Possible mechanism of anti-inflammatory activity and safety profile of aqueous and ethanolic leaf extracts of Pistia stratiotes Linn (Araceae)

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POSSIBLE MECHANISM OF ANTI-INFLAMMATORY ACTIVITY AND SAFETY PROFILE OF AQUEOUS AND ETHANOLIC LEAF EXTRACTS OF *PISTIA STRATIOTES* LINN (ARACEAE)

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ABSTRACT
Pistia stratiotes is used extensively in inflammatory disorders in several countries including Ghana. The aim of the study was to investigate the anti-inflammatory property and possible mechanism of action of aqueous and ethanolic leaf extracts of Pistia stratiotes and to ascertain its safety for use. In separate experiments, acute inflammation was induced in the right hind paws of rats using carrageenan, histamine, serotonin, prostaglandin E, and bradykinin. Paw thickness (an indication of inflammation) were measured plethysmographically. Animals were grouped and treated with diclofenac, chlorpheniramine, and granisetron (reference anti-inflammatory agents), or aqueous and ethanolic extracts of Pistia stratiotes at doses of 30, 100, and 300 mg/kg orally. Control groups received distilled water. Paw thicknesses was measured at 30 or 60 min intervals for 2.5 to 4 h for the various procedures. The extracts at all doses significantly reduced (P≤ 0.05-0.001) paw thickness in all the models of inflammation except the 300 mg/kg doses in carrageenan and serotonin-induced inflammation. The anti-inflammatory effects of the extracts were comparable to the reference drugs. Acute and delayed toxicity test revealed that the aqueous extract causes hemolysis of red blood cells (reduced count and presence of uro-bilinogen in urine) and possible acute kidney function impairment (proteinuria and microalbu-minuria). The aqueous and ethanolic extracts of P. stratiotes have anti-inflammatory activity in acute inflammation induced with carrageenan, through the inhibition of histamine, serotonin, prostaglandin, and bradykinin. Hematological profile as well as liver and kidneys function should be monitored when used orally for the treatment of inflammatory disorders.

INTRODUCTION

Inflammation is a usual protective response of living mammalian tissues to injury. It is the body's response to inactivate or raze the invading organism, to eliminate the irritant and to set the stage for repair (Bhitre *et al.*, 2008). It is an important pathophysiological parameter in the development, maintenance and aggravation of most infectious and non-infectious disorders of mammals (Sosa *et al.*, 2002; Dutta and Das, 2010). It is involved in disorders in a number of body systems e.g. uveitis (Kalariya, 2010), atherosclerosis, and asthma (Danese, 2010) and this makes an important concern to clinicians and scientists.

The current treatment of inflammation with steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) has some serious adverse effects such as immunosuppression (Leung and Bloom, 2003) delayed wound healing, osteoporosis, (Gennari, 1993) cataract formation, and increased intraocular pressure (Friedman and Kaiser, 2007) related to steroids, and gastric ulceration, induction of asthma, bleeding disorders and renal effects, (Cluett, 2011) related to NSAIDs, reduces their use in some individuals (Juni *et al.*, 2005; Pathak *et al.*, 2005; Singh *et al.*, 2010). Hence, there is the need to explore alternative means of drug treatment with novel modes of action and moderately less side effects.

Natural product-based anti-inflammatory agents with a transcriptional mode of action, good efficacy, and lower risk of side effects offer promising treatment and prevention option for inflammation-related disorders (Tripathy *et al.*, 2010). Traditionally, *Pistia stratiotes* (Family: Araceae) (Arber, 2002) commonly known as Water lettuce, Water cabbage, or Tropical duckweed has been employed in the management of opthalmia and iritis among some Ghanaians (Abbiw, 1990; Tripathi *et al.*, 2010). It has been established to have anti-inflammatory effect in carrageenan-induced model of acute inflammation in rats (Kumar, *et al.,* 2011).

This study therefore seeks to investigate the anti-inflammatory activity and possible mechanism of action of the aqueous and hydroethanolic leaves extracts of *Pistia stratiotes* and to assess its safety for use .

MATERIALS AND METHODS
Collection of Plant Material
Pistia stratiotes was collected from the Fosu lagoon, Cape Coast (5°7′ N &1°16′ W) in December 2010. It was identified and authenticated at the Faculty of Pharmacy Herbarium, KNUST. A voucher specimen bearing the number KNUST/ HM1/11/W002 was deposited in the herbarium for future reference.

Preparation of Extracts
The leaves of the plant was collected in bulk and washed with tap water to remove the dirt and then dried. The dried plant was milled into coarse powder by a mechanical grinder. The powder material was extracted using the hot extraction method. The supernatant was freeze dried to obtain a powdered sample. The percentage yield of the aqueous and hydroethanol extracts was calculated as 4.7 and 5.2 % w/w respectively. These were labeled and kept at a temperature of 4°C for subsequent use. In this study, the aqueous and hydroethanolic leaf extracts would be referred to as AQ PSE and ET PSE respectively.

Animals
Eight-week old Sprague Dawley rats of either sex (weight: 205 ± 20 g) were purchased from the Centre for Scientific Research into Plant Medicine (CSIRPM), Mampong, Ghana, and maintained in the animal house of the Department of Pharmacology, KNUST. The animals were housed in groups of eight in polyacrylic cages (34cm × 47cm× 18cm) with soft wood shaving as bedding, fed with normal commercial pellet diet (GAFCO, Tema), given water *ad libitum* and maintained under laboratory conditions (temperature $28 \pm 2^{o}C$, relative humidity 60-70 %, and normal light-dark cycle). 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, 1996). The protocols for the study were approved by the Departmental Ethics Committee.

Drugs and Chemicals
Carrageenan, histamine, serotonin-creatinine sulphate complex (Sigma and Aldrich, St. Louis MO, USA), prostaglandin E_2 and bradykinin (Sigma Life Science, USA) were the inflammogens used. Diclofenac sodium (KRKA, d.d., Novo mesto, Solvenia), a *cyclooxygenase* (COX) inhibitor; chlopheniramine (Pharmanova, Accra), a H_1 -receptor antagonist; Granisetron (Corepharma LLC, Middlesex, USA), a 5-HT_3-receptor antagonist were the reference antagonists used.

Preliminary phytochemical screening
Phytochemical screening was conducted on AQ PSE and ET PSE to ascertain the presence of starch, tannins, glycosides (sapogenetic, anthracene, and cyanogenetic), flavonoids, steroids and alkaloids (Wagner and Bladt, 1996; Harborne, 1998; Kujur *et al.*, 2010). The tannins content was determined according to the method of Glasl (1983) using pyrogallol (99.5 % HPLC) as reference compound.

Carrageenan -Induced Paw Edema
The experimental animals were fasted 24 h prior to induction of edema (water was however available *ad libitum*). Paw edema (acute inflammation) was induced by sub-plantar injection of 0.2 mg of carrageenan in distilled water into the right hind paws of the animals (Londonkar *et al.,* 2010; Ratnasooriya and Fernando, 2009). They were then put into eight groups of five. Paw edema (expressed as an increase in paw volume) was measured with an electronic von frey plethysmometer (IITC Life Science Inc., California, USA). Each group was treated with one of these; AQ PSE (30, 100, or 300mg/kg, *per os*), ET PSE (30, 100, or 300mg/kg, *per os*), Diclofenac sodium (0.93 mg/kg, i.p) 1h after induction of edema. The control group received distilled water (1 ml/kg, *per os*). Animals were deprived of water during the experimental period to ensure uniform hydration and reduce variability in edematous response. Paw edema was measured again 1, 2, and 3 h post-treatment. Percentage changes in paw volume were calculated and recorded.

Histamine –Induced Paw Edema
The experimental procedure was similar to that described in the carrageenan-induced paw edema model butpaw edema (acute inflammation) was induced with 0.1 mg of histamine (Mazumder *et al.*, 2003). The reference anti-inflammatory agent was chlorpheniramine (0.35mg/kg; *per os*). Each group was treated with either AQ PSE (30, 100, and 300 mg/kg, *per os*) or ET PSE (30, 100, and 300mg/kg, *per os*). The control group received distilled water (1 ml/kg, *per os*). Paw edema was measured at 30 min interval for 2.5 h post-treatment and the percentage changes in paw volumes calculated and recorded.

Serotonin-Induced paw Edema
The experimental procedure was similar to that described in the carrageenan-induced paw edema model but paw edema (acute inflammation) was induced with 0.1 mg of serotonin-creatinine sulphate complex (Mazumder *et al.*, 2003). The reference anti-inflammatory agent was Granisetron (28.5 mcg/kg *per os*). Each group was treated with either AQ PSE (30, 100, and 300mg/kg, *per os*) or ET PSE (30, 100, and 300mg/kg, *per os*). The control group received distilled water (1 ml/kg, *per os*). Paw edema was measured 30 min interval for 2.5 h post-treatment and the percentage changes in paw volumes calculated and recorded.

Prostaglandin -Induced Paw Edema
Prostaglandin E_2, 0.2 ml (1nM) was administered into the sub-planter region of the right hind paw of rats, in accordance with the method of Willis and Cornelsen (1973). The paw volume up to the ankle joints were measured plethysmographically before and after 30 min of the prostaglandin E_2 stimulation. Each group was treated with either AQ PSE (30, 100, and 300mg/kg, *per os*), ET PSE (30, 100, and 300mg/kg, *per os*), or Diclofenac sodium (0.93 mg/kg, i.p). The control group received distilled water (1 ml/kg, *per os*). Paw edema was measured 0.5, 1, 1.5, 2 and 2.5 h post-treatment and the percentage changes in paw volumes calculated and recorded.

Bradykinin -Induced Paw Edema
Sprague-Dawley rats were pretreated with captopril 1h before bradykinin injection to prevent kininase of the bradykinin. The stock solutions for bradykinin were prepared in phosphate-buffered saline PBS (1-10mM) in siliconized plastic tubes, maintained at 18°C just before use. Each animals was injected with 0.2ml of 10nmol per paw into the right hind paw 30 min after administering the extract and vehicle as (distilled water 1 ml/kg, *per os*) in the carragee-

nan model were administered. No selective antagonist was given in this model.

Acute and Delayed Toxicity Test

The rats were assigned to six treatment groups with five animals per group. The aqueous extract of *Pistia stratiotes* (AQ PSE) was administered at doses of 10, 30, 100, and 300 mg/kg. The control group (non-treatment group) was given 1 ml/kg distilled water. Observation for clinical and behavioral symptoms of toxicity and mortality were made hourly for 24 h and then daily thereafter for 14 days. The time of onset, intensity, and duration of these symptoms, if any, was recorded.

Blood samples from the control and treated rats were collected into EDTA treated sample tubes before treatment, 24 h after treatment, and then 10 days post-treatment for hematological assessment using the BC-3000 Plus Auto hematology Analyzer (Mindray, Shenzhen, China). Hematological parameters measured are as shown in Table 1.

A quantitative biochemical test was performed on fresh urine samples obtained from these rats in metabolic cages (Ugo Basile Biological Research Equipment, Comerio, Va., Italy) prior to treatment, 24h, and then 10 days post-treatment using H11-MA reagent strips for urinalysis (Durui, The Hague, Netherlands). Parameters measured were urobilinogen, bilirubin, ketone (acetoacetic acid), blood, protein, nitrite, leucocytes, glucose, specific gravity, pH, and microalbumin. The color, appearance and smell of the urine samples were also noted.

RESULTS

Preliminary phytochemical screening

AQ PSE has tannins, flavonoids, alkaloids, sterols and glycosides. Saponins and triterpenoids were not present in the extract. ET PSE contains tannins, sterols, glycosides, and flavonoids but had no alkaloids, saponins and triterpenoids.

Anti-Inflammatory Effect

Injection of carrageenan, histamine, serotonin, prostaglandin E, bradykinin resulted in acute inflammation (seen as paw edema) of the right hind paws of the rats within the first 0.5-1 h (Figures 1-10). Having treated the rats with the reference drugs and the extracts, it was observed then on that the 30, 100 and 300 mg/kg AQ PSE and ET PSE treated groups had significant reduction ($P \leq 0.01$-0.001) in paw edema in the histamine, prostaglandin, and bradykinin-induced inflammation (Figures 3, 4, 7-10); however only the 30 and 100 mg/kg doses showed significant effects ($P \leq 0.05$-0.01) in the carrageenan and serotonin-induced inflammation. The 300 mg/kg doses showed no significant reduction ($P > 0.05$) in paw edema (Figure 1, 2, 5, and 6). All reference drugs caused very significant reductions ($P \leq 0.001$). These observations signify that both extracts had anti-inflammatory properties comparable to the reference drugs.

Toxicological Studies

Administration of AQ PSE caused no death at all doses over the entire experimental period. There were no labored breathing, constipation, emaciation, skin eruptions, abnormal posture, hemorrhage, sedation, diarrhoea, polyuria, polydipsia, polyphagia, anorexia, rhinor-nasal congestion, loss of autonomic reflexes, neuromuscular inco-ordination and collapse, hyperesthesia, hypothermia, twitching, spasticity, convulsion, writhing, and respiratory depression. Observations of the gait did not show uncoordinated, staggering, wobbly gait, hind limbs exaggerated, overcompensating, and/or making splayed movements, feet (primarily hind feet) point outward from body, forelimbs dragging and/or showing abnormal positioning, nor walking on toes (the heels of the hind feet are perpendicular to the surface). Hematological assessments revealed significant reductions ($P \leq 0.05$-0.01) in RBC count in all treatment groups over the 10 day period (Table 1). Urine analysis of treated and untreated rat showed no significant changes in measured parameters over the experimental period except urobilinogen and microalbumin which increased significantly in all treatment groups 10 days post-treatment. Urine color changed from straw to amber with in the same period (Table 2).
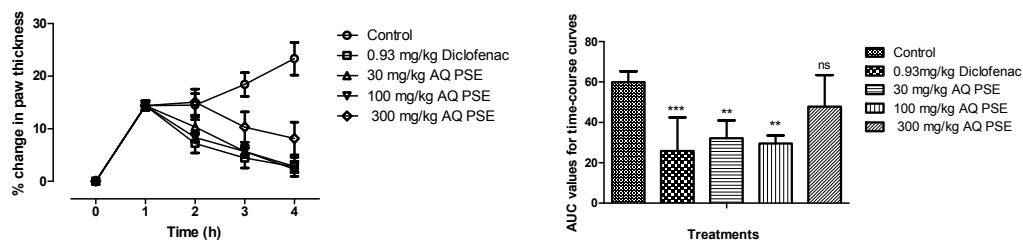
Figure 1: The effects of 30, 100, and 300 mg/kg of AQ PSE on carrageenan-induced paw edema in Sprague-Dawley rats. Data are presented as mean ± SEM (n=5). $^{ns}P > 0.05,** P< 0.01, ***P< 0.001$ are the level of significance of paw edema reduction compared to the control, analyzed by One-way ANOVA followed by Dunnet's test *post hoc*.
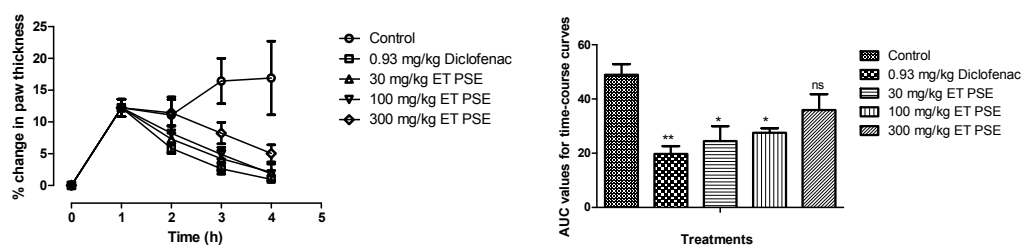

Figure 2: The effects of 30, 100, and 300 mg/kg of ET PSE on carrageenan-induced paw edema in Sprague-Dawley rats. Data are presented as mean ± SEM (n=5). $^{ns}P > 0.05,* P< 0.05, **P< 0.01$ are the level of significance of paw edema reduction compared to the control, analyzed by One-way ANOVA followed by Dunnet's test *post hoc*.

Figure 3: The effects of 30, 100, and 300 mg/kg of AQ PSE on histamine-induced paw edema in Sprague-Dawley rats. Data are presented as mean ± SEM (n=5). $** P< 0.01, ***P< 0.001$ are the level of significance of paw edema reduction compared to the control, analyzed by One-way ANOVA followed by Dunnet's test *post hoc*.
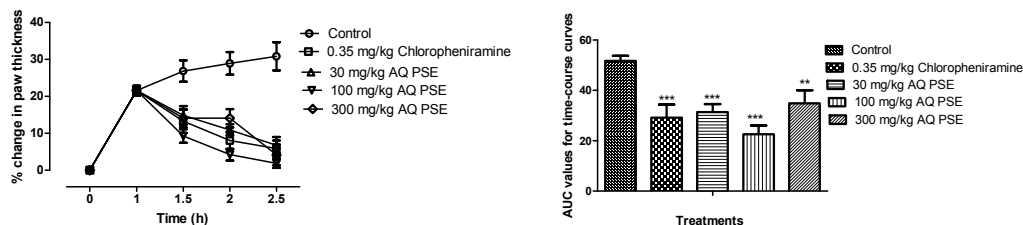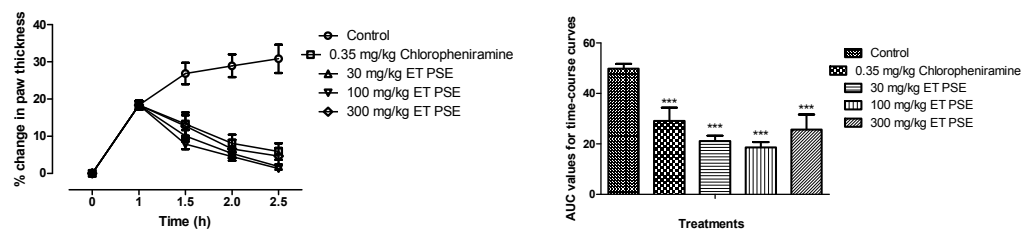

Figure 4: The effects of 30, 100, and 300 mg/kg of ET PSE on histamine-induced paw edema in Sprague-Dawley rats. Data are presented as mean ± SEM (n=5). $***P < 0.01$ is the level of significance of paw edema reduction compared to the control, analyzed by One-way ANOVA followed by Dunnet's test *post hoc*.

Figure 5: The effects of 30, 100, and 300 mg/kg of AQ PSE on serotonin-induced paw edema in Sprague-Dawley rats. Data are presented as mean ± SEM (n=5). $^{ns}P > 0.05$, $P< 0.01$, ***$P< 0.001$ are the level of significance of paw edema reduction compared to the control, analyzed by One-way ANOVA followed by Dunnet's test *post hoc*.**

Figure 6: The effects of 30, 100, and 300 mg/kg of ET PSE on serotonin-induced paw edema in Sprague-Dawley rats. Data are presented as mean ± SEM (n=5). $^{ns}P> 0.05$, ** $P< 0.01$, ***$P< 0.001$ are the level of significance of paw edema reduction compared to the control, analyzed by One-way ANOVA followed by Dunnet's test *post hoc*.
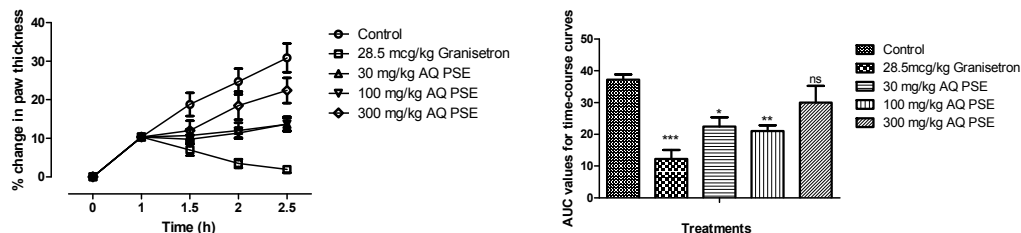

Figure 7: The effects of 30, 100, and 300 mg/kg of AQ PSE on Prostaglandin-induced paw edema in Sprague-Dawley rats. Data are presented as mean ± SEM (n=5). ** $P< 0.01$, ***$P< 0.001$ are the level of significance of paw edema reduction compared to the control, analyzed by One-way ANOVA followed by Dunnet's test *post hoc*.
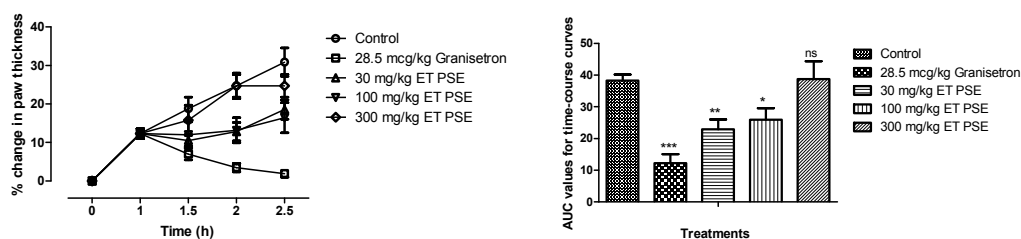

Figure 8: The effects of 30, 100, and 300 mg/kg of ET PSE on prostaglandin-induced paw edema in Sprague-Dawley rats. Data are presented as mean ± SEM (n=5). ***$P< 0.001$ is the level of significance of paw edema reduction compared to the control, analyzed by One-way ANOVA followed by Dunnet's test *post hoc*.
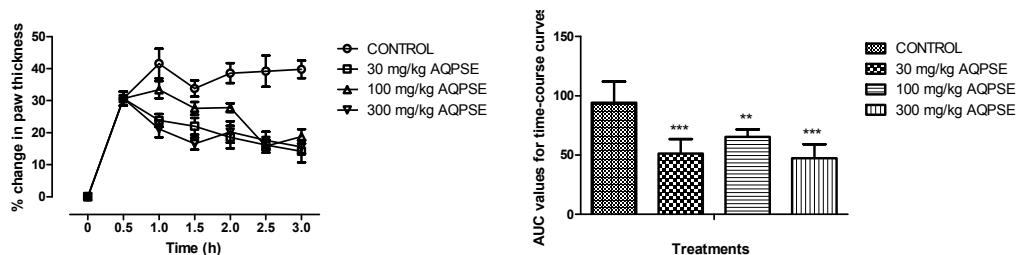
Figure 9: The effects of 30, 100, and 300 mg/kg of AQ PSE on bradykinin-induced paw edema in Sprague-Dawley rats. Data are presented as mean ± SEM (n=5). ** *P*< 0.01, ****P*< 0.001 are the level of significance of paw edema reduction compared to the control, analyzed by One-way ANOVA followed by Dunnet's test *post hoc*.
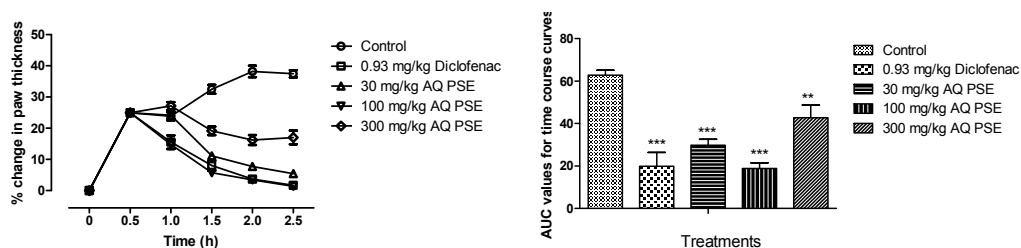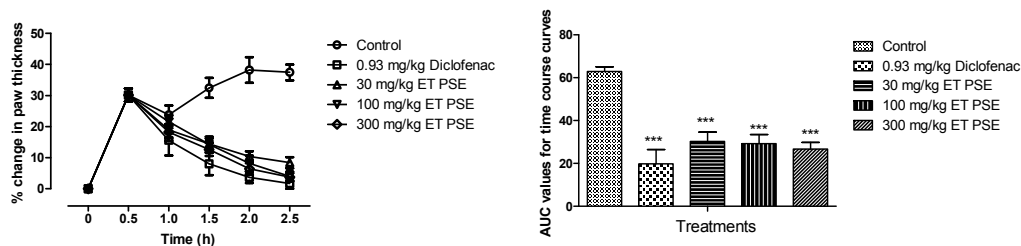

Figure 10: The effects of 30, 100, and 300 mg/kg of ET PSE on bradykinin-induced paw edema in Sprague-Dawley rats. Data are presented as mean ± SEM (n=5). ****P*< 0.001 is the level of significance of paw edema reduction compared to the control, analyzed by One-way ANOVA followed by Dunnet's test *post hoc*.
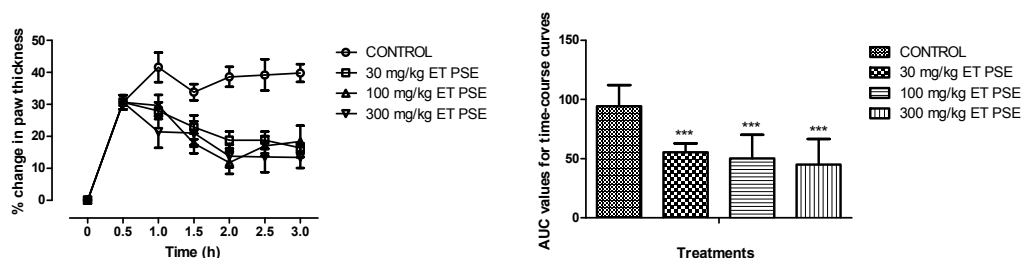
DISCUSSION

Carrageenan induced paw edema is bipbasic in nature with the first mediated by histamine and serotonin, the second mediated by prostaglandins particularly the E series and cyclo-oxygenase products which includes prostacyclins and thromboxanes. The continuity between the two phases is ensured through the action of kinins (Silva *et al.*, 2005; Perianayagam *et al.*, 2006). Inhibition of these mediators in effecting their pharmacologic activity is a sure way of curbing the incidence of inflammation at the site of injury. This study has shown that both aqueous and ethanolic leaves extracts of *Pistia stratiotes* has important anti-edematogenic effect on rat paw edema induced by carrageenan, histamine, serotonin, prostaglandin and bradykinin. Since carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents

acting by the mediators of acute inflammation (Mossai *et al.*, 1995; Sawadogo *et al.*, 2006), the results of this study are an indication that aqueous and ethanolic leaves extracts of *P. stratiotes* can be effective in acute inflammatory disorders but to further ascertain the mechanism by which these extracts may be working histamine, serotonin, prostaglandins and bradykinin were then used as mediators to induce inflammation.

The extracts significantly inhibited histamine, prostaglandin, bradykinin-mediated models of inflammation in rats at all dose levels but its anti-serotonergic activity was observed at relatively lower doses. Histamine is an important inflammation mediator, potent vasodilator substance and also increases the vascular permeability (Vasudevan, 2007). Serotonin (5-hydroxytryptamine) is a vasoactive mediator similar to histamine found in mast cells and

Table 1: Hematological assessment values obtained before and after treatment of Sprague-Dawley rats with 10, 30, 100, and 300 mg/kg of AQ PSE in an acute and delayed toxicity study

	Control	10 mg/kg		30 mg/kg		100 mg/kg		300 mg/kg	
		24 h	10 days	24 h	10 days	24 h	10 days	24 h	10 days
WBC (x10³/L)	5.63 ± 1.32	10.7 ± 2.43	9.83 ± 2.20	9.53 ± 3.35	7.90 ± 4.25	6.67 ± 2.82	7.33 ± 1.43	9.47 ± 1.02	11.47 ± 2.61 **
HGB (g/dl)	15 ± 0.72	14.43 ± 0.85	13.87 ± 0.40	13.4 ± 1.45	13.67 ± 0.40	13.8 ± 2.02	13.17 ± 0.57*	15.57 ± 0.74	14.8 ± 0.66
RBC (x10⁶/L)	8.76 ±0.24	7.64 ± 0.29 *	7.50 ± 0.14*	7.20 ± 0.67**	7.65 ± 0.31*	7.46 ± 1.27*	7.50 ± 0.79*	7.65 ± 0.55*	7.28 ± 0.55**
HCT (%)	51.47 ± 0.93	49.37 ±2.91	46.17 ± 1.13	45.18 ± 5.18	45.90 ± 2.69	45.8 ± 8.41	43.3 ± 3.35 *	52.87 ± 3.23	50.87 ± 2.03
MCV (fL)	58.7 ± 2.43	64.63 ± 1.64	61.53 ± 1.66	62.63 ± 1.77	59.83 ± 2.27	60.4 ± 2.46	61.17 ± 2.80	59.73 ± 0.81	61.53 ± 3.37
MCH (pg)	17.1 ± 0.46	18.43±0.55*	18.47 ± 0.40	18.57±0.61*	17.83 ± 0.51	18.27 ± 0.38	18.67 ± 1.60*	17.6 ± 0.46	17.8 ± 1.28
MCHC (g/dl)	29.17 ± 0.55	29.27 ± 0.23	30.03 ± 0.38	29.67 ± 0.50	29.83 ± 0.95	30.27 ± 1.38	30.47 ± 1.72	29.47 ± 0.55	28.93 ± 0.49
LYM %	74.17 ± 6.48	74.43 ± 7.91	86.7 ± 9.58	75.67± 5.52	77.03 ± 7.11	62.17 ± 1.04	63.03 ± 11.85	68.23 ± 8.65	76.60 ± 3.30
LYM#(x10³/µL)	4.20 ± 1.25	7.87 ± 1.01	7.90 ± 1.90	7.30 ± 2.86	6.23 ± 3.75	4.10 ±1.85	4.6 ± 1.14	6.53 ± 1.51	8.83 ± 2.11 *
RDW-CV (%)	12.8 ± 0.72	11.27 ± 0.67	12.5 ± 1.05	11.8 ± 1.65	12.20 ± 0.75	11.8 ± 1.55	12.61 ± 3.38	11.53 ± 0.29	12.33 ± 1.18
RDW-SD (fL)	30.97 ± 0.78	30.77 ± 0.25	30.43 ± 0.94	30.23 ± 2.06	29.77 ± 0.55	29.97 ± 2.82	31.63 ± 5.61	28.83 ± 0.78	30.37 ±0.84
PLT (x10⁵/L)	7.76 ± 1.3	6.4 ±1.5	9.3 ± 3.1	7.04 ± 0.98	5.92 ± 2.5	4.52 ± 2.5	5.53 ± 1.9	4.8 ± 2. 6	6.4 ± 1.92
MPV (fL)	6.2 ±0	6.4 ± 0.12	6.27 ± 0.21	6.5 ± 0.36	6.80 ± 0.10	6.70 ± 0.15	6.23 ± 0.40	6.97 ± 0.21	6.63 ± 0.15
PDW	7.1 ±010	7.27 ± 0.15	7.37 ± 0.35	7.57 ± 0.42	8.17 ± 0.23**	8.4 ± 0.75**	7.13 ± 0.60	8.63±0.81***	7.63 ± 0.41
P_LCR (%)	4.07 ± 0.37	5.13 ± 0.76	4.2 ± 0.69	5.13 ± 1.36	6.17 ± 0.91 **	7.07±1.01**	4.13 ± 1.20	8.03±1.22***	6.17 ± 0.25 **

Values recorded are means and standard deviations (N=5). Values obtained for the various parameters before treatment, and 24 h and 10 days post-treatment.* P ≤ 0.05, ** P≤ 0.01 are the level of significance of paw edema reduction compared to the control, analyzed by One-way ANOVA followed by Dunnet's test *post hoc*. White Blood Cell Count (WBC), Hemoglobin (HGB), Red Blood Cell Count (RBC), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Lymphocytes (LYM), Red Blood Cell Distribution Width (RDW-CV & RWD-SD), Platelet Count (PLT), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), Platelet Larger Cell Ratio (P-LCR).

Table 2: **Urinalysis values obtained before and after treatment of Sprague-Dawley rats with 10, 30, 100, and 300 mg/kg of AQ PSE in an acute and delayed toxicity study**

Parameters	Control	10mg/kg		30 mg /kg		100 mg/kg		300 mg/kg	
		24 h	10 days	24 h	10 days	24 h	10 days	24 h	10 days
UBG (umol/uL)	3.4 0± 0.00	3.40 ± 0.00	17 ± 0.00 ***	7.93 ± 7.85	17.00 ± 0.00 ***	3.4 ± 0.00	3.4 ± 0.00	12.47 ± 7.85 **	17.00± 0.00 ***
BIL (umol/L)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	17.00 ± 0.00	17.00±0.00
KET	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
BLD (Ery/uL)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
PROT (g/L)	(-)	2.1 ± 1.56	0.30 ± 1.025	0.20 ± 0.17	0.53 ± 0.40	0.30 ± 1.025	0.53 ± 0.36	0.53 ± 0.40	2.33 ± 1.15
NIT	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
LEU	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
GLU(mmol/L)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
SG	1.03 ± 0.00	1.03 ± 0.00	1.025 ± 0.00	1.02 ± 0.01	1.025 ± 0.00	1.015 ± 0.01	1.02 ± 0.76	1.025 ± 0.01	1.030 ± 0.00
pH	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.17 ± 0.58	6.5 ± 0.00	5.17 ± 0.29	6.83 ± 0.76	6.17 ± 0.29	6.00 ± 0.00
MALB(g/L)	(-)	0.15 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	0.15 ± 0.00

Values recorded are means and standard deviations (N=5). Values obtained for the various parameters before treatment, and 24 h and 10 days post-treatment. * P ≤ 0.05, ** P≤ 0.01 are the level of significance of paw edema reduction compared to the control, analyzed by One-way ANOVA followed by Dunnet's test *post hoc*. Urobilinogen (UBG);Bilirubin(BIL);Ketones(KET);Blood(BLD);Protein(PROT);Nitrite(NIT);leucocytes(LEU);Glucose (GLU);Specific gravity(SG); Microalbumin(MALB).

platelets in the GI tract and CNS. Serotonin also increases vascular permeability, dilates capillaries, and causes contraction of nonvascular smooth muscle (Borissova *et al.*, 1994). Prostaglandins act as potent pro-inflammatory mediators there by making it a desirable goal for the treatment of cancer, rheumatoid arthritis, intestinal inflammation, Alzheimer's disease and chronic musculoskeletal pain (Yedgar *et al.*, 2007). More importantly, the bradykinin-induced inflammatory response subsided rapidly in rats that were pretreated with the extracts. This inhibitory effect may have resulted from interference of the anti-inflammatory components of the extracts with the B_2 receptor-mediated mechanism by which bradykinin is reported to induce rat paw edema (Campos and Calixto, 1995).

Since the extracts effectively suppressed the edema produced by carrageenan, and subsequent exclusive mediators such as histamine, serotonin, prostaglandin and bradykinin, it showed that the extracts exhibits anti-inflammatory effects by possible inhibition of the synthesis, release or action of these inflammatory mediators.

Preliminary phytochemical screening indicated the presences of flavonoids and sterol, which could possibly be the source of the anti-inflammatory property of *P. stratiotes*. It has been shown that most flavonoids have anti-inflammatory activity (Pelzer *et al.,* 1998; Funakoshi-Tago *et al.,* 2011).The sterols have structural resemblance to steroids and are known to attenuate inflammation (by inhibiting *phospholipase A₂* which hydrolyzes arachidonic acid from membrane phospholipids, and subsequent formation of prostanoids and leucotrienes via the cyclooxygenase and lipoxygenase pathways) and immune dysfunction in experimental models (Mencarelli, 2009).

The comparable glucose concentrations between the extract-treated and control imply that the extracts do not induce gluconeogenesis in the liver to cause hyperglycemia. The elevation of urine protein could be a sign of impaired filtration as in glomerulonephritis (Pillitteri *et al.*, 2009), impaired tubular protein reabsorption and degradation capacity of renal tubules (Prakash *et al.*, 2008) or possibly the extracts could be rather rich in proteins. The elevated levels of microalbumin, however, confirms a possible acute kidney function impairment (Was´En *et al.*, 2004; Luo and Kong, 2005; LTO, 2011) as it is an important prognostic marker for kidney disease. The extract does not induce acidosis or alkalosis as urine pH was not affected. No blood in the urine indicates that the extracts do not cause kidney or bladder calculi, and/or damage to the urinary tract (Raja, 2011). Although quantities of urobilinogen in the urine are quite small, it is an important indicator of liver function and red blood cell catabolism (Troy, 2005). The elevated urobilinogen levels indicate possible hemolysis of red blood cells caused by the extract (confirmed by the significant reduction in RBC count with treatment and the amber colored urine observed).

CONCLUSION
The aqueous and ethanolic extracts of *P. stratiotes* have anti-inflammatory activity in acute inflammation induced with carrageenan, through the inhibition of histamine, serotonin, prostaglandin, and bradykinin. It is however recommended that users monitor liver and kidney function, as well as the hematological profile from time to this to ensure maximum safety with the use of this product.

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