INVESTIGATION OF THE ANTINOCICEPTIVE AND ANTI-INFLAMMATORY PROPERTIES OF *HETEROMORPHA ARBORESCENS* (APIACEAE)

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Abstract

Heteromorpha arborescens belongs to the family Apiaceae. It is commonly known as the parsley tree. One of its uses in the Eastern Cape Province of South Africa is for the treatment of abdominal pains. The therapeutic effects of the methanolic and aqueous root extracts of *H. arborescens* were investigated at two dose levels respectively on experimental models of pain and inflammation in rodents. The antinociceptive activity was evaluated using the hot-plate, abdominal constriction and formalin tests. The anti-inflammatory properties of these extracts were assessed using albumin and carrageenan as phlogistic agents. Both extracts produced significant ($P<0.05$, $P<0.01$) inhibition of thermal nociception induced by a hot plate. On chemical nociception induced by intraperitoneal acetic acid and subplantar formalin injection, both extracts significantly ($P<0.05$, $P<0.01$) decreased the number of writhing episodes and the licking time in a dose dependent manner. Treatment with the extracts at the same doses produced a significant ($P<0.05$, $P<0.01$) pain inhibition of the carrageenan induced inflammatory pain. Similarly, both extracts produced a significant ($P<0.05$, $P<0.01$) reduction of edema induced by albumin and carrageenan. These results suggest that both extracts of *H. arborescens* may act by inhibition of the mediators of inflammation. These findings seem to justify the use of the plant in traditional medicine in the management of pain and inflammation related diseases.

Key words: *Heteromorpha arborescens*, hot plate, writhing, formalin, inflammatory pain

List of non-standard abbreviation:
HA: *Heteromorpha arborescens*,
HAAE: *Heteromorpha arborescens* aqueous extract,
HAME: *Heteromorpha arborescens* methanol extract.

Introduction

The management of pain and inflammation related problems is a real challenge that people face daily. Although several drugs are available for these conditions, medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Gupta et al., 2006). South Africa has an abundance of medicinal plants, used in the treatment of various diseases on an empirical basis (McGaw et al., 1997; Lin et al., 1999). *Heteromorpha arborescens* commonly known as the parsley tree in English, "wildepitersielie" in Afrikaans and "umBangandlala" in Xhosa is a medicinal plant used to treat abdominal pains, dysmenorrhea, nervous and mental disorders as well as a vermicide in children (Palmer and Pitman, 1973). This plant is found in wooded grassland, bushveld and on forest margins in South Africa. However, up to date, there is no scientific report or verification of the use of this plant in inflammation and pain management. This study investigated the analgesic and the anti-inflammatory activity of the aqueous and methanolic extracts of *H. arborescens* using different experimental models of pain and inflammation.

Materials and methods

Plant material

Roots of *H. arborescens* were collected in the month of August 2008 in Flagstaff (South Africa - Latitude: 29° 26' 60 S, Longitude: 31° 13' 60 E). The plant material was taxonomically identified by Dr Kathleen Immelman of the Kie herbarium at the

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Walter Sisulu University and verified by the KwaZulu Natal Herbarium in Durban. A voucher specimen (Nkomo 02) was deposited in the herbarium for future reference.

Extract preparation

Root samples of *H. arborescens* were processed as outlined by Taylor et al. (1996) and Koduru et al. (2006). Briefly, the roots were chopped, air dried and pulverised. Portions of powdered plant material (50g) were shaken separately in methanol and water for 24 hours on an orbital shaker. The extracts were filtered using a Buchner funnel and whatman filter paper. The solvent from the methanol extract was evaporated using a rotor evaporator and then air-dried to yield a 17g brownish extract. On the other hand, the aqueous extracts were freeze-dried using a lyophilizer (Edwards – Modulyo), yielding 8.4g of extract.

Animals

Two months old male and female Swiss mice (30-40 g) and Wistar rats (200-250 g) were used. The animals were housed in the Physiology Department animal holding facility, with a 12 hour light/dark cycle, with access to food and tap water *ad libitum*. Animals were however, deprived of food 12 hours before each experiment. The experiments reported in this study were carried out in accordance with current ethical guidelines for the investigation of experimental pain in conscious animals (Zimmermann, 1983). Ethical clearance for this study was obtained from the Walter Sisulu University Ethics Committee Ref No: Ethics 0009-07

Writhing test

The writhing test was carried out as described by Gaertner et al. (1999) with slight modifications. Seven groups of mice (n=5/group) were treated with the aqueous extracts of *H. arborescens* (HAAE; 150 and 200 mg/kg), the methanolic extracts of *H. arborescens* (HAME; 150 and 200 mg/kg), Aspirin (100 mg/kg), Morphine (10 mg/kg) and control animals received an equivalent volume of distilled water. The writhing episodes were induced by an intraperitoneal injection of a 0.6% acetic acid solution (0.25 ml/animal) 30 minutes after treatment with the different drugs. The number of abdominal contortions was counted during the first 20 minutes after acetic acid injection. Data represents the average of the total number of abdominal contractions per group.

Hot plate test

The hot plate test was carried out as described by Wilson et al. (2005). Groups of mice (n=5) were treated with HAAE (150 and 200 mg/kg), HAME (150 and 200 mg/kg), Aspirin (100 mg/kg), Morphine (10 mg/kg) and distilled water. Mice were tested by placing them on a hot plate (Bibby Sterilin, UK) maintained at 55±1^{o}C and the reaction time in seconds for licking of hind paw or jumping was recorded. The mice which reacted within 15 sec at baseline and which did not show large variation when tested on four separate occasions were selected for studies.

Formalin test

The formalin test was carried out as described by Santos and Calixto, (1997). Groups of mice (n=5) were treated with HAAE (150 and 200 mg/kg), HAME (150 and 200 mg/kg), Aspirin (100 mg/kg), Morphine (10 mg/kg) and distilled water. Formalin (1% v/v) was injected into the sub-plantar region of the right hind paw of the animals, one hour post treatment. The duration of paw licking was measured for 0–5 minutes (neurogenic phase) and 15–30 minutes (inflammatory phase) after formalin administration.

Inflammatory pain assay

The inflammatory pain assay was carried out as described by Ferreira et al. (2001) with modifications. Groups of rats (n=5) were treated with HAAE (150 and 200 mg/kg), HAME (150 and 200 mg/kg), Aspirin (100 mg/kg) and distilled water. Rats received 0.2 ml of carrageenan subcutaneously on the plantar surface of the right hind paw to induce inflammation. Pain was assessed using the von Frey filament (Ugo Basile, Dynamic plantar Anesthesiometer, 37450). The rats were placed on a mesh-wire floor within individual plastic boxes, and were allowed to acclimatize for 30 minutes before the start of trials. The hairless plantar surface of the hind paw was probed by an electronic von Frey probe ranging from 0.01~58 g. Each monofilament was applied with sufficient force to bend. Responses were characterized by the brisk withdrawal or flinching of the tested paw. The amount of force observed indicated the mechanical pain threshold. Pain threshold was determined before the injection of carrageenan (baseline) and 0.5 H, 1, 2, 4 and 24 H after injection.

Albumin-induced inflammation

The albumin-induced hind paw oedema model was used in the determination of anti-inflammatory activity. This test was carried out as described by Okoli and Akah, (2000). Six groups of 5 rats each were allotted to different treatment groups.

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Group 1 (control) was treated with distilled water. Groups II to V were treated orally with HAAE, (150 and 200 mg/kg), HAME (150 and 200 mg/kg) respectively, while group VI was treated orally with 10 mg/kg of indomethacin and used as a reference. Thirty minutes post treatment, oedema was induced by injection of albumin (0.1 ml, 50% v/v in saline) into sub plantar tissue of the right hind paw. Paw volumes were determined plethysmographically (Hugo Basile Model No: 7140) immediately before injection of the phlogistic agents and at 30 minute, 1, 2 and 3 H after albumin injection. Percentage inhibition of inflammation (Ahmed et al. 1993; Okoli et al., 2006) was calculated using the relation: inhibition of inflammation (%) = 100 × [1- (a-x)/(b-y)], where *a* = mean paw volume of treated rats at given times after albumin injection; *x* = mean paw volume of treated rats before albumin injection; *b* = mean paw volume of control rats at given time after albumin injection; *y* = mean paw volume of control rats before albumin injection.

Carrageenan-induced inflammation

The carrageenan-induced hind paw oedema model was used to confirm anti-inflammatory activity. This test was carried out as described by Gupta et al. (2006). Six groups of 5 rats each were allotted to different treatment groups. Animals were pre-treated as in the albumin-induced experiments and 30 minutes later edema was induced by the intra-plantar injection of carrageenan (0.1 ml, 1% w/v in saline). Paw volume measurements were made immediately before injection of the phlogistic agents and at 30 minutes, 1, 2 and 3 H after albumin injection using the Ugo Basile 7140 plethysmometer. Percent inhibition of inflammation was calculated using the relation: inhibition of edema (%) = 100 × [1- (a-x)/(b-y)], where *a* = mean paw volume of treated rats at various time after carrageenan injection; *x* = mean paw volume of treated rats before carrageenan injection; *b* = mean paw volume of control rats at various time after carrageenan injection; *y* = mean paw volume of control rats before carrageenan injection.

Statistical analysis

One-way analyses of variance followed by Tukey-Kramer Multiple Comparisons Test, were done to determine differences between control and treated groups, using the software, GraphPad Instat ® version 3.00 for Windows 95, GraphPad Software, San Diego California USA, www.graphpad.com. Data are reported as mean ± SEM. $p < 0.05$ was considered significant.

Results

Writhing test

The higher doses (200 mg/kg) of both HAAE and HAME significantly ($p<0.05$) inhibited acetic-acid induced abdominal pain while the lower doses (150 mg/kg) failed to protect against this model of pain. Morphine (10 mg/kg) and aspirin (100 mg/kg), on the other hand significantly ($p<0.01$ and $p<0.05$ respectively) inhibited acetic acid-induced pain in mice (Fig 1).

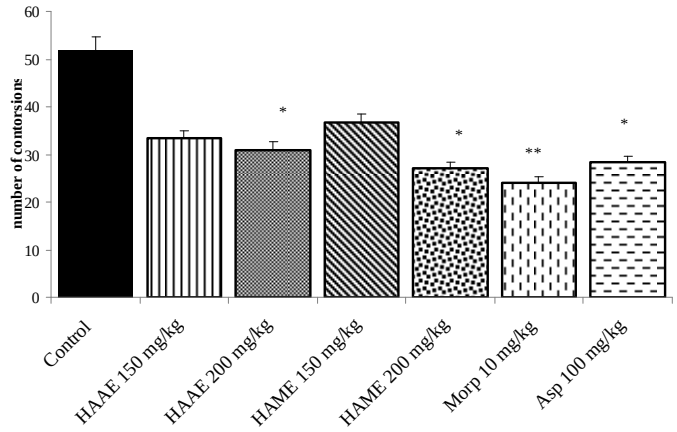

Figure 1: Antinociceptive effect of HAAE and HAME on acetic-acid induced abdominal contractions in mice. Values represent the means ± SEM. (*n*=5) *p< 0.05; **p<0.01.

Hot plate test

The extracts of HAAE and HAME showed significant ($p<0.01$) analgesic properties. However, unlike morphine which inhibited pain significantly ($p<0.01$) from the 1st hour, both doses of HAME, induced a late onset (2 hour) of analgesic activity

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and significantly increased the reaction time in treated animals. The extract of HAAE (150 mg/kg) had an even later onset of analgesic effect 3 H post-treatment which was also of short duration 4 H post-treatment (Table 1). The 200 mg/kg dose of HAAE showed significant analgesic activity in this pain model from 2 H post treatment and these effects remained significant beyond 5 H post treatment.

Table 1: Effect of HAAE and HAME on reaction time to pain induced by a hot plate

		Time difference (s)				
	Dose	1h	2h	3h	4h	5h
Control		1.20	1.00	- 0.60	- 0.20	- 1.20
HAAE	150 mg/kg	4.25	4.00	6.80**	8.20**	3.40
HAAE	200 mg/kg	4.40	9.20**	14.40**	12.20**	11.20**
HAME	150 mg/kg	4.48	7.80**	9.20**	10.40**	6.60**
HAME	200 mg/kg	5.80	9.20**	14.40**	12.00**	11.40**
Morphine	10 mg/kg	6.20**	7.20**	7.40**	5.60*	8.00

Results expressed in this table are the differences between reaction time at given time and the reaction time at baseline. Values are mean ± SEM (n = 5). *p < 0.05 and **P<0.01

Formalin test

The formalin test exhibited the characteristic biphasic response. Phase 1 response which was recorded from the time of formalin injection and 5 minutes post-injection was not affected by either extract at either dose level. Morphine however, showed significant (p<0.05) inhibition of pain in this phase (Fig 2). The second phase of the response was noted from 10 minutes to 30 minutes post formalin injection. The extracts of HAAE (150 mg/kg and 200 mg/kg) and HAME (150 mg/kg and 200 mg/kg) as well as aspirin and morphine showed significant (p<0.01) inhibition of formalin-induced pain (Fig 2). The extract of HAME 150 mg/kg significantly inhibited inflammatory pain compared to 200 mg/kg HAAE.

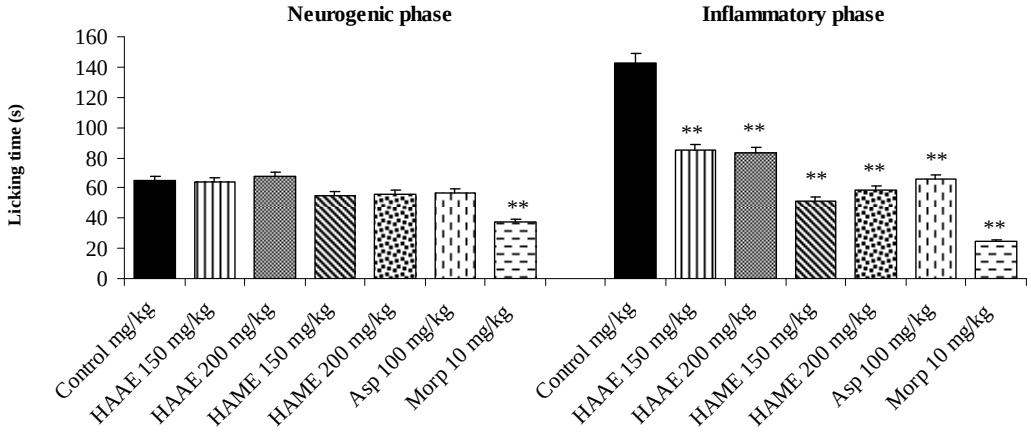

Figure 2. Antinociceptive effect of the extracts of HAAE and HAME in inhibiting formalin-induced pain. Values represent the means ± SEM. (n=5) **p<0.01.

Inflammatory pain assay

As shown in Figure 3, the administration of both HAAE and HAME significantly (P<0.05, P<0.01) increased the paw withdrawal latencies in mechanical hyperalgesia induced by carrageenan from 1 to 4 H. The analgesic effect of both extracts on inflammatory pain was comparable to that of indomethacin (10 mg/kg) a standard anti-inflammatory drug.
At the end of the 24 H post treatment, only the 200 mg/kg HAAE extract continued to show significant analgesic effects in this pain model (Fig 3).

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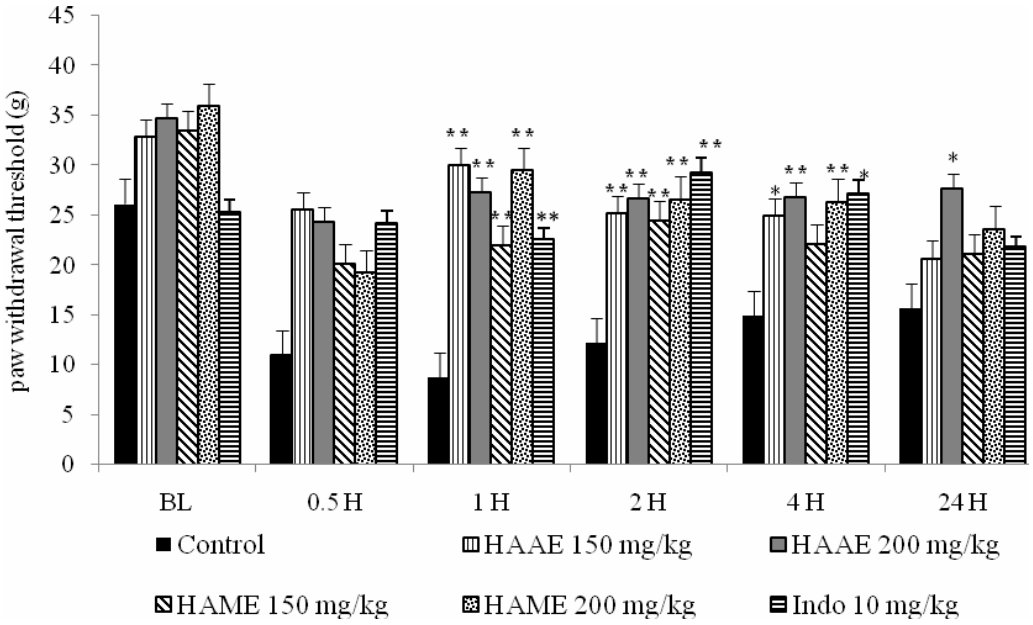

Figure 3: Antinociceptive effect of HAAE and HAME extracts on inflammatory pain induced by carrageenan. Values represent the means ± SEM. n=5; *p<0.05; **p < 0.01, Indo: Indomethacin.

Albumin induced inflammation

The anti-inflammatory activities of HAAE and HAME on albumin-induced inflammation are summarized in Table 2. The 150 mg/kg HAAE extract had significant (p<0.05 and p<0.01) analgesic properties from 1 to 3 hours post treatment though the 200 mg/kg failed to produce similar anti-inflammatory effects. With the HAME extract, significant anti-inflammatory properties were noted though the 150 mg/kg extract tended to have better anti-inflammatory properties than the 200 mg/kg dose. Indomethacin elicited analgesic properties which were comparable to the effects of 200 mg/kg HAME (Table 2).

Table 2: Effect of extracts of HAAE and HAME on albumin-induced inflammation in the rat hind paw

		Inhibition of Inflammation (%)		
	Dose	1h	2h	3h
Control	-	-	-	-
HAAE	150 mg/kg	57.8**	37.4*	44.4*
HAAE	200 mg/kg	26.7	24.8	32.1
HAME	150 mg/kg	66.9**	56.3*	73.5*
HAME	200 mg/kg	65.5**	53.4*	65.4*
Indo	100 mg/kg	67.9**	59.7*	59.3*

Values are mean ± SEM (n = 5). *p<0.05 and **p<0.01)

Carrageenan-induced inflammation

Both HAAE and HAME showed significant analgesic effects against carrageenan-induced inflammation. The lower doses (150 mg/kg) of HAAE and HAME had a later onset (2 H) while the higher dose of HAAE showed anti-inflammatory effects which were of an earlier onset and persisted after 3 H (Table 3). Unlike the extract indomethacin showed a later onset of anti-inflammatory properties which was significant from 3 hours post treatment.

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Table 3: Effect of HAAE and HAME on carrageenan induced inflammation in the rat paw

		Inhibition of Inflammation (%)		
	Dose	1h	2h	3h
control	-	-	-	-
HAAE	150 mg/kg	59	68**	50*
HAAE	200 mg/kg	57*	65**	71**
HAME	150 mg/kg	53	71**	67**
HAME	200 mg/kg	75	73**	72**
Indo	100 mg/kg	39	36	59**

Values are mean ± SEM (n = 5). *P < 0.05 and **P<0.01. Indo: indomethacin.

Discussion

The present study investigated the antinociceptive and the anti-inflammatory properties of the aqueous and methanolic extracts of roots of *H. arborescens* using various animal models. The plant extracts showed anti-nociceptive and analgesic properties thus providing proof of the efficacy of this plant in its use by the Xhosa people for the treatment of pain and inflammation.

The acetic acid-induced abdominal writhing method showed that at the higher dose levels, both HAAE and HAME had analgesic properties which were comparable with those of aspirin a peripherally acting analgesic. Acetic acid induces pain by the release of endogenous mediators of pain such as prostaglandin through the activity of cyclooxygenase (COX) (Satyanarayana et al., 2004; Ballou et al., 2000). Therefore this model of pain should be inhibited by peripheral analgesics through the inhibition of COX activity. Our results therefore show that the higher doses of HAAE and HAME have peripheral analgesic properties similar to aspirin by inhibition of the release of endogenous pain mediators. The acetic acid-induced abdominal contraction test is essentially a screening test for analgesic properties and not specific in its response, other more specific tests were performed to confirm the observed results.

The hot-plate test was utilized to assess the central antinociceptive properties of HAAE and HAME. This test is sensitive to drugs which exert their analgesic effects through the CNS. The two doses of HAME like 200 mg/kg of HAAE showed significantly increased reaction time to thermal stimulation from 2 hours to beyond 5 hours post treatment. This was not the case with 150 mg/kg of HAAE which showed analgesic effects from 3 hours to 4 hours post treatment probably because the amount of active principle in it is smaller compared to the higher dose. Morphine a central acting analgesic increased the reaction time from 1 hour post treatment. Since HAAE and HAME were able to increase the latency to thermal stimulation, they could therefore have some central analgesic properties. In order to further confirm the analgesic properties of these extracts, the formalin test was carried out.

The formalin test is said to be a model of pain which closely resembles clinical pain compared to the other nociceptive models (Tjolsen and Hole, 1997). This test has two distinct phases: the first phase (neurogenic pain) due to direct chemical stimulation of nociceptors, results from the stimulation of myelinated and unmyelinated nociceptive afferent fibers, mainly C fibers, which can be suppressed by opioid analgesic drugs like morphine (Sayyah et al., 2004). The second or late phase seems to be an inflammatory response which elicits inflammatory pain and can be inhibited by anti-inflammatory drugs (Young at al., 2005). The second phase is caused by the release of inflammatory mediators such as prostaglandins and histamine in the peripheral tissues, as well as functional changes in the neurons, of the spinal cord which may facilitate transmission in the spinal cord (Franca et al., 2001; Garcia et al., 2004). Both HAAE and HAME failed to have an effect on the neurogenic phase of the formalin test. However, in the inflammatory phase both extracts showed significant inhibition of pain. The HAME extracts showed significant inhibition of formalin induced-pain which was more profound than results obtained with HAAE. The observed effects with 150 mg/kg HAME was significantly different from results obtained with HAAE. These extracts showed analgesic properties against inflammatory pain thus suggesting that it might be acting by inhibiting the release of inflammatory mediators in the injected paw while failing to block the transmission of pain via C fibres. To further elucidate the effects of these extracts on the late phase of the formalin test, we therefore, investigated the effects of HAAE and HAME on a model of inflammatory pain induced by carrageenan.

The extracts of HAAE and HAME inhibited carrageenan-induced hyperalgesia 2 and 4 hours after treatment. Unlike HAME whose effects were observed only during the second and fourth hours post-treatment, HAAE had anti-hyperalgesic effects which were still significant 24 hours post-treatment. This anti-hyperalgesic effect of extracts of *H. arborescens* corroborates the result obtained from the late phase of formalin test suggesting a possible anti-inflammatory response. These observations suggest that *H. arborescens* may act by decreasing the production of mediators of inflammation. For further investigation, the effect of HAAE and HAME were evaluated against inflammation induced by fresh egg albumin and carrageenan.

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Extracts of both HAAE and HAME inhibited both phases of the carrageenan-induced inflammatory process indicating that the extracts have effects on both the early release of histamine and serotonin as well as the late phase involving the inhibition of cycloocygenase. Indomethacin and the other NSAIDs inhibit the second phase of this inflammatory process. Carrageenan-induced oedema has been commonly used as an experimental model for acute inflammation and is believed to be biphasic (Posadas et al, 2004). The early phase (1 - 2 hours) of the carrageenan model is mainly mediated by histamine and serotonin released in the damaged tissue surroundings (Badilla 2003). The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages (Gupta et al., 2006; Brito and Antonio, 1998). The inhibitory activity shown by both extracts of *H. arborescens* mostly after the second hour may indicate that the extract acts in the early phase probably involving neutrophils mobilization (Gupta et al., 2006; Just et al., 1998).

Based on the results of this study, it can be concluded that the aqueous and methanolic extracts of *H. arborescens* possess analgesic and anti-inflammatory properties. These findings justify the use of the plant in traditional medicine in the management of pain and inflammation related diseases.

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