80% ethanol (2,000 mL) for 72 h at room temperature (Obode *et al*., 2021). The mixtures were subsequently filtered using Whatman® filter paper, and the resulting filtrates were designated as aqueous extract (AE) and ethanol extract (EE), respectively. The filtrates were concentrated to dryness in a hot-air oven maintained at 60 °C and stored for subsequent analyses (Obode *et al*., 2021).

Acute Toxicity

Based on earlier reports, test samples were tested for potential acute toxicity in nine male rats in compliance with OECD test guideline 425 (limit dose) for oral chemical assessment. The animals received oral exposure to *Dactyloctenium aegyptium* aqueous extract (AE), or *Dactyloctenium aegyptium* ethanol extract (EE), (4000 mg/kg each), or vehicle (10ml/kg). The animals were watched for 30 minutes, 4 hours, 24 hours, and until the end of 14th day for behavioural changes and toxicity-related deaths after receiving a single dose of any test sample on the first day (OECD, 2022).

Anti-inflammatory Property Assessment

Experimental Animal

A total of twenty-four (24) albino rats weighing between 116-120 g were obtained from University of Lagos, Nigeria, Zoology Department. They were housed in a room with 25 ± 1°C temperature, 12-hour dark-light pattern and were given limitless access to water and food. Rats were maintained in the animal house at the University of Lagos for one week to acclimatise before commencement. The Federal Polytechnic Ilaro's Animal Ethics Committee in Ogun State, Nigeria, gave its prior clearance for the experiment (PREC/23). Following the experiments, all of the animals were sacrificed

Carrageenan-induced Paw Edema

Acute inflammation was induced in rats using the carrageenan-induced paw oedema model as previously described by Falade *et al*. (2019). Briefly, a 1% (w/v) carrageenan solution was prepared by dissolving 100 mg of carrageenan granules in 10 mL of 0.9% normal saline and allowing the mixture to stand for 1 h before use. One hour after oral administration of the test samples (except for Group I, the normal control), each rat received a sub plantar injection of 0.1 mL of the carrageenan solution into the right hind paw. Paw thickness was measured immediately before carrageenan injection (0 h) and subsequently at 1-h intervals for 6 h, with a final measurement taken at 24 h post-injection using a Vernier caliper (Falade *et al*., 2019). The level of inflammation was determined using the following formula:

$$\text{Percentile inflammation} = \frac{Vcar - Vo}{Vo} X\ 100 \qquad \text{eqn1}$$

Vcar denotes the paw thickness of rats after carrageenan shot. Vo denotes the paw thickness of rats in the control group.

Table 1: Experimental Design

Groups	Treatments	Number of Rats
Group 1	Distilled water	3
Group 2	Distilled water + carrageenan	3
Group 3	20 mg/kg diclofenac + carrageenan	3
Group 4	400 mg/kg aqueous extract + carrageenan	3
Group 5	400 mg/kg ethanol extract + carrageenan	3

Anti-inflammatory Activity

The percentage inhibition of paw oedema, an indicator of the anti-inflammatory activity of *Dactyloctenium aegyptium* aqueous and ethanol extracts, was calculated using the formula described by Ben Khedir *et al*. (2016).

$$\text{Percentile inhibition} = [1 - (\frac{Vd}{Vcar}) \times 100] \qquad \text{qn2}$$

Vcar denotes the edema thickness in Carr group. Vd denotes the edema thickness in test samples treated groups.

Collection of Tissue Samples

Animals were euthanized via cervical dislocation 24 hours after being given carrageenan. The soft paw tissues were promptly removed, with a portion used for oxidative stress testing and the remainder for histopathology.

Preparation of Tissue for Biochemical Analysis

A hand homogeniser was used to prepare a part of the soft paw tissues by homogenising them in a 0.1M, pH 7.4 ice-cold phosphate buffer solution. Next, for 15 minutes, the tissue suspension was centrifuged at 3000x.

Malondialdehyde (MDA) Levels

Lipid peroxidation in paw tissue homogenates was determined by measuring malondialdehyde (MDA) levels using the thiobarbituric acid (TBA) reaction, as previously described by Rotimi *et al*. (2018). The assay is based on the reaction between MDA, a product of lipid peroxidation, and thiobarbituric acid to form a coloured complex, the

absorbance of which was measured spectrophotometrically (Rotimi *et al*., 2018).

Reduced Glutathione (GSH) Activity

Reduced glutathione (GSH) levels in the paw tissue homogenates of the experimental rats were determined by measuring the absorbance associated with the conversion of NADPH to $NADP^+$, as previously described by Nwogueze *et al*. (2021). GSH concentration was expressed as µmol/mL protein.

Histological Evaluation

Rat paws were obtained 24 hours following the induction with an inflammatory agent (carrageenan) for histological analysis. By fixing the tissue slices in 10% formaldehyde, they were prepared for histological analysis of the inflamed paw.

Statistical Analysis

Mean ± standard error of the mean (SEM) was used to express the data. GraphPad Prism version 6.0 was used for statistical analysis. Two-way repeated-measures analysis of variance (ANOVA) and Dunnett's multiple comparisons test were used to assess differences between experimental groups and between measurement time points. The threshold for statistical significance was fixed at $p < 0.05$.

RESULTS AND DISCUSSION

Acute Toxicity

OECD guidelines were followed when conducting the toxicity (acute) test. There were no adverse effects from treatment with the studied plant extracts at 2000 mg/kg. Furthermore, no alterations brought on by the treatment were seen in animals' eyes, behaviour, fur examination, skin, and temperature, drink consumption and or breathing. The plant extracts examined were consequently declared safe to use at 2000 mg/kg dosage; hence, the LD50 exceeds 2000 mg/kg.

Anti-Inflammatory Activity

Table 2 depicts oedema size changes. In the inflamed groups, the oedema size and the percentage of inflammation rose quickly, peaking approximately three hours after the Carrageenan (Carr) injection. These increases, however, were not as significant in groups who received Diclofenac, *Dactyloctenium aegyptium* ethanol extract (EE), or aqueous extract (AE). Rats given AE or EE prior to carr injection showed a reduction in the thickness of their paw oedema (Table 2). By the sixth hour, percentage of inflammation and the amount of paw oedema were significantly lower in the treatment groups than in the carr group.

Fig. 1 shows that treatment with EE had the greatest paw oedema thickness reduction effect in comparison to diclofenac or AE.

Table 2: Oedema Size Changes in Experimental Rats Following CAR Injection

Time	CON	Carr grp	Carr+A.E (400 mg/kg)	Carr+E.E (400 mg/kg)	Carr+diclo
0hr	3.4±0.09	3.58±0.15	3.58 ± 0.17	3.58±0.15	3.57±0.16
1hr	$3.59±0.17^{****a}$	5.40±0.13	6.63±0.12	5.81±0.36	5.45±0.32
2hrs	$3.59±0.17^{****a}$	7.19±0.13	7.21±0.01	$5.50±0.72^{***b}$	7.17±0.10
3hrs	$3.59±0.17^{****a}$	7.98±0.64	$4.85±0.29^{****a}$	$4.28±0.17^{****a}$	$6.69±0.51^{**}$
4hrs	$3.59±0.17^{****a}$	7.83±0.58	$4.44±0.13^{****d}$	$4.04±0.14^{****c}$	$5.48±0.17^{****}$
5hrs	$3.59±0.17^{****}$	7.68±0.53	$3.69±0.18^{****}$	$3.79±0.16^{****}$	$4.26±0.30^{****}$
6hrs	$3.59±0.17^{****}$	7.74±0.30	$3.66±0.18^{****}$	$3.65±0.15^{****}$	$3.96±0.1^{****}$
24hrs	$3.59±0.17^{****}$	6.01±0.46	$3.63±0.19^{****}$	$3.63±0.14^{****}$	$3.66±0.15^{****}$

Values Shows Means ± SEM (n = 3) in Each Group.$^{****}p < 0.0001$, $^{***}p<0.001$,$^{**}p<0.01$, $^{*}p < 0.05$ Compared to Carrageenan Group; $^{a}p<0.0001$, $^{b}p<0.001$,$^{c}p<0.01$ $^{d}p < 0.05$ Compared to Diclo Group.

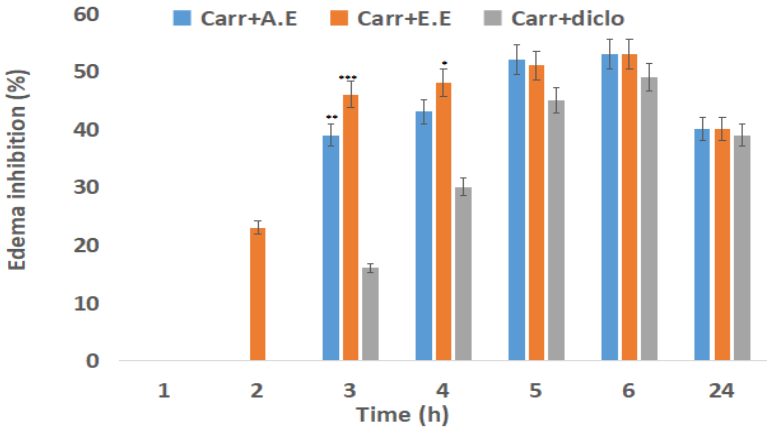


Figure 1: Percentage of oedema Suppression Caused by AE, EE, and Diclo at Different Points in Time in Rats Induced with Paw Oedema: Carr+AE Rats Received A.E. Prior to the Carr injection; Carr+E.E. Rats Received E.E. Prior to the Carr Injection; and Carr+diclo Rats Received Diclo Prior to the Carr Injection. Bars Indicates Mean ± SEM for n=3. Statistically Significant Differences Between the Groups are Indicated by (*) Symbols . * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ Versus Carr+diclo Group. SEM; Standard Error of Mean.

Changes in Lipid Peroxidation Marker; MDA Assay

Fig. 2 illustrates the measurement of paw lipid peroxidation. The figure shows that carrageenan-induced inflammation significantly ($p<0.0001$) increased paw MDA levels in comparison to the control group. However, treatments with *Dactyloctenium aegyptium* extracts particularly the aqueous extract) as well as diclofenac significantly ($p<0.0001$) reduced lipid peroxidation levels in comparison to the Carr group.

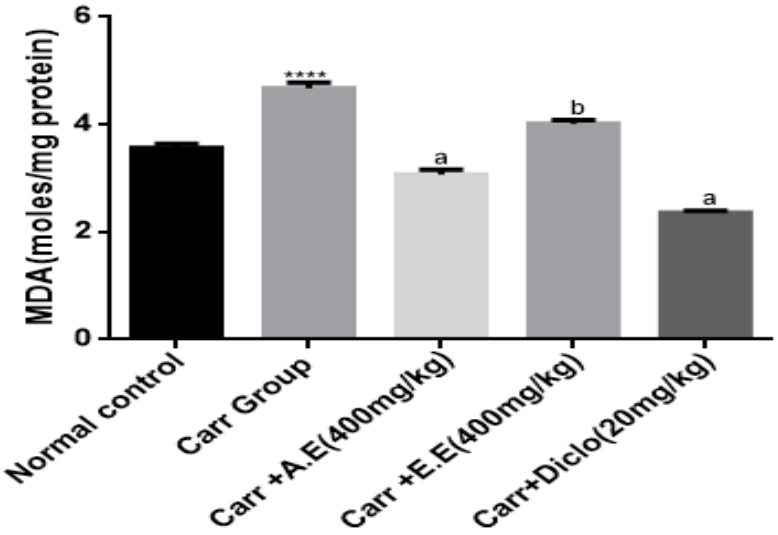


Figure 2: Levels of MDA in the Paw of the Control and Treated Rats with Carr, Diclo, *Dactyloctenium aegyptium* Aqueous and Ethanol Extracts (400 mg/kg). Values Represent Means ± SEM (n = 3) .When Comparing Carr Group to Control, ****$p < 0.0001$ Indicates Extremely Significant Increase in Level. When Comparing Treated Individual Groups to Carr group, $^{a}p<0.001$ Shows an Extremely Significant Decrease in Level , $^{b}p<0.01$ Shows a Very Significant Decrease in level,. SEM; Standard Error of Mean

Exploring the GSH Status

Carrageenan subcutaneous injection significantly decreased ($p < 0.0001$) the activity of paw GSH in Carr group rats in comparison to control group. Meanwhile, *Dactyloctenium aegyptium* ethanol extract significantly improved GSH activity ($p < 0.001$) in comparison to the Carr group and other treatment groups, as shown in Fig. 3.

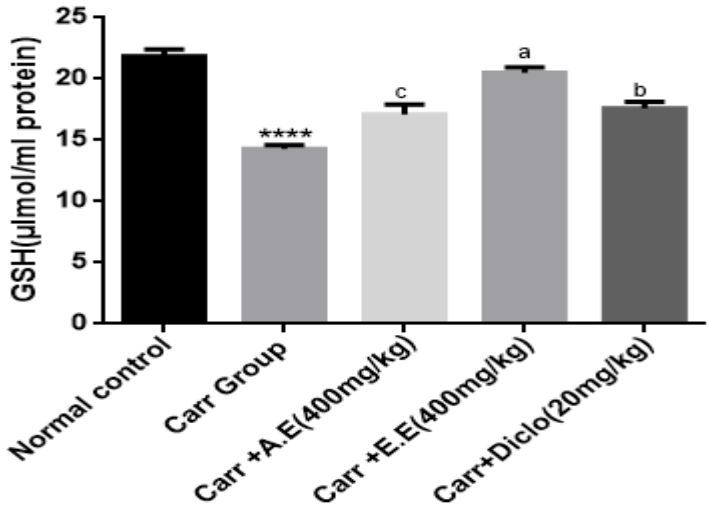


Figure 3: Tissue Paw Antioxidant Enzyme Activity of GSH in Rats. Values Denotes Means ± SEM (n = 3) in Each group. When Comparing Carr Group to Control, ****$p < 0.0001$ Indicates Extremely Significant Decrease in Level. When Comparing Treated Individual Groups to Carr Group, $^{a}p<0.001$ Shows an Extremely Significant Increase in Level , $^{b}p<0.01$ Shows a Very Significant Increase in Level,, $^{c}p<0.05$ Shows a Significant Increase in level . SEM; Standard Error of Mean

Histology of Paw Tissue

The histopathological examination of the paw tissues of each treatment group is shown in Fig. 4A-E below. Each group was assessed at 100X magnification. *Dactyloctenium aegyptium* (aqueous and ethanol extracts) and Diclofenac treatment decreased the inflammatory response in animal paw biopsies (reduction of lymphocytic infiltration). As a matter of fact, when compared to the group that received Carr treatment, inflammatory cells were less numerous and confined close to the vascular sites.

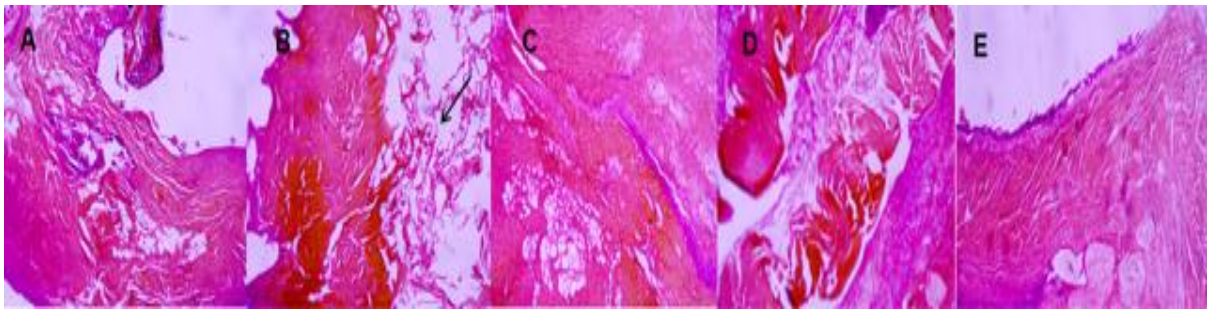


Figure 4: Impact of Extracts of *Dactyloctenium aegyptium* on the Morphological Alterations of Rats with Carr-Induced paw Edema (H&E Stain, 100×Magnification). No Inflammatory Cell Infiltration was Visible in the Control Group (A). The Carr Group (B) Showed Significant Neutrophil Infiltration in the Paw Tissue's Basal and Subcutaneous Layers (Black Arrow). By giving 400 mg/kg of *Dactyloctenium aegyptium* Aqueous Extract (C), 400 mg/kg of *Dactyloctenium aegyptium* Ethanol Extract (D), and 20 mg/kg of Diclofenac (E), Neutrophil Infiltration in the Basal Layer was Reduced

Discussion

The present study evaluated the anti-inflammatory activity of aqueous and ethanol extracts of *Dactyloctenium aegyptium* using the carrageenan-induced paw oedema model in rats and investigated their effects on the antioxidant biomarker, reduced glutathione (GSH). As expected, carrageenan administration induced acute inflammation, characterized by increased paw oedema and tissue swelling. The highest degree of oedema was observed 3 h after carrageenan injection, after which the swelling gradually declined between 5 and 6 h and further subsided within 24 h (Table 2). These findings are consistent with previous reports demonstrating that carrageenan-induced paw oedema reaches maximal intensity approximately 3 h after injection before gradually resolving (Azarbaijani *et al*., 2021; Zhang *et al*., 2022).

Paw oedema is a characteristic feature of acute inflammation induced by carrageenan and results from the release of several inflammatory mediators at the site of injury. The inflammatory response is biphasic, with the initial phase (0–1 h) mediated predominantly by histamine and serotonin, whereas the later phase (>1 h) is associated with increased prostaglandin synthesis and neutrophil infiltration into the inflamed tissue (Fatima *et al*., 2021). In the present study, both *Dactyloctenium aegyptium* extracts, particularly the ethanol extract, produced significant inhibition ($P < 0.001$) of carrageenan-induced paw oedema beginning from the second hour and continuing throughout the experimental period. The ethanol extract exhibited the greatest anti-inflammatory activity, with oedema inhibition comparable to, and slightly greater than, that of diclofenac (53%, 53%, and 49%, respectively). These findings suggest that the extracts may exert their anti-inflammatory effects by suppressing prostaglandin synthesis or inhibiting cyclooxygenase-mediated inflammatory pathways, which are the principal mechanisms of action of diclofenac (Alfaro & Davis, 2023).

Reduced glutathione (GSH) is widely recognised as a major intracellular antioxidant that protects cells against oxidative stress and inflammatory injury (Labarrere & Kassab, 2022). During inflammation, excessive production of reactive oxygen species promotes lipid peroxidation, resulting in increased production of malondialdehyde (MDA), a well-established biomarker of oxidative damage. Therefore, reductions in MDA levels together with restoration of GSH concentrations indicate attenuation of oxidative stress.

In the present study, treatment with both *Dactyloctenium aegyptium* extracts significantly reduced MDA levels compared with the untreated carrageenan group, with the aqueous extract producing the greatest reduction (Fig. 2). This effect may be attributed to the presence of saponins in the aqueous extract. Previous studies have demonstrated that saponins possess potent antioxidant properties and significantly reduce lipid peroxidation, thereby lowering MDA levels in experimental animals (Elekofehinti *et al*., 2013; Wang *et al*., 2019).

Similarly, both extracts significantly increased GSH activity compared with the carrageenan-treated group ($P < 0.001$), indicating enhancement of the endogenous antioxidant defence system. Notably, rats treated with the ethanol extract exhibited the highest GSH levels. This observation may be associated with the presence of terpenoids in the ethanol extract, as terpenoids have been reported to modulate glutathione metabolism and enhance cellular antioxidant capacity (Di Giacomo *et al*., 2023).

These findings further support our previous phytochemical investigation, which demonstrated that *Dactyloctenium aegyptium* contains flavonoids, phenolic compounds, alkaloids, tannins, cardiac glycosides, saponins, and terpenoids (Adesipe *et al*., 2023). Many of these phytochemicals possess well-established antioxidant and anti-inflammatory activities that could account for the biological effects observed in the present study. Similar anti-inflammatory effects have been reported for several medicinal plants in carrageenan-induced paw oedema, pleurisy, ear oedema, and other experimental models of acute inflammation, where antioxidant phytochemicals contributed significantly to the suppression of inflammatory responses (Nor *et al*., 2018; Nunes *et al*., 2020; Del Prado-Audelo *et al*., 2021; Zouari Bouassida *et al*., 2018).

The histopathological findings further corroborated the biochemical and pharmacological results. Treatment with both aqueous and ethanol extracts of *Dactyloctenium aegyptium* markedly reduced neutrophil infiltration and attenuated tissue damage in the carrageenan-induced inflamed paw (Fig. 4). These observations provide histological evidence supporting the anti-inflammatory activity of the plant extracts.

CONCLUSION

This work on the *in vivo* anti-inflammatory efficacy of *Dactyloctenium aegyptium* extracts and its impact on oxidative stress is the first we are aware of. According to the findings, treatment with ethanol extract of *Dactyloctenium aegyptium* produced the strongest anti-inflammatory activity. *Dactyloctenium aegyptium*'s long-standing usage in folk medicine may be supported, at least in part, by the intriguing anti-inflammatory action and antioxidant effect seen.

ACKNOWLEDGEMENTS

Not applicable.

DATA AVAILABILITY

All data are defined and accessible in the publication.

CONFLICTS OF INTEREST

The authors wish to affirm that there are no conflicts of interest to disclose.

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