ANTIINFLAMMATORY AND ANTINOCICEPTIVE ACTIVITIES OF
Loxostylis alata A.SPRENG. EX RCHB (ANACARDIACEAE)

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ABSTRACT

Loxostylis alata belongs to the family Anacardiaceae. In South Africa, the bark and leaves of *Loxostylis alata* are used in traditional medicine during childbirth and also to stimulate the immune system. This study was aimed at investigating the antiinflammatory and analgesic activities of the crude acetone extract of *Loxostylis alata*. *In vivo* experimental models that include egg albumin, prostaglandin E_2, and histamine-induced-paw oedema, and vascular permeability test were used to screen the extract for antiinflammatory effect. While formalin-induced nociception, acetic acid-induced writhing and hot plate tests were carried out to evaluate analgesic effects of the extract. The extract inhibited egg-albumin, prostaglandin and histamine-induced oedema in a dose-dependent (50-200 mg/kg) manner. It also inhibited vascular permeability cause by acetic acid in a similar fashion. Furthermore, the extract inhibited pain induced by formalin; acetic acid and heat dose-dependently (50-200 mg/kg). These findings propose the antiinflammatory and analgesic effects of *Loxostylis alata* and provide a rationale for its medicinal use against pain and inflammation.

Keywords: *Loxostylis alata*; Analgesic effect; Antiinflammatory activity; Acetone extract

INTRODUCTION

The body responds to inflammation using different mechanisms that enables it to adequately fight injurious or infectious agents. Cells of different types are involved in bringing about inflammation processes (Babu et al, 2009). Specifically, substances recruit white blood cells such as leukocytes to the damage site which subsequently play very vital roles in inflammatory progression. Though inflammation is important in the body, it could be deleterious if the stimulus persists for longer periods as it can cause certain painful inflammatory diseases such as arthritis, gastritis, e.t.c. (Simon & Green, 2005). Pain on the other hand is a sensory modality which in many cases represents the only symptom for the diagnosis of several diseases. It often has a protective function (Almeida et al, 2001). The non-steroidal antiinflammatory drugs (NSAIDs) provide relief from pain and swelling in chronic joint disease such as occurs in osteo- and rheumatoid arthritis, and in more acute inflammatory conditions such as fractures, sprains and other soft tissue injuries. They also provide relief from post-operative, dental and menstrual pain, and from the pain of headaches and migraine (Rang et al, 2003).

In spite of their effectiveness, the NSAIDs drugs have serious toxic effects, with gastro-intestinal ulceration leading to bleeding and renal disorders being the most important side effects Robert, 1976; Tapiero et al, 2002). Conversely, selective COX-2 inhibitors with little toxic effect on the gastro-intestinal tract have been associated with toxic cardiovascular effects (Dogné et al, 2005) The treatment and control of inflammatory painful conditions using NSAIDs continue to be challenging. Research to explore safer and more efficacious alternative treatments is therefore very important.

Man has been using plants primarily as source of food and medicines for centuries. Plants are very good source of drugs. Of recent, there is a renewed interest in the study of plants because of their potential to yield drugs of novel activity (Ojewole, 2007).

Loxostylis alata A.Spreng. ex Rchb belongs to the family Anacardiaceae (Coates-Palgrave, 2002). In South Africa, the bark and leaves of *L. alata* are used in traditional medicine during childbirth (Pooley, 1993) and also to stimulate the immune system (Pell, 2004). This study was aimed at evaluating the antiinflammatory and analgesic potential of acetone *Loxostylis alata* leaf extract in different *in vivo* models.

MATERIALS AND METHODS

Plant collection and processing

The leaves of *Loxostylis alata* A.Spreng. ex Rchb were collected at the Manie van der Schijff Botanical Garden of the University of Pretoria, South Africa in April 2007. Samples of the plant leaves and seeds were identified and authenticated by Lorraine Middleton and Magda Nel of the University of Pretoria. Voucher specimen of the plant with number; PRU PRU96508 was deposited at the Schweikert Herbarium of the Department of Plant Sciences, University of Pretoria, South Africa. The dried leaves were milled to a fine powder in a Macsalab mill (Model 200 LAB), Eriez®, Bramley, and stored in dark closed containers until used. Two hundred grams of finely ground plant material were extracted with 600 ml of acetone (technical grade-Merck) in a macerating bottle. The mixture was allowed to stand for 48 hours following which the tap of the percolator was opened to obtain the liquid extract. The extraction process was repeated 3 times and the extracts obtained during the extraction processes were pooled together and concentrated *in vacuo* at 50-55 ℃.

Phytochemical analysis

The acetone extract of *L. alata* was screened for presence of anthraquinones, alkaloids, carbohydrates, glycosides, flavonoids, tannins, saponins, steroids and triterpenes using methods described by Trease and Evans, (1983).

Animals

Adult Swiss albino mice (21-23 g) and Wistar rats (182-189 g) of both sexes were used for the studies. The animals were purchased from the Animal House, Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. Animals were kept in cages and pre-conditioned for two weeks in the laboratory. Wood shaving was used as beddings and changed every week throughout the period of the experiment. Animals were all fed with pelleted rat feed diet and allow free access to water. The Animal Ethics Review Committee for Animal Experimentation of Ahmadu Bello University, Zaria approved all experimental protocols described in this study.

Acute toxicity test

The LD_{50} was determined using method described by Lorke, (1983) with slight modification. Swiss albino mice were deprived of feed and water for 24 and 12 hours, respectively and randomly divided into 3 groups of 3 mice each. Mice in groups 1, 2 and 3 received *L. alata* extract at doses of 10, 100 and 1000 mg/kg, respectively. All treatments were given intraperitoneally. Similarly, a second phase of the trial was carried out using three animals per group. Twelve mice were randomly divided into 4 groups of 3 animals each. Mice in groups 1, 2, 3 and 4 were given the extract at doses of 140, 225, 370 and 600 mg/kg, respectively. In all the trials, animals were observed continuously for 1 hour after the treatment and then intermittently for 4 hours, and thereafter over a period of 24 hours. Surviving animals were further observed for up to 14 days following treatment for any signs of toxicity, and mortality. In addition, the weights of the animals that survived were recorded every other day after the administration of extract for 14 days.

Egg albumin-induced paw oedema in rats

The rat paw oedema for testing antiinflammatory agents as described by Winter et al, (1963) was employed. Twenty five rats were randomly divided into 5 groups of 5 animals each. Groups 1, 2, 3 received the extract at doses of 50, 100 or 200 mg/kg, while groups 4 (positive control group) and 5

(non-treated control group) were given piroxicam at 10 mg/kg and 5 ml/kg of normal saline, respectively. All treatments were administered by intraperitoneally (i.p.). Thirty min later, oedema was induced by injecting 100 µl of fresh egg albumin obtained from a 21-week layer hen into the right plantar surface of the hind paw of each rat. The paw volume was measured with a Plethysmometer (Ugo Basil) by water displacement method before and at 1 hour interval for 4 hours after induction of oedema (Parmar & Gosh 1978; Okoli et al. (2007). Inflammation was assessed as the difference between the zero time volume of the treated paw (V_0) and the volume at the various times (V_t) after the administration of the inflammatory agent.

Prostaglandin E$_2$-induced paw oedema in rats

The method described by Palmer and Gosh, (1978) was used to evaluate the effect of the extract on prostaglandin-induced inflammation in rats. Animals were randomly divided into five groups of five rats each (n=5 each). Animals in groups 1, 2 and 3 were treated intraperitoneally with *L. alata* extract at doses of 50, 100 or 200 mg/kg, respectively, while rats in groups 4 (treated control) and 5 (untreated control) received piroxicam (10 mg/kg) and normal saline (5 ml/kg), respectively. Thirty minutes after extract administration, 100 µl of prostaglandin E$_2$ (0.01 µg/ml) was administered into the sub-plantar surface of the right hind paw of each rat and the paw oedema was determined after 1 hour as described above.

Histamine induced paw oedema in rats

Similarly, rats were divided into five groups (n = 5 each). Animals in groups 1, 2 and 3 were treated orally with *L. alata* extract 50, 100 and 200 mg/kg, respectively, while rats in groups 4 (treated control) and 5 (untreated control) received piroxicam (10 mg/kg) and normal saline (5 ml/kg), respectively. All treatments were given by intraperitoneal route (i.p.). After 30 min of treatment, 100µl (1 mg/ml) of histamine was injected into the sub-plantar side of the right hind paw of the rats and paw oedema was determined (Whittle, 1964).

Vascular permeability test

Animals were randomly assigned into 5 groups of 5 mice each. The extract at doses of 50, 100 or 200 mg/kg was given intraperitoneally to groups 1, 2 and 3, respectively. Control animals received also by intraperitoneal injection either piroxicam (10 mg/kg) or normal saline (5 ml/kg). One hour after treatment, 0.2 ml of Evans Blue dye (0.25% in normal saline) was given intravenously through the tail vein to each mouse. Thirty minutes later, each animal was given 1 ml/100 g of acetic acid (0.6%, v/v) by intraperitoneal route. Treated animals were sacrificed 30 min after acetic acid injection and the peritoneal cavity washed with normal saline (3 ml) into heparinized tubes and centrifuged at 1500 g for 3 min. The supernatant was decanted into a clean test tube and the concentration of the dye in the supernatant was measured spectophotometrically at a wavelength of 610 nm using Thermo Helious Zeta UV/VIS Spectrophotometer (serial number UV-164617) (Whittle, 1964).

Formalin-induced nociception in rats

The formalin-induced paw licking was used as described by Hunskaar et al. (1985). Animals were divided into 5 groups of 5 rats each. The animals were treated intraperitoneally with the extract of *L. alata* at 50 mg/kg (Group 1), 100 mg/kg (Group 2) or 200 mg/kg (Group 3). Similarly, groups 4 (treated control) and 5 (untreated control) received 10 mg/kg of piroxicam and 5 ml/kg of normal saline, respectively. One hour after oral administration of extract, piroxicam or normal saline, 100 μl of formalin 3% formalin was injected into the plantar surface of the left hind foot of each rat. The time spent licking the foot by each rat is recorded during early phase (0-5 min post-injection of formalin) and late phase (20-30 min post-injection of formalin). The mean time spent licking the foot in each phase was measured.

Acetic acid-induced writhing in mice

The experiment was conducted by method described earlier by Collier et al. (1968). Mice were randomly allocated into 5 different groups (n=5 each). Groups 1, 2 and 3 received the extract of *L.*

alata at 50, 100 or 200 mg/kg, respectively, while groups 4 and 5 were given piroxicam (10 mg/kg) and normal saline (5 ml/kg), respectively. After 30 min of the intraperitoneal treatment, 0.8% acetic acid was administered through intraperitoneal route. Immediately after injection of acetic acid, each animal was placed in a transparent glass jar observation chamber and the number of writhing movements was counted for 30 min starting immediately after acetic acid administration. Percent inhibition of writhing movements was compared with positive control group.

Hot plate test

The hot plate latency assay was based on the method of Eddy et al. (1950). Fifteen Swiss albino mice were randomly divided into 5 groups of 5 animals each. Groups 1, 2, 3 received 50, 100 or 200 mg/kg of the extract of *L. alata*, while groups 4 (positive control group) and 5 (non-treated control group) were given pentazocine (10 mg/kg) and normal saline (5 ml/kg), respectively. All treatments were given intraperitoneally. Animals were placed individually on an enclosed copper hot plate maintained at 55 ± 0.5 ºC and the time between placement of a mouse on the hot plate and occurrence of either a hind-paw lick or jump-off the surface was recorded as hot plate latency. The hot plate latencies were determined after 1, 2, 3 and 4 hours.

Statistical analysis

Data were expressed as mean $\pm$ S.E.M and then analysed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. The analyses was done using Graphpad Prism version 4.0 for Windows from Graphpad software, San Diego, California, USA. Values of $p < 0.05$ were considered significant.

RESULTS

The extraction yield of acetone extract of *L. alata* gave a yield of 14.2%. Glycosides, tannins, flavonoids, triterpenes and reducing sugars were the chemical components shown to be present in the acetone extract of *L. alata*.

The extract at doses of 10, 100, 140 and 225 mg/kg did not produce any sign of toxicity or mortality in the animals. Animals treated with 370 mg/kg, showed signs of depression, decrease food intake and a loss of about $12 \pm 1.2\%$ of their body weights at the end of the trial. Animals that were given 600 mg/kg showed similar signs shown by animals in the group treated with 370 mg/kg. In addition two of the animals in the last group died after 48 h of extract administration. The LD_{50} was calculated as follows:

$$LD_{50} = (370 \times 600)^{1/2} = 471 \text{ mg/kg}$$

The degree of oedema produced by the inflammatory agent and inhibition of swelling produced by the extract at different doses is shown in Table 1. The extract produced the highest inhibition (78%) of swelling at the dose of 100 mg/kg after 4 hour. The reduction of oedema produced by the extract at all the tested doses were significantly ($p < 0.05$) lower when compared with that of untreated control group. Similarly, the extract at the dose of 100 mg/kg demonstrated greater antiinflammatory activity than piroxicam (treated control).

Table 1: Effect of the acetone extract of leaf of *L. alata* administered 30 min before the induction of inflammation with egg albumin

Treatment	Dose (mg/kg)	Oedema (ml)			
		1 h	**2 h**	**3 h**	**4 h**
L. alata	50	0.31 ± 0.04^{a} (24)	0.25 ± 0.01^{a} (42)	0.22 ± 0.03^{a} (44)	0.19 ± 0.01^{a} (49)
	100	0.29 ± 0.02^{a} (29)	0.24 ± 0.04^{a} (44)	0.17 ± 0.04^{a} (56)	0.08 ± 0.01^{b} (78)
	200	0.18 ± 0.03^{b} (56)	0.21 ± 0.01^{a} (51)	0.15 ± 0.01^{b} (62)	0.11 ± 0.02^{b} (70)
Piroxicam	10	0.27 ± 0.02^{a} (34)	0.22 ± 0.01^{a} (49)	0.24 ± 0.01^{a} (39)	0.14 ± 0.03^{a} (62)
Normal saline	5 ml/kg	0.41 ± 0.03	0.43 ± 0.01	0.39 ± 0.02	0.37 ± 0.03

Values are mean $\pm$ S.E.M. (n = 5) while those in parenthesis represent percent inhibition of oedema. Means within the same column and having the same superscript letters are not significantly (p<0.05) different.

The extract of *L. alata* exhibited dose-dependent (50 and 100 mg/kg) inhibition against prostaglandin E_2 (PG E_2)-induced paw oedema in rats (Figure 1). The extract at 100 mg/kg produced the highest percentage inhibition.
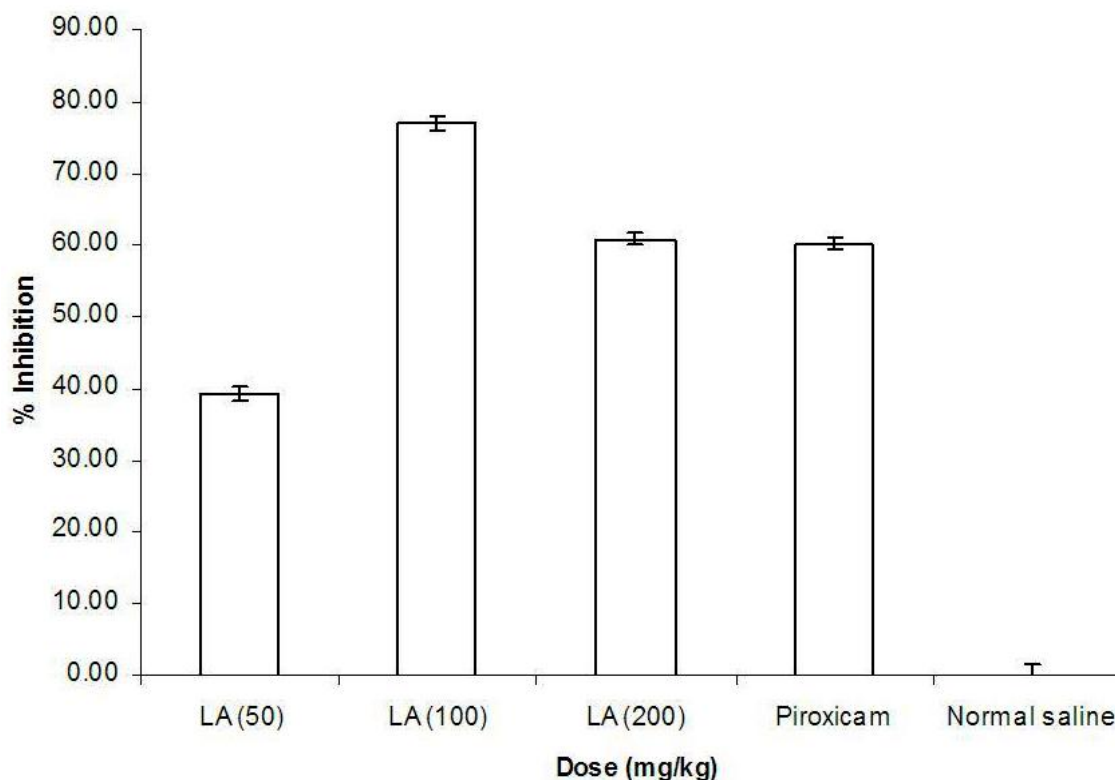

Figure 1: Inhibitory effect of acetone extract of *Loxostylis alata* administered 30 min prior to induction of inflammation using prostaglandin E_2 in rats

The extract at all the doses tested (50, 100 and 200 mg/kg) produced significantly ($p < 0.05$) and dose-dependent inhibitory effect on oedema produced by histamine when compared with group treated with normal saline at 5 ml/kg (untreated control) (Table 2).

The acetone extract of *L. alata*, at increasing doses, inhibited the access of injected blue dye into the peritoneal cavity of mice induced by acetic acid in a dose-dependent fashion (Figure 2). The extract at 100 and 200 mg/kg exhibited greater inhibitory effect than piroxicam (10 mg/kg). Results from all the treated groups were compared with the non-treated (normal saline) control group.

Table 2: Inhibitory effect of the acetone extract of *L. alata* administered 30 min prior to histamine-induced paw-oedema in rats

Treatment	Dose (mg/kg)	Percent inhibition of oedema volume			
		1 h	2 h	3 h	4 h
L. alata	50	$18 \pm 2.78^*$	$19 \pm 2.99^*$	$16 \pm 1.98^*$	$22 \pm 3.02^*$
	100	$56 \pm 3.45^{***}$	$39 \pm 1.99^{**}$	$45 \pm 3.21^{***}$	$55 \pm 2.67^{***}$
	200	$88 \pm 4.54^{***}$	$78 \pm 2.67^{***}$	$67 \pm 4.56^{***}$	$53 \pm 2.56^{***}$
Piroxicam	10	$79 \pm 2.56^{***}$	$89 \pm 4.50^{***}$	$66 \pm 2.78^{***}$	$49 \pm 1.67^{***}$

Values are mean $\pm$ S.E.M. (n = 5). $^{***}p < 0.001$, $^{**}p < 0.01$, $^*p < 0.05$ versus non-treated control group.
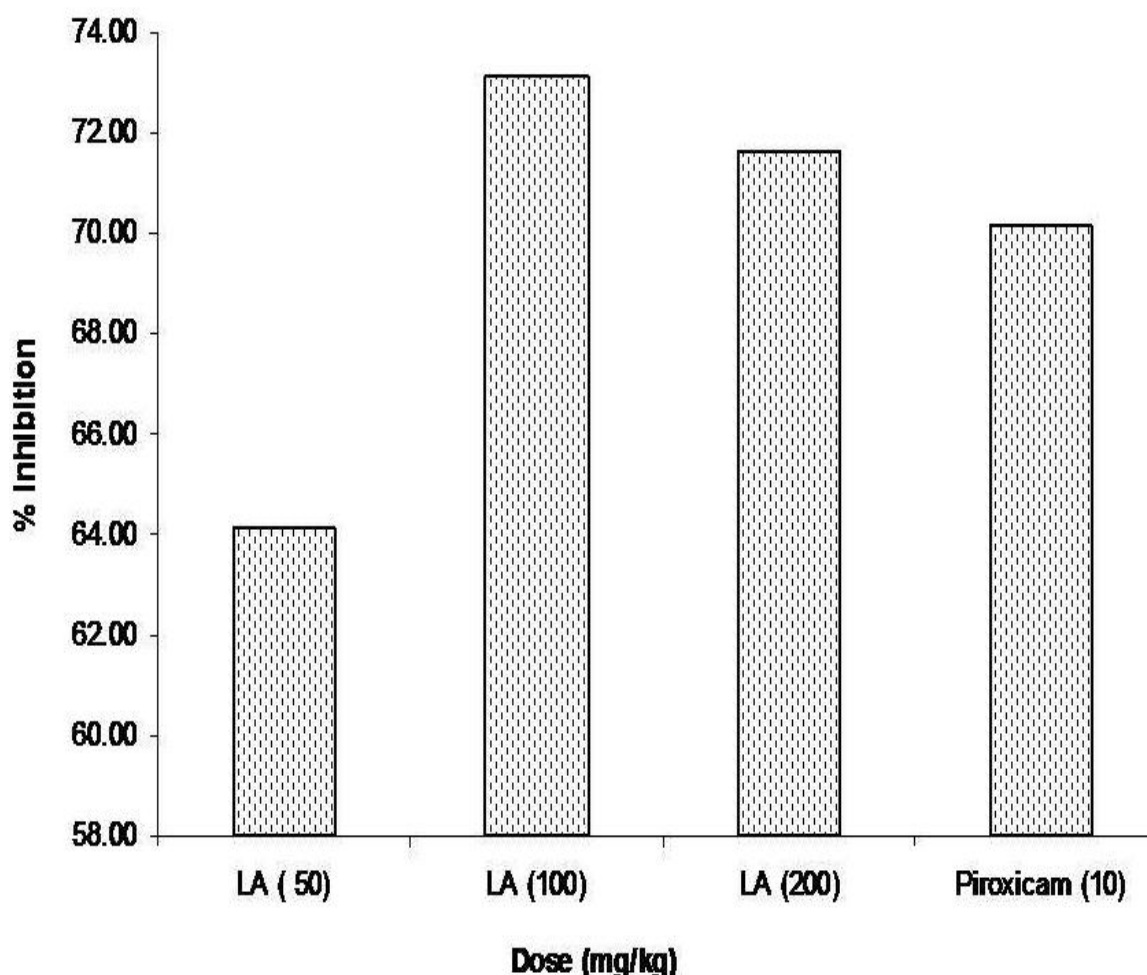

Figure 2: Inhibitory effect of *L. alata* extracts administered prior to on acetic acid-induced vascular permeability in mice.

The acetone extract of *L. alata* reduced formalin-induced pain in both early and late phases of the experiment in a dose-dependent fashion (Table 3). The inhibition produced by the extract at the early phase ranged between 37.83-66.12%. Similarly, the extract showed inhibition of formalin-induced pain in the late phase and it ranged between 41.35-60.90%. Pain inhibition shown by the extract were significantly ($p < 0.05$) higher than that shown normal saline (untreated control).

Table 3: Effect of the acetone extract of leaf of *L. alata* administered 30 min prior to the induction pain with formalin in mice

Treatment	Dose (mg/kg)	Licking time (min) (early phase)	% Inhibition	Licking time (min) (late phase)	% Inhibition
L. alata	50	1.89 ± 0.23*	37.83	1.34 ± 0.10*	49.62
	100	1.28 ± 0.11**	57.90	1.56 ± 0.24*	41.35
	200	1.03 ± 0.08**	66.12	1.04 ± 0.09**	60.90
Piroxicam	10	1.65 ± 0.21*	45.72	1.23 ± 0.26*	53.76
Normal saline	5 ml/kg	3.04	-	2.66	-

Data presented as mean ± SEM, n = 6 for all groups. * Significantly different from the control at $p < 0.05$. ** Significantly different from the control at $p < 0.01$.

The intraperitoneal injection of 0.8% acetic acid caused writhing reflex in mice. The extract at 50, 100 and 200 mg/kg significantly ($p < 0.05$; 0.01) reduced the number of writhing responses in a dose-dependent fashion within 12 min of acetic acid injection (Figure 3).

The acetone extract of *L. alata* increases the latency period of pain caused by hot plate significantly ($p < 0.01$) during all the period of observation when compared with the untreated control group. The analgesic effect was comparable and not significantly different ($p > 0.05$) from that produced by pentazocine (10 mg/kg). The greatest antinociceptive effect was produced by the extract at the dose of 100 mg/kg within 2 hours (Figure 4).
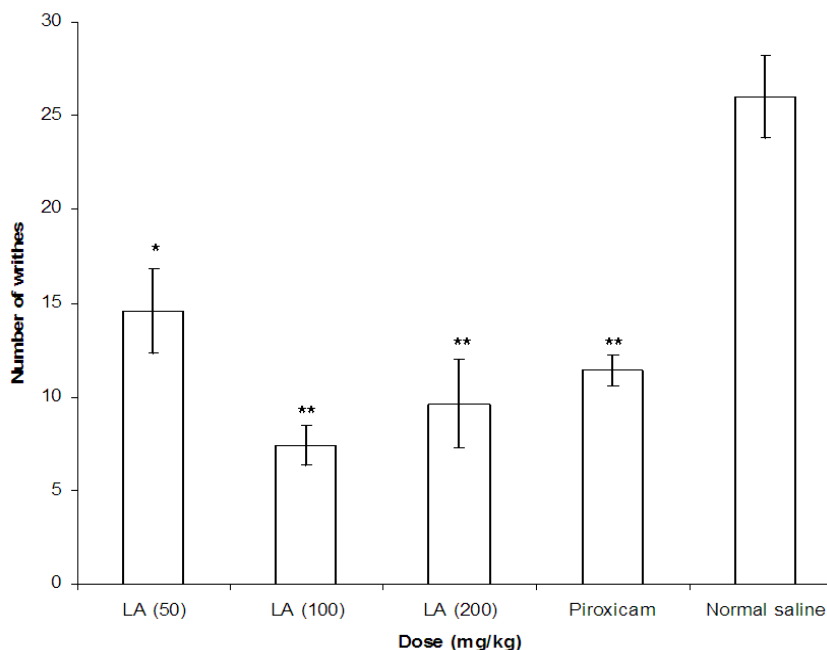
Figure 3: Effect of different doses of *L. alata* administered 30 min prior to the induction of acetic acid writhings in mice. Control group was dosed with normal saline at 5 ml/kg. Values are mean ± S.E.M. **p < 0.01 and *p < 0.05 show significant difference when compared with normal saline-treated control group.
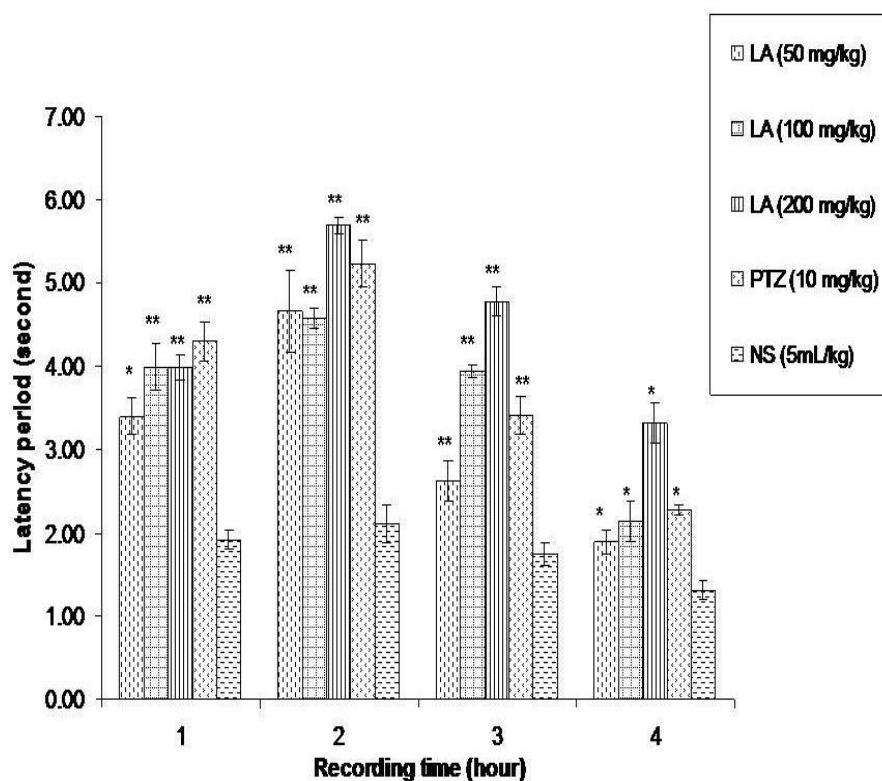

Figure 4. Antinociceptive effect of acetone extract of *L. alata* (LA) treatment administered 30 min prior to the hot plate-induced pain test. Latency periods were measured at 1, 2, 3, and 4 h after subjecting animals to temperature of 55 ± 0.5 ºC. Data are expressed as means ± S.E.M. (n = 5). *P < 0.05; **P < 0.01 compared to control group. PTZ = pentazocine; NS = normal saline

DISCUSSION

The present study had demonstrated the antiinflammatory and analgesic effects of the leaf of *Loxostylis alata* in different laboratory animal models. Furthermore, the intraperitoneal LD_{50} of the extract was calculated to be 471 mg/kg, which falls within the moderately toxic range Loomis, 1978), which results in the selection of the maximum dose of 200 mg/kg for the subsequent pharmacological assays. In a preliminary trial conducted on the acetone extract of *L. alata*, the oral LD_{50} was calculated to be over 5000 mg/kg (data not shown). The high oral LD_{50} could be attributed to the fact that orally administered drugs undergo three events: release from dosage form, transport across the gastrointestinal mucosal barrier, and passage through the liver. Each of these events has the potential to decrease the amount of drug reaching the systemic circulation unchanged and that also could lower the efficacy of pharmacological agents (Brander et al, 1991). In a similar study, oral administration of methanol extract of *Blechnum occidentale* was less potent and efficacious than when given through the intraperitoneal route, in preventing nociception induced by acetic acid (Nonato et al, 2009). Therefore in the present study, the acetone extract of *L. alata* was given through the intraperitoneal route to enhance its bioavailability and pharmacological effect. As part of the antiinflammatory tests, the extract of *L. alata* attenuated egg white, histamine and prostaglandin E_2-induced paw oedema in rats, the extract further inhibited vascular permeability of blue dye into the peritoneal cavity of mice. In addition, the extract inhibited formalin induced pain, writhing responses in the acetic acid writhing reflex and hot-plate tests in mice. The results strongly suggest the potential value of *L. alata* extract as an antiinflammatory and analgesic agent.

The rat paw oedema induced by fresh egg white is a well established model for testing antiinflammatory agents (Xu et al, 2002). Inflammatory mediators such as prostaglandins, complement, histamine, kinins and pro-inflammatory cytokines have been known to play very important roles in inflammation (Arunachalam et al, 2009). Perhaps the extract acts to inhibit or stop the action of one or more of these inflammatory mediators, which may explain the antiinflammatory mechanism of action of the extract.

Inflammation is characterized by the accumulation of a variety of mediators at the site of the injury or infection. Egg albumin causes paw oedema in a biphasic pattern with each phase having a determined period. The first phase starts from 0 to 2.5 hours, and there is predominantly histamine, serotonin and bradykinin release, while the later phase begins when prostaglandins such as PGE_2 in tissue starts to be secreted (Di Rosa, 1974). Cyclooxygenase enzyme is known to play an important role in the conversion of arachidonic acid into prostaglandins hence in the development of the later phase of inflammation. The nonsteroidal antiinflammatory agents (NSAIDs) target the enzyme so as to stop the production of prostaglandins and subsequently inflammation. The acetone extract of *L. alata* exhibited dose-dependent inhibition of oedema produced by egg albumin, prostaglandin E_2 and histamine in a way similar to that of piroxicam (treated control). In this study, PGE_2 produced immediate inflammatory response when administered unlike in the inflamed tissue where prostaglandins are produced through the action of cyclooxygenase on arachidonic acid (Rang et al, 2003). Therefore in the present study, the effect of the extract was tested an hour after PGE_2 administration onto the hind paw of tested rats. This is in contrast to other assays that require a longer duration for their effects to become grossly observable.

Brown and Roberts, (2001) reported that vascular permeability increases due to contraction and separation of endothelial cells at their boundaries which exposes the basement membrane and becomes freely permeable to plasma proteins and fluid. Exudation which is a consequence of increased vascular permeability is considered a major feature of acute inflammation (Jain et al, 1995). Acetic acid induces vascular permeability that is immediate and is sustained over 24 hours (Okoli et al, 2007). The extract produced a dose-dependent inhibition of vascular permeability produced by acetic acid. This suggests that the extract may effectively suppress the exudative phase of acute inflammation.

Formalin induces analgesia in two phases. The first 10 min, termed early phase, represents aphasic pain, while the period between 15 and 60 min, referred to as late phase represents tonic pain.

Analgesic drugs that act primarily on the CNS inhibit both phases equally while peripherally acting drugs inhibit the late phase (Tjolsen et al, 1992). For this study, the extract inhibited both phases, indicating that the extract most likely works centrally as well as peripherally.

The acetic-acid induced writhing test is widely used to study the peripheral analgesic effects of drugs; it is widely used for analgesic evaluation (Shibata et al, 1989; Ranjit et al, 2006). The extract significantly ($p < 0.05$; 0.01) reduced the number of acetic acid-induced writhes in mice. The extract at 100 mg/kg showed a higher inhibition of writhes when compared to the dose of 200 mg/kg and piroxicam (10 mg/kg) which served as the standard drug used in the study. The analgesic effect of the extract could be due to either its action on visceral receptors sensitive to acetic acid, to the inhibition of the synthesis of algogenic substances or the inhibition at the central level of the transmission of painful stimuli (Franzotti et al, 2000).

The hot plate test is a specific central antinociceptive test with response of cerebral cortex or spinal cord integration (Parkhouse and Pleuvry, 1979). Nonsteroidal antiinflammatory drugs (NSAIDs) such as aspirin and piroxicam have little central analgesic effect (Tjolsen et al, 1992). That is our main reason for using pentazocine as a treatment control. Pentazocine is a synthetically prepared prototypical mixed agonist-antagonist narcotic (opioid analgesic) drug of the benzomorphan class of opioids used to treat moderate to moderately severe pain (Rang et al, 2003). The extract showed significant ($p < 0.05$) dose- and phase-dependent analgesic effect in the hot plate test, which involves higher brain functions and consists of responses to nociceptive stimuli organized at a supraspinal level (Gardmark et al, 1978). The hot plate test is largely used to measure opioid effects (Gong et al, 1991; Plone et al, 1996) and that signifies the central involvement of the analgesic effect.

Secondary metabolites like tannins, flavonoids, glycosides and triterpenes were detected using established techniques (Trease and Evans, 1983) in the acetone extract of *L. alata*. Flavonoids have been shown to possess an antioxidant as well as anti-inflammatory properties due to their inhibitory

effects on the production of chemical mediators of pain (histamine, bradykinins) (Owoyele et al, 2005). Similarly, triterpenes (Araruna & Carlos, 2010), tannins (Owoyele et al, 2010) and glycosides (Lanhers et al, 1992) have been reported to possess anti-nociceptive and/or anti-inflammatory activities. Perhaps the presence of one of more of these secondary metabolites may be responsible for the analgesic and antiinflammatory effect of the extract.

Based on the overall effect of the extract on peripheral oedema, peripheral inflammation in the presence of moderate central analgesic effect, suggest that the effect results from the inhibition of the COX enzyme system. Interestingly, a study by Suleiman et al, (2010) demonstrated that the same extract of *L. alata* failed to inhibit platelet coagulation *in vitro*. From traditional literature it is well accepted that platelet coagulation is controlled by thromboxane and the COX I enzyme system. With the antiinflammatory activity evident *in vivo* for this study, we speculate that the plant may have preferential activity against other inflammatory system in the body.

With the exception of hot plate test, the effect of the extract at a dose of 100 mg/kg showed slight increased ($p > 0.05$) in pharmacological effects when compared with the dose of 200 mg/kg. Similar finding was reported where the extract of *Elettaria cardamomum* at a dose of 100 mg/kg produced significant inhibition of ethanol-induced gastric ulceration when compared with the same extract at a higher dose of 150 mg/kg (Jamal et al, 2006). The effect was attributed to the lower dose producing submaximal response. Perhaps similar effect was responsible for the observed effect in this study.

Lupeol has previously been isolated from the extract of *L. alata* (Suleiman, 2009). The antinociceptive and antiinflammatory activities of lupeol have been demonstrated as being non-COX related [47]. It is believed that the antiinflammatory property of lupeol often accompany its immune modulatory and antitumor action (Saleem et al, 2009). In addition, lupeol was shown to have high inhibitory effect on the production of some inflammatory mediators such as prostaglandin E2 (PGE_2), tumour necrosis factor (TNF-α), interleukin-1 β (IL-1β) (Fernández et al, 2001) It could

be possible that lupeol either singly or in combination with other compounds contained in *L. alata* may be responsible for the antiinflammatory action of the plant.

CONCLUSION

The acetone extract of the leaves of *L. alata* have antiinflammatory and central, and peripheral antinociceptive effects. The antinociceptive and antiinflammatory effects of the extract may be due to its contents of flavonoids, triterpenes or some other compounds present. Detailed *in vitro* and *in vivo* pharmacological tests are however, required to fully understand the constituent (s) responsible for the activity of the plant. Moreover, detailed toxicity trial is also required to justify the use of the plant in the treatment of pain and inflammation.

Acknowledgements

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