ORIGINAL RESEARCH article

An *in vivo* study of the anti-inflammatory activity of ethanolic extract of *Pistia stratiotes* (L) medicinal plant

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Abstract: The equatorial and tropical regions of Africa are home to the hydrophyte *Pistia stratiotes*, which is a member of the Araceae family. Recent interest in the potential therapeutic advantages of medicinal plants has been amplified by the presence of their inherent bioactive components. The purpose of this *in vivo* investigation was to determine whether *Pistia stratiotes* possesses anti-inflammatory properties. The effect of *Pistia stratiotes* extract on inflammation in rats was investigated. To categorize the rats, five sets of five rats were utilized each time. The experimental groups consisted of the following: a positive control group, a normal control group, a low-dose group (200 mg/kg), a high-dose group (400 mg/kg), and an 80 mg/kg diclofenac group. The anti-inflammatory effects were assessed through the utilization of the carrageenan-induced paw edema technique. The results demonstrate that the extract significantly decreases the production of edema. The high dose (400 mg/kg) shows a remarkable inhibition of 61.1%, surpassing both the low dose (28.5%) and the positive control (0.0%). The extract exhibits substantial anti-inflammatory properties, similar to diclofenac (73.0%). The results indicate that the ethanolic extract of *Pistia stratiotes* exhibits strong anti-inflammatory properties, making it a promising candidate for the treatment of inflammatory disorders. This study indicates an additional clinical trial and developing formulations to fully realize the medicinal potential of the plant.

Introduction

Medicinal plants have historically held significant prominence in the realm of traditional medicine. Medicinal plants continue to provide novel and inventive therapeutic interventions [1]. As a result of the frequent plant use of indigenous peoples, several pharmaceuticals that are now used to treat severe and chronic illnesses were discovered. Conventional pharmaceuticals are frequently utilized for primary healthcare in both affluent and impoverished countries due to their affordability, extensive spectrum of biological and pharmacological effects, and favorable safety margins [2-5]. On the contrary, it is beyond dispute that allopathic medications, irrespective of variety, are prone to manifesting adverse effects. As a result of its significant therapeutic potential, natural medicine has recently replaced allopathic treatment [6-8]. Throughout history, natural compounds have been employed as topical remedies for inflammation and pain, as well as in the formulation of novel pharmaceutical

substances. The primary therapeutic components of numerous potentially fatal diseases and disorders are botanical in origin [9].

Pistia stratiotes (*P. stratiotes)*, a member of the Araceae family, is a hydrophyte that floats freely and is native to subtropical and tropical regions across Asia, Africa, and the Americas [10]. In this plant, numerous nutrients are present, including vitamins A, B, and C. Sterols, terpenoids, flavone glycosides, lipids, carbohydrates, and proteins are additional constituents. The leaves of this plant have a long history of use as an antibacterial and treatment for tuberculosis and chronic diarrhea, among other traditional medicinal purposes. The plant's ashes were employed as a therapeutic intervention for ringworms of the cranium. Kurmar and others [11] documented that the leaves were utilized to treat dermatological conditions such as leprosy, ulcers, sores, and pox due to their anthelmintic properties. The literature has documented a multitude of chemical and biological investigations that have been conducted on *P. stratiotes* [10, 11]. However, research examining the anti-inflammatory and analgesic properties of these substances is partial [12]. An excruciating sensation is a defining characteristic of analgesia, which is frequently induced by harmful internal and external stimuli. Analgesics function by impeding the transmission of pain signals, which are regulated by the central nervous system [13, 14]. A classification of analgesics is as follows, as defined by Fatope and others [15] opioid analgesics and non-opioid analgesics. An imperative physiological response, inflammation functions as a protective barrier against pathogens and initiates a distinct series of events. Nevertheless, Tripathi [16] proposes a potential correlation between inflammation and discomfort. The principal objective of this *in vivo* experiment was to ascertain the anti-inflammatory properties of the ethanolic extract derived from the medicinal herb *P. stratiotes*.

Materials and methods

Plant material collection: The *P. stratiotes* (L) plants, commonly known as water lettuce were carefully collected from their natural habitat situated in Kolo Creek, Ogbia Local Government Area of Bayelsa State, Nigeria. The plant sample was identified by Inetiminebi A. Ogidi of the Department of Plant Breeding, Niger Delta University Wilberforce Island, Bayelsa State, Nigeria.

Preparation of extract: Following careful selection and harvesting, the leaves of *P. stratiotes* were subjected to a one-week sun-drying process to dehydrate them without compromising the bioactive constituents. An electric processor was employed to reduce the desiccated leaves to a powdered state. A glass container was inoculated for three days (72 hrs) using 200 g of powdered leaves and 1000 mL of 98.0% ethanol. After that, the mélange was allowed to rest at ambient temperature. By employing a sterile cheesecloth, the specimen was extracted. Utilizing the rotatory evaporator, the sample was separated at 50ºC. Following its transfer to a beaker, the filtrate was desiccated in a water bath. Then, it was preserved in a container that was refrigerated.

Animal treatment: Five groups were formed from the twenty-five Wistar Albino rats (n=5). Group II was designated as the positive control group, while Group I was assigned as the normal control group. Group III received 80 mg/kg of diclofenac orally for seven days, while Group IV received the high-dose group and Group V received monotherapy. Group III was administered an ethanolic extract of *P. stratiotes* at a dosage of 200 mg/kg, whereas the high-dose group was administered 400 mg/kg. To assess the anti-inflammatory properties of the ethanolic extract derived from *P. stratiotes* in living organisms, we performed cervical dislocation euthanasia on the rats after the 7^{th} day and obtained blood samples via direct heart puncture.

Ethics approval: The Research and Ethics Committee of the Biochemistry Department at Bayelsa Medical University's Faculty of Basic Medical Sciences, Yenagoa, Bayelsa State, Nigeria, gave their approval for this research study (Reference number: FBMS/AD/BCH/REC/29/04).

Carrageenan-induced paw edema method: To evaluate acute inflammation in living organisms, we employed the carrageenan-induced rat hind paw edema method. Through the establishment of a regulated and replicable inflammatory reaction, this approach enables a more precise evaluation of the anti-inflammatory properties exhibited by the extract of *P. stratiotes*. Rats were administered a subplantar injection containing 0.1 ml of 1.0% carrageenan suspension in normal saline, combined with 5.0% tween 80, to induce acute inflammation in the right hind paw. Following oral administration of the test items for 1.0 hr, this substance was injected into the rodents. The digits were assessed at 1, 2, 3, 4, 5, and 6 hrs after the administration of carrageenan via vernier calliper. The extract was administered orally in two different doses of 400 mg/kg body weight and 200 mg/kg body weight. The standard for anti-inflammatory drugs was diclofenac, administered orally at a dosage of 80 mg/kg in 5.0% tween 80 solution.

Statistical analysis: The data was presented in terms of the mean±standard error of the mean. One-way analysis of variance (ANOVA) was conducted for statistical analysis, followed by a Tukey post-hoc test.

Results

Anti-inflammatory activity: The anti-inflammatory results indicate that the extract effectively reduces edema formation, with the high dose (400 mg/kg) exhibiting a 61.1% inhibition, surpassing the low dose (28.5%) and the positive control (0.0%). Comparable to the diclofenac drug (73.0%), the extract demonstrates significant anti-inflammatory effects as shown in **Table 1**.

Table 1: Effect of *Pistia stratiotes* ethanolic extract on carrageenan-induced paw edema in rat

Time in hour	Group I normal control	Group II positive control	Group III low dose of *P. stratiotes* ethanolic Extract (200 mg/kg)	Group IV high dose of *P. stratiotes* ethanolic extract (400 mg/kg)	Group V diclofenac treated group (80 mg/kg)
0	0.104±0.0025	0.102±0.002	0.102±0.0025	0.104±0.00245	0.102±0.002
1.0	0.104±0.0025	0.312±0.0058	0.302±0.0066	0.18±0.00894	0.11±0.0045
2.0	0.104±0.0025	0.394±0.0147	0.344±0.0132	0.104±0.004	0.122±0.002
3.0	0.104±0.0025	0.702±0.0116	0.302±0.002	0.148±0.0049	0.148±0.0037
4.0	0.104±0.0025	0.402±0.002	0.28±0.0063	0.132±0.0049	0.114±0.0024
5.0	0.104±0.0025	0.282±0.0206	0.22±0.0089	0.112±0.0049	0.102±0.002
6.0	0.104±0.0025	0.232±0.0162	0.188±0.0049	0.106 ± 0.004	0.102±0.002
Inhibition (%)	**74.26**	**0**	**28.47**	**61.14**	**73.02**

The paw edema was measured at different time points after inducing inflammation with carrageenan. The inhibition (%) of edema volume was obtained as (1-PT/PO) . 100. PT: Paw volume of drug/extract treated group. PO: of the carrageenan-treated group

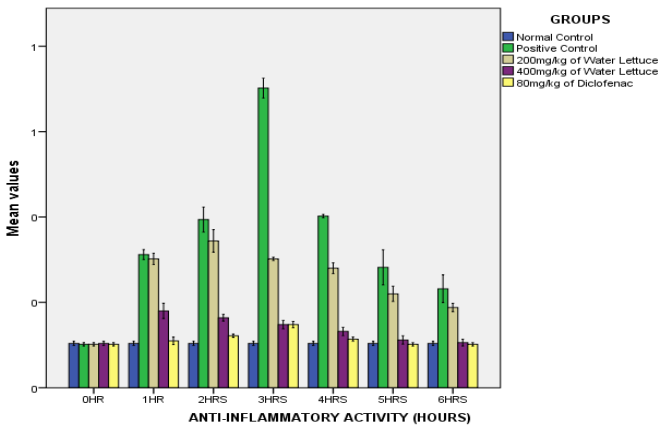

Figure 1: Anti-inflammatory activity over time

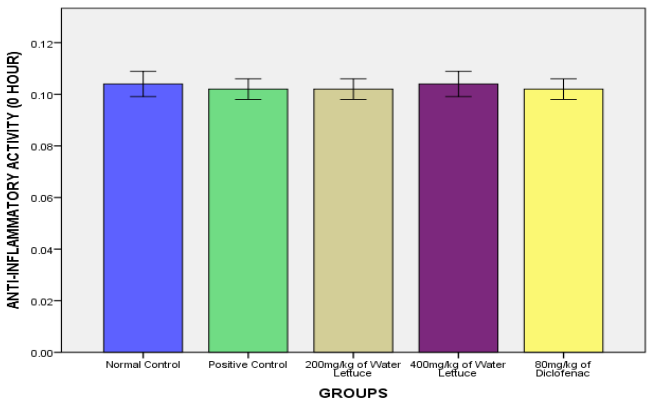

Figure 2: Anti-inflammatory activity at zero-hour

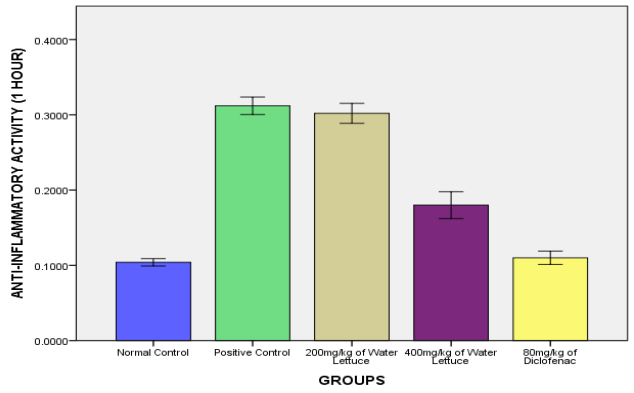

Figure 3: Anti-inflammatory activity after one hour

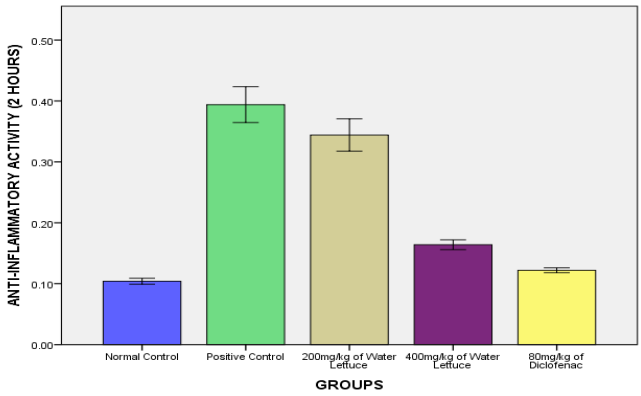

Figure 4: Anti-inflammatory activity after two hours

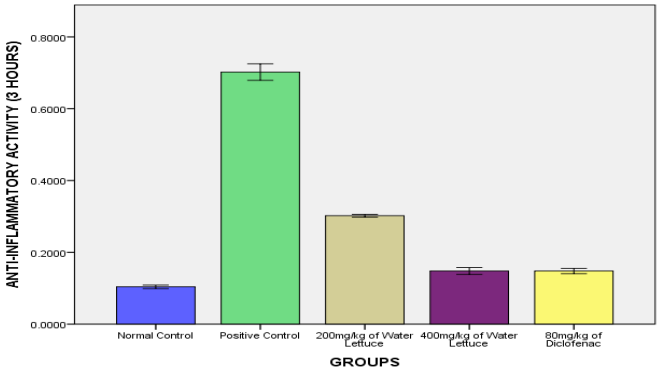

Figure 5: Anti-inflammatory activity after three hours

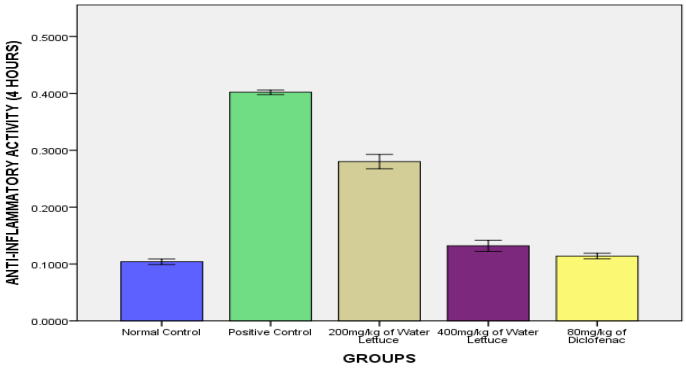

Figure 6: Anti-inflammatory activity after four hours

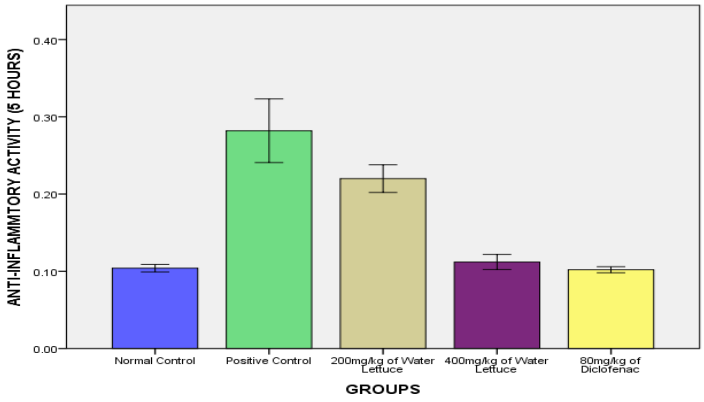

Figure 7: Anti-inflammatory activity after five hours

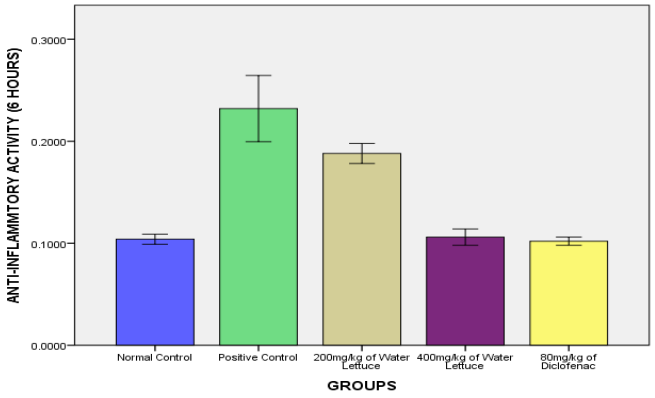

Figure 8: Anti-inflammatory activity after six hours

Discussion

The current study conducted in living organisms examined the anti-inflammatory effects of the ethanolic extract derived from the *P. stratiotes* (L) medicinal plant in rat. The study employed the carrageenan-induced paw edema method to assess the anti-inflammatory effects. The results of the anti-inflammatory assessment indicate that the ethanolic extract of *P. stratiotes* can effectively reduce edema production in albino rats. The study compared the effects of the extracts with those of a positive control, a normal control, and a standard drug (diclofenac). An investigation was conducted to study the impact of the ethanolic extract of *P. stratiotes* on carrageenan-induced paw edema in rats for a duration of six hours. The findings demonstrated a decrease in swelling in the paw over time, with notable anti-inflammatory properties evident within one hour after the extract was administered. The

effectiveness of the extract reached its highest point between one and three hours. After that, there was a progressive decrease in its ability to reduce inflammation, although it still had a substantial effect compared to the control groups even after six hours. The group with a low dose of the extract (200 mg/kg) exhibited a moderate suppression of paw edema, with a percentage inhibition of 28.5% in comparison to the positive control group which had no inhibition. This suggests that the extract's modest dose has a certain degree of anti-inflammatory effect, but at a lower level compared to the conventional medicine, diclofenac. On the other hand, the group that received a high dose of *P. stratiotes* extract (400 mg/kg) exhibited a greater percentage of inhibition (61.1%) in comparison to both the low-dose group and the positive control group. These findings indicate that the effectiveness of the extract in reducing inflammation increases as the dosage increases [16, 17]. The data suggest that the extract has similar anti-inflammatory properties as diclofenac, with the diclofenac-treated group showing a slightly greater percentage inhibition of 73.0% compared to the high dose of *P. stratiotes*-treated group. This assumption is similar to the findings of Gupta and others [18]. Koffuor and others [19] conducted a study on the ethanolic extract of *P. stratiotes* to investigate its anti-inflammatory effects. The study conducted by Koffuor and his associates [19] examined the anti-inflammatory effects of aqueous and ethanolic leaf extracts of *P. stratiotes*. The study used rat models to induce acute inflammation using different inflammatory mediators such as carrageenan, histamine, serotonin, prostaglandin E, and bradykinin. The aim was to compare the anti-inflammatory properties of the extracts with standard anti-inflammatory agents.

The findings from Koffuor et al. [19] indicate that both extracts exhibited a substantial decrease in paw thickness across all inflammatory models, demonstrating benefits that are equivalent to the reference medications. *P. stratiotes* extracts provide a wide range of anti-inflammatory properties, effectively suppressing the activity of important inflammatory mediators. Nevertheless, the research conducted by Koffuor and others [19] also showed that the aqueous extract induces hemolysis of red blood cells and may lead to acute impairment of kidney function. This emphasizes the necessity of closely monitoring for any adverse effects while utilizing this plant for medicinal intentions. The comparison of the findings obtained from the current investigation and the study conducted by Koffuor and others [19] clearly demonstrates that the ethanolic extract of *P. stratiotes* possesses strong anti-inflammatory properties, especially in cases of acute inflammation caused by carrageenan.

Conclusion: The ethanolic extract of the medicinal plant *Pistia stratiotes* (L) showed notable anti-inflammatory effects. This extract demonstrated a relationship between dosage and effects, with the high dosage showing more effectiveness in reducing paw edema compared to the low dosage. The extract exhibited anti-inflammatory properties that were equivalent to diclofenac. The findings indicate that the extract of *Pistia stratiotes* shows considerable promise as a natural therapy for reducing inflammation. Thus, it indicates the existing knowledge about natural compounds that have therapeutic characteristics, highlighting the potential of *Pistia stratiotes* as a valuable medicinal plant.

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Author contribution: EO & OIO conceived and designed the study. EMB collected data. EO & EMB contributed to data analysis and interpretation of data. All authors drafted and reviewed the manuscript for intellectual context. All authors approved the final version of the manuscript and agreed to be accountable for its contents.

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Ethical issues: The authors observed ethical issues including plagiarism, informed consent, data fabrication or falsification, and double publication or submission.

Data availability statement: The raw data that support the findings of this article are available from the corresponding author upon reasonable request.

Author declarations: The authors confirm that they have followed all relevant ethical guidelines and obtained any necessary IRB and/or ethics committee approvals.

دراسة حيوية للنشاط المضاد للالتهابات للمستخلص الإيثانولي لنبات بيستيا ستراتيوتس (L) الطبي

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ملخص: تُعدّ المناطق الاستوائية والاستوائية في أفريقيا موطنًا لنبات بيستيا ستراتيوتس المائي، وهو عضو في عائلة اللوبيا. وقد ازداد الاهتمام مؤخرًا بالمزايا العلاجية المحتملة للنباتات الطبية بفضل وجود مكوناتها الحيوية النشطة. كان الهدف من هذه الدراسة الحية تحديد ما إذا كانت بيستيا ستراتيوتس تمتلك خصائص مضادة للالتهابات. وقد تم دراسة تأثير مستخلص بيستيا ستراتيوتس على الالتهاب لدى الفئران. ولتصنيف الفئران، استُخدمت خمس مجموعات من خمسة فئران في كل مرة. تألفت المجموعات التجريبية مما يلي: مجموعة ضابطة إيجابية، ومجموعة ضابطة طبيعية، ومجموعة جرعة منخفضة (200 ملغ/كغ)، ومجموعة جرعة عالية (400 ملغ/كغ)، ومجموعة ديكلوفيناك 80 ملغ/كغ. وقُيّمت التأثيرات المضادة للالتهابات من خلال استخدام تقنية وذمة المخلب المُستحثة بالكاراجينان. وتُظهر النتائج أن المستخلص يُقلل بشكل ملحوظ من حدوث الوذمة. وتظهر الجرعة العالية (400 ملغم/كغم) تثبيطًا ملحوظًا بنسبة 61.1%، متجاوزة كل من الجرعة المنخفضة (28.5%) والتحكم الإيجابي (0.0%). يُظهر المستخلص خصائص مضادة للالتهابات قوية، تُشبه ديكلوفيناك (73.0%). تشير النتائج إلى أن المستخلص الإيثانولي لنبات بيستيا ستراتيوتس يُظهر خصائص قوية مضادة للالتهابات، مما يجعله مرشحًا واعدًا لعلاج الاضطرابات الالتهابية. تُشير هذه الدراسة إلى إجراء تجربة سريرية إضافية وتطوير تركيبات لتحقيق الإمكانات العلاجية الكاملة لهذا النبات.