African Journal of Biotechnology Vol. 8 (10), pp. 2036-2041, 18 May, 2009
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB09.090
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Anti-inflammatory, analgesic and antipyretic activities of the aqueous extract of *Hippobromus pauciflorus* (L.f) Radlk leaves in male Wistar rats

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Accepted 19 March, 2009

The aqueous extract of *Hippobromus pauciflorus* (L.f) Radlk leaves at 50, 100 and 200 mg/kg body weight were evaluated for anti-inflammatory, analgesic and antipyretic activities in male rats. Anti-inflammatory activity was studied by using carrageenan and histamine induced oedema right hind paw volume while the analgesic effect was evaluated using formalin-induced pain and tail flick nociception response. The brewer's yeast-induced pyrexia model was used for antipyretic investigation. Phytochemical screening of the aqueous extract revealed the presence of tannins, flavonoids, steroids, terpenes, cardiac glycosides and saponins. The extract at all the doses used and the indomethacin significantly inhibited both the carrageenan- and histamine-induced inflammation in a manner that was not dose dependent. The extract reduced the formalin-induced pain licking as well as prolonged the reaction time in the tail flick-induced pain. While the 50 and 100 mg/kg body weight of the extract reduced the brewer's yeast provoked elevated body temperature in rats after 60 min, that of 200 mg/kg body weight manifested from 30 min. The results suggest a potential benefit of *H. pauciflorus* leaves in treating conditions associated with inflammation, pain and fever. These properties might be adduced to the presence of the phytoconstituents.

Key words: *Hippobromus pauciflorus,* anti-inflammatory, analgesic, antipyretic, brewer's yeast, pyrexia.

INTRODUCTION

Hippobromus pauciflorus (L.f) Radlk (Sapindaceae), locally known as Ulathile in the Eastern Cape province of South Africa, is a resinous tree that grows up to 5 m high. It is widely distributed in the riverine thickets, along stream banks and at the margins of evergreen forests of South Africa (Pendota et al., 2008). The leaves are simple and are arranged in alternate fashion. Several medicinal uses of the plant have been reported. For example, the leaves of *H. pauciflorus* are used by traditional healers for the treatment of malaria (Clarkson et al., 2004) and conjunctivitis in the Eastern Cape of South Africa (Masika and Afolayan, 2003). The root is also regarded as a love charm by the Zulus and is also used in the management of dysentery and diarrhoea (Pendota et al., 2008).

Despite these, the plant's uses in the treatment of these diseases are accompanied by inflammation, pain and fever. There has not been any scientific evidence in the literature on the potential benefit of *H. pauciflorus* in the treatment of these conditions. In the present communication, we report the anti-inflammatory, analgesic and antipyretic activities of the aqueous extract of the leaves of the species in male Wistar rats. This is with a view to giving adequate scientific backing and explanations to the use of *H. pauciflorus* in the treatment of fever and other associated symptoms in the folkloric medicine of South Africa.

MATERIALS AND METHODS

Plant material and authentication

The leaves of *H. pauciflorus* were collected in August, 2008, from Sikusthwana village, near Alice, in the Eastern Cape of South Africa. The species was authenticated by Professor D. S. Grierson

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of the Department of Botany, University of Fort Hare. A voucher specimen (SC Pendota 01/2008) was deposited at Giffen, Herbarium of the University.

Chemicals

Carrageenan, indomethacin, histamine and formalin were obtained from Sigma-Aldrich Chemie Gmbh, Steinheim, Germany. All other chemicals used were of analytical grade and were supplied by Merck Chemicals (Pty) Ltd., Bellville, South Africa.

Experimental animals

Male rats (*Rattus norvegicus*) of Wistar strain weighing 206.29 ± 9.69 g were obtained from the Animal House of the Agricultural and Rural Development Research Institute, University of Fort Hare. All the animals were housed in clean metabolic cages placed in well-ventilated house conditions (temperature: 28 ± 1°C; photoperiod: 12 h natural and dark; humidity: 45 - 50%). They were allowed free access to Balanced Trusty Chunks (Pioneer Foods [Pty] Ltd., Huguenot, South Africa) and tap water. The cages were cleaned daily. This study was carried out following approval from the Ethical Committee on Animal Use and Care of the University of Fort Hare.

Preparation of extract

The leaves of the plant were air-dried at room temperature for 7 days. The dried material was pulverized with an electric blender. 100 g of the powder was extracted in 1000 ml of distilled water for 48 h on a mechanical shaker (Stuart Scientific Orbital Shaker, UK). The extract was filtered using a Buchner funnel and Whatman No. 1 filter paper. The resulting filtrate was freeze-dried with Savant Refrigerated Vapor Trap (RV T41404, USA) to give a yield of 12.47 g. This was reconstituted separately in distilled water to give the required doses used in this study.

Phytochemical screening

Phytochemical screening of the plant leaves was carried out as described for alkaloids (Harborne, 1973), steroids and terpenes (Trease and Evan, 1989), flavonoids (Awe and Sodipo, 2001), tannins (Odebiyi and Sofowora, 1978), saponins and cardiac glycosides (Sofowora, 1993).

Anti-inflammatory activity

Carrageenan-induced paw oedema test: 25 animals were grouped into five (A-E) consisting of five animals each. Groups A and B were treated with 0.5 ml of distilled water and 10 mg/kg body weight of indomethacin respectively while groups C, D and E were administered with the extract at 50, 100 and 200 mg/kg body weight respectively. 0.1 ml of 1% carrageenan solution was injected into the sub plantar region of the right hind paw of the rats, 1 h after the administration of distilled water, indomethacin and the extract (Moody et al., 2006). The paw volume was measured with a micrometer screw gauge (SMC-20326, Sterling Manufacturing Company, Ambala Cantt, India.) at 1, 2, 4 and 6 h after administration of the drug and the extract. The difference between the left and right hind paw volumes (indicating the degree of inflammation) was determined in comparison to the control animals.

The percentage inhibition of inflammation of the extract and the reference drug was calculated using the expression:

Percentage inhibition of inflammation = (X - Y/X) x 100

where X was the average degree of inflammation of the control and Y was the average degree of inflammation of the extract/indomethacin.

Histamine induced paw volume test

Adopting the method described by Perianayagam et al. (2006), the paw oedema was produced by sub-plantar administration of 0.1% freshly prepared solution of histamine into the right hind paw of the rats. The paw volume was recorded before the histamine injection. Rats (five per group) were orally administered with 0.5 ml of the extract corresponding to 50, 100 and 200 mg/kg body weight, 30 min prior to the administration of histamine. The controls were administered with 10 mg/kg body weight of indomethacin (positive control) and 0.5 ml of distilled water (negative control). Histamine was administered 1 h after the administration of the extract and indomethacin. The right hind paw volume was measured at 1, 2, 3, 4 and 6 h using a micrometer screw gauge. The anti-inflammatory activity was calculated as described earlier for carrageenan-induced oedema.

Analgesic activity

Tail immersion test: Acute nociception was assessed using the tail immersion test described by Vogel and Vogel (1997). Briefly, this method entails immersing the extreme 3 cm of the rat's tail in a water bath (Buchi water bath B-480, Buchi, Switzerland) maintained at a temperature of 55.00 ± 0.5°C. The time spent by the animal before reacting to the pain was measured with a stop watch as the initial reaction time (Tb). The various groups of the animals were orally administered with the extracts (50, 100 and 200 mg/kg body weight), indomethacin (10 mg/kg body weight) and distilled water. The response latency between the onset of immersion and the withdrawal of the tail (Ta) following the administration of the extract and the reference drug was recorded at 0.5, 1, 2, 4 and 6 h after a latency period of 30 min. The percentage analgesic activity was calculated from the expression:

Percentage analgesic activity = (Ta – Tb/Tb) x 100

Formalin-induced pain test: The procedure described by Correa and Calixto (1993) was used for the determination of response to pain induced by formalin. A 0.05 ml of 2.5% formalin solution was injected into the sub-plantar of the right hind paw of the rats. The number of times spent licking the paw was recorded and considered as indicative of pain. The animals were pre-treated with 0.5 ml of distilled water, indomethacin (10 mg/kg body weight) and extracts (50, 100 and 200 mg/kg body weight), 30 min before the administration of formalin and the responses were observed, first for 0 - 5 min and then 15 - 30 min.

Antipyretic activity

The method described by Brune and Alpermann (1983) was adopted for the determination of antipyretic activity in rats. Pyrexia was induced in the animals (that had been deprived of food for 18 h, but were supplied with water *ad libitum*) by subcutaneous administration of 15% (w/v) of brewer's yeast in 0.9% saline solution at a dose of 10 mg/kg body weight near the groin region of the animals. Following the injection, the site was massaged in order to spread the suspension uniformly beneath the skin. The rectal temperature of the rats was measured before and 18 h after the

Table 1. Effect of the administration of *H. pauciflorus* leaf extract on carrageenan-induced right hind paw oedema in Wistar rats (n = 5, x ± SD).

Treatment groups	Dose (mg/kg body weight)	Differences in right and left paw diameter (mm)				
		1 h	2 h	3 h	4 h	6 h
Control	0	0.60 ± 0.03[a] (0..00)	1.14 ± 0.05[a] (0.00)	1.84 ± 0.10[a] (0.00)	2.10 ± 0.15[a] (0.00)	2.52 ± 0.18[a] (0.00)
Extract	50	0.40 ± 0.07[b] (33.33)	0.61 ± 0.07[b] (46.49)	0.81 ± 0.08[b] (55.97)	1.42 ± 0.08[b] (32.38)	1.76 ± 0.18[b] (30.15)
Extract	100	0.40 ± 0.06[b] (33.33)	0.77 ± 0.03[c] (34.24)	0.72 ± 0.08[c] (60.86)	0.85 ± 0.07[c] (59.52)	1.15 ± 0.05[c] (54.36)
Extract	200	0.38 ± 0.07[b] (36.66)	0.73 ± 0.09[c] (35.96)	0.55 ± 0.08[d] (70.10)	0.75 ± 0.09[d] (64.28)	1.10 ± 0.21[c] (56.34)
Indomethacin	10	0.12 ± 0.07[c] (65.00)	0.34 ± 0.04[d] (70.17)	0.46 ± 0.04[e] (75.00)	0.50 ± 0.07[d] (76.19)	0.62 ± 0.08[e] (75.39)

Percentage inhibitions are indicated in brackets; values carrying superscripts different from the control down the group for each hour are significantly different ($p < 0.05$).

Table 2. Effect of the administration of *H. pauciflorus* leaf extract on histamine-induced right hind paw oedema in Wistar rats (n = 5, x ± SD).

Treatment groups	Doses (mg/kg body weight)	Differences in right and left paw diameter (mm)				
		1 h	2 h	3 h	4 h	6 h
Control	0.5 ml	0.73 ± 0.03[a]	1.25 ± 0.11[a]	1.65 ± 0.18[a]	2.35 ± 0.21[a]	2.79 ± 0.24[a]
Extract	50	0.65 ± 0.08[b] (10.95)	0.72 ± 0.05[b] (42.40)	0.80 ± 0.03[b] (51.51)	0.93 ± 0.04[b] (60.40)	1.07 ±0.12[b] (61.64)
Extract	100	0.60 ± 0.02[b] (17.80)	0.75 ± 0.04[b] (40.00)	0.87 ± 0.03[b] (47.27)	0.99 ± 0.09[b] (57.87)	0.12 ± 0.01[c] (59.85)
Extract	200	0.57 ± 0.08[b] (21.91)	0.66 ± 0.04[c] (47.20)	0.74 ± 0.04[c] (55.15)	0.88 ± 0.02[c] (62.55)	0.97 ± 0.08[d] (65.23)
Indomethacin	10	0.24 ± 0.04[b] (67.12)	0.32 ±0.03[d] (74.40)	0.46 ± 0.06[d] (72.12)	0.52 ± 0.02[d] (77.87)	0.60 ± 0.07[e] (78.49)

Percentage inhibitions are indicated in brackets; values carrying superscripts different from the control down the group for each hour are significantly different ($p < 0.05$).

brewer's yeast injection by inserting a clinical thermometer (Panamedic Corporation, Cheonan Choongnam, Korea), 3 - 4 cm into the rectum. Only rats that showed an increase of at least 0.5°C rise in temperature were used for the study. The animals were thereafter administered orally with the extract (50, 100 and 200 mg/kg body weight), distilled water (negative control) and the reference drug, indomethacin (10 mg/kg body weight) and allowed for a latency period (30 min) before their rectal temperature were recorded at 0.5 - 6 h post-dosing.

Statistical analyses

Data were presented as mean ± SD. Statistical differences between the control and the treated groups were tested by student's t-test. The differences were considered significant at $p<0.05$.

RESULTS

Phytochemical screening of the aqueous extract of *H. pauciflorus* leaves revealed the presence of tannins, flavonoids, steroids, terpenes, cardiac glycosides and saponins.

The anti-inflammatory effect of the extract on carrageenan-induced oedema right hind paw volume in rats is depicted in Table 1. There was a gradual increase in the oedema paw volume in the distilled water treated control group throughout the period of the experiment. The extract at 50, 100 and 200 mg/kg body weight as well as indomethacin significantly reduced the oedema paw volume in a manner that was not dose dependent. There was also substantial inhibition against the oedema induced paw volume in the extract and drug treated animals.

The injection of histamine to the hind paw volume of the negative control increased significantly throughout the 6 h experimental period (Table 2). In contrast, the extract at 50, 100 and 200 mg/kg body weight reduced the histamine-induced right hind paw volume in a manner that was not dose-dependent. The indomethacin treated (positive control) animals produced the highest inhibition of histamine-induced oedema but this was not comparable to the extract treated animals.

Although, the extract decreased the formalin-induced number of licks in the first phase (0 - 5 min) in a manner that was inversely proportional

Table 3. Effect of the administration of *H. pauciflorus* leaf extract on formalin-induced pain in Wistar rats (n = 5, x ± SD).

Treatment groups	Doses (mg/kg body weight)	Number of licks			
		0 - 5 min		15-30 min	
		Score of pain	% inhibition	Score of pain	% inhibition
Control	0	14.00 ± 1.41[a]	0	19.25 ± 2.41[a]	0
Extract	50	5.00 ± 0.06[b]	64.29	12.66 ± 10.03[b]	32.24
Extract	100	6.25 ± 0.04[c]	55.36	11.66 ± 0.16[b]	39.43
Extract	200	7.33 ± 0.05[d]	47.64	8.00 ± 0.19[c]	54.44
Indomethacin	10	5.25 ± 0.04[b]	62.50	8.00 ± 0.10[c]	54.44

Values carrying superscripts different from the control down the treatment groups are significantly different ($p < 0.05$).

to the doses, the decrease in the same parameter in the second phase (15 - 30min) was dose dependent (Table 3). The indomethacin treated animals produced the greatest reduction in the number of licks as well as inhibitory effect on the formalin-induced pain in the animals. In addition, the 200 mg/kg body weight also produced analgesic effect in the second phase that compared favourably with the indomethacin (Table 3).

The extract also significantly prolonged the reaction time of the animals to the warm sensation from the water bath in a manner that was not dose related (Table 4).

The effects of the administration of *H. pauciflorus* leaf extract on the brewer's yeast elevated body temperature in rats are shown in Table 5. Whereas the distilled water control group remained hyperpyretic throughout the experimental period, the extract and indomethacin dosed animals had their body temperature lowered. The lowering of elevated body temperature for 50 and 100 mg/kg body weights manifested after 1 h while that of the 200 mg/kg body weight began after half an hour. The antipyretic effect was sustained throughout the remaining period of the experiment in a manner similar to indomethacin.

DISCUSSION

The results from the present study show that the leaf extract of *H. pauciflorus* exhibited activities in various degrees against inflammation, pain and fever. By activating the cyclooxygenase, the levels of prostaglandin, especially PGE_2, increases markedly and its production provokes inflammation, pain and fever (Dannhardt and Kiefer, 2001). Therefore, we assume that some active metabolites of the extract in this study could inhibit cyclooxygenase activity.

The most widely used primary test to screen anti-inflammatory agent is to measure the ability of a compound to reduce local oedema induced in rat paw following the injection of irritants such as carrageenan and histamine (Winter et al., 1962).

The carrageenan-induced paw oedema model in rats is known to be sensitive to cyclooxygenase (COX) inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents (Rao et al., 2005). It is also suitable for assessing the anti-oedematous effect of natural products and is believed to be biphasic (Adedapo et al., 2008). The first phase of 1 h involves the release of serotonin and histamine while the second phase of the next 1 h is mediated by prostaglandin (Perianayagam et al., 2006). The significant reduction as well as inhibitory effect of the extract on the carrageenan-induced oedema paw volume is an indication of the anti-inflammatory potentials of the plant. Therefore, the result of this study supports the use of the plant in folklore medicine for the management of acute inflammation. The suppression of paw oedema in the last phase could probably be due to inhibition in the release of early mediators such as histamine, serotonins and kinins (Amresh et al., 2007) as well as cyclooxygenase (Seibert et al., 1994).

Histamine is an important inflammation mediator as well as a potent vasodilator which increases vascular permeability (Linardi et al., 2002). The anti-histaminic activity of the extract is an indication that it has the potential to inhibit the biosynthesis, release or the activity of prostaglandin. This further corroborates our findings in this study on the carrageenan-induced paw oedema model.

The phytoconstituents in the extract have also been implicated in several studies to exhibit anti-inflammatory and analgesic activities (Calixto et al., 2000; Sabu and Kath, 2002; and Silva et al., 2005). Triterpenes, flavornoids and steroids found in this extract could be responsible for the observed anti-inflammatory, analgesic and antipyretic activities. The ability of quercetin, to inhibit nitric oxide synthase, 5-lipooxygenase, phospholipase A_2 and C as well as cyclooxygenase-2, all of which are pro-inflammatory enzymes have been reported (Chiesi and Schwaller, 1995; De Pascual-Teresa et al., 2004). Therefore, the anti-inflammatory activity of *H. pauciflorus* leaves may be attributed to the presence of flavonoids.

The formalin test is considered a suitable model for chronic pain (Dubuisson and Dennis, 1977). In the formalin-induced licking, animals present two distinct nociceptive behavioural phases, which probably involve different stimuli. The first phase (neurogenic) initiates immediately after formalin injection and lasts for 3 - 5 min, resulting

Table 4. Effect of administration of *H. pauciflorus* leaf extract on tail flick nociception response in rats (n = 5, x ± SD).

Treatment groups	Dose (mg/kg body weight)	Latency periods (h)					
		0	0.5	1	2	4	6
Extract	50	2.45 ± 0.13[a]	3.37 ± 0.13[b]	3.35 ± 0.11[b]	3.80 ± 0.24[b]	3.25 ± 0.19[b]	4.27 ± 0.11[c]
Extract	100	2.50 ± 0.10[a]	3.10 ± 0.13[b]	3.65 ± 0.09[c]	3.42 ± 0.05[c]	4.10 ± 0.14[d]	4.27 ± 0.17[d]
Extract	200	2.60 ± 0.10[a]	3.33 ± 0.05[b]	3.20 ± 0.10[b]	3.80 ± 0.10[c]	3.93 ± 0.10[c]	4.43 ± 0.05[d]
Indomethacin	10	2.35 ± 0.11[a]	2.75 ± 0.07[b]	3.65 ± 0.10[c]	3.20 ± 0.12[c]	3.65 ± 0.17[c]	3.80 ± 0.05[b]

[a-d]Test values are significantly different from the control at 0 ($p < 0.05$).

Table 5. Effect of administration of *H. pauciflorus* leaf extract on brewer's yeast induced pyrexia in rats (n = 5, x ± SD).

Treatment groups	Doses (mg/kg body weight	Test period (h)						
		0	18	18.5	19	20	22	24
Control	0.5 ml	35.97 ± 0.84	37.60 ± 0.54	37.22 ± 0.15	37.05 ± 0.05	37.10 ± 0.14	36.97 ± 0.12	36.72 ± 0.12
Extract	50	36.17 ± 0.54	37.72 ± 0.08[a]	37.92 ± 0.09[a]	37.72 ± 0.09[a]	36.60 ± 0.06[b]	36.22 ± 0.03[c]	36.18 ± 0.02[c]
Extract	100	36.70 ± 0.09	37.82 ± 0.04[a]	37.42 ± 0.02[a]	37.80 ± 0.04[a]	36.81 ± 0.06[b]	36.77 ± 0.05[b]	36.56 ± 0.05[c]
Extract	200	35.97 ± 0.08	37.05 ± 0.04[a]	36.85 ± 0.03[b]	36.47 ± 0.05[b]	36.37 ± 0.02[b]	36.08 ± 0.08[c]	35.90 ± 0.08[c]
Indomethacin	10	35.92 ± 0.06	37.90 ± 0.06[a]	37.10 ± 0.06[a]	36.40 ± 0.02[b]	36.30 ± 0.09[b]	36.50 ± 0.07[b]	36.20 ± 0.06[b]

[a-c]Test values for each column are significantly different from the control ($p < 0.05$).

from chemical stimulation of nociception. The second phase (inflammatory pain) begins from 15 to 20 min after formalin injection, lasts for 20 -40 min and depends on both peripheral and central mechanisms (Ferreira et al., 2004). While substance P and bradykinin are involved in the first phase, histamine and prostaglandins are involved in the second phase (Ferreira et al., 2004). Centrally acting analgesics such as narcotics inhibits both phases equally while peripherally acting drugs, such as steroids and non-steroidal anti-inflammatory drugs (NSAIDS) like aspirin suppresses mainly the late phase (Shibata et al., 1989; Adzu et al., 2003; Trongsakul et al., 2003). Although, *H. pauciflorus* extract did not inhibit both phases equally, it may still be logical to assume that it produced analgesic effect on the two phases. This may thus suggest that the extract is a centrally acting analgesic.

The tail flick or tail immersion model is an index that is used to evaluate acute pains in animals (Franzotti et al., 2002). Tail flick response is predominantly considered to be selective for centrally acting analgesics while peripherally acting ones are known to be inactive on this kind of painful stimulus (Srinivasa et al., 2003). The prolonged reaction time which was not dose dependent is an indication of analgesic potential of the extract. Since centrally acting analgesics are known to elevate pain threshold in animals arising from heat and pressure (Adeyemi et al., 2004), the aqueous extract of *H. pauciflorus* leaves may be a centrally acting analgesic.

Fever may be as a result of infection or one of the sequelae of tissue damage, inflammation, graft rejection or other diseased states (Devi et al., 2003). Regulation of body temperature requires a delicate balance between the production and loss of heat. The present results show that the aqueous extract of *H. pauciflorus* leaves possesses significant antipyretic effect on brewer's yeast provoked elevation of body temperature in rats. The reduction in the brewer's yeast induced fever by the extract in this study suggests some influence on the prostaglandin biosynthesis since it is believed to be a regulator of body temperature (Dascombe, 1985).

The result of this study confirmed that *H. pauciflorus* leaves could be beneficial in the management of inflammations, pains and fever. These activities may be due, in part, to the

presence of phytochemicals such as tannins, flavonoids, steroids and or terpenes.

ACKNOWLEDGEMENT

The authors are grateful to the National Research Foundation (NRF) of South Africa for supporting this work.

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