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# Analgesic and Anti-inflammatory Effects of Methanolic Extract of *Pausinystalia Macroceras* Stem-Bark in Rodents

[1]Paul A. Nwafor, [2]T.W. Jacks and [2]A.U. Ekanem
[1]Department of Pharmacology, [2]Department of Anatomy,
College of Medical Sciences, University of Maiduguri,
P.M.B. 1069 Maiduguri, Borno State, Nigeria

**Abstract:** The effect of methanolic extract of *Pausinystalia macroceras* stem-bark was investigated in chemically-induced inflammation in rodents. The extract dose-dependently (17.5-350.0 mg $kg^{-1}$) inhibited acetic acid-induced writhing, formalin-induced pain licking and carrageenin-induced hind paw oedema in rodents. The extract also inhibited both the fresh egg albumin and prostaglandin $E_2$-induced inflammations as well as capsaicin-induced nociception in rats. These inhibitions were statistically significant ($p<0.01$-$0.001$). This effect may in part involve suppression of capillary permeability through neurogenic and non-neurogenic pathways.

**Key words:** *Pausinystalia macroceras*, methanolic extract, non-neurogenic, inflammation

## INTRODUCTION

*Pausinystalia macroceras* (Rubiaceae) is used for the treatment of male infertility. It is reported for its powerful aphrodisiac properties for which it is even currently being abused (Leboeuf *et al*., 1981). It has enjoyed much reputation among the pigmy tribes of Sangolema and Yoko of southern Cameroun and the Bakassi in Southern Nigeria (Margolis and Leslie, 1966). The plant is known as *Idagbon* in Yoruba, *Burantasi* in Hausa, *Ikeagwughnwankpi* among the Etches of Nigeria and *Duala djombe wa* among the Cameroun. Jacks *et al*. (2004) had earlier reported its acute toxicity in rats. Recently, pilot review of the extract by the authors in our laboratory revealed its potential in the treatment of inflammatory disorders. Based on these observations and cognizance of high incidence of inflammatory reactions among local populace in Nigeria who are daily involved in farming, fishing and grazing among other related occupations and aware of lack of literature report on the use of the plant on inflammatory conditions, the authors decided to investigate its potential in the experimentally-induced inflammation in rodents with a view to ascertaining the usefulness of the plant in the treatment of inflammatory disorders and encouraging further investigations on elucidation of its mechanisms of action.

## MATERIALS AND METHODS

**The plant material:** The plant material used was collected from Southern Cameroun in December, 1999. The plant was identified and authenticated by Dr. S.S. Sanusi of the Department of Biological Sciences, University of Maiduguri, Maiduguri, Nigeria. A specimen voucher (CMS 021) has been made and deposited at the Department of Pharmacology Laboratory, University of Maiduguri. The dried stem-bark was pulverized by grinding using pestle and mortar. Thereafter, 59 g of the ground stem-bark was subjected to exhaustive soxhlet extraction in 250 mL methanol for 72 h at 60°C. The extract was stored at -4°C until required for use.

**Animal stock:** Adult albino mice and rats (weighing 25-30 g and 165-200 g, respectively) were used in this study. All the animals were housed in a cross ventilated room with temperature of 22±2.5°C, 12 h light/12 h dark cycle) and were fed with standard mash (Unfailing Veterinary Service, Uyo, Nigeria) and water *ad libitum*.

**Acetic acid-induced writhing in mice:** The abdominal constrictions resulting from intraperitoneal (i.p.) injection of 3% acetic acid consisting of the contraction of abdominal muscle together with the stretching of hind limbs, were carried out according to the standard

**Corresponding Author:** Paul A. Nwafor , Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, P.M.B. 1017, Uyo Akwa Ibom State, Nigeria

procedure (Santos *et al.*, 1994; Correa *et al.*, 1996; Nwafor *et al.*, 2002). The animals were divided into six groups of six mice per group. Group 1 served as control while groups 2-4 were pretreated with 17.5, 175 and 350 mg $kg^{-1}$ of *Pausinystalia macroceras* extract (i.p.), respectively. Group 5 was administered with acetyl salicylic acid (100 mg $kg^{-1}$, i.p.) only, while group 6 received acetyl salicylic acid (100 mg $kg^{-1}$, i.p.) and 10 min later, was treated with the extract (175 mg $kg^{-1}$, i.p.). After 30 min, acetic acid was administered to all groups (except group 1). The numbers of writhing movements were counted for 45 min. Antinociception (analgesia) expressed as the reduction of the number of abdominal constrictions between control animals (acetic acid treated mice) and mice pretreated with the extract.

**Formalin-induced hind paw licking in rats:** The procedure used was essentially similar to that described previously by Hunskaar and Hole (1987), Correa and Calixto (1993) and Gorski *et al.* (1993). The animals were used to analyze the first phase of formalin-induced licking. Twenty microlitre (2 μL) of 2.5% formalin solution (0.9% formaldehyde) made up in phosphate buffer solution (PBS concentration: NaCl, 137 mM; KCl, 2.7 mM and phosphate buffer, 10 mM) was injected subcutaneously under the surface of the right hand paw. The amount of time spent licking the injected paw was timed and was considered as indicative of pain. The first of the nociceptive response normally peaks 5 min after injection and the second phase 15-30 min after formalin injection, representing the neurogenic and inflammatory pain responses, respectively (Hunskaar and Hole, 1987). The animals in groups 2-4 were pretreated with extract (17.5; 175 and 350 mg $kg^{-1}$, i.p, respectively). Group 5 received acetyl salicylic acid (100 mg $kg^{-1}$, i.p) only while group 6 received acetyl salicylic acid (100 mg $kg^{-1}$, i.p.) plus extract (175 mg $kg^{-1}$, i.p.) 10 mins later. After 30 min, all animals were challenged with buffered formalin. Group 1 served as the control and all responses were observed for 30 min after the injection of formalin (i.p.) into the hindpaw.

**Carrageenin-induced rat hind paw oedema:** Increase in the rat hind paw linear circumference induced by sub-plantar injection of the phlogistic agent was used as the measure of acute inflammation (Winter *et al.*, 1962). Adult albino rats of either sex were used after 24 h fast and deprived of water only during experiments. Inflammation of the hind paw was induced by injection of 0.1 mL of freshly prepared 1% carrageenin suspension in normal saline into the sub-plantar surface of the hind paw. The linear circumference of the injected paw was measured before and 0.5-5 h after administration of phlogistic agent at an interval of 30 min. For routine drug testing, the increase in paw circumference 0.5-5 h after administration of phlogistic agent was adopted as the parameter for measuring inflammation (Ekpendu *et al.*, 1994; Besra *et al.*, 1996; Nwafor *et al.*, 2002). Oedema (inflammation) was assessed as the difference in paw circumference between the control and 0.5-5 h after administration of the phlogistic agent (Hess and Milonig, 1972). The extract 17.5, 175 or 350 mg $kg^{-1}$ was administered intraperitoneally (i.p.) to groups 2-4, respectively, 30 min before induction of inflammation. Group 5 animals received acetyl salicylic acid (100 mg $kg^{-1}$; i.p.) while group 6 received acetyl salicylic acid (100 mg $kg^{-1}$, i.p.) and 10 min later, was treated with extract (175 mg $kg^{-1}$, i.p.). Control rats received carrageenin. The average (mean oedema was assessed by measuring with vernier calipers.

**Fresh egg albumin-induced inflammation in rats:** Increase in the rat hind paw linear circumference induced by sub-plantar injection of a phlogistic agent was used as the measure of acute inflammation (Winter *et al.*, 1962). The phlogistic agent employed in this study was fresh egg albumin (Akah and Nwanbie, 1994). Adult albino rats of either sex were used for this study. They were fasted for 24 h before use and were only deprived of water during experiment. Inflammation (oedema) of the hind paw was induced by injecting 0.1 mL of fresh egg-albumin into the subplantar surface of the hind paw. Oedema (inflammation) was assessed as the difference in paw circumference between the control and 1, 2, 3, 4 and 5 h after administration of the phlogistic agent (Hess and Milonig, 1972). Animals groups 2-4 received extract doses of (17.5, 175 or 350 mg $kg^{-1}$, i.p.), respectively, while group 5 received acetyl salicylic acid (100 mg $kg^{-1}$ i.p.), 10 min later with extract (175 mg $kg^{-1}$, i.p.) treatment. Group 6 received only acetyl salicylic acid. Each rat received 0.1 mL of fresh egg-albumin intraperitoneally (i.p.) 30 min post extract and drug treatment into the right paw. The linear circumference of the paw was measured after every hour for 5 h using vernier calipers. Group 1 served as the control and treated with only the egg-albumin.

**Prostaglandin $E_2$-induced hind paw oedema in rats:** Inflammation of the rat hind paw was induced by injecting prostaglandin-$E_2$, a phlogistic agent into the right hind paw sub-plantar surface of adult albino rats. They were used after 24 h fast and deprived of water only during experiment. The linear circumference of the injected paw was measured before and 1, 2, 3, 4 and 5 h after administration of phlogistic agent (Hess and Milonig, 1972). The drugs were administered intraperitoneally

(i.p.) before inducing inflammation. Group 1 received prostaglandin $E_2$ (100 ng $kg^{-1}$, i.p.) groups 2-4 received 17.5, 175 or 350 mg $kg^{-1}$ of extract, respectively. Group 5 received indomethacin (60.0 mg $kg^{-1}$, i.p.) while group 6 was injected with extract (175 mg $kg^{-1}$, i.p.) followed 30 min later with indomethacin. The average mean oedema (inflammation) was measured using vernier calipers.

**Capsaicin-induced inflammation in rats:** To provide evidence on the possible analgesic effect of the extract on the neurogenic pain, the extract was investigated on its ability to antagonize capsaicin-induced oedema in rat. The procedure of Sakurada *et al.* (1992) was adopted with slight modification. Oedema was assessed as the difference in paw circumference between the control and 0.5-5 h after administration of the phlogistic agent. The extract (17.5-350 mg $kg^{-1}$, i.p.) was administered intraperitoneally (i.p.) to groups 2-4, 30 min before the induction of inflammation. Group 5 received acetyl salicylic acid (100 mg $kg^{-1}$, i.p.), while group 6 was injected with extract (175 mg $kg^{-1}$, i.p.), followed 30 min later with capsaicin. Group 1 received capsaicin (5 μg $kg^{-1}$, i.p.) the average (mean) oedema was assessed by measuring with Venier Callipers.

**Statistical analysis:** Multiple comparisons of mean± SEM were carried out by one way analysis of variance (ANOVA), followed by Tukey-Krammar multiple comparisons tests. A probability level of less than 5% was considered significant.

## RESULTS

**Acetic acid-induced writhing in mice:** The extract (17.5-350 mg $kg^{-1}$, i.p.) dose-dependently reduced acetic acid-induced abdominal constrictions and stretching of hind limbs. The reduction was statistically significant ($p<0.001$) (Table 1).

**Formalin-induced hind paw licking in rats:** The extract pretreated animals showed a significant ($p<0.001$) dose related reduction of hind paw licking caused by formalin (Table 2).

**Carrageenin-induced hind paw oedema in rats:** The extract showed a good anti-inflammatory activity against acute inflammation (Table 3). It suppressed in a dose-related manner the increase in the rat paw oedema caused by carrageenin. The inhibition was statistically significant ($p<0.05$-$0.001$).

**Fresh egg albumin-induced inflammation in rats:** The extract showed significant anti-inflammatory activity against acute inflammation ($p<0.01$-$0.001$). It suppressed in a dose-related manner the increase in the rat paw oedema caused by egg albumin (Table 4).

**Prostaglandin $E_2$-induced hind paw oedema in rats:** Pretreatment of animals with the extract caused a dose-dependent decrease in prostaglandin $E_2$-induced inflammation in rats. This decrease was statistically significant ($p<0.01$-$0.001$) (Table 5).

Table 1: Effect of *Pausinystalia macroceras* on acetic acid-induced writhing in mice

| Dose (mg $kg^{-1}$) | 5 min | 10 min | 15 min | 20 min | 25 min | 30 min | 35 min | 40 min | 45 min |
|---|---|---|---|---|---|---|---|---|---|
| Control (acetic acid) | 0.50±0.37 | 20.80±0.86 | 18.83±0.21 | 35.67±0.43 | 64.67±0.64 | 43.00±0.29 | 41.83±0.99 | 36.00±0.38 | 18.33±0.54 |
| 17.50 | 0.50±0.24 | 10.67±0.36$^c$ | 16.50±0.25$^c$ | 33.00±0.49$^c$ | 60.83±0.52$^c$ | 38.67±0.23$^c$ | 35.67±0.23$^c$ | 31.33±0.23$^c$ | 16.17±0.44$^c$ |
| 175.00 | 0.33±0.36 | 7.50±0.24$^c$ | 15.83±0.18$^c$ | 31.00±0.55$^c$ | 30.83±0.65$^c$ | 28.23±0.23$^c$ | 23.67±0.36$^c$ | 19.83±0.71$^c$ | 15.17±0.18$^c$ |
| 350.00 | 0.33±0.23 | 5.67±0.36$^c$ | 13.00±0.28$^c$ | 27.67±0.23$^c$ | 25.00±0.74$^c$ | 23.33±0.23$^c$ | 21.20±0.36$^c$ | 17.14±0.36$^c$ | 14.33±0.23$^c$ |
| ASA (100.0) | 0.18±0.21 | 3.04±0.31$^c$ | 5.40±0.13$^c$ | 17.45±0.03$^c$ | 15.22±0.34$^c$ | 12.04±0.44$^c$ | 9.36±0.15$^c$ | 7.64±0.11$^c$ | 6.25±0.23$^c$ |
| 175+ASA | 0.05±0.11 | 0.15±0.14$^c$ | 1.44±0.22$^c$ | 11.38±0.26$^c$ | 10.06±0.18$^c$ | 8.64±0.41$^c$ | 6.66±0.35$^c$ | 5.00±0.26$^c$ | 2.66±0.42$^c$ |

Significance relative to control: $^c p<0.001$; ASA = Acetyl salicylic acid; Values represent mean±SEM (n = 6)

Table 2: Effect of *Pausinystalia macroceras* extract on formalin-induced hind paw licking in rats

| Dose (mg $kg^{-1}$) | 5 min | 10 min | 15 min | 20 min | 25 min | 30 min |
|---|---|---|---|---|---|---|
| Control | 18.00±0.17 | 7.67±0.02 | 10.50±0.21 | 16.17±0.11 | 14.50±0.01 | 12.33±0.01 |
| 17.50 | 10.83±0.50$^a$ | 7.17±0.01$^a$ | 07.67±0.10$^a$ | 12.17±0.05$^a$ | 11.50±0.13$^a$ | 11.50±0.30$^a$ |
| 175.00 | 14.67±0.20$^a$ | 0.50±0.02$^a$ | 07.16±0.02$^a$ | 07.83±0.02$^a$ | 09.00±0.12$^a$ | 8.67±0.31$^a$ |
| 350.00 | 11.33±0.30$^a$ | 0.00±0.00$^a$ | 04.33±0.14$^a$ | 07.83±0.13$^a$ | 07.17±0.11$^a$ | 5.00±0.21$^a$ |
| ASA (100) | 7.83±0.30$^a$ | 1.83±0.02$^a$ | 01.33±0.06$^a$ | 04.17±0.24$^a$ | 04.33±0.40$^a$ | 3.83±0.52$^a$ |
| 175+ASA | 7.00±0.02$^a$ | 1.33±0.02$^a$ | 01.83±0.05$^a$ | 02.83±0.30$^a$ | 04.17±0.44$^a$ | 2.83±0.44$^a$ |

Significance relative to control: $^a p<0.001$; ASA = Acetylsalicylic acid; Values represent mean±SEM (n = 6)

Table 3: Effect of *Pausinystalia macroceras* extract on carrageenin-induced hind paw oedema in rats

| Dose (mg $kg^{-1}$) | Initial measurement (mm) | 30 min | 1 h | 2 h | 3 h | 4 h | 5 h |
|---|---|---|---|---|---|---|---|
| Control | 0.41±0.01 | 0.59±0.02 | 0.64±0.05 | 0.75±0.01 | 0.69±0.01 | 0.64±0.01 | 0.59±0.01 |
| 17. 50 | 0.45±0.00 | 0.56±0.01$^a$ | 0.60±0.01$^a$ | 0.67±0.01$^b$ | 0.67±0.02 | 0.62±0.01$^b$ | 0.58±0.02 |
| 175.00 | 0.44±0.00 | 0.58±0.02 | 0.60±0,00$^a$ | 0.65±0.02$^b$ | 0.64±0.02$^b$ | 0.60±0.01$^b$ | 0.56±0.02$^a$ |
| 350.00 | 0.40±0.03 | 0.56±0.01$^a$ | 0.58±0.01$^b$ | 0.61±0.01$^b$ | 0.61±0.01$^b$ | 0.58±0.00$^b$ | 0.54±0.01$^b$ |
| ASA (100) | 0.50±0.02 | 0.56±0.01$^a$ | 0.58±0.01$^b$ | 0.61±0.00$^b$ | 0.58±0.00$^b$ | 0.55±0.00$^b$ | 0.51±0.01$^b$ |
| 175+ASA | 0.45±0.01 | 0.55±0.01$^b$ | 0.57±0.01$^b$ | 0.59±0.01$^b$ | 0.56±0.00$^b$ | 0.53±0.00$^b$ | 0.50±0.01$^b$ |

Significance relative to control: $^a p<0.05$; $^b p<0.001$; ASA = Acetylsalicylic acid; Values represent mean±SEM (n = 6)

Table 4: Effect of *Pausinystalia macroceras* extract on fresh egg albunin-induced hind paw oedema in rats

| Dose (mg $kg^{-1}$) | 1 h | 2 h | 3 h | 4 h | 5 h |
|---|---|---|---|---|---|
| Control (0.1 mL egg alb) | 7.08±0.12 | 6.02±0.18 | 6.15±0.20 | 5.93±0.22 | 5.30±0.15 |
| 17.50 | 6.87±0.06 | 5.95±0.05 | 5.50±0.40[b] | 5.10±0.37[b] | 4.78±0.36[b] |
| 175.00 | 6.15±0.33[b] | 6.53±0.20 | 5.62±0.20[a] | 5.28±0.22[b] | 4.72±0.18[b] |
| 350.00 | 5.72±0.26[b] | 5.52±0.10 | 5.37±0.10[b] | 5.07±0.04[b] | 4.90±0.04[a] |
| 175+ASA | 5.62±0.28[b] | 4.77±0.17[b] | 4.60±0.13[b] | 4.50±0.12[b] | 4.22±0.07[b] |
| ASA (100) | 4.95±0.20[b] | 4.92±0.12[b] | 4.60±0.06[b] | 4.32±0.09[b] | 4.22±0.07[b] |

Significance relative to control: [a]p<0.01; [b]p<0.001; ASA = Acetylsalicylic acid; Values represent mean±SEM (n = 6)

Table 5: Effect of *Pausinystalia macroceras* extract on Prostaglandin $E_2$-induced hind paw oedema in rats

| Dose (mg $kg^{-1}$) | 1 h | 2 h | 3 h | 4 h | 5 h |
|---|---|---|---|---|---|
| $PGE_2$ ($1.0\times10^{-4}$) | 6.90±0.07 | 6.35±0.11 | 6.05±0.10 | 5.75±0.08 | 5.22±0.07 |
| 17.50 | 6.83±0.03 | 6.35±0.11 | 5.90±0.07 | 5.35±0.11 | 4.90±0.07[b] |
| 175.00 | 6.40±0.06[b] | 6.15±0.09 | 5.68±0.13[b] | 5.15±0.09[a] | 4.18±0.08[b] |
| 350.00 | 6.20±0.12[b] | 5.80±0.13[b] | 5.28±0.12[b] | 4.95±0.10[b] | 4.13±0.07[b] |
| Indo (60.00) | 5.72±0.14[b] | 5.28±0.12[b] | 4.80±0.13[b] | 4.28±0.12[b] | 3.80±0.01[b] |
| Indo+175Extr. | 5.38±0.20[b] | 4.97±0.20[b] | 4.53±0.18[b] | 3.90±0.15[b] | 3.55±0.05[b] |

Significance relative to control: [a]p<0.01; [b]p<0.001; $PGE_2$ = Prostaglandin $E_2$ ; Indo = Indomethacin Values represent mean±SEM (n = 6)

Table 6: Effect of *Pausinystalia macroceras* extract on capsaicin-induced hind paw inflammation in rats

| Dose ($mg^{-1}$ kg) | Initial measurement (mm) | 30 min | 1 h | 2 h | 3 h | 4 h | 5 h |
|---|---|---|---|---|---|---|---|
| Control | 0.45±0.01 | 0.55±0.00 | 0.57±0.00 | 0.60±0.01 | 0.57±0.01 | 0.54±0.01 | 0.51±0.01 |
| 17.50 | 0.46±0.01 | 0.53±0.00 | 0.55±0.00 | 0.58±0.00[b] | 0.56±0.00 | 0.54±0.01 | 0.50±0.01 |
| 175.00 | 0.44±0.01 | 0.49±0.00 | 0.51±0.00 | 0.55±0.01[c] | 0.52±0.01 | 0.51±0.01[c] | 0.49±0.01[a] |
| 350.00 | 0.43±0.00[a] | 0.48±0.00[c] | 0.49±0.01[a] | 0.52±0.00[c] | 0.48±0.00[c] | 0.45±0.01[c] | 0.43±0.01[c] |
| 0.01[c] ASA (100) | 0.41±0.01[c] | 0.46±0.10[a] | 0.48±0.01[b] | 0.48±0.01[c] | 0.47±0.00[c] | 0.44±0.00[c] | 0.48±0.01[c] |
| [c]175+ASA (100) | 0.41±0.00[c] | 0.45±0.00[b] | 0.46±0.00[c] | 0.46±0.00[c] | 0.44±0.00[c] | 0.44±0.00[c] | 42.00± 0.01[c] |

Significance relative to control: [a]p<0.05; [b]p<0.01; [c]p<0.001; ASA = Acetyl salicylic acid; Values represent mean±SEM (n = 6)

**Capsaicin-induced hind paw inflammation in rats:** The extract reduced the inflammation induced by phlogistic agent (capsaicin) in a dose related manner (Table 6). This reduction was significant (p<0.05-0.001).

## DISCUSSION

The extract caused a dose and time-dependent antinociception against chemically-induced nociception (pain) in rodents. Acetic acid causes inflammatory pain by inducing capillary permeability (Amico-Roxas *et al*., 1984; Nwafor and Okwuasaba, 2003), formalin exhibits neurogenic and inflammatory pain (Vaz *et al*., 1996, 1997) while capsaicin induces neurogenic pain mediated by neuropeptides such as calcitonin gene-related peptide, substance P, neurokinin A and vasoactive intestinal peptide, which are released from capsaicin-sensitive neurons (Blazso and Gabor, 1995). That the extract showed significant effect in these types of pain induction suggests that its analgesic effect may in part be related to its anti-inflammatory neurogenic and non-neurogenic properties.

Carrageenin induction of inflammation involves three distinct phases of mediators release including histamine and 5-hydroxytryptamine in the first phase, kinins in the second phase and prostaglandin in the third phase (Di Rosa *et al*., 1971; Singh *et al*., 1996). Prostaglandin in particular, is known to cause or enhance the cardinal signs of inflammation (Singh *et al*., 1996). Fresh egg albumin-induced inflammation appears to be similar to carrageenin induced inflammation in rats (Nwafor and Okwuasaba, 2003). The extract progressively reduced oedema induced by these chemicals. Due to a primary stimulus, two mechanisms contribute to the development of oedema caused by increased vascular permeability. One induced by local release or formation of various autacoids and another induced neurogenically by stimulation of primary sensory neurons and subsequent mediator (substance P) released from peripheral endings of these fibers (Gabbiani *et al*., 1970; Gamse *et al*., 1980; Amico-Roxas *et al*., 1984). According to Lembeck and Holzer (1979), the neurogenic component plays an important role in maintaining the non-neurogenic plasma extravasation since the stimulation of peripheral neurons and subsequent release of substance P from peripheral sensory endings causes further release of histamine from mast cells. It therefore means that the possible specific action of this extract in blocking the neurogenic component of the stimulated vascular permeability can stop the series of pathogenetic events locally evoked by noxious stimuli.

In conclusion, although the exact mechanism of antinociceptive properties of the extract is not fully understood, it may not be unrelated to the present study, which involves suppression of capillary permeability through neurogenic and non-neurogenic pathways.

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