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# ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS FRUIT PULP EXTRACT OF *HUNTERIA UMBELLATA* K. SCHUM IN ACUTE AND CHRONIC INFLAMMATION

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**Abstract:** The anti-inflammatory effect of the aqueous fruit pulp extract of *Hunteria umbellata* K. Schum (Apocynaceae) was evaluated using the carrageenan- and dextran-induced rat paw edema, xylene-induced ear edema and formalin-induced arthritis inflammation tests. Oral administration of the extract produced significant ($p < 0.05$) antiedematogenic effect with a dose of 500 mg/kg throughout the period of the experiment in the dextran induced paw edema and at the 3 h in the carrageenan model. The extract (250 and 500 mg/kg) exhibited a dose-related and significant ($p < 0.01$) inhibition of xylene induced ear edema and the effect was similar to that produced by dexamethasone (1 mg/kg). In the chronic inflammation (formalin induced arthritis) the extract did not show any significant anti-inflammatory activity. Oral acute toxicity assays did not show any mortality at 15 g/kg of the plant extract. The results indicate that the aqueous extract of *H. umbellata* possesses acute inflammatory activity which may be mediated by either inhibition or by blocking the release of prostaglandins and histamine, thus supporting the usage of the plant in traditional medicine treatment of inflammation.

**Keywords**: anti-inflammatory, arthritis, edema, *Hunteria umbellata*

Various herbal medicines derived from plant extracts are being used in the treatment of a wide variety of clinical diseases, though relatively little knowledge about their mechanisms of action is known (1). Many herbal preparations are being prescribed widely for the treatment of inflammatory conditions (2). There is a need for research and developmental work in herbal medicine because apart from the social and economic benefits, it has become a persistent aspect of present day healthcare in developing countries.

*Hunteria umbellata* K. Schum (Apocynaceae) is a tree, about 15-22 m in height, found in west and central Africa. In Nigeria, it is known as Osu (Edo), erin (Yoruba) and nkpokiri (Ibo). The leaves have been described as broad, abruptly acuminate and broadly lineate. The fruit is about 5-25 cm and consists of two separate globose mericaps 3-6 cm long, yellow, smooth, 8-25 seeded embedded in a gelatinous pulp (3). Various parts of the plant have been used in herbal medicine for the treatment of diabetes, peptic ulcers, piles, yaws, dysmenorrhea, fevers, infertility, and helminthic infections (4-6). Chemical constituents such as saponins, steroids, tannins, volatile oils, phenols and copious amounts of alkaloids have been reported in the fruits of *H. umbellata* (7). Members of the genus *Hunteria* have been a subject of chemical/biological investigations and they have been widely employed in traditional herbal medicine for treatment of pains and inflammations. The crude alkaloid stem bark extract from *Hunteria zeylanica* (Retz.) Gardner ex Thwaites inhibited acute inflammation in experimental animals (8). Antinociceptive and antipyretic effects of alkaloids extracted from stem bark of *H. zeylanica* have also been reported (9). Corymine, an alkaloid extracted from the leaves of *H. zeylanica* has been reported to potentiate convulsion-induced by either picrotoxin or strychnine through inhibitory effects on glycine receptors (10). Although the aqueous extract of the fruit pulp of *H. umbellata* has been used traditionally in treatment of various fevers and

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inflammations, there is no scientific evidence in support of this therapeutic use. The present study was therefore undertaken to evaluate the anti-inflammatory activity of the aqueous fruit pulp extract of this plant, using various experimental animal models of inflammation.

## MATERIALS AND METHODS

### Plant material and extraction

The ripe fruits of *H. umbellata* were collected from Okhoro village in Benin City, Nigeria in the month of October, 2007. The plant was first identified by Prof. MacDonald Idu of the Department of Botany, Faculty of Life Sciences, University of Benin, Benin City and was later authenticated by the Forest Research Institute of Nigeria (Ibadan, Nigeria) where a herbarium sample with voucher number FHI 107678 has been deposited.

The seeds were removed from the ripe fruits and the fruit pulp was sun-dried to a constant weight over a 14-day period. The dried fruit pulp was then powdered using a mechanical grinder. The powdered fruit pulp (500 g) was boiled in 2 L of distilled water for 30 min. The material was then filtered, concentrated to dryness under reduced temperature and pressure in a vacuum evaporator (yield = 38%). The dried extract was stored in air-tight clean glass container at 4$^{o}$C until use.

### Phytochemical screening

The aqueous extract of *H. umbellata* was subjected to various tests in order to determine the classes of the various chemical constituents present in the extract, by using standard methods (11).

### Animals

Experiments were performed using either Swiss albino mice (17-23 g) or Wistar rats (150-190 g). The animals were obtained from the Laboratory Animal Centre, College of Medicine, University of Lagos, Nigeria. The animals were fed with standard rodent cubes obtained from Ladokun Feeds Ltd. (Ibadan, Nigeria) and had free access to tap water. All animals were fasted overnight before the beginning of each experiment. Animals were exposed to natural lighting conditions and were handled according to standard experimental protocols approved by the Faculty of Pharmacy Animal Ethics Committee, University of Benin, Nigeria.

### Drugs and chemicals

λ-Carrageenan, dextran-A, indomethacin and diphenhydramine were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A). Xylene and formaldehyde were obtained from BDH Chemicals, UK, while dexamethasone was obtained from Vardhman Exports, India.

### Acute toxicity study

Overnight-fasted Swiss albino mice (17-23 g) of either sex were used for the study. The animals were divided into five groups of five animals each. Groups A to D received orally 1, 5, 10 and 15 g/kg of the extract, respectively, while the control (group E), received distilled water (3 mL/kg) by the same route. General symptoms of toxicity and mortality in each group were observed within 24 h. Animals that survived after 24 h were observed for any signs of delayed toxicity for two weeks.

### Anti-inflammatory activity

### Carrageenan induced paw edema

Male Wistar rats (150-190 g) were divided into four groups of five animals each. The test groups received 250 and 500 mg/kg, *p.o.* of the extract. The reference group received indomethacin (10 mg/kg, *p.o.*) while the control group received 3 mL/kg of distilled water. After 1 h, 0.1 mL, 1% w/v carrageenan suspension in normal saline was injected into the subplantar tissue of the right hind paw (12). The paw thickness was measured at hourly interval for 5 h using a vernier caliper (13).

### Dextran induced paw edema

Male Wistar rats (150-190 g) were divided into four groups of five animals each. The different groups of animals received extract (250 and 500 mg/kg) or diphenhydramine (60 mg/kg) or distilled water (3 ml/kg) orally. The animals were treated 1 h before injection of 0.1 mL of 1.5% w/v dextran in normal saline into the subplantar tissue of the right hind paw (14). Paw thickness was measured using vernier calipers at 0, 1, 2, 3, 4, and 5 h.

### Xylene induced ear edema

Swiss albino mice were divided into four groups of five animals each. Animals were treated orally with the extract (250 and 500 mg/kg), dexamethasone (1 mg/kg) and distilled water (3 mL/kg). Thirty minutes later, edema was induced in each mouse group by applying a drop of xylene to the inner surface of the right ear. After 15 min, the animals were sacrificed under ether anaesthesia and both ears cut off, sized and weighed (15). The anti-inflammatory activity was expressed as the percentage inhibition of edema in the treated mice in comparison with the control mice.

Table 1. Effect of aqueous extract of *H. umbellata* on xylene-induced ear edema in mice

| Treatment | Dose (mg/kg) | Weight of right ear(mg) | Weight of left ear (mg) | Difference (mg) | Inhibition % |
|---|---|---|---|---|---|
| Control | 3 mL/kg | 39.76 ± 0.74 | 23.72 ± 0.62 | 16.04 ± 0.54 | - |
| *H. umbellata* | 250<br>500 | 28.02 ± 0.62<br>26.16 ± 0.44 | 22.00 ± 0.50<br>21.30 ± 0.39 | 62.47<br>4.86 ± 0.33** | <br>69.70 |
| Dexamethasone | 1 | 30.26 ± 0.46 | 26.18 ± 0.50 | 4.08 ± 0.36** | 74.56 |

Data are the mean ± SEM values for five mice in each group. *p < 0.05, **p < 0.01 as compared to the control

**Formalin induced arthritis inflammation**

Male Wistar rats (150-190 g) were divided into four groups of five animals each. Inflammation was produced by subaponeurotic injection of 0.1 mL of 2 % w/v formalin in normal saline in the right hind paw of the rats on the first and third day. The extract (250 and 500 mg/kg) and distilled water (3 mL/kg) were administered orally once a day for 10 days. Indomethacin (5 mg/kg) given orally, was used as standard. The rat paw thickness was measured daily for 10 days using vernier calipers (16). The percentage inhibition of the mean increase in the paw edema of each group was calculated on the tenth day and compared with the control.

**Statistical analysis**

Data were expressed as the mean ± SEM. The data were analyzed using one way analysis of variance (ANOVA) followed by Tukey's test. Differences between two means were detected using the Student's *t*-test. Data were considered different at significance level of $p < 0.05$.

## RESULTS

Acute toxicity studies showed that all the doses (1, 5, 10, and 15 g/kg) of the *H. umbellata* extract used for the study were non-toxic.

The preliminary phytochemical screening of the aqueous fruit pulp extract of *H. umbellata* revealed the presence of carbohydrates, alkaloids, reducing sugars, saponins, tannins, steroidal components and flavonoids, further corroborating previous reports (7)

In the carrageenan-induced paw edema (Fig. 1), the aqueous fruit pulp extract of *H. umbellata* (500 mg/kg) significantly ($p < 0.05$) inhibited paw edema at the 3rd h compared with the control animals

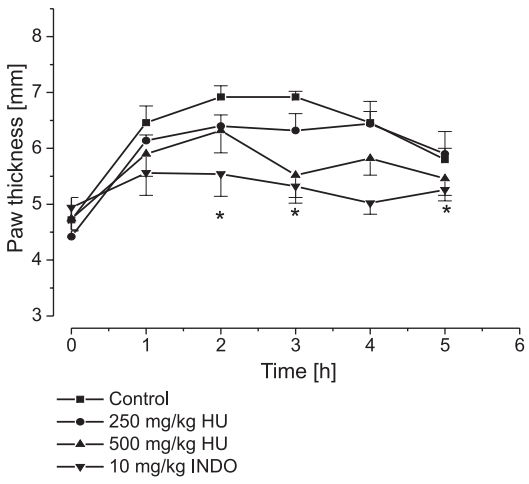


Figure 1. Effect of aqueous extract of *H. umbellata* on carrageenan-induced paw edema in rats. HU = *H. umbellata*, INDO = indomethacin, *p < 0.05, as compared to the control (n = 5 for each group)

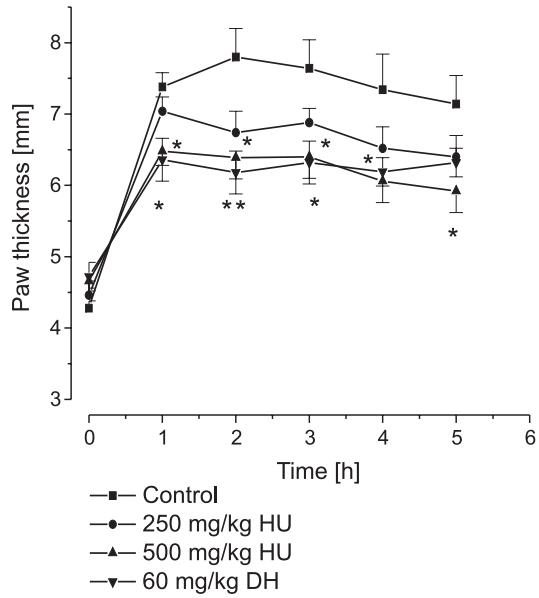


Figure 2. Effect of aqueous extract of *H. umbellata* on dextran-induced paw edema in rats. HU = *H. umbellata*, DH = diphenhydramine *p < 0.05, **p < 0.01 as compared to the control (n = 5 for each group)

Table 2. Effect of aqueous extract of *H. umbellata* on formalin induced arthritis inflammation in rats

| Treatment | Dose (mg/kg) | Paw thickness on day 10 (mm) | Inhibition (%) |
|---|---|---|---|
| Control | 3 mL/kg | 7.34 ± 0.14 | - |
| *H. umbellata* | 250<br>500 | 6.92 ± 0.10<br>7.08 ± 0.28 | 5.72<br>3.54 |
| Indomethacin | 5 | 5.44 ± 0.23* | 25.89 |

Data are the mean ± SEM values for five rats in each group. *$p < 0.05$ as compared to the control

which received distilled water. This effect was comparatively less than indomethacin. In Figure 2, the extract (500 mg/kg) produced a significant ($p < 0.05$) inhibitory effect on the dextran induced paw edema sustained throughout the period of the experiment. The extract's inhibitory effect was greater than the positive control, diphenhydramine at the 4th and 5th h of the experiment but there was no significant difference ($p > 0.05$).

On xylene-induced ear edema (Table 1), the extract at doses of 250 and 500 mg/kg showed significant percentage inhibitions of 62.47 and 69.70%, respectively. The inhibitory effect at a dose of 500 mg/kg was comparable to that of dexamethasone, with an inhibition of 74.56%. In Table 2, the aqueous extract produced no significant ($p > 0.05$) inhibitory effect in formalin-induced arthritis at doses of 250 and 500 mg/kg.

## DISCUSSION

In the present study, the anti-inflammatory activity of the aqueous fruit extract of *H. umbellata* has been evaluated in both acute and chronic inflammatory models. The inhibition of carrageenan-induced inflammation in rats is an established model for evaluating anti-inflammatory drugs, which has been used frequently to assess anti-edematous effect of natural products. The development of carrageenan-induced edema is biphasic (17); the first phase occurs within one hour of carrageenan inflammation and is attributed to the release of cytoplasmic enzymes, histamine and serotonin, from the mast cells. The second phase (> 1.0 h) is mediated by an increased release of prostaglandins in the inflammatory area and continuity between the two phases is provided by kinins. Since the extract significantly inhibited paw edema induced by carrageenan in the second phase, this finding suggests a possible inhibition of cyclooxygenase synthesis by the extract, because the carrageenan inflammatory model basically reflects the actions of prostaglandins (18, 19). This effect is similar to that produced by non-steroidal anti-inflammatory drugs such as indomethacin, whose mechanism of action is inhibition of the cyclooxygenase enzyme, which catalyses the synthesis of cyclic endoperoxides important in the formation of prostaglandins.

Dextran-induced paw edema has been reported to be mediated mainly by histamine and serotonin released by the mast cells (20). The release of these inflammatory mediators results in marked vascular changes: including vasodilation, increased permeability and an increase of blood flow, eventually leading to an increase in paw size. The extract was found to be more effective in dextran-induced edema than the carrageenan-induced edema, suggesting that the extract may also interfere with histamine release or its activity.

The xylene ear edema model permits the evaluation of anti-inflammatory steroids and is less sensitive to non-steroidal anti-inflammatory agents (21). Histopathologically, severe vasodilation, edematous changes of skin and infiltration of inflammatory cells are detected as signs of acute inflammation after topical application of xylene (22). In the present study, the increases in ear weight were inhibited in a dose-related manner by the extract, suggesting a likely indication of the antiphlogistic effects of the extract.

It is well known that inhibition of edema induced by formalin in rats is one of the most suitable test procedures to screen anti-arthritic and anti-inflammatory agents, as it closely resembles human arthritis (23). Arthritis induced by formalin is a model used for the evaluation of an agent with probable antiproliferative activity. The results of the formalin tests ruled out a possible effect of the extract on formalin induced cell damage and accordingly, arthritic conditions.

The presence of the reported phytochemical constituents in the fruit pulp extract may contribute to its observed anti-inflammatory activity. Many flavonoids and alkaloids have been found to exhibit anti-inflammatory effects (24). Acute toxicity stud-

ies revealed no mortality at a dose of 15 g/kg. Hence, the extract was classified as "relatively harmless" when administered orally (25).

In conclusion, the aqueous fruit pulp extract of *H. umbellata* has been shown to be effective against acute inflammation (carrageenan- and dextran-induced paw edema, and xylene-induced ear edema) in a dose related manner but without any significant inhibitory effect on chronic inflammation (formalin-induced arthritis). This present study supports the claim in the use of the fruit pulp extract of *H. umbellata* in traditional medicine for the treatment of inflammatory conditions.

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**REFERENCES**

1. Ratheesh M., Helen A.: Afr. J. Biotech. 6, 1209 (2007).
2. Bagul M.S., Srinivasa H., Kanaki N.S., Rajani M.: Ind. J. Pharm. 37, 399 (2005).
3. Keay R.W.J, Onochie C.F.A, Stanfield D.P.: Nigerian Trees, vol II. p. 378, Federal Department of Forest Research, Ibadan 1964.
4. Sofowora A.: Medicinal plants and traditional medicine in Africa. 1st ed., p. 168, John Wiley and Sons Ltd., Chichester 1982.
5. Oluwemimo A., Usifoh C.O.: Pak. J. Sci. Ind. Res. 44, 286 (2001).
6. Boone, M.J.: *Hunteria umbellata* (K. Schum.) Hallier f. in Schmelzer G.H., Gurib-Fakim A. Eds. Prota 11: Medicinal plants/Plantes médicinales. [CD-Rom]. PROTA, Wageningen, Netherlands (2006).
7. Falodun A., Nworgu Z.A.M., Ikponmwosa M.O.: Pak. J. Pharm. Sci. 19, 256 (2006).
8. Reanmongkol W., Matsumoto K., Watanabe H., Subhadhirasakul S., Takaya H., Sakai S.: Biol. Pharm. Bull. 18, 33 (1995).
9. Reanmongkol W., Matsumoto K., Watanabe H., Subhadhirasakul S., Sakai S.: Biol. Pharm. Bull. 17, 1345 (1994).
10. Leewanich P., Tohda M., Matsumoto K., Subhadhirasakul S., Takayama H., Aimi N., Watanabe H.: Eur. J. Pharmacol. 332, 321 (1997).
11. Trease G.E., Evans M.C.: in A Textbook of Pharmacognosy 13th ed, p. 520, Bailiere Tindall, London 1989.
12. Winter C.A., Risley E.A., Nuss G.W.: Proc. Soc. Exp. Biol. Med. 111, 544 (1962).
13. Thambi P.T., Kuzhivelil B., Sabu M.C., Jolly C.I.: Ind. J. Pharm. Sci. 68, 352 (2006).
14. Glauce S.B.V., Tiago G.V., Rao V.S.N., Matos F.J.A.: Pharm. Biol. 36, 347 (1998).
15. Akindele A.J., Adeyemi O.O.: Fitoterapia 78, 25 (2007).
16. Hosseinzadeh H., Younesi H.: BMC Pharmacol. 2, 1 (2002).
17. Vinegar R., Truax J.F., Selph J.H., Johnston P.R., Venable A.L., McKenzie K.K.: Fed. Proc. 46, 118 (1987).
18. Di Rosa M., Giroud J.P., Willoughby D.A.: J. Pathol. 104, 15 (1971).
19. Ferreira S.H., Flower R.J., Parsons M.F., Vane J.R.: Prostaglandins 8, 433 (1974)
20. Lo T.N., Almeida A.P., Beaven M.A.: J. Pharmacol. Exp. Ther. 221, 261 (1982).
21. Zaninir J.C., Medeiros Y.S., Cruz A.B., Yunes R.R.A., Calixto J.B.: Phytother. Res. 6, 1, 1992
22. Kou J., Ma R., Zhu D., Yan Y.: Zhong Yao Cai 26, 268 (2003).
23. Greenwald R.A.: Methods Find. Exp. Clin. Pharmacol. 13, 75 (1991).
24. Martini N.D., Katerere D.R.P., Eloff J.N.: J. Ethnopharmacol. 93, 207 (2004).
25. Loomis T.A.: Essentials of Toxicity, 3rd ed., p. 198, Lea and Febiger, Philadelphia 1978.

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