Ocular anti-inflammatory effect of aqueous and ethanolic leaf extracts of *Pistia stratiotes* Linn (Araceae) in endotoxin-induced uveitis

Samuel Kyei¹,², George A. Koffuor¹, Johnson N. Boampong³, Osei Owusu-Afriyie⁴

ABSTRACT

Background: Despite the numerous studies conducted on the anti-inflammatory effect of *Pistia stratiotes*, its traditional use in the treatment of conjunctivitis and iritis has not been scientifically evaluated. **Materials and Methods:** This study, therefore, investigated the anti-inflammatory effect of aqueous and ethanolic leaf extracts of *P. stratiotes* in endotoxin-induced uveitis. Uveitis was induced in Sprague-Dawley rats with lipopolysaccharides. Body temperature as well as erythrocyte sedimentation rate and C-reactive proteins of blood in *Pistia* extracts-treated, prednisolone-treated, and normal saline-treated (control) rats were measured. Clinical signs of uveitis were observed using a slit lamp and histopathological assessments of inflammation in ocular tissue made. Polymorphonuclear neutrophils and total proteins in the aqueous humor of the eyes were estimated. **Results:** A rise in body temperature after lipopolysaccharide inoculation was found to be associated with corresponding high erythrocyte sedimentation rate and elevated C-reactive protein levels. There were significant reductions in vasodilatation of iris vessels, exudation, polymorphonuclear neutrophil (inflammatory cells), and total proteins into the anterior chamber of the eyes in rats treated with the *Pistia* extracts compared to the untreated (control). Histopathological assessments revealed resolution of uveitis in treatment groups but not in the untreated. All effects exhibited by the extracts were similar to that of Prednisolone; the reference to the untreated (control). **Conclusion:** This study has shown that curative oral administration of aqueous and ethanolic leaf extracts of *P. stratiotes* have ocular anti-inflammatory effect in Sprague-Dawley rats.

Key words: Erythrocyte sedimentation rate, inflammation, lipopolysaccharides, polymorphonuclear neutrophil count, uveitis

INTRODUCTION

Uveitis is a major vision-threatening intraocular inflammatory disorder that can lead to complete blindness if not well managed.¹ A recent study indicates an incidence of 52.4/100,000 person and a period prevalence of 115.3/100 000 persons.² The age distribution of 25-50 years among suffers of uveitis makes uveitis a group of ocular diseases an important socioeconomic impact as most sufferers are in the active period of their working life.³ Although the available literature on its etiology is not definite, causes may be attributed to autoimmune disorders, exposure to toxins, microbial infections and idiopathic factors.⁴ Endotoxin-induced uveitis (EIU) stimulated by injection of lipopolysaccharide (LPS) is a useful animal model for acute ocular inflammation. LPS found in the cell wall of gram-negative bacteria has been demonstrated to be an important immunostimulatory agent in autoimmune disorders such as Freud’s adjuvant arthritis and uveitis.⁵

Steroidal anti-inflammatory drugs, which are the mainstream treatment for uveitis, are associated with severe adverse effect such as cataract, increased intraocular pressure, delayed wound healing, fluid retention, mood changes (depression or euphoria), hypertension, diabetes, osteoporosis, nausea, increased appetite, and weight gain.⁶-⁸ Therefore, the need for alternative drugs with minimal adverse effects is urgently desirable.

*P. stratiotes* (Family: Araceae) commonly known as water lettuce, great duck-weed, or Sudd plant⁹ has been used traditionally to treat ocular inflammatory disorders.¹⁰,¹¹ Although studies conducted by Sundeen Kumar et al.¹² and Kyei et al.¹³ have revealed the anti-inflammatory and anti-arthritic
effect of *P. stratiotes*, its reported traditional use in the treatment of conjunctivitis and iritis has not been scientifically evaluated to ascertain its importance for use. It is against this backdrop that this study has been conducted to investigate the anti-inflammatory effect of aqueous and ethanolic leaf extracts of *P. stratiotes* in Sprague-Dawley rats.

**MATERIALS AND METHODS**

**Plant collection**

*P. stratiotes* was collected from the Fosu lagoon, Cape Coast in the Central Region of Ghana (5°7´ N and 1°16´ W) in December 2010. It was identified and authenticated by Mr. G H Sam of the Department of Herbal Medicine, CHS, KNUST, where a voucher specimen bearing the number KNUST/HM1/11/W002 has been deposited in the herbarium for future reference.

**Preparation of pistia extracts**

The leaves of *P. stratiotes* were washed thoroughly with tap water and sun-dried. The dry leaves were milled into coarse powder by a hammer mill (Schutte Buffalo, New York, USA). In preparing the aqueous leaf extract of *P. stratiotes*, 700 g of the leaf powder was mixed with 1 L of water. The mixture was maintained at 80°C (in a round-bottom flask fitted with a reflux condenser) in a thermostatically controlled water bath for 24 h and then filtered. The filtrate was frozen dried in a Hull freeze dryer/lyophilized 140 SQ FT (model 140FS275C, USA) into a powder (percentage yield 4.7%) and stored at a temperature of 4°C in a refrigerator. This powder was reconstituted in normal saline to a desired concentration and labeled as AQ PSE for dosing in this study. Similarly, 700 g of the leaf powder was soaked with one liter of 70% ethanol at room temperature (27-29°C) for 72 h and filtered. The filtrate obtained was freeze-dried into powder (percentage yield 5.2%). Quantities of this powder was reconstituted in normal saline at desired concentrations to be referred to and used in this study as the ethanolic leaf extracts of *P. stratiotes* or ET PSE.

**Drugs and chemicals used**

LPS from *Escherichia coli* (Axxora, LLC, San Diego, USA) was used to induce uveitis. BCA Protein Assay Reagent kit (Pierce, Rockford, IL, USA) was used to determine total proteins in aqueous humor. Prednisolone (Letap Pharmaceuticals Ltd., Accra, Ghana) was the reference anti-inflammatory agent in this study. Ketamine Hydrochloride (Fabriqué par Laborario Sanderson S.A, Chile) was the anesthetic used and Normal Saline Solution (Intravenous Infusions Ltd., Koforidua, Ghana) was the vehicle in which other drugs were dissolved in.

**Animals and husbandry**

Six week-old male Sprague-Dawley rats (100-120 g) purchased from the Centre for Scientific Research into Plant Medicine (CSIRPM), Mampong-Akwapim, Ghana, were maintained in the Animal House of Department of Pharmacology, KNUST, Ghana. The animals were housed in polyacrylic cages (34 cm × 47 cm × 18 cm) with soft wood shaving as bedding, under ambient laboratory conditions (temperature 28±2°C, relative humidity 60-70%, and normal light-dark cycle). Females were non-pregnant. They were fed with normal commercial pellet diet (GAFCO, Tema) and water *ad libitum*. All procedures and techniques used in these studies were in accordance with the National Institute of Health for the Care and Use of Laboratory Animals (NIH, Department of Health and Human Services Publication No. 85-23, revised 1985). The protocols for the study were approved by the Departmental Ethics Committee.

**Dosing of drugs**

Doses of AQ PSE and ET PSE were selected based on preliminary experiments at establishing activity. Six groups (*n* = 6) of rats with LPS-induced uveitis were treated with 10, 30, 50, 100, 300 and 500 mg/kg of AQ PSE or ET PSE. They were assessed for signs of inflammation to select the dose with activity. The dose of prednisolone used was calculated from treatment doses for uveitis recorded in official compendia.[14] Dosing was done once daily at a volume of 10 ml/kg body weight. Individual dose volumes were calculated based on the animal’s most recent recorded body weight.

**Preliminary phytochemical screening**

Screening was performed on AQ PSE and ET PSE to ascertain the presence of phytochemicals using standard procedures described by Wagner and Bladt,[15] Glasl,[16] Harborne,[17] and Kujur *et al.*[18]

**Pyrexia, erythrocyte sedimentation rate and C-reactive protein determination**

After baseline body temperatures (taken from the rectum of Sprague-Dawley rats) were recorded, the rats were inoculated with 200 µg LPS intraplantarly (100 µg per hind footpad). Two hours after LPS inoculation the body temperatures were again recorded. Six of the rats that showed an increase in body temperature of 0.5°C and more were euthanatized and blood samples were collected into trisodium citrate erythrocyte sedimentation rate (ESR) tubes (Chengdu Rich Science Industry Co., Ltd., Sichuan, China) for estimation of ESR using the Westergren method.[19] Blood was also collected from another six into glass tubes and centrifuged (temperature 25°C, speed 4000 g) for 5 minutes using a Mikro 220R (Hettich Zentrifuge, Tuttlingen, Germany) machine to obtain the plasma, which was used to estimate C-reactive protein (CRP) levels using an enzyme-linked immunosorbent
Endotoxin-induced uveitis

Rats which showed an increase in temperature of 0.5°C and more after 2 h of LPS inoculation were selected and grouped into eight groups (n = 6). They were randomized for treatment with 30, 100, or 300 mg/kg of AQ PSE or ET PSE, 30 mg/kg Prednisolone, or 10 ml/kg of Normal (uveitic control) saline by oral gavage. A normal control group was kept under experimental conditions with no LPS inoculation. After 24 h, the animals were anesthetized with Ketamine HCl and the eyes assessed for clinical signs of vasodilation and exudation using an SL500 Shin Nippon Slit Lamp (Ajinomoto Trading Inc., Tokyo, Japan) fitted with a digital microscope camera (Olympus, Tokyo, Japan) to take photographs of the anterior chamber of the rats. The clinical score of inflammation was determined from the photographs using a scale of 0-4 (0 = normal; 1 = mild; 2 = moderate; 3 = severe; and 4 = very severe). The animals were euthanatized and the anterior chamber was punctured with a 30 gauge needle, and the aqueous humor was collected from both eyes (15 µl/rat).

Estimation of total protein concentration

A 1:10 dilution (Diluent: Turk solution) of the aqueous humor in the eye of the rats was introduced onto the counting chamber of the Improved Neubauer Haemocytometer (Depth 0.1 mm, Area: 1/400 mm²; Yancheng Cordial Lab Glassware Co. Ltd., Jiangsu, China) fitted with a digital microscope for fluorescence (Medline Scientific limited, UK) under objective magnification of ×40. The number of PMNs was determined per mm³ of aqueous humor (taking into consideration the dilution factor).

Polymorphonuclear neutrophil count

A BCA protein assay kit (Pierce, Rockford, IL, USA) was used to establish the total protein concentration in the aqueous humor obtained from the enucleated rat eyes. The protein concentration was determined by pipetting 10 µl of aqueous humor from; LPS inoculated rats treated with 30, 100, and 300 mg/kg of AQ PSE or ET PSE, 30 mg/kg prednisolone, 10 ml/kg normal saline; normal rats without LPS challenge, and standard bovine serum albumin (BSA) into a 96-well microplate. A 200 µl volume of working reagent, constituted according to the manufacturer’s instructions, was mixed thoroughly with the content of each well and shaken for 30 seconds. It was then incubated at 37°C for 30 minutes and allowed to cool to room temperature. Absorbances of the mixtures were measured at 562 nm using an ELx800 absorbance microplate reader (BioTek Instruments, Inc., USA). Each determination was in triplicate.

Histopathological assessment

The enucleated eyes of the animals were fixed in 4% phosphate-buffered paraformaldehyde, and embedded in paraffin. Sections were made and stained with hematoxylin and eosin and fixed on glass slides for microscopic examination at the Pathology Department of the Komfo Anokye Teaching Hospital, Kumasi, Ghana for histopathological assessment by a Specialist Pathologist.

RESULTS

Phytochemical screening

Preliminary phytochemical screening revealed the presences of tannins, flavonoids, sterols in both AQ PSE and ET PSE. Alkaloids were present only in the AQ PSE [Table 1].

Pyrexia, erythrocyte sedimentation rate and C-reactive protein

Intra-plantar injection of LPS was characterized by pyrexia, elevated serum levels CRP and high levels of ESR two hours after injection [Table 2]. Elevation of these parameters was considered a satisfactory indication of an inflammatory response.

Endotoxin-induced uveitis

There was significant reduction in vasodilation of the iris vessels and exudation into the anterior chamber relative to the control; seen as significant decrements (P ≤ 0.001) in clinical scores of inflammation [Table 3] graded from photographs taken upon slit lamp examination [Figure 1]. The extent of inflammation (including vasodilation and exudation into the anterior chamber) determined upon slit lamp examination were scored on a scale of 0-4 (0 = normal; 1 = mild; 2 = moderate; 3 = severe; and 4 = very severe). This grading constituted the clinical scores of inflammation.

Polymorphonuclear neutrophil count

There were significant reductions (P ≤ 0.001) in the number of polymorphonuclear neutrophil in the reference drug, AQ PSE and ET PSE-treated groups

<table>
<thead>
<tr>
<th>Table 1: Results obtained after preliminary phytochemical screening of AQ PSE and ET PSE</th>
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<tbody>
<tr>
<td>Tannins</td>
</tr>
<tr>
<td>AQ PSE</td>
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<td>ET PSE</td>
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*: Present, -: Absent
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Total protein concentration
Total protein concentration was significant lower (*P* ≤ 0.001) in prednisolone-treated, as well as the AQ

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Clinical score of inflammation</th>
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<tbody>
<tr>
<td>Uveitic control</td>
<td>3.33±0.211</td>
</tr>
<tr>
<td>Normal control</td>
<td>0.16±0.167***</td>
</tr>
<tr>
<td>30 mg/kg AQ PSE</td>
<td>0.50±0.224***</td>
</tr>
<tr>
<td>100 mg/kg AQ PSE</td>
<td>1.66±0.333***</td>
</tr>
<tr>
<td>300 mg/kg AQ PSE</td>
<td>1.66±0.333***</td>
</tr>
<tr>
<td>30 mg/kg ET PSE</td>
<td>0.66±0.211***</td>
</tr>
<tr>
<td>100 mg/kg ET PSE</td>
<td>1.50±0.342***</td>
</tr>
<tr>
<td>300 mg/kg ET PSE</td>
<td>1.16±0.307***</td>
</tr>
<tr>
<td>30 mg/kg prednisolone</td>
<td>0.66±0.333***</td>
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Values are means ±SEM (*n* = 6). Significant differences between the clinical score (from a scale of 0-4, 0: Normal, 1: Mild, 2: Moderate, 3: Severe, and 4: Very severe) of inflammation of the drug-treated and normal control and the uveitic control were established using one-way analysis of variance followed by Dunnett’s post-hoc test. *** *P* ≤ 0.001
**Histopathological assessment**

The histopathological assessment did not show any remarkable signs of inflammation in anterior uvea in all rats treated with Prednisolone, AQ PSE, ET PSE-treated uveitic rats, and the normal control. However, there were histopathological signs of inflammation characterized by neutrophil infiltration into the uveal tissues of the uveitic control rats [Figure 4].

**DISCUSSION**

EIU stimulated by injection of LPS is a useful animal model for acuteocular inflammation. The mechanism by which inflammation and its associated fever is produced by infectious noxa, e.g. bacterial endotoxic LPS challenge is through the activation of mononuclear phagocytes that then produce and release pyrogenic cytokines, including IL-1β and TNF-α.\(^{[22-25]}\) LPS stimulation triggers cellular inflammatory response causing the release of nitric oxide (NO), prostaglandin E2 and tumor necrosis factor (TNF)-α.\(^{[26-29]}\) TNF-α indirectly trigger the production of prostaglandin, which mediates the process of inflammation, which includes uveitis.\(^{[30,31]}\) LPS could also induce nuclear factor-kappaB (NF-κB) activation and monocyte chemo-attractant protein-1 (MCP-1) expression resulting in inflammation.\(^{[32]}\) LPS footpad challenge marked by pyrexia, elevated serum levels of CRP and high ESR was associated with infiltration of inflammatory cells, vasodilation of the iris vessels, protein exudation into the aqueous humor; classical signs of uveitis.

The curative oral administration of AQ PSE and ET PSE showed anti-uveitic activity indicating the ocular
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anti-inflammatory effect. This conforms to earlier studies in which aqueous and ethanolic extracts of *P. stratiotes* have been demonstrated to have anti-inflammatory and anti-arthritic effects.\[^{12,13}\] It managed well vasodilatation associated with inflammation; seen as significant decrements in clinical scores of inflammation graded from photographs taken upon slit lamp examination. The significant decrease in polymorphonuclear neutrophil infiltration into the aqueous also shows anti-inflammatory activity due to inhibition of vasodilatation. During the beginning (acute) phase of inflammation, neutrophils are one of the first-responders of inflammatory cells to migrate towards the site of inflammation.\[^{33}\] They migrate through the blood vessels, then through interstitial tissue, following chemical signals such as Interleukin-8 and other inflammatory mediators in a process called chemotaxis.\[^{34}\]

Neutrophils also release an assortment of proteins (Lactoferrin, Cathelicidin, Myeloperoxidase, bactericidal/permeability-increasing protein (BPI), Defensins, and the serine proteases neutrophil elastase and cathepsin G, cathepsin, and gelatinase) by a process called degranulation.\[^{35}\] This increases the total proteins in an area of inflammation. Treatment with AQ PSE and ET PSE resulted in very significant decrements in total protein concentration compared to the uveitic control. This again indicates that inflammation has been managed.

Histopathological studies also confirmed the anti-inflammatory activity of the extract in a similar manner as prednisolone as seen in the photomicrographs of the histology of uveitic and the uveitic but treated rats. Prednisolone is a corticosteroid drug with predominant glucocorticoid activity, making it useful for the treatment of a wide range of inflammatory and condition.\[^{36}\] The anti-inflammatory action of prednisolone is thought to involve phospholipase A2 inhibitory proteins, collectively

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<table>
<thead>
<tr>
<th>Controls</th>
<th>AQ PSE-treated</th>
<th>ET-PSE treated</th>
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</thead>
<tbody>
<tr>
<td>Normal</td>
<td>30 mg/kg</td>
<td>30 mg/kg</td>
</tr>
<tr>
<td>Uveitic</td>
<td>100 mg/kg</td>
<td>100 mg/kg</td>
</tr>
<tr>
<td>Prednisolone-treated</td>
<td>300 mg/kg</td>
<td>300 mg/kg</td>
</tr>
</tbody>
</table>

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Figure 4: Photomicrographs of the anterior uvea of, normal, uveitic, and uveitic rats treated with 30, 100 and 300 AQ PSE, and 30, 100 and 300 ET PSE
called lipocortins. Lipocortins, in turn, control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes by inhibiting the release of the precursor molecule arachidonic acid. The extracts possibly could be exerting their ocular anti-inflammatory effect via a similar mechanism. Earlier studies with the extracts conducted by Kyei et al., 2012, to establish its anti-arthritic effect revealed that AQ PSE and ET PSE had similar effects as dexamethasone (a corticosteroid) and diclofenac (a COX inhibitor).

The presence of biologically active phytochemicals present in both the aqueous and ethanolic extracts of *P. stratiotes* could have contributed to the anti-inflammatory activity. Tannins, flavonoids, sterols, and glycosides have been documented to have anti-inflammatory effect via several mechanisms.

**CONCLUSION**

*P. stratiotes* has ocular anti-inflammatory effect in EIU-induced uveitis in Sprague-Dawley rats.

**REFERENCES**


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