Anti-Inflammatory Activity of Ethanol Root Extract of Panicum maximum

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Authors’ contributions

This work was carried out in collaboration among all authors. Author JEO designed the study, performed the statistical analysis and wrote the protocol. Author JAU wrote the first draft of the manuscript. Authors DNO and UAE managed the analyses of the study. Author JAU managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Panicum maximum root is used routinely to treat ailments such as malaria, fever, pains and inflammatory diseases by traditional medicine practitioners.

Aim: The study evaluated the anti-inflammatory effect of P. maximum root so as to validate its uses by practitioners of traditional medicine.

Methodology: The root of P. maximum (dried powdered material) was extracted in ethanol using cold maceration technique. The root crude extract (137 –547 mg/kg) of P. maximum was investigated for anti-inflammatory activity using various experimental models; carrageenan, egg albumin and xylene - induced edema models.

Results: The root extract of P. maximum caused significant (p<0.05 – 0.001) reduction of inflammation induced by the phlogistic agents in a dose-dependent fashion. The recorded anti-inflammatory effects were comparable to those initiated by 100 mg/kg acetyl salicylic acid (ASA, standard drug) used in some of the models here. The anti-inflammatory effect of this plant may be attributed to the phytochemical constituents of the plant.

Conclusion: The findings from this research confirm the ethnomedical use of Panicum maximum root in treating inflammatory conditions.

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1. INTRODUCTION

Panicum maximum Jacq. (Poaceae), a perennial tuft grass with a short, creeping rhizome, regarded as a very valuable fodder plant, is also used in hay making. It is a robust grass with stem that can reach up to 2 m in height, with leaf sheath found at the bases of the stems which are covered in fine hairs. Leaf blades can reach 35 mm in width, ending with tapering point. P. maximum has large multi-branched inflorescence with whorl-like lower branches. There is male lower floret, fertile female upper lemma, and green to purple spikelets. It preferably grows in fertile soil, in shaded, damp areas under trees and shrubs, along rivers and open woodland. As a tropical grass, it is widely distributed in Africa and other tropical regions of the world [1]. The Ibibios of Akwa Ibom State, South South Nigeria use the leaves ethnomedically to treat several diseases including malaria, microbial infections, rheumatism pain, inflammation and diabetes. Antidiabetic [2], antimalarial and analgesic [3], antibacterial [4,5,6], anti-inflammatory and antipyretic [7], antifungal [8], anticancer, antioxidantive burst and antileishmanial [9] activities of the leaf extract have been reported. Also, Panicum maximum root extract has earlier been reported to have analgesic and antimalarial properties [10]. LD_{50} value of 2738.1 mg/kg and antidepressant and anticonvulsant activities [11]. Phytochemical components such as alkaloid, flavonoid, tannins, terpenes, saponin, and cardiac glycosides [10] have also been found. In this study, we investigated the anti-inflammatory effect of ethanol root extract of P. maximum.

2. MATERIALS AND METHODS

2.1 Plants Collection

The plant material, Panicum maximum (root), was collected in compounds from a farmland in Uyo metropolis, Akwa Ibom State, South South Nigeria in August, 2018. Its identification and authentication was done in the Department of Botany and Ecological Studies, University of Uyo, Nigeria, while a specimen was kept at the Herbarium of Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Nigeria.

2.2 Extraction

The roots of the plant were washed, allowed to drain and then dried in the shade for 14 days. The dried roots were converted to powder form with the use of mortar and pestle, and then macerated in 50 % ethanol. The fluid content of the filtrate resulting from the maceration was then eliminated, by drying the liquid filtrate itself in a rotary evaporator in-vacuo at 40 °C. The final concentrate was then kept at -4 °C in a refrigerator until when required to be used for the experiment.

2.3 Animals

Male and female Albino Swiss mice (19 - 28 g) from the Department of Pharmacology and Toxicology Animal House, Faculty of Pharmacy, University of Uyo, Nigeria, were kept under standard atmospheric and laboratory conditions, and given access to pelleted rodents feeds and water ad libitum. Permission and ethical approval for animal use was obtained from the Experimental Ethics Committee on Animal Use of the Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.

2.4 Evaluation of Anti-Inflammatory Activity of the Extract

2.4.1 Carrageenan-induced mice hind paw oedema

Adult albino male mice fasted for 24 hours were used for the experiment. The animals were deprived of water only during the experiment. Hind paw inflammation was induced by injection of 0.1 mL of freshly prepared carrageenan suspension in normal saline into the sub plantar surface of the hind paw. Prior to induction, the linear circumference of the hind paw was measured using vernier calipers. The measurement was repeated at 0.5, 1, 2, 3, 4 and 5 hours post administration of the phlogistic agent. The post administration increase in paw circumference of the phlogistic agent was used as a measure for inflammation [12,13]. Inflammation was assessed using the difference in the injected paw circumference of the test groups (0.5, 1, 2, 3, 4 and 5 hours after administration of the phlogistic agent [14]. The extract (137, 273 and 547 mg/kg i.p.) was given to different groups containing 6 mice each, 1 hour before inflammation was induced. The negative control group received distilled water (10 mL/kg p.o.) while positive control (reference) group was given 100 mg/kg body weight of ASA. Vernier calipers measurements were used to assess the average (mean) edema.
2.4.2 Egg albumin-induced inflammation

Induction of inflammation in mice was done by injecting egg albumin (0.1 mL, 1% in normal saline) into the sub plantar tissue of the mice right hind paw [15-17]. Prior to induction, the linear circumference of the hind paw was measured using vernier calipers. The measurement was done before, and at 0.5, 1, 2, 3, 4 and 5 hours after applying the phlogistic agent. Exactly 1 hour before the induction of inflammation, the 24 hours fasted mice (6 per group) were administered intraperitoneally 137, 273 and 547 mg/kg body weight of *P. maximum* extract and orally, 100 mg/kg of ASA (reference group). The control group was given 10 mL/kg of distilled water orally. The difference in paw circumference between the control and extract treated groups after application of the phlogistic agent was used to assess inflammation [17]. The average (mean) edema was measured and recorded.

2.4.3 Xylene – induced ear oedema

Induction of inflammation was done by topically applying 2 drops of xylene to the inner surface of the mice right ear, and keeping it to act for 15 min. However, The extract (137, 273 and 547 mg/kg i.p), dexamethasone (4 mg/kg) and distilled water (0.2 mL/kg) were all orally given to different groups of mice containing 6 mice each, 1 hour before inflammation was induced. The animals were then sacrificed under light anaesthesia, after which the left ears were cut off. The difference between the weights of the ears accounted for inflammation or edema induced by the xylene [16,18].

2.5 Statistical Analysis and Data Evaluation

In this study, all data were statistically analyzed using ANOVA (one-way). This was then followed by Tukey-Kramer multiple comparison test. The differences between means were considered to be significant at 5 % level of significance i.e. P = .05.

3. RESULTS

3.1 Carrageenan-Induced Oedema in Mice

Table 1 shows the effect of ethanol root extract of *P. maximum* on carrageenan-induced oedema. The extract (137-547 mg/kg) exerted a significant anti-inflammatory effect in a non dose–dependent manner that was shown to be comparable to the standard drug ASA, 100 mg/kg (Tables 1a and 1b).

3.2 Egg Albumin-Induced Edema

Result for egg albumin-induced edema is as presented in Table 2. *P. maximum* root extract (137-547 mg/kg) caused significant non dose-dependent anti-inflammatory effect against egg albumin-induced inflammation. The highest effect was seen at 3-5 hours. At 547 mg/kg extract (highest dose), recorded effect was comparable to that of 100 mg/kg ASA (standard drug) (Tables 2a and 2b).

3.3 Xylene-Induced Ear Edema

The root extract of *P. maximum* exerted a significant (P=.05) dose dependent anti-inflammatory effect against xylene-induced ear edema. At the highest dose (547 mg/kg), the extract exerted a potent anti-inflammatory effect that exceeded that of the standard drug, dexamethasone (4.0 mg/kg) (Table 3).

4. DISCUSSION

*P. maximum* root is used by Ibibio traditional medicine practitioners to treat certain disease conditions like fever, pains, swellings and various arthritic and/or inflammatory conditions. This study evaluated the anti-inflammatory effect of *P. maximum* root extract using different established experimental models.

Table 1a. Effect of *P. maximum* root extract on carrageenan-induced oedema in rats

<table>
<thead>
<tr>
<th>Treatment/ dose (mg/kg)</th>
<th>0.5hr</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
<th>4hr</th>
<th>5hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.16±0.09</td>
<td>0.62±0.06</td>
<td>0.47±0.01</td>
<td>0.33±0.06</td>
<td>0.15±0.04</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td>Extract 137</td>
<td>1.01±0.09</td>
<td>0.60±0.06</td>
<td>0.43±0.08</td>
<td>0.33±0.06</td>
<td>0.11±0.03</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>Extract 273</td>
<td>1.35±0.18</td>
<td>0.74±0.12</td>
<td>0.50±0.06</td>
<td>0.27±0.07</td>
<td>0.14±0.03</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>Extract 547</td>
<td>0.89±0.11</td>
<td>0.42±0.09</td>
<td>0.26±0.10</td>
<td>0.27±0.06</td>
<td>0.05±0.01</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>ASA 100</td>
<td>1.08±0.12</td>
<td>0.65±0.12</td>
<td>0.31±0.10</td>
<td>0.15±0.04</td>
<td>0.07±0.01</td>
<td>0.03±0.01</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SEM. Significant at *p*<0.05 when compared to control. n = 6*
mediators of acute inflammation

Carrageenan

Extract

ASA

Control

Extract

ASA

Treatment

Data are expressed as mean ± SEM. Significant at *p< 0.05, **p< 0.01 when compared to control. n = 6

Table 2a. Effect of P. maximum root extract on egg albumin-induced oedema in mice

Table 2b. Effect of P. maximum root extract on egg albumin-induced oedema in rats

Table 3. Effect of P. maximum root extract on xylene-induced ear oedema in mice

Carrageenan-induced edema model which is normally used to predict the presence of mediators of acute inflammation has two separate phases. Serotonin and histamine are released during the first phase which lasts for 1-2 hours, while prostaglandins, leucotrienes and
cyclooxygenase products account for the activities recorded during the second phase. There is an intermediate connecting phase between the two phases mediated by kinins. In this study, the extract (137-547 mg/kg) exerted a considerable, though statistically non significant effect, during the early stage of inflammation (1-2 hour). This suggest that the plant extract may probably inhibit the actions of histamine, serotonin and kinins which are pro-inflammatory mediators known to be involved in the early phase of carrageenan-induced oedema [17,19]. The root extract also significantly reduced the later stage of the oedema, and this may be attributed to its inherent ability to inhibit prostaglandin, leukotrienes and cyclooxygenase products which are involved in the second stage of carrageenan-induced inflammation [17,19]. Similarly, ASA (100 mg/kg), which is a prototype non steroidal anti-inflammatory drug (NSAID) and known cyclooxygenase inhibitor that inhibits prostaglandin, also caused significant anti-inflammatory effect as was seen in the paw swelling in this study.

Egg albumin-induced edema is biphasic, consisting of early and late phases. The early phase occurs from histamine, serotonin (5-HT) and kinins mediation, while the late phase is from the release of bradykinin, leukotrienes and prostaglandins from tissue macrophages. In this study, *P. maximum* extract inhibited the edema caused by egg albumin, clearly showing that its inflammatory effect is mediated through the inhibition of histamine and 5-HT release, which are released by egg albumin [20]. However, it was noted that ASA, a cyclooxygenase inhibitor, also caused significant reduction of the oedema induced by egg albumin.

In xylene induced edema, phospholipase A2 (PLA2) is involved in the pathophysiology of the resultant inflammation. PLA2 catalyzes the breakdown of membrane phospholipids to produce arachidonic acid and a lysophospholipid which are precursors of inflammatory mediators like prostaglandins, leukotrienes and platelet activating factors (PAF). The extract significantly inhibited the xylene-induced oedema at all the doses that were given, most probably by inhibiting PLA2 [21]. However, dexamethasone, a steroid anti-inflammatory agent significantly reduced the mean right ear weight of the positive control rats thereby demonstrating PLA2 inhibition.

Okokon *et al.* [10] has reported that *P. maximum* root extract contains alkaloids, saponins, tannins, phlobatannins, flavonoids and cardiac glycosides among others which might have contributed to the observed anti-inflammatory activity in this study.

Flavonoids are known anti-inflammatory agents that act by inhibiting the cyclo-oxygenase pathway [22]. Some flavonoids are reported to block both the cyclo-oxygenase and lipoxygenase pathways of the arachidonate cascade at relatively high concentrations, while at lower concentrations they only block lipoxygenase pathway [23]. Flavonoids also exhibit inhibitory effects against PLA2 and phospholipase C, [24] and cyclo-oxygenase and/or lipoxygenase pathways [25].

5. CONCLUSION

The root of *P. maximum* has been reportedly used in Ibibio traditional medicine for the management of inflammatory conditions. From the results and interpretations made thus far, this study demonstrates that *P. maximum* root possesses anti-inflammatory property. The observed biological effect may be due to the presence of phytochemical constituents such as polyphenolics, flavonoids, monoterpenes, sesquiterpenes and triterpenes in the plant. Therefore, the extract may be exploited as an adjuvant in the management of inflammatory conditions.

**ETHICAL APPROVAL**

All necessary ethical considerations as regard the use of animals in research were satisfactorily met. The care and use of animals was conducted in accordance with the National Institute of Health Guide for the Use of Laboratory Animals (NIH, 1996). Moreover, ethical approval for animal use was obtained from the Experimental Ethics Committee on Animal Use of the Faculty of Pharmacy, University of Uyo, Nigeria.

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**COMPETING INTERESTS**

Authors have declared that no competing interests exist.
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