ORIGINAL ARTICLE

The efficacy of aqueous and ethanolic leaf extracts of *Pistia stratiotes* Linn in the management of arthritis and fever

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Arthritic pain and disability are at or near the top of the list of reasons adult patients seek medical attention. This study therefore attempts to establish the efficacy of an aqueous and ethanolic leaf extract of *Pistia stratiotes* Linn (Araceae) in a rodent experimental model of arthritis and fever to ascertain its importance in the traditional management of this inflammatory disorder. The aqueous and ethanolic extracts of *P. stratiotes* at doses of 30, 100, and 300 mg/kg as well as 0.3 mg/kg methotrexate, 0.46 mg/kg diclofenac and 1 mg/kg dexamethasone were administered to formalin-induced arthritic rats. The same doses of the extracts in comparison to 150 mg/kg acetaminophen were also administered to rats in which fever had been induced with lipopolysaccharides. Data obtained was analyzed using GraphPad Prism 5.0. The results obtained indicated significant reduction (P ≤ 0.05 - 0.01) in paw thickness of formalin-induced arthritic animals treated with both aqueous and ethanolic leaf extracts with effects comparable to that of methotrexate, diclofenac, and dexamethasone. Lipopolysaccharide-induced fever in rats was also significantly reduced (P ≤ 0.05-0.01) at all dose levels of aqueous and ethanolic treated animals in a manner similar to that of acetaminophen. The aqueous and ethanolic leaf extracts of *P. stratiotes* have anti-arthritic and antipyretic effect in formalin-induced arthritis and LPS-induced fever in Sprague-Dawley rats.

Keywords: Formaldehyde-induced arthritis, Fever, Lipopolysaccharide, Prostaglandin

INTRODUCTION

Arthritis is a form of joint disorder that involves inflammation of one or more joints characterized by varied levels of pain, swelling, joint stiffness, and sometimes a constant ache around the joint(s) (CDC, 2011). The world prevalence of arthritis is estimated be around 0.3–1.2 % (Silman and Horchberg, 1993). At least 47.8 million US residents have arthritis. In Europe, the magnitude of the problem is similar, affecting 8 million in the United Kingdom and 108 million across the continent (VanItallie, 2010). The story is no different in Africa. It is the number one disabling disease in South Africa; affecting 132 Million East Africans have minimal rheumatological care (Arthritis Africa, 2012). Although there is no study on the prevalence of arthritis in Ghana the situation is projected to be no better (WHO, 2000). Arthritis makes it very difficult for individuals to be physically active and many become home bound. Arthritic pain and disability are at or near the top of the list of reasons adult patients seek medical attention. Arthritis makes it very difficult for individuals to be physically active and many become home bound.

Although non-steroidal anti-inflammatory agents remains the mainstay treatment for this degenerative inflammatory disorder, its prolong clinical use elicits numerous side effects, notable amongst them are gastric erosion, ulceration, hemorrhage, bronchospasm, kidney and liver dysfunction (Lin et al., 2004). Studies have shown that asymptomatic mu-
cosal damage is initially evident in 80% of subjects after non-steroidal anti-inflammatory drug (NSAID) therapy (Ehsanullah et al., 1988) however, upon continuous use of NSAIDs 15-20% of treated patients develop ulcer (Singh et al., 1996), and 1-3% received hospital treatment for gastrointestinal (GI) bleeding or perforation. Fever is one of the most prominent systemic manifestations of acute inflammation, especially when an inflammation is associated with infection (Romanovsky et al., 2005).

A population based study projects a phenomenal increase in the consumption of non-steroidal anti-rheumatic drug from 380 million to 600 million consumers in the next two decades among the geriatric population alone (Steineyer, 2000). This consolidates the need for an urgent search for new safer and efficacious anti-inflammatory agents. One medicinal plant commonly used traditionally for inflammatory disorders is *Pistia stratiotes*. Commonly known as water lettuce or water cabbage, it is an aquatic plant, stoloniferous, floating on lakes, streams, and stagnant water ponds. It is distributed in the tropical and subtropical region of Asia, Africa, and America. (Arber, 2002). Several medicinal prowess have been ascribed to this plant. These include anti-helminthic, anti-microbial and anti-fungal properties (Prem Kumar and Shyamsundar, 2005; Achola and Indalo, 1997; Sundeep Kumar et al., 2000). The anti-inflammatory and anti-pyretic activity of ethanolic extract of *P. stratiotes* has been demonstrated using carrageenan, cotton-pellet-induced granuloma model and brewer's yeast fever model (Sundeep Kumar et al., 2001).

This study therefore attempts to establish the efficacy of an aqueous and ethanolic leaf extracts of *Pistia stratiotes* in a rodent formalin-induced model of arthritis as it closely depict corresponding human disease state (Greenwald, 1991) and lipopolysacharides-fever model to enhance understanding into the possible mechanism of action. This is to ascertain its importance in the traditional management of inflammatory disorders and to predict its possible mechanism in curbing fever commonly associated with inflammation (Abbiw, 1990).

**MATERIALS AND METHODS**

**Plant Collection**

*Pistia stratiotes* was collected from the Fosu lagoon, Cape Coast in the Central Region of Ghana (5°7’ N &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 at the herbarium for future reference.

**Preparation of 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 litre 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 freeze dried with a Hull freeze dryer /lyophilizer 140 SQ FT (model 140FS275C, USA) into 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 extract of *P. stratiotes* or ET PSE.

**Drugs and Chemicals**

Formaldehyde (Yash Chemicals, India) was used to induce arthritis while LPS (Sigma-Aldrich, USA) was used to induce pyrexia. Diclofenac sodium (KRKA, d.d., Novo mesto, Solvenia), dexamethasone sodium (Anhui Medihel Co. Ltd), and methotrexate sodium (Dabur Pharma, New Delhi, India) were the reference anti-inflammatory agent in this study. Acetaminophen (Simpex Pharmchem Inc. USA) was the reference antipyretic.
Preparation of Reference Drugs
The reference anti-inflammatory drugs were dissolved in normal saline for the study. The drugs were freshly prepared as follows: 0.3 mg/kg methotrexate, 0.46 mg/kg, 1 mg/kg dexamethasone which was administered in volumes not exceeding 10 ml/kg.

Animals
Six to eight-week-old Sprague Dawley rats of either sex (180-200 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 ºC, relative humidity 60-70 %, and normal light-dark cycle). Females were non-pregnant. They were fed with normal commercial pellet diet (GAFCO, Tema) 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.

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 (1996), Glasl (1983), Harborne (1998), and Kujur et al., (2010).

Formaldehyde-Induced Arthritis and Treatment
The test was performed according to the technique developed by Brownlee in 1950. Pedal inflammation was induced by injecting 0.1 ml of 4 % formalin solution below the plantar aponeurosis of the right hind paw of the rats after measuring their paw thickness. The arthritic animals were divided into ten groups of five and treated with either 30, 100, or 300 mg/kg AQ PSE or ET PSE, orally 30 minutes after intra-plantar injection with formalin on day 1, and then daily), 0.3 mg/kg methotrexate intraperitoneally (i.p every four days), 0.46 mg/kg diclofenac (i.p, daily), 1 mg/kg dexamethasone (i.p, every other day), 1 ml/kg normal saline (p.o, daily), the control, over the experimental period.

Lipopolysaccharide-Induced Fever and Treatment
The method of Santos and Rao (1998) was modified and used for the assessment of the anti-pyretic activity of the aqueous and ethanolic extracts of *P. stratiotes*. Animals were fasted overnight prior to induction of fever, but given water ad libitum. Rectal temperature was measured using a lubricated ECT-1 digital thermometer (Estar Electronic And Instrument Co., Ltd., ZheJiang, China) inserted 3 cm deep into the rectum of the rats. Fever was induced by injecting intramuscularly, 1 mg/kg of LPS into the right thigh of each rat. Rectal temperature was measured again and animals that showed an increase in temperature of 0.5 ºC and more were selected for the study. The animals with fever were put into eight groups of five and were treated with either 30, 100, or 300 mg/kg AQ PSE or ET PSE, 150 mg/kg acetaminophen, or 1 ml/kg normal saline solution (the control), orally, two hours after LPS-induced fever. Rectal temperature was measured at 1 h intervals for 6 h. All experiments were carried out between 08.00 h and 18.00 h in a quiet laboratory with an ambient temperature of 25 ± 2 ºC.

Statistical Analysis
Results were analyzed using one way analysis of variance (ANOVA) followed by Dunnet’s multiple comparisons test by using GraphPad Prism; version 5.03. Values were expressed as mean ± SEM and P values ≤ 0.05 were considered statistically significant.

RESULTS
Preliminary Phytochemical Screening
Results for the initial phytochemical screening are as shown in Table 1.

Formaline-induced Arthritis
There were significant reductions (P ≤ 0.01-0.001) in paw thickness of formalin-induced arthritic ani-
Table 1: Results of phytochemical screening of the aqueous and ethanolic extracts of *P. stratiotes* Linn

<table>
<thead>
<tr>
<th>Components</th>
<th>AQ PSE</th>
<th>ET PSE</th>
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<tbody>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Alkaloids</td>
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<td>Sterols</td>
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<tr>
<td>Saponins</td>
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<tr>
<td>Triterpenoids</td>
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“+” implies present, “-“ implies absent

Table 1: Results of phytochemical screening of the aqueous and ethanolic extracts of *P. stratiotes* Linn

In arthritis and fever

Kyei et al.,

mals treated with both aqueous and ethanolic leaf extracts of *P. stratiotes* compared to the normal saline-treated arthritic animals. Similar significant reductions (P ≤ 0.001) in paw thicknesses were observed among the methotrexate, diclofenac, and dexamethasone treated arthritic animals (Figure 1, 2 and 3).

Lipopolysaccharide –Induced Fever

Lipopolysaccharide-induced fever in rats was significantly reduced (P ≤ 0.01-0.001) at all dose levels of AQ PSE and ET PSE treatment; the effect was similar to that observed for acetaminophen treatment (Figures 4 and 5).

Figure 1: Plots of (A) the time-course curves and (B) the area under the time-course curves (AUC) of the effects of 30, 100, and 300 mg/kg of AQ PSE on formalin-induced arthritis in Sprague-Dawley rats. Data are presented as mean ± SEM (n=5). ** implies P ≤ 0.01, *** implies P ≤ 0.001: the level of significance of paw thickness reduction (compared to the control) analyzed by One-way ANOVA followed by Dunnet’s test post hoc. Percentage change in paw thickness was computed using the formula V=(Vt-Vo)/Vo×100 where V is percentage in paw thickness, Vt is paw thickness after formalin challenge, Vo is the initial paw thickness before formalin challenge.

Figure 2: Plots of (A) the time-course curves and (B) the area under the time-course curves (AUC) of the effects of 30, 100, and 300 mg/kg of ET PSE on formalin-induced arthritis in Sprague-Dawley rats. Data are presented as mean ± SEM (n=5). *** implies P ≤ 0.001; the level of significance of paw thickness reduction (compared to the control) analyzed by One-way ANOVA followed by Dunnet’s test post hoc. Percentage change in paw thickness was computed using the formula V=(Vt-Vo)/Vo×100 where V is percentage in paw thickness, Vt is paw thickness after formalin challenge, Vo is the initial paw thickness before formalin challenge.
DISCUSSION

It is established that inhibition of formalin-induced paw oedema in rats is one of the most appropriate modus operandi to screen for anti-arthritic and anti-inflammatory agents as it closely resembles human arthritis (Greenwald, 1991). Injection of formalin subcutaneously into hind paw of rats produces localized inflammation and pain. The nociceptive effect of formalin is biphasic, an early neurogenic component followed by a later tissue mediated response (Wheeler-Aceto and Cowan, 1991). Thus formalin-induced arthritis is a model used for the evaluation of an agent with probable anti-proliferative activity. This experiment is associated with the proliferative phase of inflammation (Banerjee et al., 2000).

The reference drugs and both extracts of *P. stratiotes* significantly suppressed formalin-induced arthritis. Dexamethasone is acknowledged to inhibit the release of pro-inflammatory cytokines (TNF-α, Tumor Necrosis Factor- α and IL-1β, interleukin-1β), which are known to play a central role in the pro-
Acetaminophen is a reputable antipyretic analgesic agent, often administered therapeutically to ease pain and fever (Ayoub et al., 2004). The main mechanism proposed is the inhibition of COX, and recent findings suggest that it is highly selective for COX-2 (Hinz et al., 2008). Paracetamol reduces the oxidized form of the COX enzyme, preventing it from forming pro-inflammatory chemicals (Roberts et al., 2001; Högestätt et al., 2005). This leads to a reduced amount of Prostaglandin E2 in the CNS, thus lowering the hypothalamic set-point in the thermoregulatory centre.

Oral administration of both aqueous and ethanolic leaf extracts of *P. stratiotes* as earlier indicated could possibly be inhibiting COX-2 and subsequent production of prostaglandins thereby exhibiting potent hypothermic effect in LPS-induced fever in Sprague-Dawley rats at much lower doses. (Sundeep Kumar...
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 (Mota *et al.*, 1985; Owoyele *et al.*, 2010), flavonoids (Borissova *et al.*, 1994; Hämäläinen *et al.*, 2007), sterols (Bouic *et al.*, 1996; Bouic, 1998; Akihisa *et al.*, 2007), alkaloids (Barbosa-Filho *et al.*, 2006) and glycosides (Odontuya *et al.*, 2005; Liu and Wang, 2011) have been documented to have anti-inflammatory effect via several mechanisms.

**CONCLUSION**

*P. stratiotes* has anti-arthritic and antipyretic effect in formalin-induced arthritis and LPS-induced fever in Sprague-Dawley rats.

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Pistia stratiotes in arthritis and fever
Kyei et al.,