International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 4 (1): 51-55, 2008
ISSN 1811-7775

Analgesic and Anti-Inflammatory Effects of Ethanolic Root Extract of *Hippocratea africana*

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Abstract: The ethanolic root extract of *Hippocratea africana* (200-600 mg kg^{-1}) was evaluated for analgesic, anti-inflammatory and antipyretic properties. The extract dose dependently inhibited acetic acid-induced writhing, formalin-induced paw licking and thermally -induced pain in mice. The extract also inhibited fresh egg albumin, carrageenin and xylene-induced inflammation in mice. These inhibitions were statistically significant ($p<0.05$) when compared to control. The roots extracts was also found to reduce pyrexia in rats. The analgesic, anti-inflammatory and antipyretic activities of the extract may be related to its active constituents such as tannins, saponins, steroid and flavonoids.

Key words: *Hippocratea africana*, anti-nociception, anti-inflammatory, antipyretic

INTRODUCTION

Hippocratea africana (Willd.) Loes (Hippocrateaceae) is a green forest perennial climber without hairs (glabrous) and reproducing from seeds (Dalziel, 1956). The plants is widely distributed in tropical Africa. The root of the plant is used traditionally by the Ibibios of the Niger Delta region of Nigeria in the treatment of various ailments such as fever, malaria, body pains, diabetes and diarrhea (Okokon *et al*., 2006). The plant (root) has been reported by Okokon *et al*. (2006) to possess *in vivo* antiplasmodial activity with LD_{50} of 2.45 g kg^{-1}. Report of scientific studies on *Hippocratea africana* are few and there is no data on the anti-inflammatory and analgesic activity of the extract. So we investigated the analgesic and anti-inflammatory activities of the roots extract of *Hippocratea africana*.

MATERIALS AND METHODS

Plant materials: Fresh roots of *H. africana* were collected in November, 2006 at Nyan forest in Uruan, Akwa Ibom State, Nigeria. The plant was identified and authenticated by Dr. Margaret Bassey, a taxonomist in the Department of Botany, University of Uyo, Uyo, Nigeria. Hebarium specimen was deposited at Faculty of Pharmacy Hebarium. The fresh rootbark (2 kg) of the plant were dried on laboratory table for 2 weeks and reduced to powder. The powder 100 g was macerated in 95% ethanol (300 mL) for 72 h. The liquid filtrate obtained was concentrated in vacuo at 40°C. The yield was 2.08% w/w. The extract was stored in a refrigerator at 4°C until used for experiment reported in this study.

Animals: Albino wistar rats (105-165 g) and albino mice (20-25 g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics committee, University of Uyo.

Acetic acid-induced writing in mice: The abdominal constriction resulting from intraperitoneal (ip) injection of acetic acid (3%) consisting of the contraction of abdominal muscle together with a stretching of hind limbs, were carried out according to the procedure of Santos *et al*. (1994), Correa *et al*. (1996) and Besra *et al*. (1996). The animals were divided into five groups of 6 mice per group. Group 1 served as control while groups 2-4 were pretreated with 200-600 mg kg^{-1} i.p., of *H. africana* extract. Acetyl Salicylic Acid (ASA) 100 mg kg^{-1} was given to the reference group. After 30 min, acetic acid was administered by the same route. The numbers of writhing movements were counted for 30 min. Antinociception was expressed as the reduction of the number of abdominal constrictions between control animals treated with saline and mice pretreated with the extract.

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Formalin paw licking in mice: The method similar to that of Hunskaar and Hole (1987), Gorski *et al.* (1993) and Correa and Calixto (1993) was used. The animals were pretreated with *H. africana* extract (200-600 mg kg^{-1} i.p) and ASA (100 mg kg^{-1}) before being challenged with buffered formalin and the responses were observed for 30 min.

The animals were used to analyse the first phase of formalin-induced licking and 20 μL of 2.5% formalin solution (0.9% of 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 hind paw. The amount of time spent licking the injected paw was timed and was indicative of pain. The first phase of the nociceptive response normally peaked at 5 min after formalin injection and the second phase 15-30 min after formalin injection, representing the neurogenic and inflammatory pain responses, respectively (Hunskaar and Hole, 1987).

Thermally-induced pain in mice: The effect of the extract on hot plate -induced pain was investigated in adult mice. The hot plate test was used to measure response latencies according to the method of Vaz *et al.* (1996).The hot plate was set at 45±1°C. The animals were divided into 5 groups of 5 mice per cage. Group 1 animals served as the control and received only saline. Group 2, 3 and 4 were pretreated with 200, 400 and 600 mg kg^{-1} of *H. africana* root extract i.p., respectively 30 min prior to the placement on the hot plate, while group 5 animals received 100 mg kg^{-1} of acetyl salicylic acid by the same i.p., route. Animals were placed into a glass beaker of 50 cm diameter on the heated surface and the time(s) between placement and shaking or licking of paw or jumping was recorded as the index of response latency. An automatic 30 sec cut off was used to prevent tissue damage.

Carrageenin-induced mice hind paw edema: Increase in the mice hind paw linear circumference induced by sub-planar injection of the phlogistic agent was used as the measure of acute inflammation (Winter *et al.*, 1962). Adult albino mice of either sex were used after 24 h fast and deprived of water only during experiment. The extract (200-600 mg kg^{-1}) was administered i.p., to various groups of mice, 1 h before inducing inflammation. Control mice received carrageenin while reference group received ASA (100 mg kg^{-1} i.p.,). The average (mean) edema was assessed by measuring with Vernier Calipers. 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 planar surface of the hind paw. The linear circumference of the injected paw was measured before and 0.5, 1, 2, 3, 4 and 5 h after administration of phlogistic agent for routine drug testing, the increase in paw circumference 0.5, 1, 2, 3, 4 and 5 h after administration of phogistic agent was adopted as the parameter for measuring inflammation (Winter *et al.*, 1962; Akah and Nwambie, 1994; Ekpendu *et al.*, 1994; Besra *et al.*, 1996). Edema (inflammation) was assessed as the difference in pain circumference between the control and 0.5, 1, 2, 3, 4 and 5 h after administration of phlogistic agent (Hess and Milonig, 1972).

Egg albumin induced inflammation: Inflammation was induced in mice by the injection egg albumin (0.1 mL, 1% in normal saline) into the subplantar tissue of the right hind paw (Akah and Nwambie, 1994). The linear circumference of the injected paw was measured before and 0.5, 1, 2, 3, 4, 5 h after the administration of phlogistic agent. The root extract (200-600 mg kg^{-1}, i.p.,) and ASA (100 mg kg^{-1}, orally) were administered to the mice 1 h before the induction of inflammation. Control group received 10 mL kg^{-1} distilled water orally. Edema(inflammation) was assessed as the difference in paw circumference between the control and 0.5, 1, 2, 3, 4, 5 h after the administration of the phlogistic agent (Hess and Milonig, 1972). The average (mean) edema was assessed by measuring with vernier calipers.

Xylene-induced car oedema: Inflammation was induced in mice by topical administration of 2 drops of xylene at the inner surface of the right ear. The xylene was left to act for 15 min. *H. africana* extract (200-600 mg kg^{-1}), dexamethasone (4 mg kg^{-1}) and distilled water (0.2 mL kg^{-1}) were orally administered to various groups of mice 30 min before the induction of inflammation. The animals were sacrificed under light anaesthesia and the left ears cut off. The difference between the ear weights was taken as the oedema induced by the xylene (Tjolsen *et al.*, 1992).

Antipyretic test: The rats (120-166 g) deprived of food for 24 h were used for the experiment. At 0 h the basal temperature of the rats were taken using digital clinical thermometer. Thereafter each animal was administered subcutaneously with 50% w/v aqueous suspension of yeast at a volume of 100 mL kg^{-1} (Gural *et al.*, 1955). At suitable intervals beginning 1 h after yeast injection, rectal temperature of animals were taken, animals with increase of 1°C were selected and grouped for the study. The extract understudy was administered intraperitoneally after the pyrogen at the dose of 200, 400 and 600 mg kg^{-1} to respective groups of rats. The control group received the vehicle (5 mL kg^{-1}) and the reference group administered with 100 mg kg^{-1}

ASA both intraperitoneally. The rectal temperature was taken for the next 4 h after treatment. The mean temperature of the groups after 4 hours were taken and compared with that of the control group.

RESULTS

Acetic acid induced writhing in mice: The extract (200-600 mg kg^{-1}) dose dependently reduced acetic acid-induced abdominal constrictions and stretching of hind limbs. The reduction was statistically significant ($p<0.05$) when compared to control (Table 1). The effect of the extract was more than that of the reference drug, ASA (100 mg kg^{-1}).

Formalin induced hind paw licking in mice: The extract pretreated animals showed a significant ($p<0.01$) dose related reduction of hind paw licking caused by formalin when compared to control (Table 2).

Hot plate-induced pain in mice: Rats pretreated with *H. africana* (200-600 mg kg^{-1}, i.p.,) demonstrated a dose-dependent increase in latency of response in the hot plate test. The increases in the latency responses (analgesic effect) were statistically significant ($p<0.05$-0.001) (Table 3).

Fresh egg-albumin induced inflammation in mice: The *H. africana* extract showed a significant ($p>0.05$) anti-inflammatory activity against acute inflammation (Table 4) compared to control. The extract (200-600 mg kg^{-1}) suppressed in a dose dependent manner the increase in the mice paw edema caused by egg albumin. The activity was comparable to that of the standard drug, ASA (100 mg kg^{-1}).

Carrgeenin-induced inflammatory in mice: The root extract of *H. africana* exert a significant ($p<0.01$) dose-dependent anti-inflammatory effect against carragenin induced inflammation when compared to control (Table 5). The anti-inflammatory activity of the root extract was comparable to that of the reference drug ASA (100 mg kg^{-1}).

Xylene-induced Oedema: Administration of root extract of *H. africana* (200-600 mg kg^{-1}) to mice significantly ($p>0.05$) prevented oedema induced by

Table 1: Effect of ethanolic root extract of *H. africana* on egg albumin-induced inflammation

Treatment/Dose (cm kg^{-1})	0 h	30 min	1 h	2 h	3 h	4 h	5 h
Extract (mg kg^{-1})							
200	0.238±0.04	0.296±0.02*	0.298±0.02*	0.293±0.02*	0.270±0.02*	0.266±0.02*	0.263±0.02*
400	0.233±0.03	0.274±0.04*	0.287±0.03*	0.276±0.03*	0.270±0.03*	0.253±0.03*	0.250±0.03*
600	0.212±0.04	0.256±0.02*	0.278±0.03*	0.263±0.02*	0.246±0.02*	0.232±0.02*	0.230±0.02*
Control	0.243±0.04	0.363±0.04	0.332±0.04	0.331±0.03	0.320±0.03	0.313±0.03	0.304±0.03
ASA (100 mL)	0.221±0.02	0.256±0.04*	0.263±0.04*	0.238±0.04*	0.231±0.04*	0.226±0.02*	0.222±0.01*

Results are expressed as Mean±SEM. *$p<0.01$ compared to control n = 6

Table 2: Effect of ethanolic root extract of *H. africana* on Carrageenan-induced inflammation

Treatment/Dose (cm kg^{-1})	0 h	30 min	1 h	2 h	3 h	4 h	5 h
Extract (mg kg^{-1})							
200	0.231±0.03	0.289±0.02*	0.286±0.04*	0.281±0.02*	0.278±0.03*	0.275±0.04*	0.269±0.03*
400	0.242±0.04	0.293±0.03*	0.288±0.02*	0.275±0.04*	0.272±0.04*	0.268±0.04*	0.256±0.04*
600	0.235±0.04	0.282±0.04*	0.279±0.02*	0.273±0.03*	0.267±0.02*	0.253±0.02*	0.242±0.04*
Control	0.228±0.02	0.331±0.03	0.335±0.04	0.332±0.02	0.325±0.04	0.321±0.04	0.315±0.02
Indomethacin	0.224±0.04	0.280±0.02*	0.276±0.03*	0.262±0.03*	0.258±0.03*	0.241±0.02*	0.233±0.02*

Results are expressed as Mean±SEM. *$p<0.01$ compared to control n = 6

Table 3: Effect of *H. africana* extract on thermally induced pain

Treatment	Dose (mg kg^{-1})	Time (sec)
Control	-	2.98±0.16
H. africana extract	200	3.70±0.27*
	400	4.10±0.18*
	600	4.30±0.20*
ASA	100	16.23±0.28*

Results are expressed as Mean±SEM. *$p<0.05$,**0.001 compared to control n = 6

Table 4: Effect of ethanolic root extract of *H. africana* on xylene-induced inflammation

Treatment	Dose (mg kg^{-1})	Weight of right ear (g)	Weight of left ear (g)	Increase in ear weight (g)	Inhibition (%)
Extract	200	0.062±0.01	0.041±0.01	0.020±0.01*	44.73
	400	0.058±0.01	0.045±0.01	0.023±0.01*	53.39
	600	0.048±0.01	0.038±0.01	0.010±0.00*	57.23
Control		0.078±0.01	0.040±0.00	0.038±0.01	
Dexamethasone	4.0	0.045±0.01	0.036±0.01	0.009±0.00*	54.38

Results are expressed as mean±SEM. *$p<0.05$ significant different from control n = 6

Table 5: Effect of ethanolic root extract of *H. africana* on acetic acid induced writhing

Treatment/Dose (cm kg^{-1})	10 min	20 min	30 min	40 min	50 min	Total
Control extract	54.50±0.34)	48.50±0.34	35.0±0.40	25.5±0.15	19.50±0.03	183.0±1.36
200	11.60±0.14*	35.30±0.31*	30.6±0.90*	25.0±3.10*	17.00±3.10*	119.5±1.36*
400	2.66±0.01*	23.30±0.63*	19.6±0.50*	24.3±5.40*	16.00±2.78	85.86±1.80*
600	0.00±0.00*	3.30±0.30*	10.6±0.70*	29.3±0.10*	2.66±0.05*	45.86±1.26*
ASA mg kg^{-1}	36.50±0.02*	31.00±0.60*	18.5±0.10*	10.5±0.34*	7.50±0.34*	104.0±1.47*

Results are expressed as Mean±SEM (n = 6). *p<0.05 significantly different from control

Table 6: Effect of ethanolic root extract of *H. africana* on formalin induced pain

Treatment/Dose (cm kg^{-1})	0-5 min	Inhibition (%)	15-30 min	Inhibition (%)
Control	31.2±4.31		39.40±7.28	
Extract (mg kg^{-1})				
200	20.3±1.93*	34.940	15.00±2.23*	61.92
400	15.8±2.01*	49.358	10.30±1.96*	73.85
600	11.6±1.92*	62.820	7.00±2.81*	82.23
ASA 100 mg kg^{-1}	12.6±2.01*	59.930	9.02±3.12*	77.15

Results are expressed as Mean±SEM. *p<0.01 compared to control n = 6

Table 7: Antipyretic effect of ethanolic root extract of *H. africana*

Treatments (Dose mg kg^{-1})		Mean temperature after 4 h
Control		37.20±0.25
Extract		
	200	36.75±0.13*
	400	36.05±0.29**
	600	36.03±0.17**
ASA	100	36.64±0.08**

Results are expressed as Mean±SEM. *p<0.01, **0.001 compared to control n = 6

tropical application of xylene when compared to control (Table 6). This effect was comparable to that of the standard drug, ASA (100 mg kg^{-1}).

Antipyretic activity: Administration of the root extract of *H. africana* (200, 400 and 600 mg kg^{-1}) in the presence of pyrogen caused a dose dependent reduction in temperature which though significant when compared to control was observed to be less effective than that produced by Acetic Salicylic Acid (ASA) (Table 7).

DISCUSSION

The root extract caused a dose-and time-dependent antinociception against acetic acid and formalin induced nociception (pain) in mice. Acetic acid causes inflammation pain by inducing capillary permeability (Amico-Roxas *et al*., 1984), formalin exhibits neurogenic and inflammatory pain (Vaz *et al*., 1997), while hot plate-induced pain indicates narcotic involvement (Besra *et al*., 1996). Okokon *et al*. (2006) had reported that the plant extract contains alkaloids, flavonoids, tannins, terpenes cardiac glycosides, anthraquinones and saponin. The observed analgesic effect of the extract suggests that the activity is related to its anti-inflammatory, neurogenic and narcotic properties. The root extract also progressively reduced, in a dose-dependent fashion, oedema of the mice hind paw induced by carrageenin and egg-albumin as well as xylene induced ear oedema in mice. These effects were significant (p<0.05) when compared to control. This finding correlate well with significant anti-inflammatory activities reported of *Hippocratea excelsa* (Perez *et al*., 1995) and *Hippocratea indica* (Ogbole *et al*., 2007), species of the same genus. Carrageenan oedema consists of two distinct phases; an initial release of histamine, 5HT and kinins and finally a second phase, the mediator is suspected to be prostaglandins (Vane and Booting, 1987). The same mediators are involved in egg-albumin induced oedema, whereas xylene induced oedema is linked to phospholipase A_2 (Lin *et al*., 1992). The anti-inflammatory activity of the root extract is due to its ability to inhibit histamine, 5HT, kinnins, prostaglandins and phospholipase A_2; this could be due to the presence of flavonoid in the extract (Parmer and Ghosh, 1978). The root extract demonstrated a significant (p<0.05) antipyretic activity and fever is known to be associated with the production of prostaglandins in the hypothalamus. The extract has been suggested above to act by inhibiting the production of prostaglandins. It's antipyretic action is believed to result from this action. The root extracts which had been reported to possess antiplasmodial activity (Okokon *et al*., 2006) and the findings of the present study suggests its ability to alleviate malarial symptoms like inflammation, pains and fever.

CONCLUSION

The result obtained in this study shows that *H. africana* possess anti-inflammatory, analgesic and antipyretic properties which are probably mediated via inhibition of various autocoids formation and release. Further studies are needed to elucidate the exact mechanism by which *H. africana* inhibits inflammation and pains.

ACKNOWLEDGMENT

The authors are grateful to Ms. Sifon Akpan of Department of Pharmacology and Toxicology, University of Uyo, Uyo for her assistance.

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