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Full Length Research Paper

Antinociceptive and anti-inflammatory properties of *Gunnera perpensa* (Gunneraceae)

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Gunnera perpensa, **which belongs to the Gunneraceae family, is used in folk medicine to relieve rheumatoid pain, facilitate childbirth and for healing wounds. In this study, the antinociceptive and anti-inflammatory properties of this plant extracts were evaluated using the abdominal constriction, hot-plate, formalin, hyperalgesia and fresh egg albumin-induced inflammation. The extracts were administered orally at the test doses of 150 and 200 mg/kg prior to the above-mentioned assays. Both extracts produced significant (P < 0.05, P < 0.01) inhibition of thermal nociception induced by hot plate respectively. Chemical nociception induced by intraperitoneal and sub plantar injections of acetic acid and formalin respectively, were significantly (P < 0.05, P < 0.01) reduced by the extracts in a dose independent manner. The extracts also showed significant antihyperalgesia and anti inflammatory properties (P < 0.05, P < 0.01) respectively. Our findings suggest that *G. perpensa* possesses both antinociceptive and anti inflammatory activity supporting its traditional use for pain management.**

Key words: *Gunnera perpensa,* hot plate, writhing, formalin, inflammation, pain.

INTRODUCTION

South Africa has an abundance of medicinal plants, used in the treatment of various diseases on an empirical basis (Hutchings and van Staden, 1994; Hutchings et al., 1996; Jäger et al., 1996; Salie et al., 1996; McGaw et al., 1997).

The management of pain is a daily challenge in modern medicine, despite the currently available wide range of analgesics. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects on pain (Gupta et al., 2006; Farnsworth, 1889). *Gunnera perpensa* is widely used by the rural population in South Africa for the treatment of several diseases including dysmenorrhoea. Aqueous decoctions of this plant, relieve rheumatoid pain, facilitate childbirth and are believed to treat female infertility (Hutchings et al., 1996). A decoction of the rhizomes of *G. perpensa* is applied as a dressing for wounds and psoriasis (Watt and Breyer-Brandwijk, 1962, Grierson and Afolayan, 1999).

Previous studies have showed that *G. perpensa* has both antibacterial and antioxidant properties, as well as stimulate fibroblast growth in wound healing (Steenkamp et al., 2004). Drewes et al. (2005) showed that Z-venusol, a phenylpropanoid glucoside and two new, simple, 1,4 benzoquinones isolated from *G. perpensa* had antibac-terial activity as well as contracted both ileal and uterine smooth muscles. However, up to date, there is no scien-tific validation of the use of this plant in the management of pain.

This study investigated the analgesic and anti-inflammatory activity of aqueous and methanolic extracts of *G. perpensa* using several experimental animal moleds of pain to validate its traditional uses.

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Abbreviations: GPAE, *Gunnera perpensa* aqueous extract; **GPME,** *Gunnera perpensa* methanolic extract.

MATERIALS AND METHODS

Plant material

Rhizomes of *G. perpensa* were collected on the 14th of August

2008, 40 Km North East of Lusikisiki (Eastern Cape- South Africa). The plant material was taxonomically identified by Dr Kathleen Immelman of the Kie herbarium at Walter Sisulu University. A voucher specimen (Nkomo 01) has been deposited in the herbarium for future reference.

Extract preparation

Rhizome samples of *G. perpensa* were chopped, air dried and ground to powder. 50 g portions of each dried plant were shaken separately in methanol and water for 24 h on an orbital shaker. The extracts were filtered using a Buchner funnel and Whatman No. 1 filter paper, with the methanol filtrates concentrated to dryness under reduced pressure at a maximum of 40°C using a rotating evaporator to afford a brownish paste yielding 17 g of crude methanolic extract. The aqueous filtrates were freeze- dried, yielding an 8.4 g brown powder (Taylor et al., 1996; Koduru et al., 2006).

Animals

Two month old Swiss mice (30 - 40 g) and Wistar rats (200 - 250 g) were obtained from the South African Vaccine Products-Johannesburg and kept in the Department of Physiology and Animal holding facility.

The animals were housed with a 12 h light/dark cycle and fed standard rat chow and tap water *ad libitum*. 12 h before each experiment animals would receive only water. 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 assay

The writhing test was carried out as described by Gaertner et al. (1999). Groups of mice (n = 5) were pre-treated orally with *G. perpensa* aqueous extract (GPAE) (150 and 200 mg/kg), *G. perpensa* methanolic extract GPME (150 and 200 mg/kg), Aspirin (100 mg/kg), Morphine (10 mg/kg) and distilled water. The writhing episodes were induced by an intraperitoneal injection of a 0.6% acetic acid solution (0.25 ml /animal) 30 min after the pre-treatment. The number of writhes/contortions was counted during the first 20 min after acetic acid injection.

Hot plate assay

The hot plate test was carried out as described by Wilson et al. (2003). Groups of mice (n = 5) were treated orally with GPAE (150 and 200 mg/kg) and GPME (150 and 200 mg/kg), Aspirin (100 mg/kg), Morphine (10 mg/kg) and distilled water. Mice were placed on a hot plate (Bibby Sterilin, UK) maintained at 55 ± 1°C and the reaction latency (in seconds) for licking of hind paw or jumping noted.

The mice which reacted within 15 s and which did not show large variation when tested on four separated occasions were selected for studies. Recordings were taken before treatment with the different drugs and 1, 2, 3, 4, 5 h post treatment. Results were expressed as the difference between the baseline reaction latency and the reaction latency at recorded times.

Formalin assay

The formalin test was carried out as described by Santos and Calixto, (1997). Groups of mice (n = 5) were treated orally with GPAE and GPME (150 and 200 mg/kg), Aspirin (100 mg/kg), Morphine (10 mg/kg) and distilled water. Formalin (1%) was injected into the sub-plantar region of the right hind paw of the animals 30 min post- treatment. The number of times paw was licked/bitten within the time frames of 0 - 5 min (neurogenic phase) and 15 - 30 min (inflammatory phase) after formalin administration was counted.

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 orally with GPAE and GPME (150 and 200 mg/kg), Indomethacin (10 mg/kg) and distilled water. Rats received sub-plantar injections of 0.2 ml of carrageneen in the right hind paw 30 min post-treatment. Mechanical stimulation was performed using von Frey filament (Ugo Basile, Dynamic plantar Anesthesiometer, 37450).

The rats were placed on a mesh-wire floor within individual plastic cubicles and were allowed to acclimatize for 30 min. The 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 cause them to bend. Animals responded to pain by a brisk withdrawal or flinching of the tested paw. Baseline measurements were performed before carrageneen injection while post-treatment measurements were carried out 1, 2, 3, 4 and 24 h after treatment.

Albumin inflammatory pain assay

The albumin- induced hind paw edema model was used in the determination of anti-inflammatory activity. Six groups of 5 rats each were allotted to different treatment groups. Groups of animals were treated orally with one of the following: GPAE (150 and 200 mg/kg) GPME (150 and 200 mg/kg) indomethacin (100 mg/kg) and an equal volume of distilled water, 30 min after pre-treatment, edema was induced by injection of fresh egg albumin (0.1 ml, 50% v/v in saline) into sub plantar tissue of the right hind paw. Measurements were made immediately before injection of the phlogistic agents and at 30 min, 1, 2 and 3 h after albumin injection using the Ugo Basile 7140 plethysmometer. Percentage inhibition of inflammation was calculated as described by Ahmed et al. (1993) and Okoli et al. (2006).

Statistical analysis

The software package, GraphPad InStat 3 was used for data analyses. One way analysis of variance (ANOVA) followed by Dunnett's test was used for data analyses. Results are reported as mean ± SEM. $p < 0.05$ was considered significant.

RESULTS

Writhing assay

The response to acetic acid-induced writhing was signify-cantly ($p < 0.05$) reduced by both extracts and reference drugs. Figure 1 shows that aqueous GPAE reduced acetic acid-induced abdominal contractions in a dose dependent manner with the higher dose producing significant results ($p < 0.05$). On the other hand, both doses of the methanolic extracts significantly ($p < 0.01$)

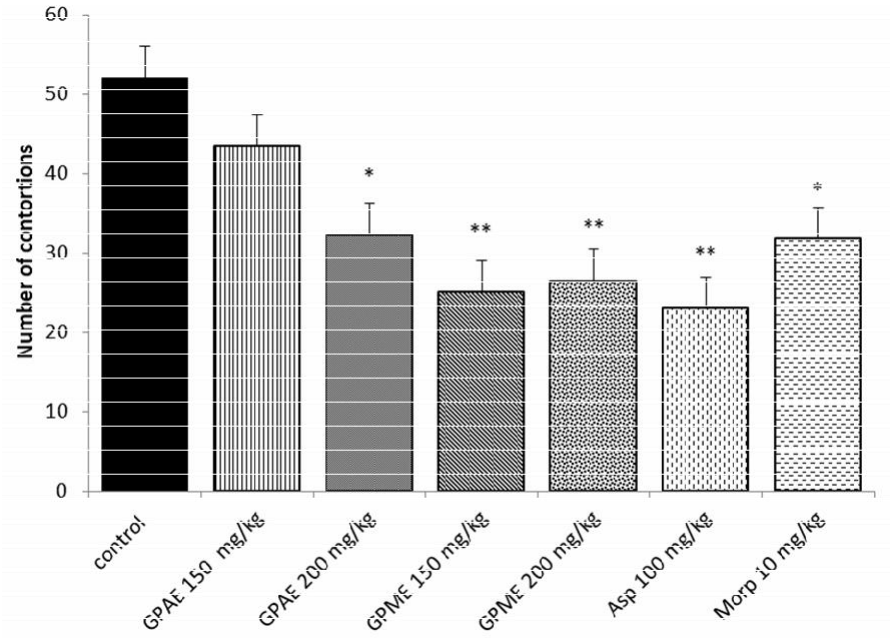

Figure 1. Antinociceptive effect of *G. perpensa* on acetic -acid induced abdominal contractions in mice. The values represent the means ± SEM. n=5; *p< 0.05; **p< 0.01, significantly different compared to control. Asp, Aspirin; Morp, Morphine.

Table 1. Effect of *G. perpensa* on hot plate reaction time in mice.

	Dose (mg/kg)	1 h	2 h	3 h	4 h	5 h
Control		1.2	1	- 0.6	- 0.2	- 1.2
GPAE	150	8.3 ± 0.9**	12.5 ± 1.6**	12.75 ± 1.0**	11.0 ± 1.4	9.0 ± 0.9**
GPAE	200	5.6 ± 1.2	8.2 ± 0.4**	12.2 ± 0.8**	9.0 ± 0.6**	9.0 ± 0.3**
GPME	150	8.6 ± 1.2	9.6 ± 1.4*	11.4 ± 1.8**	15.0 ± 2.1**	12.4 ± 2.2**
GPME	200	8.4 ± 1.0	8.6 ± 1.3**	17.0 ± 0.8**	19.0 ± 0.7**	12.0 ± 0.6**
Aspirin	100	4.4 ± 0.5	11.0 ± 2.3**	11.6 ± 1.4**	11.6 ± 1.0**	11.0 ± 1.1**
Morphine	10	7.2 ± 0.6*	7.2 ± 1.3*	7.4 ± 1.4**	5.6 ± 0.8*	8.0 ± 0.6

Values are mean ± SEM (n = 5). These values were obtained by computing the difference between the latency at given times post treatment and the baseline latency. Experimental groups were compared with the control (*P < 0.05 and **P<0.01).

inhibited acetic acid-induced writhing. These results were comparable with results obtained with aspirin. The analgesic effects of GPAE (200 mg/kg) were similar to the effects of morphine ($p < 0.05$).

Hot plate assay

As shown in Table 1, GPAE and GPME produced signify-cant ($p < 0.05$; $p < 0.01$) analgesic activity respectively from the 2nd till the 5th hour post treatment compared to to controls. Aspirin (100 mg/kg) and morphine (10 mg/kg) also showed significant ($p < 0.01$; $p < 0.05$) analgesic effects although the effects of morphine had an earlier onset.

Formalin assay

The formalin-induced pain occurred in the characteristic two phases. The first phase also known as the neuro-genic phase occurred during the first 5 min followed by a relative pain-free period. 10 min after formalin injection, the second phase known as the inflammatory phase started and lasted beyond 30 min post injection. The extracts of *G. perpensa* significantly ($p < 0.01$) inhibited the second phase of the formalin-induced pain response though these extracts failed to inhibit the first phase (Figure 2).

Morphine on the other hand, significantly ($p < 0.01$) attenuated the pain responses in both the neurogenic and inflammatory phases, while aspirin (100 mg/kg) like

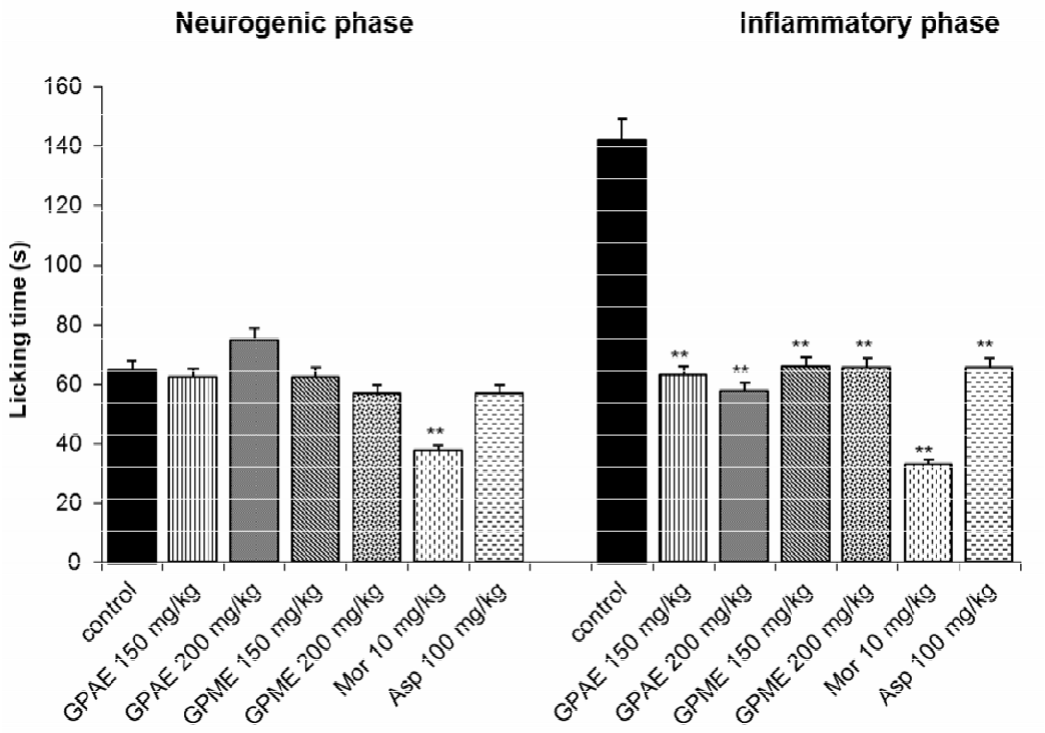

Figure 2. Antinociceptive effect of *G. perpensa* on formalin-induced pain. The values represent the means ± SEM (n=5). **P* < 0.05; ***P* < 0.01, significantly different compared to control. Asp, Aspirin; Morp, Morphine.

the extracts had a significant ($p < 0.01$) analgesic effect only on the inflammatory phase. The effect of morphine on the inflammatory phase was significantly ($p < 0.05$) better than the inhibitory effects of both extracts and aspirin.

Hyperalgesia assay

As shown in Figure 3, the administration of *G. perpensa* significantly ($P < 0.05$) reduced mechanical hyperalgesia induced by carrageneen 1 and 2 h post treatment in a non-dose dependent fashion. Indomethacin showed significant inhibition of carrageneen-induced hyperalgesia from 1 to 4 h post- treatment. However, beyond the 4th hour after treatment none of the drugs showed protective effects against this model of inflammatory pain.

Albumin-induced inflammation assay

The anti-inflammatory activity of GPAE and GPME against acute paw inflammation induced by egg albumin is summarized in Table 2. The higher doses of both GPAE and GPME significantly ($p < 0.01$) inhibited albumin-induced inflammation and had an earlier onset compared to the lower doses whose effects were delayed (1 h post treatment). Indomethacin on the other hand had an earlier onset of anti-inflammatory effect which was still significant after the third hour.

DISCUSSION

In this study, we evaluated the analgesic and anti-inflammatory effect of the aqueous and methanolic extracts of *G. perpensa.* Both extracts of *G. perpensa* demonstrated analgesic activities which were not dose-dependent. In the acetic acid- induced writhing test, both doses of GPME significantly reduced abdominal contortions. These results were similar to those induced by aspirin. However, only the 200 mg/kg of the GPAE had significant analgesic effects on this model of pain. Although morphine significantly reduced acetic acid-induced pain, yet its effects were weaker compared to the effects of aspirin and GPME. The acetic acid-induced writhing is a visceral pain model which is generally used for screening plants and new agents for analgesic properties (Gene et al., 1998; Tjolsen and Hole, 1997). It is able to determine antinociceptive effects of compounds at dose levels that might appear inactive in other methods like the tail-flick test (Bentley et al., 1981).

However, it has been shown that the acetic acid-induced writhing test is a non-specific nociceptive model (Collier et al., 1968; Bighetti et al., 1999; Sánchez-Mateo

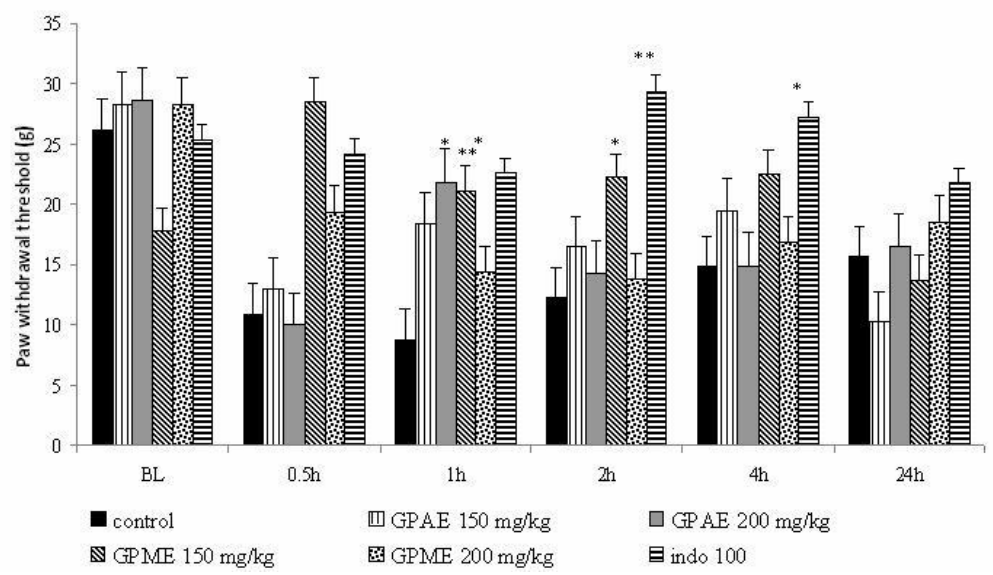

Figure 3. Antinociceptive effect of *G. perpensa* on inflammatory pain induced by carrageneen. The values represent the means ± SEM.; *n*=5; **P* < 0.05; "**P" < 0.01; significantly different compared to control. Indo, indomethacin; BL, baseline.

Table 2. Effects of G. perpensa on rat right hind paw volume.

		% Inflammation inhibition			
	Dose (mg/kg)	**0.5 h**	**1 h**	**2 h**	**3 h**
Control		-	-	-	-
GPAE	150	40.0 ± 12.5	56.4 ± 3.9**	59.2 ± 12.2*	42.6 ± 14.3
GPAE	200	59.2 ± 5.2**	54.4 ± 4.6**	29.6 ± 10.9	11.7 ± 11.2
GPME	150	20.0 ± 10.3	44.6 ± 3.1**	42.2 ± 8.4	51.2 ± 12.1
GPME	200	51.6 ± 10.0**	49.0 ± 10.9**	51.0 ± 9.5	50.6 ± 11.3
Indo	10	43.2 ± 7.7*	67.9 ± 5.3**	59.7 ± 9.7*	59.3 ± 9.2*

Values are mean ± SEM (n = 5). Experimental groups were compared with control (*P < 0.05 and **P<0.01) Indo: Indomethacin. Percentage inhibition of inflammation was thus calculated: inhibition of inflammation (%) = 100 × [1- (a-x)/(b-y)], where *a* = mean paw volume of treated rats at various time after egg albumin injection; *x* = mean paw volume of treated rats before egg albumin injection; *b* = mean paw volume of control rats at various time after egg albumin injection; *y* = mean paw volume of control rats before albumin injection.

et al., 2006). Generally, this test is used to elucidate peripheral activity of drugs. The intra-abdominal injection of acetic acid induces the release of prostaglandins as well as cytokines which may be the cause of visceral pain. (Deraedt et al., 1980; Ikeda et al., 2001). *G. perpensa* extracts may be acting by inhibiting the release of these mediators. However, the results of this writhing alone were unable to ascertain whether the antinociception was central or peripheral.

The hot-plate test was used to assess the central antinociceptive properties of *G. perpensa*. Both doses of GPAE and GPME significantly increased the reaction time to thermal stimulation. The GPME extracts like aspirin, had a later onset of analgesic effects which lasted beyond the 5th hour post treatment. Under-standably, the effects of morphine had an earlier onset which was similar to the effects of the lower dose of GPAE. The hot plate test is used to distinguish between peripheral and central acting analgesic agents (Ramabadran et al., 1989). Our results are suggestive of central acting antinociceptive effects of GPAE and GPME.

The formalin test is believed to resemble clinical pain more closely in comparison with other tests that employ mechanical or thermal stimuli (Tjolsen and Hole, 1997). This test induced a biphasic response in all animals. Neither of the extracts nor aspirin affected the pain intensity in the first phase although morphine significantly attenuated pain behaviour in this phase. However, during the inflammatory phase both doses of GPAE and GPME significantly reduced pain. Aspirin and morphine were also effective against this phase of inflammatory pain, the

effects of morphine were significantly greater. The first phase of the formalin- induced pain is due to direct chemical stimulation of nociceptors whose effects are transmitted via C fibers, which can be suppressed by opioid analgesic drugs such as morphine (Sayyah et al., 2004). On the other hand, the late phase is inflammatory and thus sensitive to the NSAIDs such as aspirin (Hunskaar and Hole, 1987; Rosland et al., 1990; Young et al., 2005). The late phase, results from the action of inflammatory mediators in the peripheral tissues, such as prostaglandins, serotonin, histamine and bradykinin, as well as functional changes in the neurons, which promote facilitation of synaptic transmission at the spinal level (Franca et al., 2001; Garcıa et al., 2004). To verify the effect of *G. perpensa* on the late phase of the formalin test, the analgesic efficiency was investigated on inflamematory pain tests induced by carrageneen and albumin respectively. In the hyperalgesia test, the GPAE and GPME significantly increased paw withdrawal latencies during the first and second hours post carrageneen injection thus confirming previous results which showed that these extracts inhibited inflammatory pain. The analgesic effects of indomethacin, however, were significant from the first hour through the fourth hour post treatment. Carrageenan induced acute inflammatory pain results from the sensitization of primary sensory nociceptive neurons. Tissue injury around these neurons causes the release of primary mediators which lower the nociceptor threshold and increase neuronal membrane excitability thus increasing the perception of pain (Cunha et al., 2005).

Results obtained from the albumin test showed that all the extracts at all the doses exhibited inhibition of inflammation after one hour of albumin injection, while both 200 mg/kg doses began inhibition after 0.5 h while indomethacin showed significant inhibition of inflammation at all the time intervals studied. Indomethacin like the other NSAIDs prevents inflammation by inhibition of cyclooxygenase conversion of arachidonic acid into prostaglandins (Toshihiro et al., 2001). The plant extract could be acting by a similar mechanism to prevent albumin induced inflammation. Based on the results of this study, it can be concluded that GPAE and GPME extracts have both analgesic property and anti-inflammatory properties. These findings seem to justify the use of the plant in traditional medicine in the management of pain.

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