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Anti-Inflammatory and antipyretic effects of an ethanolic extract of *Palisota hirsuta* K. Schum roots

E. Boakye-Gyasi¹, E. Woode¹*, G. K. Ainooson¹, D. D. Obiri¹, C. Ansah¹, M. Duwejua² and A. Donkoh³

¹Department of Pharmacology, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
²Department of Clinical and Social Pharmacy, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
³Department of Animal Science, College of Agriculture and Renewable Resources, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

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The effect of *Palisota hirsuta* root ethanolic extract, a herbal preparation used in Ghana for pain and inflammatory disorders, was assessed in acute inflammation in carrageenan-induced foot oedema in chicks and brewer's yeast-induced pyrexia in rats. Two paradigms were used for the inflammation assessment; effect of the extract on established inflammation (curative protocol) and effect before the induction of inflammation (preemptive protocol). *P. hirsuta* extract (50 - 400 mg/kg, p.o) dose-dependently reduced foot oedema with maximal effect of 58.90 ± 11.38% (prophylactic) and 62.52 ± 4.73% (curative). Similarly, the NSAID, diclofenac (10 - 100 mg/kg, i.p.) used as a reference drug, dose-dependently reduced the oedema with a maximal effect of 96.82 ± 3.64% (prophylactic) and 60.74 ± 5.56% (curative). The steroidal anti-inflammatory drug, dexamethasone (0.5 - 2 mg/kg, i.p), inhibited the oedema with a maximal effect of 86.51 ± 2.61% (prophylactic) and 55.76 ± 9.56% (curative). In terms of potency, the ethanolic extract of *P. hirsuta* exhibited similar potency when it was administered 1 h before (ED₅₀ 178.00 ± 56.8 mg/ kg) and 1 h after (ED₅₀ 181.10 ± 49.89 mg/kg) carrageenan injection and this was found to be less potent than both dexamethasone and diclofenac in both the prophylactic and curative protocols. Also, PHE caused a significant dose-dependent decrease in yeast-induced pyrexia in rats (IC₅₀: 265.10 ± 63.73 mg/kg) which was = 15 times less potent than the standard, paracetamol (IC₅₀: 18.05 ± 4.08 mg/kg). The results thus confirm the use of the plant for inflammatory disorders in traditional medicine.

Key words: *Palisota hirsuta*, carrageenan, yeast, chicks, rats.

INTRODUCTION

Inflammation is a complex localized response to foreign substances such as bacteria or in some instances to internally produced substances (Laupattarakasem et al., 2003; Schmid-Schönbein, 2006) with fever usually presenting as one of its sequelae (Okumura et al., 2006) inflammation underlies almost all disease conditions (Erlinger et al., 2004; Lucas et al., 2006; Schmid-Schönbein, 2006) and it is fundamentally a protective response the ultimate goal of which is to rid the organism of both the initial cause of cell injury (for example microbes and toxins) and the consequences of such injuries (necrotic cells and tissues) (Serhan, 2004; Schmid-Schönbein, 2006). Various medicinal plants provide relief of symptoms comparable to that obtained from allopathic medicines (Gagnier et al., 2004) The majority of clinically important medicines belong to steroid or non-steroidal anti-inflammatory drugs (Choi and Hwang, 2003). Though these drugs have potent activity, they have various and severe adverse effects such as gastrointestinal disturbances and body fat redistribution. Therefore, agents of natural origin with fewer side effects are required as substitute chemical therapeutics (Verpoorte, 1998). In Ghana, several medicinal plants are
used as combination therapy with orthodox medicine in the treatment of pain and inflammation. 

*P. hirsuta* K. Schum. ([Family: Commelinaceae), known locally in Ghana as *somenini* or *mpentemi* (Twi), *sombenyin* (Fante) and *sumbe* (Ewe), is one of such plants. It is a robust herb found in forest re-growths and is about 2 - 4 m high. Different parts of the plant are used for various conditions. The roots are used to treat dysentery, anemia and rheumatism. Whole plant is used as an analgesic and antiseptic, a leaf decoction for colic, juice of roasted leaves for ear-ache, roots to hasten expulsion of placenta after childbirth, roots as enema for stomach pains and indigestion, and the powdered roots for gonorrhea (Abbiwi, 1990; Ayensu, 1978; Mshana et al., 2000). Apart from work on the anti-viral properties (Anani et al., 2000; Hudson et al., 2000), not much has been reported on this plant.

In the present paper therefore, we report on the anti-inflammatory effect of the ethanolic extract of the root in carrageenan- induced oedema in chicks and its antipyretic effects in rats as an attempt to validate its traditional uses.

**MATERIAL AND METHODS**

**Plant material**

Roots of *P. hirsuta* were collected from the Botanic Gardens, KNUST, Kumasi, between January and February, 2006. After the roots have been authenticated by Mr. Amissah, the curator of the garden, a voucher specimen was kept in the Faculty of Pharmacy Herbarium (No. FP 10081). The roots were then air-dried indoors for a week and pulverized with a hammer-mill. A hydro-alcoholic extract of the powder was obtained by soxhleting with 70% v/v of ethanol for 12 h. The extract was evaporated to a brown syrupy mass under reduced pressure in a rotary evaporator, air-dried and kept in a desiccator till required (yield = 6%). This is subsequently referred to as PHE or extract.

**Phytochemical analysis**

The presence of saponins, tannins, alkaloids, triterpenes, flavonoids, glycosides, and reducing sugars were tested by simple qualitative and quantitative methods of Trease and Evans (1989) and Sofowora (1993).

**Drugs and chemicals**

Diclofenac (Troge, Hamburg, Germany), dexamethasone (Pharm-Inter, Brussels, Belgium), carrageenan sodium salt (Sigma Chemicals, St. Louis, MO, USA).

**Animals**

Cockerel (*Gallus gallus*; strain Shaver 579, Akropong Farms, Kumasi, Ghana) were obtained 1-day post-hatch and were housed in stainless steel cages (34 x 57 x 40 cm³) at a population density of 12 – 13 chicks per cage. Food (Chick Mash, GAFCO, Tema, Ghana) and water were available *ad libitum* through 1-quart gravity-fed feeders and water trough. Room temperature was maintained at 29°C, and overhead incandescent illumination was maintained on a 12 h light–dark cycle. Daily maintenance of the cages was conducted during the first quarter of the light cycle. Chicks were tested at 7 days of age. Group sample sizes of 5 – 6 were used throughout the study.

Male Spraque-Dawley rats (150 – 200 g) were also purchased from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, and maintained in the Animal House of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. The animals were housed in groups of six in stainless steel cages (34 x 47 x 18 cm³) with soft wood shavings as bedding and fed with normal commercial pellet diet (GAFCO, Tema) with water given *ad libitum*. The room was maintained under laboratory conditions of temperature 24 - 28°C, relative humidity 60 - 70%, and 12 h light-dark cycle. All procedures and techniques used in these studies were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health and Human Services publication No. 85 - 23, revised 1985) and were approved by the Departmental Ethics Committee (Reference No. P/col/C1202).

**Carrageenan-induced oedema**

Carrageenan foot oedema model of inflammation in the chick (Roach and Sufka, 2003) was used with some modifications (Woode et al., 2007) to evaluate the anti-inflammatory properties of the extract. Chicks were randomly divided into groups of six. Foot volumes were measured by water displacement plethysmography as described by Fereidoni et al. (2000). Inflammation was induced by a subplantar injection of carrageenan (10 µl of a 2% solution in saline) into the right footpad of the chicks. Chicks were then randomly selected to perform one of the study groups: control (vehicle-treated); PHE (50, 100, 200 and 400 mg/kg, p.o.); diclofenac (10, 30 and 100 mg/kg, i.p.); and dexamethasone (0.5, 1.0 and 2.0 mg/kg, i.p.). Two protocols were used in this study—*a preemptive*; where drugs were given 30 min for i.p. route and 1 h for oral route before carrageenan injection and *b) curative*; where drug were administered 1 h after the induction of inflammation. Foot volumes were measured at hourly intervals for 5 h. The oedema component of inflammation was quantified by measuring the difference in foot volume before carrageenan injection and at the various time points. The extract was prepared in 2% tragacanth mucilage. Diclofenac and dexamethasone, a non-steroidal and steroidal drug respectively, were used as positive controls.

**Induction of Brewer's yeast pyrexia**

Hyperthermia was induced in rats as previously described (Panthong et al., 2007; Teotino et al., 1983). Male rats weighing 180 – 300 g were used for the experiment. Animals were fasted overnight and during the entire duration of the experiment but given water *ad libitum*. Initial rectal temperatures were recorded before induction of pyrexia with a lubricated digital thermometer inserted about 3 cm into the rectum of each rat. To induce pyrexia, the rats were given 10 ml/kg of 20% aqueous suspension of brewer's yeast (Reference No. P/col/C1202).
tial temperatures were determined before and at hourly intervals up to 4 h after extracts/drugs administration.

Analysis of data

Raw scores for right foot volumes were individually normalized as percentage of change from their values at time 0, then averaged for each treatment group. The time-course curves for foot volume was subjected to two-way (treatment × time) repeated measures analysis of variance with Bonferroni's post hoc t test. Total foot volume for each treatment was calculated in arbitrary unit as the area under the curve (AUC) and to determine the percentage inhibition for each treatment, the following equation was used.

\[
\% \text{ inhibition of oedema} = \left( \frac{AUC_{\text{control}} - AUC_{\text{treatment}}}{AUC_{\text{control}}} \right) \times 100
\]

Data from the antipyretic studies were treated like that in the anti-tal temperatures were determined before and at hourly intervals up to 4 h after extracts/drugs administration.

RESULTS

Phytochemical analysis

The phytochemical analysis of *P. hirsuta* showed it contains alkaloids, flavonoids, tannins and terpenoids with alkaloids and flavonoids being the most dominant chemical constituents.

Effect of extract on carrageenan-induced foot oedema in chicks

After preliminary studies, 10 µl of 2% carrageenan was selected and found to induce a moderate inflammation with with a maximal oedema in 7 day old chicks peaking at 2 – 3 h as described by Roach et al. (2003).

Figure 1 shows the time course curve and AUC for the effect of *P. hirsuta* extract (PHE), diclofenac and dexamethasone prophylactically on carrageenan-induced oedema. From the time course curves, two-way ANOVA (treatment × time) revealed a significant and dose-dependent effect of PHE \([F_{1,118} = 12.20, P < 0.01]\) (Figure 1a). Furthermore, when total oedema over the period of the experiment is represented arbitrary as AUC of the time course curves, PHE (50 - 400 mg/kg, p.o) significantly reduced total oedema with maximal inhibitory effect of 58.90 ± 11.38% at 200 mg/kg as shown in (Figure 1b). Diclofenac (10 - 100 mg/kg, i.p.), an NSAID, also showed significant effect on the time course \([F_{1,98} = 3.43, P < 0.05]\) (Figure 1c); and the total oedema (AUC), with maximal inhibitory effect of 96.82 ± 3.64% (Figure 1d) at 100 mg/kg. Dexamethasone treatment also exhibited a significant effect \([F_{1,98} = 5.16, P < 0.05]\) on the time course of carrageenan-induced oedema (Figure 1e) with maximal inhibitory effect of the total oedema by 86.51 ± 2.61% (Figure 1f) at 2 mg/kg.

As depicted in figure 2, all the drugs used showed significant effects when administered curatively on the time course curve and AUC of carrageenan-induced oedema. Two-way ANOVA (treatment × time) revealed a significant effect of PHE treatment on the time course of carrageenan-induced oedema \([F_{4,120} = 25.15, P < 0.001}\) (Figure 2a) PHE (50 - 400 mg/kg, p.o) significantly reduced total oedema in the curative protocol with maximal inhibitory effect of 62.52 ± 4.73% at 200 mg/kg (Figure 2b). Diclofenac (10 - 100 mg/kg, i.p.), also showed significant effect on the time course \([F_{1,98} = 21.18, P < 0.001}\) (Figure 2c) and the total oedema (AUC), with maximal inhibitory effect of 60.74 ± 5.58% (Figure 2d) at 100 mg/kg. Dexamethasone (0.5 - 2.0 mg/kg, i.p.) also exhibited a significant effect \([F_{4,120} = 15.15, P < 0.001}\) (Figure 2e) curatively on the time course of carrageenan-induced oedema with maximal inhibitory effect of the total oedema 55.76 ± 9.56% (Figure 2f) at 2 mg/kg.

Based on ED_{50} values (Table 1) obtained from dose-response curves (Figure 3), PHE showed similar potency \((F_{1,24} = 7.16, P = 0.99)\) in both the prophylactic (ED_{50} 178.00 ± 56.8 mg/kg, Figure 3a) and the curative (ED_{50} 181.10 ± 49.89 mg/kg, Figure 3b) treatment. Dexamethasone also showed similar potency \((F_{1,16} = 3.07, P = 0.12)\) in the prophylactic (ED_{50} 0.55 ± 0.21 mg/kg, Figure 3a) and the curative (ED_{50} 1.36 ± 0.28 mg/kg, Figure 3b) treatment. However diclofenac exhibited a significantly greater potency \((F_{1,16} = 10.4< 0.01)\) in the prophylactic (ED_{50} 10.86 ± 3.7 mg/kg, Figure 3a) compared to the curative (ED_{50} 32.73 ± 5.14 mg/kg, Figure 3b).

Effect of extract on brewer’s yeast-induced pyrexia

Rectal temperatures before yeast injection ranged from...
Figure 1. Effect of PHE (50 - 400 mg/kg; p.o.), diclofenac (10 - 100 mg/kg; i.p.) and dexamethasone (0.5 - 2 mg/kg; i.p.) on time course curve (a, c and e respectively) and the total oedema response (b, d and f respectively) in prophylactic protocol of carrageenan-induced paw oedema in chicks. Values are means ± S.E.M. (n = 5). ***P < 0.001; ** P < 0.01; *P < 0.05 compared to vehicle-treated group (Two-way ANOVA followed by Bonferroni’s post hoc test).

36.2 to 37.3°C with an overall mean ± S.E.M of 36.59 ± 0.03°C (n = 42). Subcutaneous injection of 20% yeast increased significantly (t = 16.8; paired t-test), the rectal temperature to between 36.8 to 38.3°C with a mean of 37.52 ± 0.06. Mean differences in the pre- and post-injection temperatures was 0.93°C with a 95% confidence interval of 0.82 - 1.05. PHE (30, 100 and 300 mg/kg) administered orally, dose-dependently and significantly \([F_{3, 80} = 6.14; P = 0.0039]\); two-way ANOVA (treatment group × time) reduced the increase induced by subcutaneous injection of the yeast as shown in Figure 4a. Furthermore, PHE produced a significant \([F_{3, 20} = 6.41; P = 0.0032]\) and dose-dependent decrease in total pyrexia represented as AUCs in Figure 4b. Similarly, a two-way ANOVA (treatment group × time) of time course curves revealed a significant treatment effect for paracetamol \([F_{3,80} = 21.81; P < 0.0001]\) (Figure 4c). Also, paracetamol significantly \([F_{3,80} = 21.81; P < 0.0001]\) and dose-dependently decreased the area under the curves of the time course curves compared to that of vehicle-treated group (Figure 4d). On analysis of data by non-linear regression, the IC\(_{50}\)s obtained were 265.10 ± 63.73 and 18.05 ± 4.08 mg/kg respectively for PHE and paracetamol. Thus the extract was 15 times less potent than the standard, paracetamol \([F_{1,34} = 85.17; P < 0.0001]\).

**DISCUSSION**

The results presented in this study show that an ethanolic extract has antipyretic effects in rats and anti-inflammatory properties in chicks when administered preemptively and curatively. Carrageenan-induced acute footpad oedema in laboratory animals was first introduced by Winter et al. (1962). It has been widely used to screen new anti-inflammatory drugs and remains an acceptable preliminary screening test for anti-inflammatory activity (Niemegeers et al., 1975; Singh et al., 2000).
Figure 2. Effect of PHE (50 - 400 mg/kg; p.o.), diclofenac (10 - 100 mg/kg; i.p.) and dexamethasone (0.5 - 2 mg/kg; i.p.) on time course curve (a, c and e respectively) and the total oedema response (b, d and f respectively) in curative protocol of carrageenan-induced paw oedema in chicks. Values are means ± S.E.M. (n = 5). ***P < 0.001; ** P < 0.01; *P < 0.05 compared to vehicle-treated group (Two-way ANOVA followed by Bonferroni’s post hoc test). †††P < 0.001; ††P < 0.01; †P < 0.05 compared to vehicle-treated group (One-way ANOVA followed by Newman-Keul’s post hoc test).

It is commonly used to evaluate non-steroidal anti-inflammatory drugs (NSAID) (Di Rosa and Willoughby, 1971). In the present study we have used chicks instead of the commonly used rodents. Carrageenan-induced oedema has been validated in the chicks by Roach and Sufka (2003), and is much more economical than rodent models. Furthermore, chicks are easier to handle. Studies have demonstrated that intraplantar injection of carrageenan in the 7-day-old chick elicits a measurable, reliable and relatively short-lasting state of oedema, that is differentially attenuated by the systemic administration of typical anti-inflammatory compounds (Roach and Sufka, 2003) and compares favorably with the more commonly used rodent models (rat and mice) in the screening of drugs with anti-inflammatory activities. Th
Table 1. ED$_{50}$ values for the effect of the PHE, dexamethasone and diclofenac in carrageenan-induced oedema in chicks.

<table>
<thead>
<tr>
<th>Drug</th>
<th>ED$_{50}$ (mg/kg)</th>
<th>Preemptive Treatment</th>
<th>Curative Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palisota extract</td>
<td>178.00±56.8</td>
<td></td>
<td>181.10±49.89***</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>0.55±0.21</td>
<td></td>
<td>1.36±0.28*</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10.86±3.79</td>
<td></td>
<td>32.73±5.14**</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. (n = 5). ***P < 0.001; ** P < 0.01; *P < 0.05 compared to respective preemptive values (F test).

Figure 3. Dose response curves for dexamethasone (0.5 - 2.0 mg/kg, i.p), diclofenac (10 - 100 mg/kg i.p) and PHE (50 - 400 mg/kg p.o.) on carrageenan-induced foot oedema in the chick for the prophylactic (a) and curative protocol (b).

The ability of the extract to inhibit inflammation in both the prophylactic and curative protocols as observed for the reference drugs (diclofenac and dexamethasone) shows it can be employed before the onset (preemptive) and after the onset (curative) of inflammation. It is worth commenting that preemptive treatment using anti-inflammatory agents is not uncommon in clinical practice especially in preemptive analgesia (Kelly et al., 2001b). Preemptive analgesia is the process of providing antinociceptive treatment that prevents establishment of altered central processing of afferent input from injuries (Kelly et al., 2001b; Muratani et al., 2002; Vallejo et al., 2006).

Anti-inflammatory agents (e.g. NSAIDs, corticosteroids) are used preemptively as adjunct in multimodal therapy
Figure 4. Effect of PHE (30 - 300 mg/kg p.o) and paracetamol, Pmol (10 - 100 mg/kg p.o) on time course curves (a and c) and the total change in rectal temperature (b and d) in baker’s yeast-induced fever in rats. Values are means ± S.E.M (n = 5). *P < 0.05, **P < 0.01, ***P < 0.001 compared to respective controls (two-way repeated measures ANOVA followed by Bonferroni’s post hoc); †P < 0.05, ††P < 0.01, †††P < 0.001 (one-way ANOVA followed by Newman-Keuls post hoc)

(mainly with opioids, local anesthetics, alpha-2 agonist, N-methyl-D-aspartate receptor antagonist) in the management of post-operative pain (Kelly et al., 2001b; Vallejo et al., 2006). The aim of preemptive administration of anti-inflammatory agents is to prevent (or inactivate) the release of the various neurotransmitters and inflammatory mediators, which sensitize the peripheral nociceptors (Kelly et al., 2001a, b). The anti-inflammatory drugs block the nociceptive response to endogenous mediators of inflammation and in this way can reduce post-operative inflammatory pain (Kelly et al., 2001a; Muratani et al., 2002; Vallejo et al., 2006) associated with surgery.

Surprisingly in this study, when the highest dose of the extract (400 mg/kg) was administered, the anti-inflammatory effect of the extract decreased. This could be explained by the presence of possible pro-inflammatory compounds in the crude extract which might have become predominant as the concentration of the extract was increased and thus masking anti-inflammatory activity (Damas et al., 1985; Vieira et al., 2001). This is possible because the crude extract comprises of several chemical constituents which could be acting via contradicting mechanisms.

Although the actual mechanism of action of PHE is not known, it is possible that, the anti-inflammatory activity exhibited by the extract could be attributed to the inhibition of the synthesis, release or action of Inflamma-
flammatory mediators that are known to be involved in carrageenan-induced inflammation which include cytoplastic enzymes and serotonin from mast cells and also bradykinin, prostaglandins and other cyclooxygenase products.

Moreover, flavonoids, which were identified in this study as one of the constituents of the plant extract, are known to target prostaglandins which are involved in the late phase of acute inflammation (Damas et al., 1985; Rajnarayanan et al., 2001). Hence, the presence of flavonoids may contribute to the anti-inflammatory activity of the ethanolic root extract of _P. hirsuta_.

In addition to its anti-inflammatory properties, the extract exhibited antipyretic activity in yeast-induced pyrexia in rats. It is currently accepted that prostaglandin _E_2 (PGE_2_) is the final fever mediator in the brain, specifically in the preoptic area of the anterior hypothalamus (Li et al., 2008), thus it may be plausible to conclude that the extract inhibits the synthesis of prostaglandins. However, it must be noted that several biochemical events occur leading ultimately to the synthesis of PGE_2_. Fever is believed to result from a finely tuned, complex event that involves both the peripheral immune system and the brain, through which a series of inflammatory and metabolic processes are regulated (Inoue et al., 2008; Roth et al., 2006). It is established that there are two pathways leading to the transcription and induction of cyclooxygenase (COX)-2, the rate limiting enzyme for prostaglandin (PGE_2_) synthesis (Inoue et al., 2008). Both pathways are activated by cytokines e.g. IL-1α, IL-6 and tumor necrosis factor (TNF) which trigger central mechanisms that act via the transcription factors such nuclear factor (NF)κB and signal transducer and activator of transcription (STAT-3) (Inoue et al., 2008). It may therefore be worthwhile to investigate the exact point in the biochemical events where the extract exerts its antipyrhetic effect.

**Conclusion**

In conclusion, we have demonstrated that the ethanolic extract from the roots of _P. hirsuta_ has anti-inflammatory activity in the chick model of acute inflammation comparable to the NSAID diclofenac as well as an antipyretic effect in rats and hence may be potentially useful in the management of inflammatory conditions in humans, a validation of its traditional use as anti-inflammatory agent in Ghana.

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