Anti-Histaminic Potential of the Fractions from *Hedranthera Barteri* [(Hook F.) Pichon] Root in Laboratory Rodents

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Summary: *Hedranthera barteri* L. (HB) is used in the treatment of painful conditions and oedema amongst its folkloric use. The hexane and ethyl acetate fractions of the root of *H. barteri* were investigated for anti-nociceptive and anti-inflammatory properties and probable mechanism of action. Hot plate, tail flick, formalin-induced oedema and acetic acid-induced writhing tests were employed to investigate the anti-nociceptive activity while the anti-inflammatory activity was investigated using carrageenan-induced paw oedema. Anti-histaminic potential of HB root extracts on the rat peritoneal mast cells (RPMCs) was explored through spectrofluorometric method. The root was screened for its phytochemical components. The HB root contains alkaloids, cardenolides and saponins. HXHBR exhibited higher anti-inflammatory potentials (P<0.001). HXHBR dose-dependently (P<0.01) reduced the histamine release from the rat peritoneal mast cells which is comparable with a standard anti-histaminic drug, ketotifen. These results showed that EAHBR and HXHBR possess anti-nociceptive and anti-inflammatory activities, and suggested its mechanism of action through the inhibition of histamine, an inflammatory mediator, usually released during the early phase of allergic responses and chronic phase of inflammatory pain. Flavonoids, alkaloids and/or saponins present in HB root may be responsible for its anti-nociceptive and anti-inflammatory properties.

Keywords: *Hedranthera barteri, anti-inflammatory activity, anti-nociceptive activity, anti-histaminic activity.*

INTRODUCTION

Hedranthera barteri, HB (Family: Apocynaceae) is a shrub found in damp situations of the closed forest in Ghana, North and South Nigeria, West Cameroon and Zaire (Congo Brazzaville). The fruit has been used to prevent abortion in women (Thomas, 1910). The leaf exudate has been used to treat gonorrhea, as a femifuge and to suppress painful tumor (Ainslie, 1937). The leaf decoction is drunk by Igbo of South Nigeria to treat dizziness (Thomas, 1967). The plant has been reported to be rich in alkaloids like amataine, beninine, goziline, owerreine, subsessiline, isoquinoline and vobstusine (William and Li, 1970). Studies have shown that its alkaloids have antibacterial properties (Ogunlana and Ramstad, 1975). In a previous *in-vitro* study aimed at screening some Nigerian medicinal plants, HB extract was reported to have anti-inflammatory, anti-malarial and antibacterial activities (Chukwujekwu *et al.,* 2005) while *in vivo* studies reported its anti-nociceptive and anti-inflammatory (Onasanwo and Elegbe, 2006; Onasanwo *et al.,* 2008). Beta-sitosterol has been suggested to be partly responsible for its anti-inflammatory pain remedy (Onasanwo *et al.*, 2008).

The root extracts of medicinal plants are therapeutically active and appear to induce fewer side effects than the extracts from the leaves and stems of the herb. More than 70 compounds have been identified in the roots of natural products including diterpinoids, triterpinoids, sesquiterpinoids, alkaloids, beta-sitosterol, dulcitol and glycosides (Tao and Lipsky, 2000) which are known for their medicinal purposes. Most of the active compounds have been speculated to be extracted through various solvents apart from hexane but recent finding revealed that many active anti-inflammatory compounds are

present in hexane fractions of many medicinal plants. Also, mast cell plays a role in the immune and inflammatory responses by sensing a variety of pathogenic patterns through cell-surface receptors, and contains many granules rich in histamine. Histamine is an essential mediator of the pathophysiology of many allergic diseases. It has been implicated as one of the inflammatory mediators released in the first 2-3 hours of inflammatory responses.

In this study, the anti-nociceptive and anti-inflammatory activities of hexane and ethyl acetate fractions of *HB* root were investigated and the probable mechanism of action, using the assay of histamine release was explored.

MATERIALS AND METHODS

Plant material and extractions

The roots of HB used for this study were purchased from the Herbarium Department, Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria and authenticated voucher specimen (FHI-108375) was deposited there for future reference by Mr. Felix Usani. The root of HB was extracted with hexane and ethyl acetate by soxhlet extraction yielding 12.4% and 16.4% respectively. The solvents were removed at 40^0C under reduced pressure in a rotavapor. The semi-solid samples of the extract were stored at 4^0C until when needed. The extracts were prepared as suspensions with 2.5% tween 80/normal saline, and were administered 60 mins before each experimental model.

Animals

The experiments were performed using male Sprague Dawley (SD) rats (180±5)g and balb/c mice (25±5)g. The animals were kept in standard environmental conditions, subjected to a 12 h light–dark cycle and 25±2 °C relative humidity 50–60%, fed with pellets of rodent feed (Ashirwad Industries, Chandigarh, India) and water *ad libitum*. The Institutional Animal Care Committee of the Indian Institute of Integrative Medicine, Jammu-Tawi, India approved the experimental protocols.

Chemicals and Drugs

All chemicals/reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA) except otherwise stated. Paracetamol, tween 80, glacial acetic acid, formaldehyde were purchased from S.D. Fine Chemical Pvt. Ltd., Mumbai, India. The standard drugs were suspended in 2.5% tween 80/normal saline.

Phytochemical Screening

The phytochemical screening for alkaloids, cardenolides, anthraquinone, saponins, tannins and flavonoids was done on the dried root of the plants

Anti-nociceptive activity

Hot plate test in mice: Pain reflexes in response to a thermal stimulus were measured using a hot plate apparatus (Ugo Basile, Italy) as described by Eddy (1950) with slight modification. The control group of mice received normal saline (10ml/kg, p.o.). The test group mice were treated with HXHBR and EAHBR (25, 100, 200 mg/kg, p.o.) and paracetamol (500 mg/kg, p.o.). Mice were habituated to the apparatus for 1min before the start of the test. They were placed on the hot plate of 25.4cm x 25.4cm at $(55±1.0)^0$C, which is surrounded by an opened-top acrylic cage (19cm tall), with the start/stop button on the timer. A 30sec cut-off time was used to prevent tissue damage. The latency measures were taken both before and 60min after drug/extract administration, as the time elapsed between placing the mice on the hot plate and forepaw lick, hind paw flick or jump. Each mouse was immediately removed from the hot plate and returned to its home cage.

Tail flick test in rats: The rat tail-flick latency was assessed by applying radiant heat on a selected spot on the tail, 2.0-2.5cm from the tail tip, using tail flick apparatus (Ugo-Basile, Italy) as described by D'Amour and Smith (1941). The different test groups received various doses of HXHBR and EAHBR (25, 100, 200mg/kg, p.o.) and paracetamol (500mg/kg, p.o.), reference drug, while the control group received distilled water (2ml/kg, p.o.). The distal part of the tail was placed over a heated nichrome wire and the time taken for tail-flick response was recorded both before and after drug administration (0, 60, 120 and 180min). A cut-off time of 20sec was followed to avoid undue tissue damage of the tail by the radiant heat.

Formalin-induced paw licking test in rats:

20µL of 2.5% formalin was injected into the plantar surface of the left hind-paw of the rat (Hunskar and Hole, 1997) 60mins after treatment with the extract. The test was carried out in a transparent plastic chamber (30 x 30 x 30cm) with a mirror placed at the base (bottom) of the chamber to allow an unobstructed view of the rats. The time that the rat spent licking the injected hind paw was measured as an index of pain or nociception. The initial, acute nociceptive response (0-5min) after formalin injection indicated the first phase while (15-30min) indicated the second's chronic phase.

Acetic acid-induced abdominal writhing test in mice:

The mice were injected, intraperitoneally, with

0.1ml/10g body weight of 3% acetic acid solution one hour after treatment with the extract, which induced the characteristic writhing (Koster *et al.,* 1959). The number of writhing was observed between (5-15min). The data were collected and computed according to the following formula:

% Inhibition =
$$\frac{[(\text{Mean no of writhing test})_{control} - (\text{Mean no writhing test})_{test}]}{(\text{Mean no of writhing test})_{control}} \times 100\%$$

Anti-inflammatory activity

Carrageenan-induced paw oedema in rats: Pedal inflammation in male albino rats was produced by an injection of 0.1ml of 1% carrageenan into the right hind foot of the rat under the subplantar aponeurosis (Winter *et al.,* 1962). The animals were treated with the extract, orally 60min before carrageenan injection. The inflammation was quantified by measuring the volume displaced by the paw, using a plethysmometer (Ugo Basile, Italy) at time 0, 1, 3, and 24hrs after carrageenan injection. Ibuprofen (100mg/kg) was given as reference drug. The inhibiting activity was calculated according to the formula:

$$\% \text{ inhibition} = \frac{[(C_t - C_o)_{control} - (C_t - C_o)_{test}]}{(C_t - C_o)_{control}} \times 100$$

where C_o = Mean paw size in the control group and C_t = Mean paw size in the treated group.

Preparation of rat peritoneal mast cells (RPMCs)

RPMCs were isolated as described by Kim (2000). In brief, rats were anesthetized by ether and quickly injected with 20ml of Tyrode buffer B (137mM NaCl, 5.3mM glucose, 12mM NaHCO3, 2.7mM KCl, 0.3mM NaHPO4) containing 0.1% gelatin, into the peritoneal cavity and the abdomen was gently massaged for about 90sec. The peritoneal cavity was carefully opened and the fluid containing peritoneal cells was aspirated using a Pasteur pipette. Thereafter, the peritoneal cells were sedimented at 150*g* for 10min at room temperature and re-suspended in Tyrode buffer B. Mast cells were separated from the major components of rat peritoneal cells, i.e. macrophages and small lymphocytes (Yurt *et al.,* 1977).

Compound 48/80-induced histamine release

Purified RPMCs were re-suspended in Tyrode buffer A (10mM HEPES, 130mM NaCl, 5mM KCl, 1.0mM MgCl$_2$, 1.4 mM CaCl$_2$, 5.6mM glucose, 0.1% BSA) and were pre-incubated with HB extracts (25, 50 and 100µg/ml) for 10 min at 37 °C. The cells were further incubated for 20min with compound 48/80 (100µL). The cells were then incubated for 20min at 37^{0}C before centrifugation at 2000g for 10min.The cells were separated from the released histamine. 400µL of 1N NaOH was added to each tube of the cells and stirred constantly on addition of 400µL of 0.2% O-pthaldehyde in methanol. The tubes were allowed to stand for 2min after addition of 200µL of 3N HCl into the tubes. Then, 3N HCl was added into the tubes again.

Assay of histamine release

Histamine content was measured by the OPA spectro-fluorometric method according to Shore *et al.* (1959), with excitation and emission wavelengths of 350 and 450, respectively (slit 2.5nM). The inhibition percentage of histamine release was calculated using the following equation: % Inhibition = (A-B)/A x 100% *where A is the histamine released without HB and B is the histamine released with HB.*

Statistical analysis

Data are represented as the standard error of the mean (S.E.M.) of the different experiments under the same conditions. Student's *t*-test was used for statistical comparison of results. For multiple comparisons, one way analysis of variance (ANOVA) was used to make a statistical comparison between the groups followed by Newman-Keuls Multiple Comparison Test. The results with *P*<0.05 were considered statistically significant.
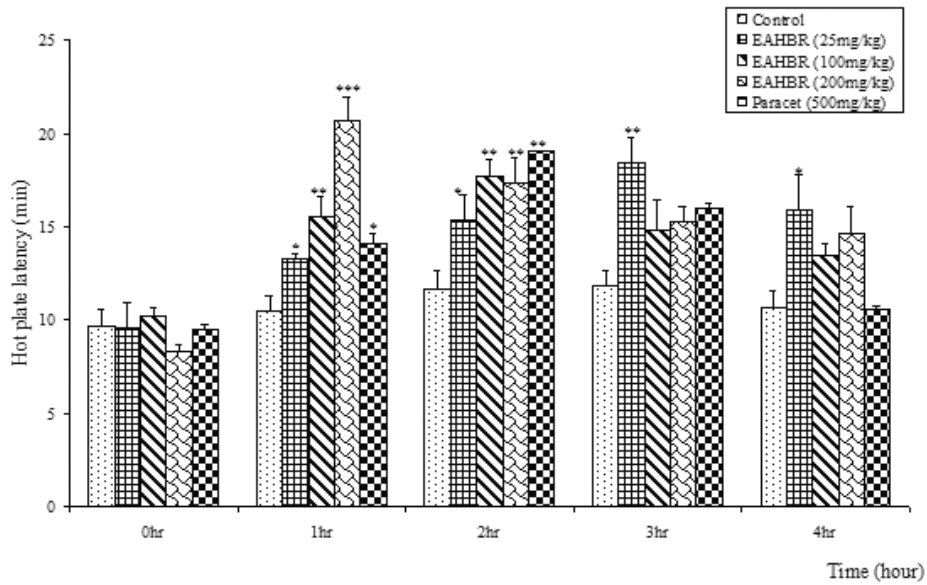
RESULTS

The anti-nociceptive effects of EAHBR on hot plate latency assay in mice

The anti-nociceptive potential of EAHBR on hot plate test using balb/c mice is shown in figure 1. EAHBR showed a statistically significant anti-nociceptive property within the first 1hr with 200mg/kg at 20.68 ± 1.33sec (P<0.001), 100mg/kg at 15.56 ± 1.07sec (P<0.01) and 25mg/kg at 13.31 ± 0.26sec (P<0.05). This revealed a dose-dependent manner of its anti-nociceptive property. The paracetamol group tolerated pain for 14.12 ± 0.56sec. Similar dose-dependent anti-nociceptive property was observed at the second hour of the scoring but better pronounced with paracetamol (19.04 ± 0.62sec), P<0.01, compared with EAHBR at 25, 100 and 200mg/kg which tolerated pain for 15.32 ± 1.44sec (P<0.05), 17.74 ± 0.97sec (P<0.01) and 17.36 ± 1.31sec (P<0.01) respectively. 25mg/kg of EAHBR attained the peak of its activity within three hours (18.42 ± 1.44 sec), P<0.01; suggesting its long acting property at a lower dose compared with its higher dose. The significance of the higher and lower doses of the extract at the 1st and 3rd hours respectively, were significantly higher compared with the standard drug, paracetamol (P<0.001, P<0.01). The effect of the EAHBR and paracetamol subsided after the 3rd

hour of their administration except for 25mg/kg of EAHBR.

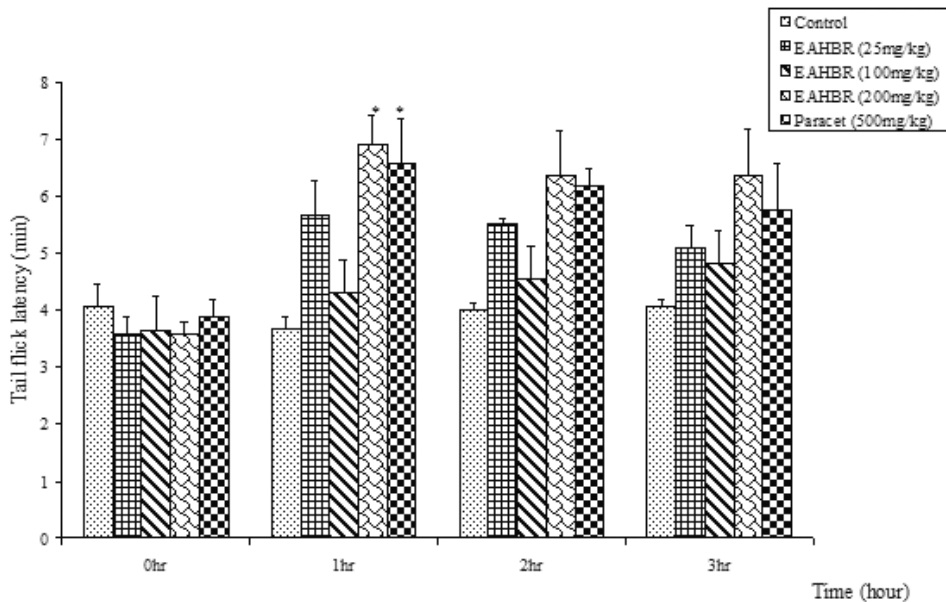*The anti-nociceptive effects of EAHBR on tail flick assay in albino rats*

The anti-nociceptive properties of various doses of EAHBR are shown in figure 2. EAHBR (200mg/kg) showed a statistically significant anti-nociceptive property (6.92 ± 0.59sec), P<0.05, together with paracetamol (7.18 ± 1.00 sec), P<0.05; after 60mins.

Figure 1:

The anti-nociceptive effects of EAHBR on hot plate latency assay in mice

Each value is presented as the mean ± S.E.M. (n = 8 mice). *P < 0.05, **P<0.01 or ***P<0.001; significantly different from the control value.

Figure 2:

The anti-nociceptive effects of EAHBR on tail flick assay in rats.

Each value is presented as the mean ± S.E.M. (n = 8 rats). *P<0.05; significantly different from the control value.

Hedranthera barteri-derived anti-histaminic potential

The anti-nociceptive effects of HXHBR on tail flick assay in albino rats

The anti-nociceptive properties of various doses of HXHBR are shown in figure 3. HXHBR at 25, 100 and 200mg/kg showed significant tolerance to pain for 11.00 ± 0.84sec (P<0.05), 10.00 ± 0.72sec (P<0.05) and 12.00 ± 0.73sec (P<0.01) respectively which were comparable to that of paracetamol (11.60 ± 1.00sec), P<0.01. The anti-nociceptive property of HXHBR (200mg/kg) was significantly higher than that of paracetamol. The anti-nociceptive potential of 100mg/kg HXHBR reached its peak at the 2nd hour after its administration (11 ± 1.30 sec), P<0.01.

The anti-nociceptive effects of EAHBR and HXHBR on formalin-induced paw licking in albino rats

All the doses of the EAHBR and HXHBR showed dose dependent anti-nociceptive properties, both at the early and late phases of the paw licking in albino rats with the exception of 25mg/kg and 100mg/kg of EAHBR at the early phase as shown in figure 4.
At the early phase, the licking time for EAHBR (50, 100 and 200mg/kg) were 28.20 ± 2.80sec, 33.20 ± 3.97sec and 25.00 ± 3.29sec respectively (P<0.05) while for aspirin it was 10.60 ± 0.87sec (P<0.001). At the late phase, the licking time of EAHBR (50, 100 and 200mg/kg) was 144.80 ± 13.36sec (P<0.05),

102.60 ± 10.27sec (P<0.01) and 59.80 ± 6.28sec (P<0.001) respectively while aspirin group was 44.00 ± 1.79sec (P<0.001).

At the early phase of the anti-nociceptive property of HXHBR in formalin test, the licking time for EAHBR (50, 100 and 200mg/kg) were 24.20 ± 1.80sec (P<0.05), 23.20 ± 3.97sec (P<0.05) and 19.00 ± 1.29sec (P<0.01) respectively while aspirin group is 10.60 ± 0.87sec (P<0.01). At the late phase, the licking time for HXHBR (50, 100 and 200mg/kg) was 83.80 ± 5.36sec (P<0.01); 69.60 ± 10.27sec (P<0.001) and 63.80 ± 6.280sec (P<0.001) respectively while aspirin group is 33.20 ± 1.79sec (P<0.001).

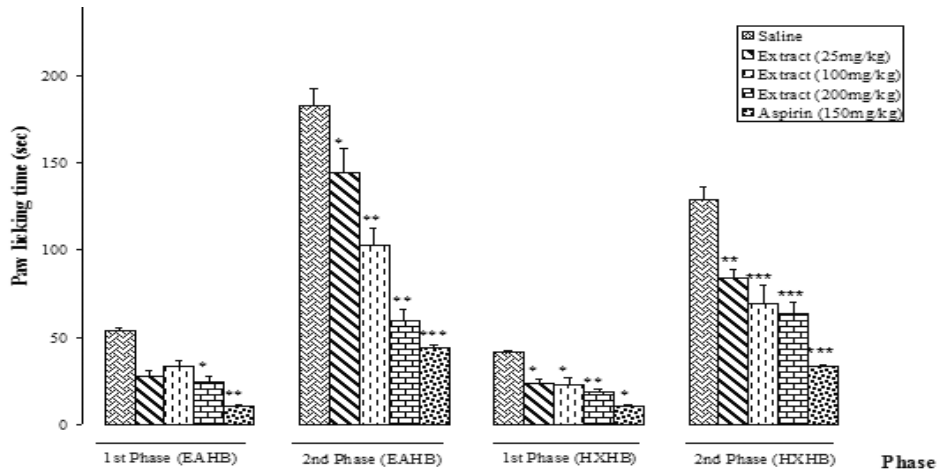*The anti-nociceptive effects of EAHBR and HXHBR on acetic acid-induced abdominal writhing in mice*

All doses of EAHBR and HXHBR showed dose dependent anti-nociceptive properties in acetic acid-induced abdominal writhing in mice as shown in table 1. The inhibition of the abdominal writhing produced by 200mg/kg of EAHBR (68.72%) was significantly higher than that of 150mg/kg aspirin (66.52%) but 200mg/kg of HXHBR produced an inhibition of 72.05% comparable with aspirin (78.12%).
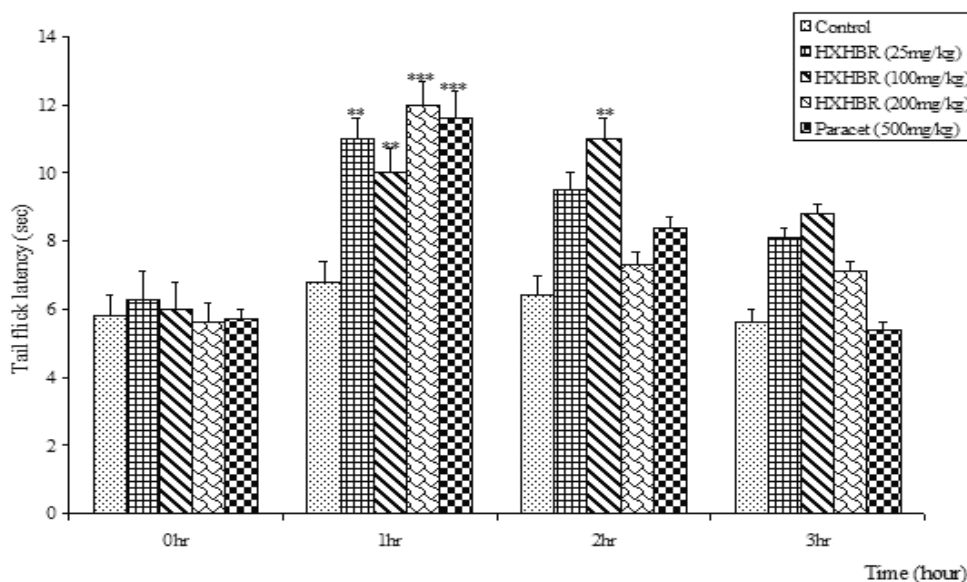
Figure 3:
Anti-nociceptive effects of HXHBR on tail flick assay in albino rats
Each value is presented as the mean ± S.E.M. (n=8rats). **P<0.01 or ***P<0.001; significantly different from the control value.
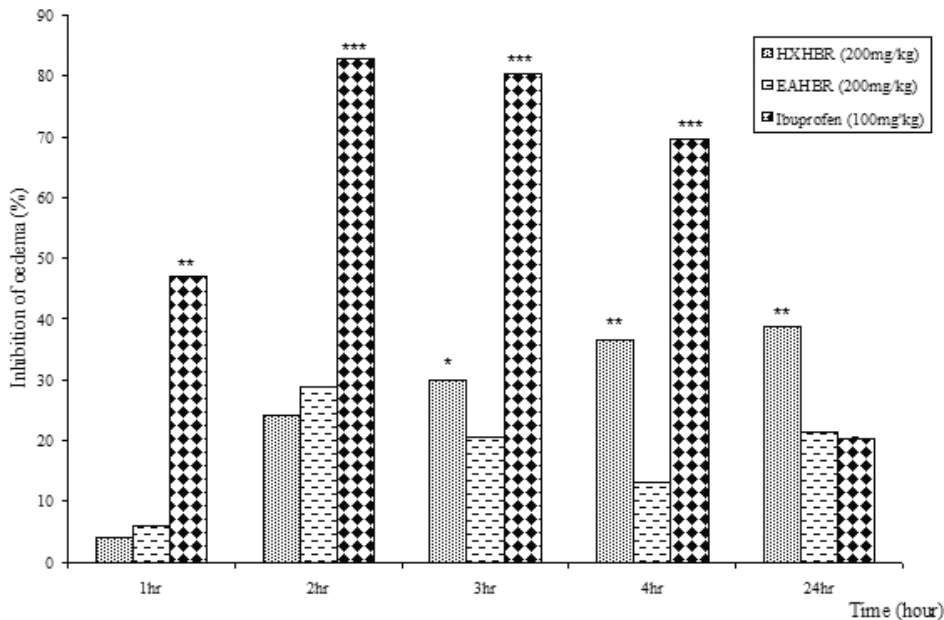
Figure 4:
Anti-nociceptive effects of EAHBR and HXHBR on formalin-induced paw licking in albino rats in albino rats'
Each value is presented as the mean ± S.E.M. (n = 8 rats). *P<0.05, **P<0.01 or ***P<0.001; significantly
different from the control value.

Table 1:
The effects of EAHBR and HXHBR on acetic acid-induced abdominal writhing in mice.

Treatment/	EAHBR		HXHBR	
Dose(mg/kg)	Number of writhing	% Inhibition	Number of writhing	% Inhibition
Normal Saline	45.4 ± 1.50	0	59.4 ± 1.47	0
25	24.2 ± 1.42**	46.7	24.6 ± 2.24	58.59
100	21.8 ± 1.77***	51.98	21.4 ± 1.28***	63.97
200	14.2 ± 0.96***	68.72	16.6 ± 1.07***	72.05
Aspirin (150)	15.2 ± 1.39***	66.52	13.0 ± 1.18***	78.12

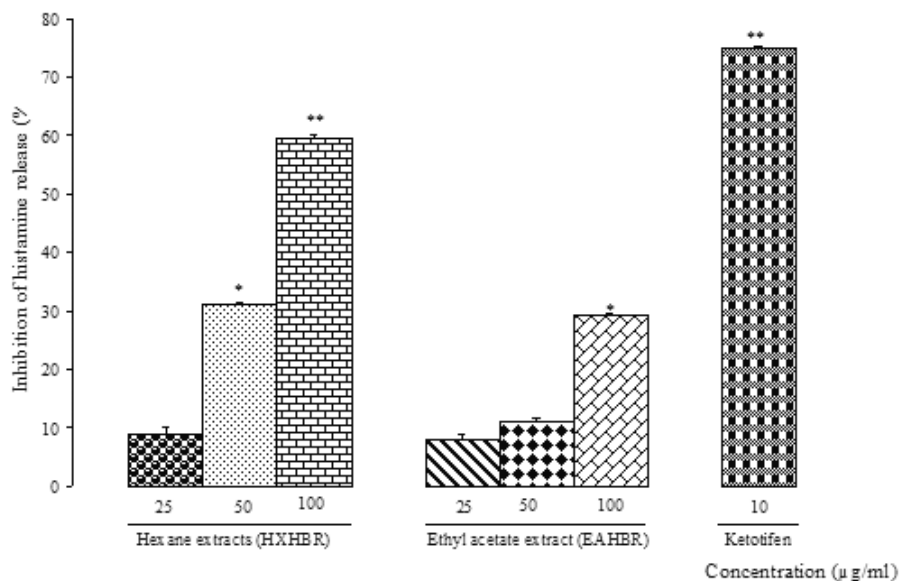*Values are expressed as mean ± S.E.M. (n = 8mice). **P<0.01, ***P<0.001; significantly different from the control value.*

Figure 5:
Effects of HXHBR and EAHBR on carrageenan-induced paw oedema in albino rats.
Values are expressed as mean ± S.E.M. (n = 8 rats), *P < 0.05, **P < 0.01, ***P < 0.001; significantly different from the
control value.

Hedranthera barteri-derived anti-histaminic potential

Figure 6:
Effect of HXHBR and EAHBR on compound 48/80-induced histamine release from the rat peritoneal mast cells of albino rats. Each value is expressed as mean ± S.E.M. of six independent experiments. *P < 0.05 or **P < 0.01; significantly different from the control value.

Anti-inflammatory effects of HXHBR and EAHBR on carrageenan-induced paw oedema in albino rats

The effects of HXHBR and EAHBR on carrageenan-induced paw oedema in albino rats are shown in figure 5. HXHBR (200mg/kg) showed anti-oedematous effect of 29.9%, 36.5% and 38.8% at the 3rd, 4th and 24th hours respectively when compared with the zero hour paw volume after carrageenan administration. But EAHBR showed no significant anti-inflammatory potential. The reference compound, Ibuprofen (100mg/kg) showed anti-oedematous effect of 47.1%, 82.8% , 80.4% and 69.6% at the 1st , 2nd , 3rd and 4th hours respectively when compared with the zero hour paw volume after carrageenan administration. The anti-oedematous effect induced by ibuprofen reached the peak effect at the 2nd hour and progressively decreased.

Effect of HXHBR and EAHBR on compound 48/80-induced histamine release from the rat peritoneal mast cells (RPMCs) of albino rats

The inhibitory effects of HXHBR and EAHBR on compound 48/80-induced histamine released from RPMCs of albino rats are shown in figure 6. HXHBR and EAHBR dose-dependently inhibited compound 48/80-induced histamine release. HXHBR (50 and 100μg/ml) showed a significant inhibition rate in histamine release with 100μg/ml HXHBR inhibition of 59.6% (P < 0.01) compared to 74.8% histamine inhibition by 10μg/ml ketotifen (P < 0.01). EAHBR (100μg/ml) had the lowest inhibition of 29.2% (P < 0.05).

DISCUSSION

In our previous study, the leave extracts of *Hedranthera barteri* (HB) and their fractions have been reported to possess anti-inflammatory and anti-nociceptive properties (Onasanwo and Elegbe, 2006; Onasanwo *et al.*, 2008) but in this piece of research work, the hexane (HXHBR) and ethyl acetate (EAHBR) fractions of HB root were explored for their anti-inflammatory and anti-nociceptive properties. The earlier study suggested the involvement of β-sitosterol in the anti-inflammatory property of the HB leaf (Onasanwo *et al.*, 2008) and we decided to use the hexane-extracted fraction of the root (basically of fatty compounds) and the ethyl acetate-extracted fraction for the analgesic and anti-inflammatory studies of the root. The present study revealed that the HXHBR and EAHBR have protections against nociception and inflammation through thermally and chemically-induced models and the HXHBR presented better significant anti-histaminic property.

In the hot plate latency assay, only EAHBR showed anti-nociceptive property (figure 1) and no significant protection was observed in HXHBR (results not shown). Hot plate assay represent the thermal model for inducing peripheral pain. The trend of the anti-nociceptive effect of EAHBR revealed its dose-dependent manner and observations from these suggested that the lower dose of EAHBR (25mg/kg) has a long duration acting potential while the high dose (200mg/kg) has a short duration action.

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The tail flick test involves the central nervous system and the HXHBR may have its action mediated through the central nervous system more than the peripheral level when compared with the EAHBR (figures 2 and 3). The significant protection of HXHBR (200mg/kg) after 60 min of exposure to thermal ray was comparable with paracetamol (figure 3). The significant effects of HXHBR and EAHBR extracts on tail flick response of the rats to nociception suggest its central effect since the tail flick assay is of a spinal reflex, and is considered to be selective for centrally acting analgesic compounds (Ramabadran *et al.,* 1989; Srinivasan *et al.,* 2003)

Intraplantar injection of formalin into the rat hind paw produces a biphasic pain response that consists of an early, acute phase and a late, tonic phase that is manifested behaviorally as flinching and licking of the affected paw. The two phases represented the direct effect on nociceptors and inflammatory nociceptive responses, respectively (Hunskaar and Hole, 1997; Rosland *et al*., 1990; Tjolsen *et al.,* 1992). Formalin-induced paw licking in albino rats represent a chemically-induced burning pain. Both the EAHBR and HXHBR extracts showed a dose dependent manner of anti-inflammatory potentials which were comparable with aspirin (standard drug).

Acetic acid-induced abdominal writhing represents the very sensitive model for screening compounds with anti-nociceptive potentials. The writhing activity consists of a contraction of the abdominal muscles together with a stretching of the hind limbs which is considered to be nociceptive (Hern´andez-P´erez and Rabanal, 2002). They suggested that where a substance or compound has a long latency, as observed with acetic acid, it might have acted indirectly, possibly by liberating an endogenous substance that excites pain endings. Many of the endogenous substances that elicit this response have been shown to induce nociceptive responses in the dog (Guzman *et al.,* 1962), guinea-pig (Collier and Lee, 1963), and rat (Deffenu *et al*., 1966; Blane, 1967). Both extracts showed dose dependent activities while the 200mg/kg of EAHBR showed a significantly higher anti-nociceptive property ($P<0.001$) than aspirin (table 1).

In the carrageenan-induced paw oedema model of inflammation in rat, anti-inflammatory activity of HXHBR was significantly higher with all the doses, when compared with EAHBR. Only 200mg/kg of HXHBR showed significant protection at the 3rd and 4th hours while EAHBR extract produced no significant inhibition. Ibuprofen, a known analgesic and anti-inflammatory drug, produced a significant anti-inflammatory potential, as much as 82.8% within the first 2 hours and almost double (69.5%) the anti-inflammatory potential of the HXHBR extract (36.5%) within 4 hours after carrageenan injection.

Indeed, carrageenan has been used mostly to study anti-inflammatory effect of both $P<0.001$ steroidal and non-steroidal anti-inflammatory drugs because of inflammatory mediators involved both at the initial and late phases (Al-Rehaily *et al.,* 2001). Vascular permeability of histamine, serotonin and bradykinin has been implicated at the initial phase (Vinger *et al.,* 1987).

Histamine, a biogenic amine, amongst other mediators, plays a very vital role at the early phase of allergic responses and inflammation. Both the HXHBR and EAHBR showed antihistaminic potentials in a dose dependent manner with more significant inhibition in hexane extract, depicting the presence of more potent anti-inflammatory metabolite or compound in HXHBR. This corroborates the reports of some researchers whose findings have shown hexane extracts of natural products to possess analgesic and anti-inflammatory properties (Alcaraz *et al.,* 1989; Garcı´a *et al.,* 1999; Dongmo *et al.,* 2001; Jorge *et al.,* 2004; Delporte *et al.,* 2005). Although, the EAHBR extract showed no significant inhibition, *in-vivo,* in carrageenan-induced paw oedema, but the sensitivity of the *in-vitro* assay compared to the *in-vivo* experiment was observed. Since the β-sitosterol has been suggested as likely compound with anti-inflammatory properties present in HB leaf extract (Onasanwo *et al.,* 2008), further chromatographic analysis of the HB root extract will be needed for its probable presence in the root. Also, compound 48/80 has been implicated in the conductance of chloride ion channel and increase in the permeability of the lipid bi-layer membrane, causing a threat to membrane integrity (Tasaka *et al.,* 1986; Lau and Wan, 2000) in the process of mast cell degranulation. The potentials of HXHBR extracts in inhibiting these membrane threats by compound 48/80 may not be ruled out. Studies are needed in this direction to elucidate these claims.

The phytochemical screening of the root sample of *Hedranthera barteri* has revealed the presence of alkaloids, cardenolides, saponins and flavonoids. Medicinal plants with flavonoids and saponins (Calixto *et al*., 2000; Guardia *et al.,* 2001; Rotelli *et al.,* 2003) have been reported to possess analgesic and anti-inflammatory properties. The anti-nociceptive and anti-inflammatory effects observed in the present work may be due to the presence of these compounds, though the chromatographic separation of these compounds is needed to ascertain this speculation.

Conclusively, these results showed that hexane (HXHBR) and ethyl acetate (EAHBR) extracts of the HB root possess anti-nociceptive and anti-inflammatory activities. It is suggested that its mechanism of action may be through the inhibition of histamine, an inflammatory mediator, usually

released during the early phase of allergic responses and chronic phase of inflammatory pain amongst some other factors. Flavonoids and saponins present in HB root may be involved in its anti-nociceptive and anti-inflammatory properties.

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