Journal of Medical and Biomedical Sciences (2012) 1(2): 29-37

ORIGINAL ARTICLE

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

S. Kyei[1], G. A. Koffuor[1] and J. N. Boampong[2]

[1]Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, KNUST, Kumasi, Ghana; [2]Department of Human Biology, School of Biological Sciences, University of Cape Coast, Cape Coast, Ghana.

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 ($P \leq 0.05$-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. Lipopolysacharride-induced fever in rats was also significantly reduced ($P \leq 0.05$-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.
Journal of Medical and Biomedical Sciences (2012) 1(2), 29-37

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 an estimated one in every seven people. About 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-steriodal 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 live dysfunction (Lin *et al.,* 2004). Studies have shown that asymptomatic mu-

Correspondence: *G.A. Koffuor, Department of Pharmacology, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. E*-mail: gkoffuor@yahoo.com

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 prowesses have been ascribed to this plant. These include anti-helmintic, 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.,* 20011).

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 1litre of water. The mixture was maintained at 80 ^{o}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 ^{o}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 ^{o}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 Pharamchem 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 (34cm × 47cm× 18cm) 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) 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 3cm 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 6h. All experiments were carried out between 08.00 h and 18.00 h in a quiet laboratory with an ambient temperature of 25 ± 2ºC.

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.

Formalin-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

Components	AQ PSE	ET PSE
Tannins	+	+
Flavonoids	+	+
Alkaloids	+	-
Sterols	+	+
Glycosides	+	+
Saponins	-	-
Triterpenoids	-	-

"+" implies present, "-"implies absent

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

Lipopolysacharride –Induced Fever

Lipopolysacharride- induced fever in rats was significantly reduced ($P \leq 0.01$-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).

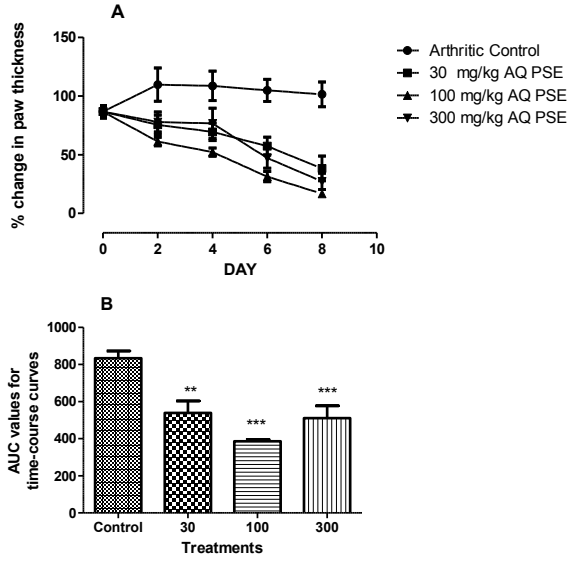

Figure 1: Plots of (A) the time-course curves and (B) the area under the time-course curves (AUC) of the effects of 30, 100, and 300 mg/kg of AQ PSE on formalin-induced arthritis in Sprague-Dawley rats. Data are presented as mean ± SEM (n=5). ** implies $P \leq 0.01$, ***implies $P \leq 0.001$: the level 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

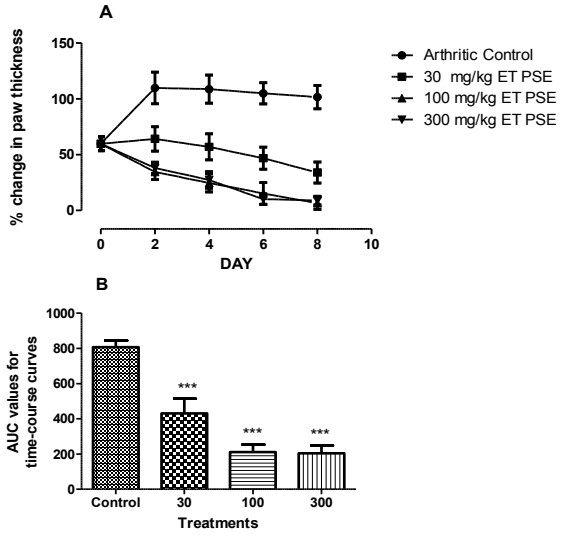

Figure 2: Plots of (A) the time-course curves and (B) the area under the time-course curves (AUC) of the effects of 30, 100, and 300 mg/kg of ET PSE on formalin-induced arthritis in Sprague-Dawley rats. Data are presented as mean ± SEM (n=5). * implies $P \leq 0.001$; the level of significance of paw 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**

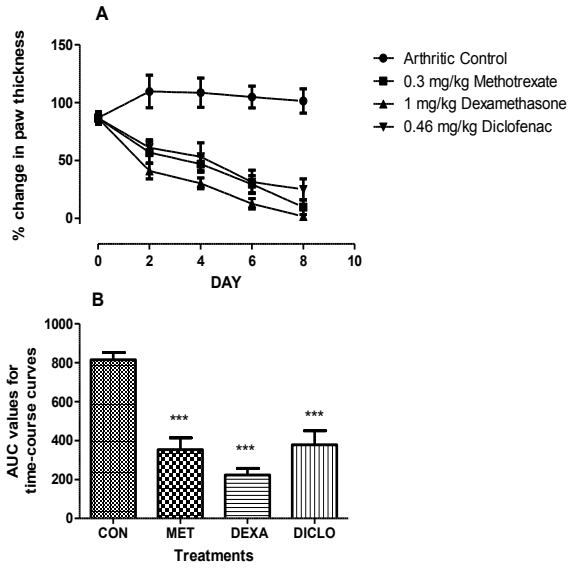

Figure 3: Plots of (A) the time-course curves and (B) the area under the time-course curves (AUC) of the effects of 0.3 mg/kg methotrexate, 1 mg/kg dexamethasone, and 0.46 mg/kg diclofenac on formalin-induced arthritis in Sprague-Dawley rats. Data are presented as mean ± SEM (n=5). * implies $P \leq 0.001$; 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**

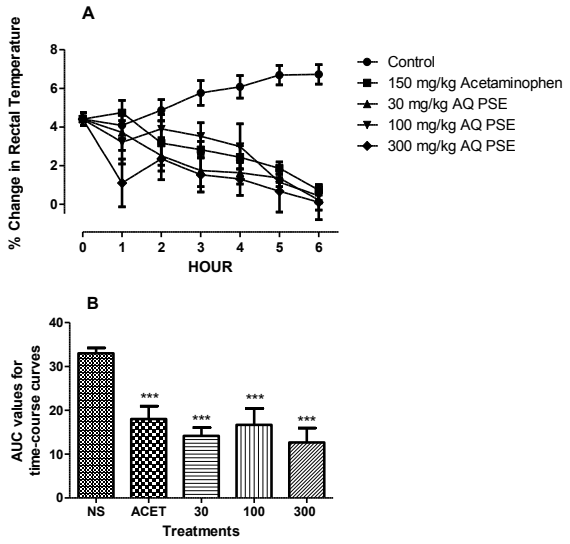

Figure 4: Plots of (A) the time-course curves and (B) the area under the time-course curves (AUC) of the effects of 30, 100, and 300 mg/kg of AQ PSE and 150 mg/kg acetaminophen on LPS-induced fever in Sprague-Dawley rats. Data plotted are means ± SEM (n=5). ***implies $P \leq 0.001$; the level of significance of rectal temperature reduction (compared to the normal saline-treated) analyzed by One-way ANOVA followed by Dunnet's test *post hoc*. Percentage change in rectal temperature was computed using the formula T=(Tt-To)/ To × 100 where T is percentage in rectal temperature, Tt is rectal temperature after LPS challenge, To is the initial rectal temperature before LPS 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 prop-

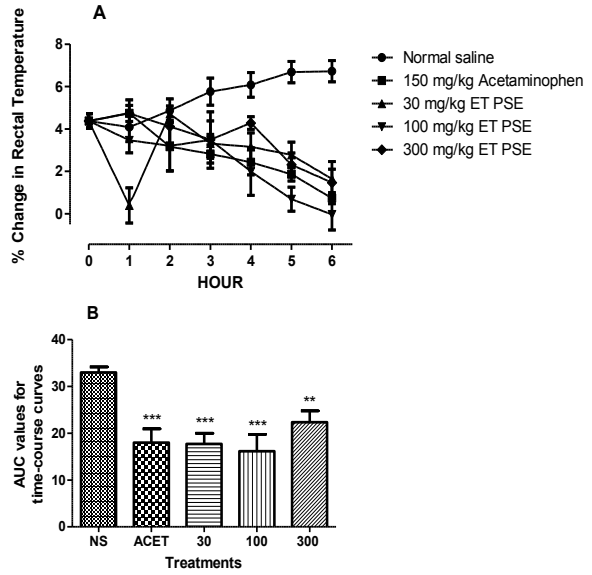

Figure 4: Plots of (A) the time-course curves and (B) the area under the time-course curves (AUC) of the effects of 30, 100, and 300 mg/kg of ET PSE and 150 mg/kg acetaminophen on LPS-induced fever in Sprague-Dawley rats. Data plotted are means ± SEM (n=5). ** implies P ≤ 0.01; ***implies *P* ≤ 0.001: the level of significance of rectal temperature reduction (compared to the normal saline-treated) analyzed by One-way ANOVA followed by Dunnet's test *post hoc*. Percentage change in rectal temperature was computed using the formula T=(Tt-To)/ To × 100 where T is percentage in rectal temperature, Tt is rectal temperature after LPS challenge, To is the initial rectal temperature before LPS challenge

agation of the disease process in arthritis thus its ability to arrest paw swelling (Issekutz and Issekutz, 1991). Methotrexate inhibits proliferation of the lymphocytes and other cells responsible for inflammation in the joint (Gubner, 1951). The anti-inflammatory effect of diclofenac is mediated mainly through inhibition of COX and prostaglandin production (Furst and Manning, 2001).

Fever is one of the most prominent systemic manifestations of acute inflammation, especially when an inflammation is associated with infection (Romanovsky *et al.*, 2005). These reactions represent the primary host defense response to infection; collectively called the "acute-phase reaction"(Blatteis, 1992). The usual view of the mechanism by which infectious fevers are produced stipulates that infectious noxa e.g. bacterial endotoxic lipopolysaccharides (LPS) that invade the body activate mononuclear phagocytes that then produce and release pyrogenic cytokines including IL-1 β and TNF-α. These are transported via the bloodstream to the ventromedial preoptic area of the anterior hypothalamus, the "fever producing center", where they operate (Saper, 1998; Roth and De Souza, 2001; Dunn, 2002; Dinarello, 2004). It is, however, doubtful how cytokines, as hydrophilic peptides, could penetrate the brain. That is to say, it is generally believed that, rather than acting directly, the cytokines induce the local generation and release of prostaglandin E_2, a lipid mediator that is obviously thermogenic when injected centrally (Blatteis, 1997; Ivanov and Romanovsky, 2004). Its production is dependent on the activation of two enzymes, cyclooxygenase (COX)-2 and microsomal PGE synthase-1, which catalyze its conversion from arachidonic acid present in the membranes of cells (Ivanov *et al.,* 2002).

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

et al., 2011).

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.

ACKNOWLEDGEMENT

The Authors are grateful for the technical assistance offered by Mr. Thomas Ansah of the Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana for his technical assistance and Mr. G H Sam of the Department of Herbal Medicine, CHS, KNUST, Kumasi, Ghana for identification and authentication of the plant.

REFERENCES

Abbiw DK (1990).Useful plants of Ghana: West African uses of wild and cultivated plants. Intermediate Technology Publications and the Royal Botanic Gardens Kew.

Achola KJ, Indalo AA. Pharmacologic activities of *Pistia stratiotes* (1997). *Pharmaceut Biol 35(5): 329-333.*

Akihisa T, Nakamura Y, Tagata M, Tokuda H, Yasukawa K, Uchiyama E, Suzuki T, Kimura Y (2007).Anti-inflammatory and anti-tumor-promoting effects of triterpene acids and sterols from the fungus Ganoderma lucidum. *Chem Biodivers. 4(2):224-31.*

Arber A (2002). The Vegetative Morphology of Pistia and the Lemnaceae*Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character.* 91, :636 pp. 96-103 The Royal Society .Available from URL: http://www.jstor.org/stable/80788

Ayoub SS, Botting RM, Goorha S, Colville-Nash PR, Willoughby DA, Ballou LR (2004). Acetaminophen-induced hypothermia in mice is mediated by a prostaglandin endoperoxide synthase 1 gene-derived protein. *Proc Natl Acad of Sci USA 101:30;11165–11169*

Banerjee S , Kumar Sur T, Mandal S, Chandra Das P, Sikdar S (2000). Assessment of the anti-inflammatory effects of *Swertia chirata* in acute and chronic experimental models in male albino rats. *Indian Journal of Pharmacol 32: 21-24*

Barbosa-Filho JM, Piuvezam MR, Moura MD, Silva MS, Batista Lima KV, Leitão da-Cunha EV Fechine IM, Takemura OS (2006).Anti-inflammatory activity of alkaloids: a twenty-century review .*Rev. bras. farmacogn. 16 (1)*

Blatteis CM (1992). The pyrogenic action of cytokines Interleukin-1 in the Brain. Oxford: Pergamon Press; 93–114.114eds. Rothwell NJ and Dantzer RD.

Blatteis CM. (1997) Prostaglandin E2: a putative fever mediator. In: Mackowiak P.A. (Ed.), *Fever: Basic Mechanisms and Management* (2nd ed.). Lippincott-Raven, Philadelphia, PA, pp. 117–145

Borissova P, Valcheva S, Belcheva A(1994). Anti-inflammatory effect of flavonoids in the natural juice from Aronia melanocarpa, rutin and rutin-magnesium complex on an experimental model of inflammation induced by histamine and serotonin. *Acta Physiol Pharmacol Bulg. 20(1):25-30.*

Bouic PJD (1998). Sterols/Sterolins: The natural, nontoxic immuno-modulators and their role in the control of rheumatoid arthritis. *The Arthritis Trust;Summer:3-6.*

Bouic PJD, Etsebeth S, Liebenberg RW, Albrecht CF, Pegel K, Van Jaarsveld PP(1996).Beta-sitosterol and beta-sitosterol glycoside stimulate human peripheral blood lymphocyte proliferation: implications for their use as an immunomodulatory vitamin combination. *Int J Immunopharmacol 18:693-700.*

Brownlee G. (1950). Effect of deoxycortone and

ascorbic acid on formaldehyde induced arthritis in normal and adrenalectomised rats. *Lancet. 1, 157-l 59*

Centre of Disease Control and Prevention. Arthritis Basics. Division of adult and community health, National Centre for Chronic Disease Prevention and Health Promotion (2011).

Dinarello CA. (2004) Infection, fever, and exogenous and endogenous pyrogens: some concepts have changed. *J. Endotoxin Res., 10: 201–222.*

Dunn AJ (2002). Mechanisms by which cytokines signal the brain. *Int. Rev. Neurobiol., 52: 43–65.*

Ehsanullah RS, Page MC, Tildesley G, Wood JR (1988). Prevention of gastroduodenal damage induced by non-steroidal anti-inflammatory drugs: controlled trial of ranitidine. *Br Med J 297:1017-21.*

Furst DE, Manning DC (2001). Future directions in pain management. *Clin. Exp. Rheumatol. 19:71-76.*

Glasl H (1983). Zur Photometrie in der Drogenstandiserung. DAZ, 123:1979-1987

Greenwald RA (1991). Animal models for evaluation of arthritic drugs. *Meth Find Clin Pharmacol;**13**:75-83.*

Gubner R (1951). Effect of aminopterin on epithelial tissues. *Arch. Dermatol. 64, 688-699.*

Hämäläinen M, Nieminen R, Vuorela P, Heinonen M, Moilanen E (2007). Anti-inflammatory effects of flavonoids: genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-kappaB activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-kappaB activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. *Mediators Inflamm. 2007:45673.*

Harborne JB (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 3rd ed. London. Chapman and Hall. p 302

Hinz B, Cheremina O, Brune K (2008). Acetaminophen (paracetamol) is a selective cyclooxygenase-2 inhibitor in man. *The FASEB 22 (2): 383–390.*

Högestätt ED, Jönsson BA, Ermund A, Andersson DA, Björk H, Alexander JP, Benjamin F. Cravatt BF, Basbaum AI and Zygmunt PM (2005). Conversion of acetaminophen to the bioactive N-acylphenolamine AM404 via fatty acid amide hydrolase-dependent arachidonic acid conjugation in the nervous system. *J. Biol. Chem. 280 (36): 31405–12.*

Issekutz AC. and Issekutz TB (1991). Quantitation and kinetics of polymorphonuclear leukocyte and lymphocyte accumulation in joints during adjuvant arthritis in the rat. *Lab. Invest., 64: 656-663.*

Ivanov AI and Romanovsky AA (2004). Prostaglandin E2 as a mediator of fever: synthesis and catabolism. *Front. Biosci.9: 1977–1993.*

Ivanov AI, Pero RS, Scheck AC, Romanovsky AA (2002). Prostaglandin E (2)-synthesizing enzymes in fever: differential transcriptional regulation. *Am. J. Physiol., 283: R1104–R1117*

Kujur RS, Singh V, Ram M, Yadava HN, Singh KK, Kumari S, Roy BK (2010). Antidiabetic activity and phytochemical screening of crude extract of *Stevia rebaudiana* in alloxan-induced diabetic rats. *Phcog Res 2:258-63*

Lin J, Zhang W, Jones A, Doherty M (2004). Efficacy of topical non-steroidal anti-inflammatory drugs in the treatment of osteoarthritis: meta-analysis of randomised controlled trials. *Br Med J;329:324*

Liu X, Wang J. Anti-inflammatory effects of iridoid glycosides fraction of Folium syringae leaves on TNBS-induced colitis in rats (2011). *J Ethnopharmacol. 27;133(2):780-7.*

Mota ML, Thomas G, Barbosa Filho JM (1985). Anti-inflammatory actions of tannins isolated from the bark of Anacardium occidentale L. *J Ethnopharmacol. 13(3):289-300.*

National Institutes of Health, Office of Science and Health Reports,Guide for care and use of laboratory animals. Publication no. 83-23.Office of Science and Health Reports, Department of Health and Human Services. Bethesda, 1996 MD

Odontuya G, Hoult JRS, Houghton PJ (2005).

Structure-Activity Relationship for Anti-inflammatory Effect of Luteolin and its Derived Glycosides. *Phytother. Res. 19, 782–786*

Owoyele BV, Negedu MN, Olaniran SO, Onasanwo SA, Oguntoye SO, Sanya JO Oyeleke SA, Ibidapo AJ, Soladoye AO (2010). Analgesic and anti-inflammatory effects of aqueous extract of Zea mays husk in male Wistar rats. *J Med Food. 13(2):343-7*.

Prem kumar VG, Shyamsundar D. Antidermatophytic activity of pistia stratiotes (2005). *Indian J Pharmacol.37:127-8*

Roberts LJ, Marrow, J.D (2001). "Analgesic-antipyretic and Antiinflammatory Agents and Drugs Employed in the Treatment of Gout" in, "Goodman & Gilman's The Pharmacological Basis of Therapeutics 10th Edition" by Hardman, J.G. & Limbird, L.E. Published by McGraw Hill. pp.687–731

Romanovsky AA, Almeida MC, Aronoff DM, Ivanov AI, Konsman JP, Steiner AA, Turek VF (2005). Fever and hypothermia in systemic inflammation: Recent discoveries and revisions. *Front. Biosci. 10: 2193-2216*

Roth J, De Souza, GE (2001). Fever induction pathways: evidence from responses to systemic or local cytokine formation. *Braz. J. Med. Biol. Res., 34: 301–314*.

Santos FA, Rao VS (1998). A study of the antipyretic effect of quinine, an alkaloid effective against cerebral malaria, on fever induced by bacterial endotoxin and yeast in rats. *J Pharm Pharmacol 50: 225-9*.

Saper CB (1998). Neurobiological basis of fever. *Ann. N.Y. Acad. Sci., 856: 90–94*.

Silman AJ, Horchberg MC (1993). Rheumatoid arthritis In: Silman AJ, Horchberg MC eds. Epidemiology of the rheumatic diseases. Oxford: Oxford Medical Publications, 7–*68*.

Singh G, Ramey DR, Morfeld D, Shi H, Hatoum HT, Fries JF (1996). Gastrointestinal tract complications of nonsteroidal anti-inflammatory drug treatment in rheumatoid arthritis: a prospective observational cohort study. *Arch Intern Med. 156(14):1530-6*.

Steinmeyer J (2000).Die medikamentöse Therapie der Arthrose. *Sport Orthop Traumatol **16**:19-25*

Sundeep Kumar HK, Anindya Bose, Arundhuti Raut, Sujit Kumar Sahu S and Raju M. B. V (2000)**.** Evaluation of Anthelmintic Activity of *Pistia stratiotes* Linn. *J Basic and Clin Pharm, JBCP 1: (2) (2000)*

Sundeep Kumar HK, Raju MBV, Dinda SC, Sahu SK and Banerjee M (2011). Analgesic, Anti-inflammatory and Antipyretic Activity of *Pistia Stratiotes* L. *RASAYAN J. Chem.4 (3) ;506-511*

Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography. 2nd ed. New York. Springer Verlag, 1996

Wheeler-Aceto H, Cowan A (1991). Neurogenic and tissue mediated components of formalin-induced oedema. *Agents Actions;**34**:264-*9.

WHO (2003). The burden of Musculoskeletal conditions at the start of the new millennium. Report of a WHO Scientific Group WHO Technical Report Series 919. Geneva

ISSN 2026-6294