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Full Length Research Article

Anti-nociceptive and anti-inflammatory properties of the leaf extracts of *Hedranthera barteri* in rats and mice

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ABSTRACT

Hedranthera barteri, HB (Apocynaceae) is a shrub in the closed-forest in some parts of West Africa and used among the natives for inflammatory pain relief. This study was carried out to assess the anti-nociceptive and anti-inflammatory effects of its leaf extracts to confirm folkloric claims. Phytochemical screening and acute toxicity were carried out on the leaf of the plant. Aqueous (AEHB), methanol (MEHB) and chloroform (CEHB) extracts of the leaf were assessed for anti-nociceptive and anti-inflammatory properties. The probable mechanism of action of the extracts in analgesia was assessed using naloxone. Student's *t*-test was used to test for statistical significance. Phytochemistry of the extracts revealed the presence of alkaloids, cardenolides, saponins and flavonoids. The rats tolerated thermal pain significantly more ($P < 0.05$) with the extracts than the control. The inhibitory rates of the extracts on acetic acid-induced writhing, formalin-induced paw licking (late and early phase) and carrageenan-induced paw oedema when compared with the control were significant. Graded doses of MEHB tolerated thermal pain more significantly ($P < 0.05$), compared with the control. Likewise, the inhibition produced by the graded doses of MEHB on acetic acid-induced writhing, formalin-induced paw licking (early and late phases) and carrageenan-induced paw oedema were significant compared with the control ($P < 0.05$). Pre-treatment with naloxone partially prevented the analgesia induced by MEHB in thermally and chemically induced pains. *Hedranthera barteri* reduced nociception and inflammation in dose-dependent manner. Interactions with naloxone depicted its partial mediation through opioid receptors. (*Afr. J. Biomed. Res.* 9:109 - 118, May 2006)

Keywords: *Hedranthera barteri*, naloxone, nociception, inflammation

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INTRODUCTION

Hedranthera barteri, HB (family: Apocynaceae) is a shrub 2m high, found in damp situations of the closed-forest in Ghana, North and South Nigeria, West Cameroon and also in (Congo Brazzaville) Zaire. The fruit has been used to prevent miscarriage in women (Thomas, 1910). The fruit is taken in Nigeria for treatment of gonorrhoea, as a femifuge and the exudates from the leaf to suppress painful tumor (Ainslie, 1937). The leaf decoction is drunk by Igbo of South Nigeria in treating dizziness (Thomas, 1967). The plant has been reported to be rich in alkaloids like amataine, beninine, goziline, owerreine, subessiline isoquinoline and vobstusine (William and Li, 1970) and studies have shown that its alkaloids have antibacterial properties (Ogunlana and Ramstad, 1975).

In a previous study aimed at screening some Nigerian medicinal plants, HB was reported to have potent anti-inflammatory, antimalarial and antibacterial activities (Chukwujekwu et al, 2005). In the present study, the anti-nociceptive and anti-inflammatory activities of different extracts of HB leaf are investigated.

MATERIALS AND METHOD

Animals

Matured male Sprague Dawley albino rats (150-220g) and Swiss mice (30 –35g) kept at a well-ventilated Central Animal house, College of Medicine, University of Ibadan, Nigeria, were used. They were kept in a photo period-controlled environment (12 hours light/dark cycle).

Plant material and extractions

The leaves of HB used for this study were purchased from the Herbarium Department, Forest Research Institute of Nigeria (F.R.I.N.), Ibadan, Nigeria and authenticated voucher specimen (FHI-106485) was deposited in the same place. The leaves of HB were exhaustively extracted with water, methanol and chloroform by Soxhlet extraction yielding 12.1%, 10.3% and 9.4% respectively. The solvents were removed at 52°C under reduced pressure in a rotavapor. The solid samples of the extract were stored in the refrigerator until when needed. The extracts were prepared as suspensions with 2.5%

tween 80/saline for the physiological and pharmacological experiments.

Phytochemical Screening

The phytochemical screening for alkaloids, cardenolides, anthraquinone, saponins, tannins and flavonoids was carried out on the dried leaf of the plants (Trease and Evans, 1999).

Toxicity studies

The toxicity studies were carried out using New Approach to Practical Acute Toxicity Testing (Lorke, 1983). Swiss mice (25-35 grams) were used. Fifty mice were used to determine the LD₅₀. The animals were divided into 10 groups of 5 mice each. They were fasted for 24 hours and different doses of the extract were administered orally to each group. They were observed for a period of 72 hours for any signs of toxicity like posture, reactive activities, obvious physiological signs and death.

Analgesic studies

Tail immersion latency assay in rats

About 5cm of rat's tail was immersed into warm water maintained at 50 ± 1 °C and the tail withdrawal reflex period (latency/pain threshold) were taken at 0, 30, 60 and 90 minutes after the administration of the extract or drug, p.o. (Uma-Devi et al, 1999).

Formalin-induced paw licking in mice and 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 30) cm with a mirror placed at the base (bottom) of the chamber to allow an unobstructed view of the rats. The time that the animal spent licking the injected paws was measured as an index of pain or nociception. The initial nociceptive response (0-5min) after formalin injection indicated the first phase while (15-30min) indicated the second's phase.

Acetic acid-induced abdominal writhing in mice:

Koster et al (1959) was used. The mice were injected intraperitoneally with 0.2ml of 3% acetic acid solution 1hour after treatment with the extract, which induced the characteristic writhing. The number of writhing was observed between (5-15min). The data were

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collected and computed according to the following formula:

$$\text{Percentage Inhibition} = \frac{\text{Mean of writhing test (control)} - \text{Mean writhing test (test)}}{\text{Mean number of writhing test (control)}} \times 100$$

Anti-inflammatory test

Carrageenan-induced paw oedema:

Pedal inflammation in male albino rats was produced according to Winter *et al* (1962). An injection of 0.1 ml 1% carrageenan was delivered into the right hind foot of the rat under the subplantar aponeurosis. The animals were treated with the extract, orally 1 hour before carrageenan injection. Measurement of the extent of oedema was carried out by the method Bamgbose and Noamesi (1981). The measurement was done 3 hours after carrageenan injection. The inhibiting activity was calculated according to the formula:

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

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

Statistical analysis

The results of the experiment were expressed as mean

± S.E.M. The statistical significance of differences was estimated by Student's *t*-test for unpaired comparison. When $P < 0.05$, the difference was considered to be significant.

RESULTS

Phytochemical studies

The Phytochemical screening of the leaf extract of *Hedranthera barteri* revealed the presence of alkaloids, cardenolides, saponins and flavonoids.

Acute toxicity

There was no death amongst the graded dose groups but various obvious physiological activities were recorded from the groups and 25, 50 and 100 mg/kg doses were used for this study.

Analgesic and anti-inflammatory effects of single dose of AEHB, MEHB and CEHB

Tail immersion latency assay

As shown in figure 1, 100 mg/kg of AEHB, MEHB and CEHB with standard drug, morphine had significant analgesic effects on tail flick response in rats with MEHB having most potent analgesic activity amongst the extracts.

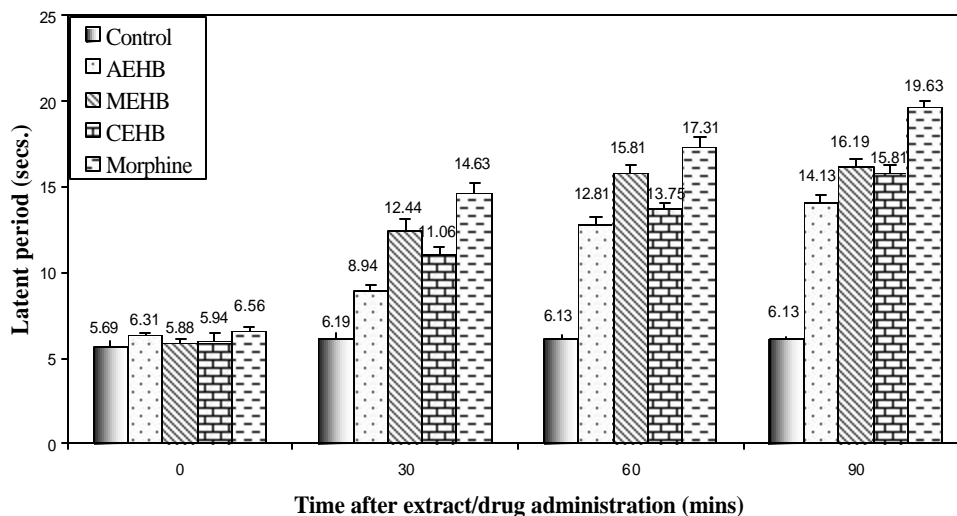


Figure 1: Effects of AEHB, MEHB and CEHB on tail immersion in albino rats. Values are expressed as mean ± S.E.M of eight rats, * $P < 0.05$ (Student's *t*-test)

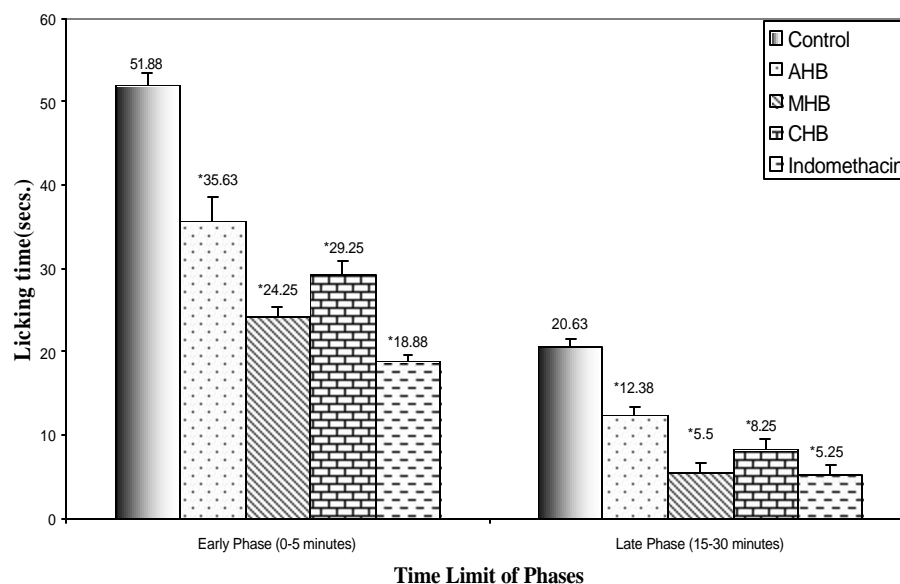


Figure 2: Effects of AEHB, MEHB and CEHB on formalin-induced paw licking in albino rats. Values are expressed as Mean \pm S.E.M of eight rats, *P < 0.05 (Student's t-test).

Table 1: Effects of the leaf extracts of AEHB, MEHB and CEHB on acetic acid-induced writhing in mice

Group	Dose (mg/kg)	Number of Writhing (per 10 min.) ^(y)	% Inhibition
NS	10ml/kg	41.00 \pm 1.13	0
AEHB	100	20.50 \pm 1.12	*50.0
MEHB	100	11.38 \pm 0.65	**74.1
CEHB	100	13.25 \pm 1.05	*67.7
Indomethacin	5	9.25 \pm 1.49	**77.4

*P < 0.05, **P < 0.001 (Student's t-test).

^(y) Values are expressed as mean \pm S.E.M of eight mice. NS = Normal Saline, AEHB, MEHB and CEHB refer to aqueous, methanol and chloroform extracts of *Hendrathera berteri*

Acetic acid-induced abdominal writhing test

The anti-nociceptive properties of AEHB, MEHB and CEHB on mice using the acetic acid writhing model is shown in table 1 with MEHB having most potent inhibitory activity amongst the extracts.

Formalin-induced paw licking test

AEHB, MEHB and CEHB reduced the paw licking time on formalin-induced paw licking in albino rats as shown in figure 2 with MEHB having most potent analgesic activity amongst the extracts comparable with morphine.

Carrageenan-induced paw oedema test

The result of the carrageenan-induced paw oedema test is shown in table 2 with MEHB having most potent anti-inflammatory activity amongst the extracts.

Analgesic and anti-inflammatory studies of graded doses of MEHB

Tail immersion latency assay

Figure 3 shows that the tail flick response values in rats treated with graded doses of MEHB in a dose-dependent manner with 25mg/kg of the extract producing the highest pain threshold or latency.

Acetic acid-induced abdominal writhing test

The dose-response relationship for the anti-nociceptive properties of 25, 50, and 100 mg/kg of MEHB using

the acetic acid writhing model is shown in Table 3 with analgesic properties of the extracts produced in a dose-dependent manner.

Formalin-induced paw licking test

Figure 4 shows the analgesic effects of graded doses of MEHB on formalin-induced paw licking in albino rats in a dose-dependent manner at both the early and late phases in a dose-dependent manner.

Carrageenan-induced paw oedema test

The dose-dependent anti-inflammatory potency of the graded doses of MEHB is shown in table 4 using the carrageenan-induced paw oedema test.

Table 3:

Effects of graded doses of MEHB on acetic acid-induced abdominal writhing in mice.

Group	Dose (mg/kg)	Number of writhing (per 10min.) ^(y)	% Inhibition
Normal saline	10ml/kg	41.00 ± 1.13	0
MEHB	25	15.75 ± 0.96	*61.6
MEHB	50	13.38 ± 0.60	*67.4
MEHB	100	8.13 ± 1.92	**80.2
Indomet h	5	9.25 ± 1.49	**77.4

*P < 0.05, **P < 0.001 (Student's t-test)

^(y)Values are expressed as Mean ± S.E.M of 8 mice.

Table 2:

Effects of AEHB, MEHB and CEHB on carrageenan-induced paw oedema in albino rats

Group	Dose (mg/kg)	Paw sizes before & after injection ^(y)		Change in paw size (t ₃ - t ₀)	Percentage Inhibition (%)
		t ₀ (cm)	t ₃ (cm)		
Normal Saline	10ml/kg	2.04 ± 0.04	3.16 ± 0.06	1.12	0
AEHB	100	2.09 ± 0.04	2.49 ± 0.06	0.40	*64.3
EHB	100	2.04 ± 0.04	2.31 ± 0.03	0.27	**75.9
CEHB	100	2.05 ± 0.03	2.44 ± 0.04	0.39	*65.2
Indomethacin	5	2.00 ± 0.04	2.20 ± 0.04	0.20	**82.1

^(y) Values are expressed as Mean ± S.E.M of eight rats, *P < 0.05, **P < 0.001 (Student's t-test)

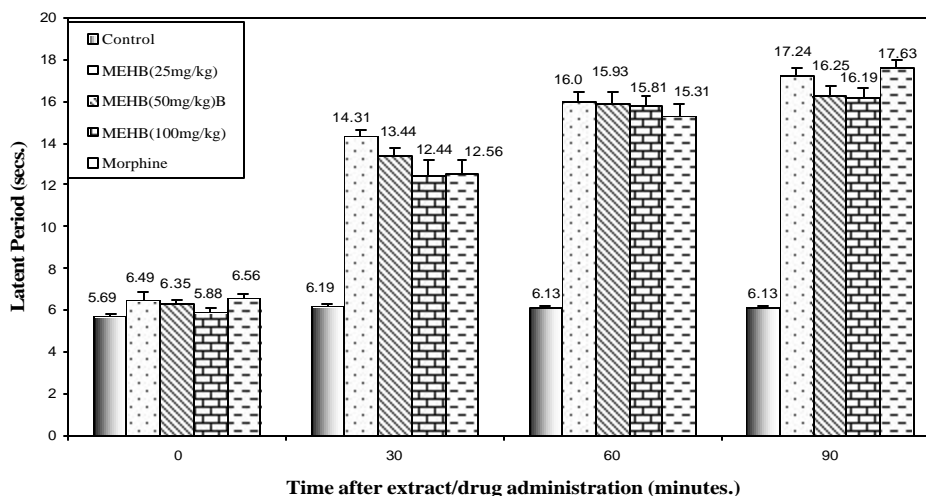


Figure 3:

Tail flick response to tail immersion in albino rats treated with graded doses of MEHB. Values are expressed as Mean ± S.E.M of eight rats, *P < 0.05 (Student's t-test)

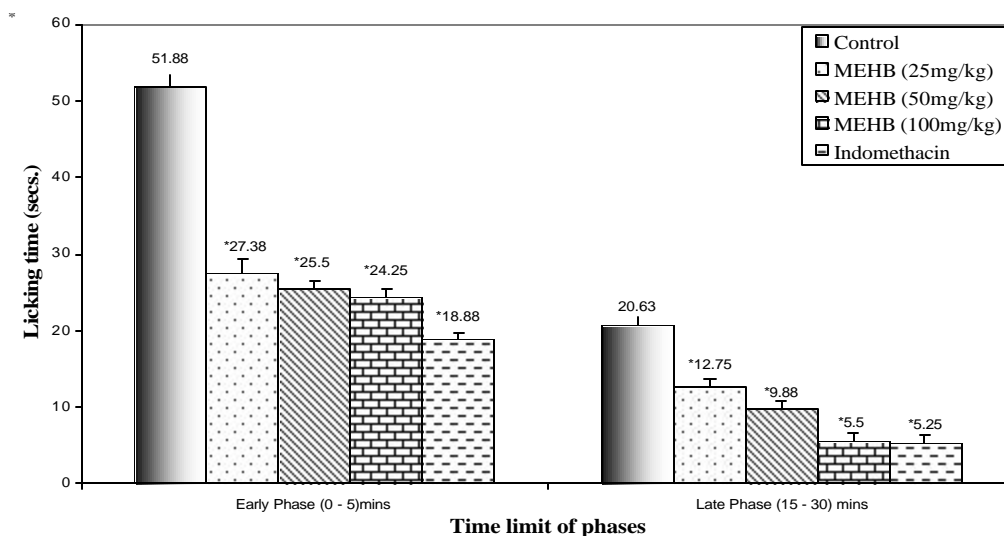


Figure 4:

Effect of graded-doses of MEHB on formalin-induced paw licking in albino rats. Values are expressed as Mean \pm S.E.M of eight rats, * $P < 0.05$ (Student's t-test)

Table 4:

Change in paw size by carrageenan-induced paw oedema in albino rats treated with graded doses of *Hedranthera barteri* leaf extracts (MEHB).

Group	Dose (mg/kg)	Paw sizes before & after injection ^(y)		Change in paw size ($t_3 - t_0$)	Percentage Inhibition (%)
		t_0 (cm)	t_3 (cm)		
Normal Saline	10ml/kg	2.04 \pm 0.04	3.16 \pm 0.06	1.12	0
MEHB	25	2.01 \pm 0.02	2.51 \pm 0.04	0.50	*55.4
MEHB	50	1.99 \pm 0.05	2.36 \pm 0.05	0.37	*67.0
MEHB	100	2.04 \pm 0.04	2.31 \pm 0.03	0.27	**75.9
Indomethacin	5	2.00 \pm 0.04	2.20 \pm 0.04	0.20	**82.1

^(y) Values are expressed as Mean \pm S.E.M of eight rats. * $P < 0.05$, ** $P < 0.001$ (Student's t-test)

Mode of action of leaf extracts of *Hedranthera barteri* through the opioid receptors

Tail immersion mechanism

Figure 5 shows that the tail flick responses in rats treated with extract and naloxone. The extract-treated rats tolerated thermal pain more significantly than with naloxone-treated rats and the control.

Formalin-induced paw licking mechanism

Protections against paw-licking in both phases by the extract-treated rats (51.0 and 37.8 %) and naloxone-

treated rats (38.3 and 26.6 %) are higher than the control ($P < 0.05$).

DISCUSSIONS

The presence of flavonoids, alkaloids, cardenolides and saponins in the plant extracts of *Hedranthera barteri* supports the claim that these compounds have anti-nociceptive and anti-inflammatory properties since alkaloids, flavonoids and saponins have been found in other natural products with analgesic and anti-inflammatory properties (Kerber, 1999; Fernanda *et al*, 2002).

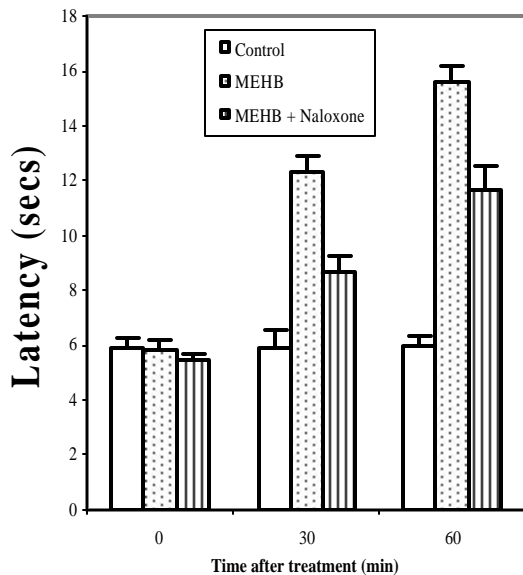
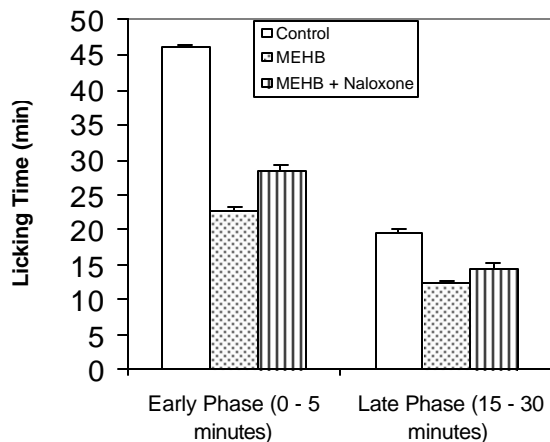


Fig. 5. Effect of Naloxone on the action of leaf extract of HB on tail immersion in rats. Values are expressed as Mean + SEM of eight (8) rats. * $P < 0.05$



The low toxicity of the plant observed in this study seems to suggest that the plant extract is relatively safe for consumption and did not affect any of the parameters measured. The present study has shown that aqueous (AEHB), methanol (MEHB) and chloroform (CEHB) extracts of *Hedranthera barteri* have analgesic and anti-inflammatory properties. Highest level of analgesic and anti-inflammatory

activities was observed in MEHB compared to the other extracts and comparable to standard drugs, indomethacin and morphine in each experiment. Also, the analgesic and anti-inflammatory properties of methanol extract are dose dependent as revealed in the experiment with doses ranging from 25mg/kg to 100 mg/kg of MEHB. This suggests dose-dependent manner of the medicinal plant extracts in the treatment of pain and inflammation (Oriowo, 1982; Olajide *et al*, 2000).

Tail immersion technique was employed which replaced the hot plate model (Uma-Devi *et al*, 1999). About 5cm of the tail was immersed in order to determine the level of latency in rats by tail withdrawal from warm water at a specified temperature of $50 \pm 1^{\circ}\text{C}$. Actually, it was noticed at the beginning of the experiment that some rats showed latency for about 2 to 3 seconds. This may be due to strange environment but after the animals have been allowed to relax and adjust to the vicinity for some minutes, they showed reasonable latent periods equivalent to the antinociceptive potency of the extracts. However, the data from this experiment showed that AEHB, MEHB, and CEHB administered orally at the dose of 100mg/kg demonstrated significant inhibition on the thermally induced nociceptive responses in rats on time-dependent manner when compared with the control group ($P < 0.05$).

Taber *et al* (1969) and Berkenkopf and Weichman (1988) reported that several chemicals (e.g. phenylquinone, acetic acid) could induce a writhing response in laboratory animals. Intraperitoneal injection of acetic acid in this experiment produced abdominal writhing responses by activating the chemo-sensitive nociceptors in the animals and the protective strength of the methanol extract (74.1 %) is comparable with the standard drug, indomethacin (77.4 %). Other extracts, AEHB and CEHB extracts significantly alleviate pain in this model of visceral pain (Vycklicky, 1979) with percentage protection of 50.0 % and 67.0 % respectively.

The formalin-induced licking response was used as a model for evaluating new analgesic (Hunskar *et al*, 1985). Mice were subcutaneously administered with 0.02 ml of 2.5% formalin on the dorsal part of the mice hind paw. This will definitely tell whether the licking is genuinely due to formalin injected into the paw because at times, the animals lick the forepaw

under normal physiological condition. However, formalin test is sensitive to non-steroidal anti-inflammatory drugs and other mild analgesics. The test possesses two distinct phases, possibly reflecting different stages of pain. Formalin produced a distinct biphasic response. The early phase reflects a direct effect of formalin on nociceptors (non-inflammatory pain), whereas the late phase reflects inflammatory pain (Elisabetsky et al, 1995; Hunskar and Hole, 1997). All the extracts produced very significant analgesic effects in both phases. This suggests that the anti-nociceptive effect of the extract was mediated by both neurogenic and inflammatory mechanisms.

All the extracts at 100mg/kg were found to significantly inhibit the carrageenan-induced rat paw oedema, a test which has significant predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation (Mossa et al, 1995). Carrageenan induced inflammation is useful in detecting orally active anti-inflammatory agents (DiRosa et al, 1971). Carrageenan- induced hind limb oedema had a pronounced biphasic reaction in rats with different inflammatory mediators being mediated at different periods. The first phase is related to the release of histamine and like-substances like serotonin (Crunkhon and Meacock, 1971). The second phase is associated with the activation of tissue prostaglandins and plasma kinins. This phase is sensitive to most clinically effective anti-inflammatory drugs (Vinegar et al, 1969; DiRosa et al, 1971). Likewise, it was suggested that prostaglandin release could be detected in rat paw exudates as early as 1 hour after carrageenan injection (Oriowo, 1979).

Graded doses of MEHB were tested on pain and inflammation. Tail immersion durations revealed that the lower dose makes the animals to tolerate thermal pain than the medium and higher doses. The effect produced by the lower dose is comparable with the standard drug, indomethacin. This simply suggests that the lower dose will be more effective in treating thermal nociceptive responses in pain. Contrary to the effect of MEHB on thermal pain, the extract inhibited the acetic acid-induced writhing test with the high dose showing the highest percentage inhibition, which is even more significant than indomethacin. It is likely that the extract has central nervous system and depressant effect since the central nervous depressants and antihistaminic are known to reduce the number of

writhing. Likewise, in the formalin-induced paw licking test, the high dose (100 mg/kg) significantly reduce the licking time when compared with other doses (25 and 50 mg/kg). The inhibition at the late phase is comparable with that of the standard drug.

In carrageenan-induced paw oedema test, the highest inhibitory property compared to the other dose was exhibited by the high dose (100 mg/kg). This simply depicts that the higher the dose, the more the inhibition of the oedema. Hence, an inhibitory property of the extract is dose-dependent.

In the elucidation of the probable mechanism of action of the extracts, whether it is mediated through the opioid receptors, naloxone hydrochloride (Narcan^R) was administered with the extract. The extract-treated rats showed a latency of 15.60 ± 0.26 sec in tail immersion test compared with extract-naloxone-treated rats, 11.69 ± 0.35 sec and the control, 5.98 ± 0.11 sec. Also, in formalin-induced paw licking, protections in both phases by the extract treated groups (51.1% and 37.8%) compared with extract-naloxone-treated group (38.3% and 26.6%) are significantly higher than the control. This suggests partial mediation of the anti-nociceptive activities of the extract through the opioid receptors amongst other pain receptors. The mechanisms of action of the secondary metabolites present in *Hedranthera barteri* may be comparable with that of the compounds discovered in the work by Kerber (1999) and Fernanda *et al* (2002).

These results from the activities of the crude extracts of *H. barteri* suggest that the extract has been shown to be relatively non-toxic in mice and rats.

Further studies to systematically extract the active metabolites from the leaf of HB will be needed. This will help to ascertain their analgesic and anti-inflammatory properties separately. Also, the mechanism of action of this plant at the molecular level will throw more light on its mode of action.

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