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Research Article

Anti-inflammatory and Analgesic Properties of *Albizia altissima* (Hook f.) Hutch Dandy leaves in mice

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Abstract

Albizia altissima (Hook f.) is used in traditional system in the treatment of pain, rheumatism, inflammation, fever, arthritis and mental illness. The antinociceptive properties of the plant have not been explored; hence the present work was carried out to evaluate the anti-inflammation and analgesic properties of *Albizia altissima* in mice. The anti-inflammation and analgesic properties of *Albizia altissima* was investigated; using carrageenan-induced paw oedema, histamine-induced paw oedema, formalin test, tail immersion test and acetic acid-induced abdominal constriction test in mice. The results showed that the crude extract of *Albizia altissima* (100 – 400 mg/kg; p.o) dose independently produced anti-oedematogenic activity in both carrageenan and histamine-induced paw oedema in mice. In the acetic acid-induced writhing model, *Albizia altissima* produced a dose dependent analgesic effect characterized by reduction in the number of abdominal writhes when compared to control. The extract also produced analgesic effect by producing a dose dependent decrease in formalin-induced paw licking time in mice.
It was concluded that *Albizia altissima* possessed anti-inflammatory and analgesic properties.

Keywords: Albizia altissima, anti-inflammation, analgesic, toxicity, indomethacin.

INTRODUCTION

Albizia altissima (Hook f.) Hutch et Dandy is a tree in the Leguminosae-Mimosoideae family, growing to a height of 15 m, which bole to 25 cm in diameter, low branching and bearing a spreading flat-topped crown. It is found in the riverine forest and secondary jungle extending from Sierra Leone to West Cameroons and across Tropical Africa to Sudan, Uganda and Angola (Burkill, 1985). Phytochemistry of the bark, root and leaves of different species of *Albizia* shows that the plant contains alkaloids, glycosides, saponins and steroids. The crude saponin fraction from the root of *Albizia adianthifolia* has two new triterpenoidal, namely prosapogenins 1 and 2 (Haddad *et al*., 2002). The plant has been shown to possess nitrogen fixing capability, and hence it is used in agroforestry and rehabilitation of degraded sites (Swaine *et al*., 2005). Bioassay-guided fractionation of a methanol extract of *Albizia subdimidiata* specie led to the isolation of two active saponins 1 and 2 of which albiziatrioside and the other showed significant cytotoxicity against A2780 cell line (Maged *et al*., 2001). Methanol extract of leaves of another specie *Albizia grandibracteata* contains three new oleanane-type triterpene saponins named grandibracteosides A-C which showed significant inhibitory activity against KB and MCF7 tumor cell lines in vitro (Sabrina *et al*., 2005). New triterpenoid saponins acylated with monoterpenic acid was also isolated from the root of *Albizia adianthifolia* that is adianthifoliosides C, D, E and F (Haddad *et al*., 2004). The plant *Albizia altissima* is claimed to be useful in traditional system of medicine for the treatment of pain, rheumatism, inflammation, fever, arthritis and mental illness. However, there are no substantial experimental studies to justify its use in the treatment of pain and inflammation. Hence the present study was embarked upon to evaluate the anti-inflammatory and analgesic effects of the crude extract of *Albizia altissima* leaves in mice.

MATERIALS AND METHODS

Plant Materials

Albizia altissima leaves were collected from Amassoma, Bayelsa state in June, 2009. It was identified by Mr. Oladele the Curator of the Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University (OAU), Ile-Ife. It was further identified and authenticated by Dr. H. C Illoh of Department of Botany, O.A.U. Ile – Ife. A voucher specimen with number UHI 16180 was deposited at the herbarium of the Department of Pharmacognosy and Herbal Medicine, Niger Delta University.

Preparation of Plant Materials

The plant was air dried for two weeks at room temperature. The dried leaves were pulverized and 100 g of the powder was extracted with 0.6 liters of fifty percent (50%) ethanol for 48 hr. The marc was re-extracted twice and the combined extract

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was concentrated *in vacuo* at a temperature of 30ºC to yield 25 g crude extract. The crude extract was prepared by dissolution in normal saline.

Animals

The animals used for this experiment were Swiss albino male mice. All the animals were bred and housed in well lit and aerated room in the animal house, College of Medicine, Niger Delta University, Amassoma. They were maintained under natural daylight/night condition. All animals had free access to drinking water and standard commercial diet (Guinea feeds brand, Bendel Feeds Nigeria). All experiment was carried out in accordance with NIH guide for the care and use of laboratory animals.

Drugs

Acetylsalicylic acid (Sigma-Aldrich, St. Louis, MO, USA), morphine (Sigma – Aldrich, St. Louis, MO, USA), acetic acid, carrageenan, indometachin, histamine and formalin (BDH Chemicals Limited, UK) were used in the study.

Methodology

Toxicity Test: The method described by Lorke (1983) was used to determine the LD_{50}, which is the index of acute toxicity. Swiss albino male mice (20 - 25 g) were used. This method involved an initial dose finding procedure, in which the animals were divided into three groups of three animals per group. Doses of 10,100 and 1000 mg/kg of *Albizia altissima* leaves extract were administered orally (p.o.), one dose for each group. The treated animals were monitored for 24 h mortality and general behavior. From the results of the above step, four different doses (2000, 3000, 4000 and 5000 mg/kg) of the extract were chosen and administered p.o. respectively to four groups of one mouse per group. The treated animals were monitored for 24 h. The LD_{50} was then calculated as the geometric mean of the lowest dose showing death and the highest dose showing no death.

Anti-inflammatory Activity

Carrageenan-induced paw oedema in mice:

The anti-inflammatory activity was studied using carrageenan-induced paw oedema (acute inflammation) method in mice (Winters *et al*., 1962; Adeyemi *et al*., 2002). Twenty-five mice were randomly divided into five groups (n=5). Mice in group 1 (control) were administered with normal saline orally (10 ml/kg), while mice in group 2–4 received oral doses of the crude extract (100, 200 and 400 mg/kg) respectively, and mice in group 5 were treated with indomethacin (10 mg/kg, p.o.). One hour later, 1% carrageenan (0.05 ml) was injected into the sub-planter surface of right hind paw of each of all the mice in all the groups. Measurement of paw size was done by using a vernier caliper (Olajide *et al*., 2000; Yin *et al*., 2003). The measurement was carried out at time 0, 1, 2, 3, 4 and 5 h respectively.

Histamine-induced paw oedema:

The method used was the same as that of carrageenan-induced paw oedema. Twenty-five mice were randomly divided into five groups (n=5). Mice in group 1 (control) were give normal saline (10 mg/kg, p.o.), while mice in group 2–4 were orally administered with doses of the crude extract (100, 200 and 400 mg/kg) respectively and mice in group 5 received oral administration of indomethacin (10 mg/kg). One hour later, 0.05 ml of 1 mg/kg of histamine was injected into the sub-planter surface of right hind paw of each of all the mice in all the groups. Measurement of paw size was done by using a vernier caliper; the measurement was carried out at time 0, 1, 2, 3, 4 and 5 h respectively (Parma and Gosh, 1978)

Analgesic Activity

Tail Immersion Test:

This test was performed as described by Sewell and Spencer (1976) and as modified by Furst *et al*., (1993). Mice were divided into five groups (n=5-7). Group (1) received normal saline (0.2 ml/20 g, p.o.), while the crude extract (100, 200, 400 mg/kg, p.o) was given to groups (2-4). All substances were administered 30 min before the beginning of the experiment. The analgesic activity was evaluated at 30 min interval for 2 h (30, 60, 90 and 120 min). The tail (up to 5 cm) was dipped into a water bath maintained at 55.0 ± 0.5ºC. The time in seconds taken for a mouse to withdraw its tail clearly out of the water was taken as the reaction time. Group 5 received morphine (5 mg/kg, i.p.) which served as the reference drug.

Acetic acid-Induced Writhing Test:

The acetic-acid induced abdominal writhing test was performed as described by Koster *et al*., (1959). The mice were fasted for 12 hr and had water *ad libitum*. They were randomly divided into five groups (n=5-7). Group (1) received normal saline (0.2 ml/20 g, p.o.), while crude extract (100, 200, 400 mg/kg, p.o.) was given to groups (2-4). Each mouse was given 1.0 % aqueous solution of acetic acid and then placed in an observation box. The animals were pretreated for 30 min before acetic acid injection. Nociception was evaluated by counting the number of abdominal constriction for 20 min after the administration of acetic acid. Antinociceptive activity was expressed as the percentage reduction or inhibition of the number of abdominal writhes. Acetylsalicylic acid (ASA, 150 mg/kg, p.o.) group (5) served as reference drug.

The percentage inhibition of writhing was calculated as follows

$$\% \text{ Inhibition} = \frac{Wc - Wt}{Wc} \times 100$$

Where

Wc =Mean value of writhing in control mice

Wt =Mean value of writhing in treated mice

Formalin Test:

The method used was that described by Elisabetsky *et al*. (1995) and Hunskaar and Hole (1997) with little modification. Five groups of mice consisting of 5 mice each were randomly selected. Mice in group 1 (control) was administered with normal saline (10 ml/kg, p.o.) while mice in groups 2–4 were treated with the crude extract (100, 200 and 400 mg/kg, p.o.). Mice in groups 5 was treated with Indomethacin (80 mg/kg, p.o.) for one hr prior to administration of 0.05ml of 1% formalin into the sub-planter space of the right hind paw and the duration of paw licking was determined 0–5 minutes (1st Phase or neurogenic phase) and 20–30 mins (2nd phase or inflammatory phase) after formalin administration. The 1st phase is regarded as the neurogenic mechanism and the 2nd phase is the inflammatory mechanism (Elisabetsky *et al*., 1995; Hunskaar and Hole, 1997).

Statistical analysis
The results obtained were presented as means ± SEM and analyzed using one-way analysis of variance (ANOVA) followed by Student Newman Keul test. The level of significance was set at 95%, $p<0.05$ for all treatment carried out compared to control group using the Primer of Biostatistics by Stanton A. Glantz (version 3.01) copyright (C) 1992 by Mc Graw-Hill Inc

RESULTS

The LD_{50} of *Albizia altissima* was found to be 2078 mg/kg, p.o. This shows that the extract is very safe at this dose.

Effect of *Albizia altissima* on paw oedema induced by carrageenan in mice
The crude extract of *Albizia altissima* (100 – 400 mg/kg, p.o) produced a significant reduction in the volume of paw oedema at 3 h after carrageenan injection in mice (Fig 1). The anti-inflammatory effect was dose independent.

Effect of *Albizia altissima* on paw oedema induced by histamine in mice
The crude extract of *Albizia altissima* (100 – 400 mg/kg, p.o) produced a significant reduction in the volume of paw oedema at 3 h after histamine injection in mice (Fig 2). The anti-inflammatory effect was dose independent.

Effect of *Albizia altissima* on formalin test in mice
The crude extract of *Albizia altissima* (400 mg/kg, p.o) was active in both early and late phases of formalin-induced pain in mice, while the 200 mg/kg was active only in the late phase. The decrease in licking time was significant [$F(4, 20) = 10.3$, $P < 0.001$] in early phase when compared to control. The decrease in licking time was also significant [$F(4, 20) = 14.3$, $P < 0.001$] in late phase when compared to control (Fig 3).

Effect of *Albizia altissima* on tail immersion test in mice
The crude extract of *Albizia altissima* (100 - 400 mg/kg, p.o) increased reaction time in tail immersion test. The increase reaction time was significant [$F(4, 20) = 10.3$, $P < 0.001$] when compared to control (Fig 4).

Effect of *Albizia altissima* on acetic acid – induced writhing in mice
The crude extract of *Albizia altissima* (100 - 400 mg/kg, p.o.) produced a significant [$F(4, 20) = 418.5$, $P < 0.001$] dose dependent inhibition in the number of acetic acid- induced writhes in mice when compared to control (Table 1).

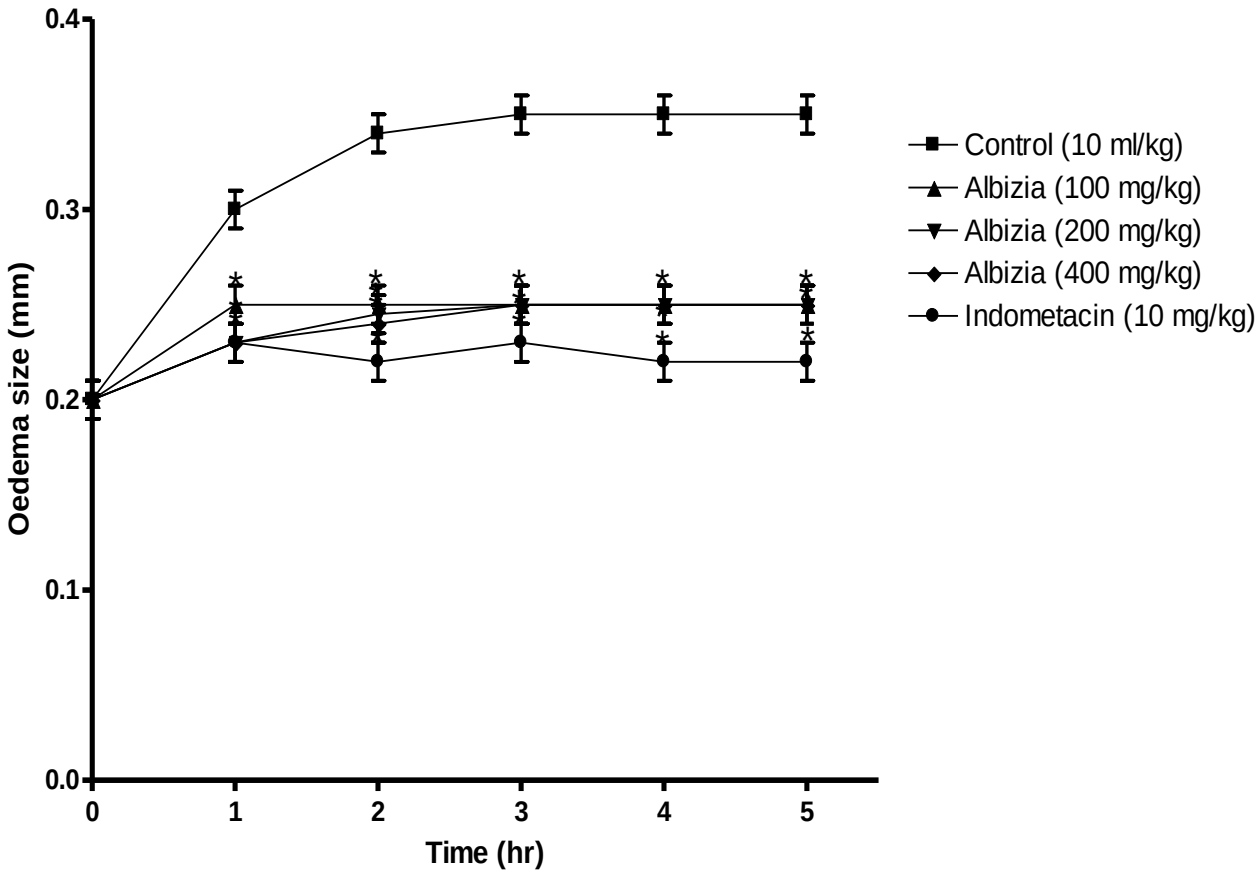

Fig 1:
Effect of the crude extract of *Albizia altissima* on paw oedema induced by carrageneen in mice. The results are expressed as mean ± S.E.M, (n = 5 – 7). The significant difference between control and various treatment groups are indicated by P< 0.05. Student t-test. *Indicate significant difference from normal saline. $P < 0.05$

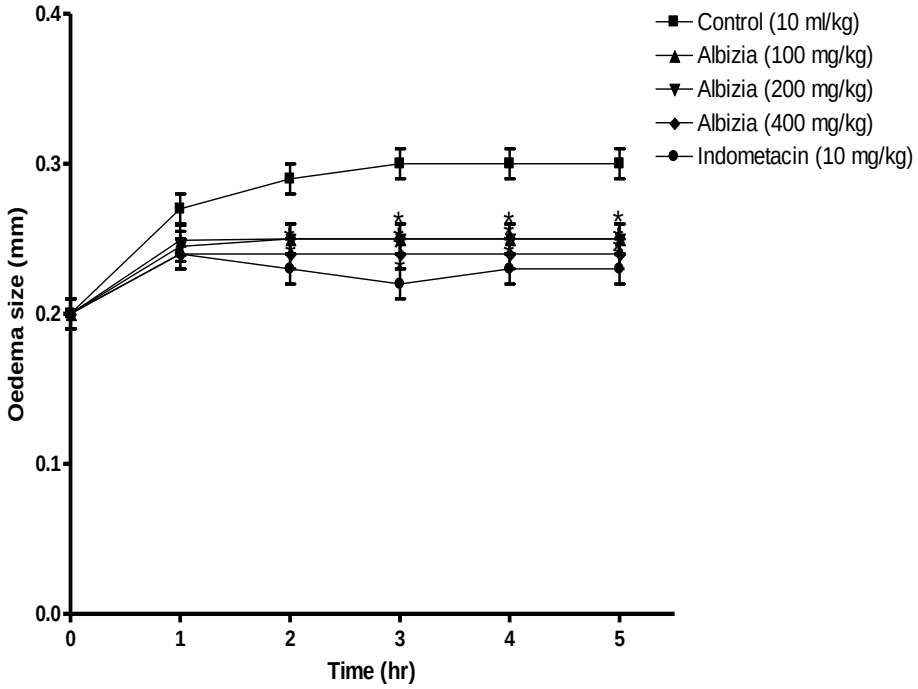

Fig 2: **Effect of the crude extract of *Albizia altissima* on paw oedema induced by histamine in mice**
The results are expressed as mean ± S.E.M, (n = 5 – 7). The significant difference between control and various treatment groups are indicated by P< 0.05. Student t-test. *Indicate significant difference from normal saline. P < 0.05

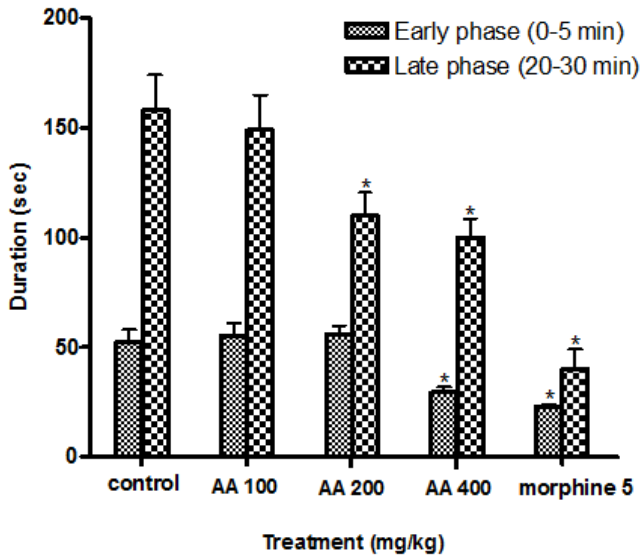

Fig 3
Effect of the crude extract of *Albizia altissima* (AA) on the formalin test (early and late phases). Results are expressed as Mean ± S.E.M, (n = 5-7). One way ANOVA revealed that there is significant [F (4, 20) = 14.7, P < 0.001] difference between various treatment groups. *indicate significant difference from control. P < 0.05.

DISCUSSION

The results of this study revealed that the crude extract of *Albizia altissima* possesses anti-inflammatory property as shown by its dose independent inhibitory activity against carragennan-induced paw oedema in mice in a highly significant manner. The doses of the crude extract used in this study produced a reduction in inflammation at the 1-5 hr similar to that obtained with the reference drug; indometacin. Inflammation is a complex condition involving various processes which includes; arrays of enzyme activation, release of mediators, extravasations of fluid, migration of cells, tissue damage and repair (Vane, 1995).

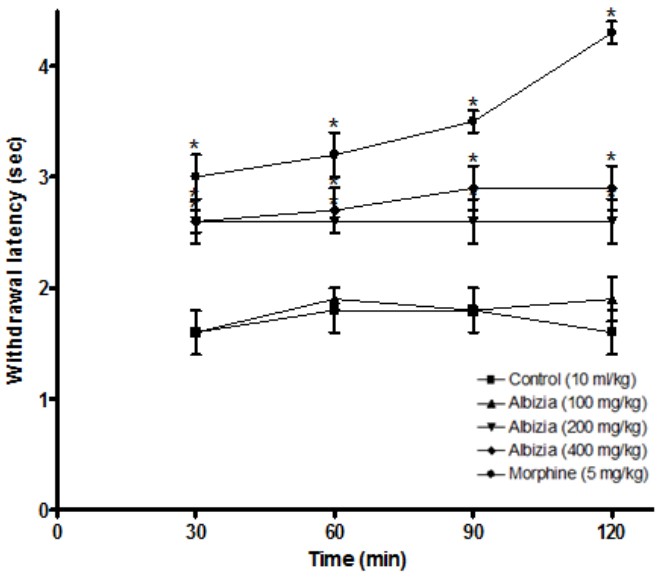

Fig 4
Effect of *Albizia altissima* on tail immersion test in mice. The results are expressed as Mean ± S.E.M, (n = 5 – 7). One way ANOVA revealed that there is significant difference between the various treatment groups. Morphine was used as standard reference drug. *Indicate significant difference from normal saline. P < 0.05

Table 1
Effect of *Albizia altissima* on acetic acid – induced writhing in mice

Treatment	Dose (mg/kg i.p.)	No of Writhes/20min	% Inhibition
Control	0.2ml/20g	40.1±2.7	0.00
Albizia	100	24.5±1.0*	38.9
Albizia	200	14.4±0.6*	64.1
Albizia	400	8.6±0.4*	78.6
Aspirin	150	5.4±0.7*	86.5

The results are expressed as mean ± S.E.M, (n = 5 – 7). One way ANOVA revealed that there is significant difference between various treatment groups. Aspirin was used as standard reference drug. *Indicate significant difference from normal saline. $P < 0.05$

Carragennan-induced paw oedema is a useful inflammatory model in the preclinical evaluation of anti-inflammatory compounds. Injection of carragennan into mice paw induces a cascade of events leading to the release of inflammatory mediators such as histamine, serotonin, kinins and prostaglandins (Di Rosa *et al*., 1971; Okpo *et al*., 2001). The reduction in paw volume produced by carragennan shows that the crude extract of *Albizia altissima* may be inhibiting histamine, serotonin and prostaglandin synthesis. A strong involvement of its effect on prostaglandins release and action is suggested based on the fact that the late phase of carrageenan-induced oedema is associated with the release of prostaglandin-like substances (Vineger *et al*., 1969; Zakaria *et al*., 2008). Likewise, histamine is a potent microvascular dilator which directly increases vascular permeability and produced oedema in mice. The extract also produced a decrease in paw oedema induced by histamine thus suggesting that the extract exhibits anti-inflammatory effect which may be manifested via the reduction of histamine production in mice.

Pain is a subjective, individualized, unpleasant sensory and emotional experience associated with actual or potential tissue damage. Formalin test which is a useful tool for obtaining neurogenic inflammation and continuous pain produces a distinct biphasic response with the action of analgesia different at both the early and late phase (Yuh-Fung Chen *et al*., 1995; Zakaria *et al*., 2008). The early phase seems to be caused predominantly by activation of C-fibre subsequent to peripheral stimulation. The late phase that describes the peripheral component has been shown to involve pro-inflammatory mediators such as histamine, prostaglandins, nitric oxide and bradykinin (Hunskaar and Hole, 1997). Centrally acting drugs like narcotic analgesics inhibit both phases, while peripheral acting drugs such as aspirin only inhibit the late phase (Chan *et al*., 1995). The crude extract caused an inhibition in both the early and late phase showing that it has both central and peripheral effect. The fact that the activity of the extract in the second phase was greater than that produced in the first phase suggests greater involvement of peripheral mechanism in its anti-nociceptive action. This shows that the extract is effective against nociceptive and inflammatory pains with more effect against inflammatory pains. The crude extract also showed a dose dependent effect, as the higher dose gave better anti-nociceptive and anti-inflammatory action than the lower dose.

The tail immersion method is very effective for evaluating substances possessing analgesic property which act centrally (Di Rosa *et al*., 1971; Okpo *et al*., 2001). It involves the spinal reflex (Pini *et al*., 1997) and measures the complex response to a non-inflammatory, acute nociceptive input (Zakaria *et al*., 2008). The crude extract produced an increase in reaction time when compared to control. The analgesic activity of *Albizia altissima* resembles that shown by some plants such as *Cleome viscose*, *Careya arborea*, which also produce analgesic properties in the tail immersion test as stated in literature (Vineger *et al*., 1969; Di Rosa *et al*., 1971).

Acetic acid – induced writhing is another useful and highly sensitive animal model for evaluating compounds possessing analgesic effects or activity peripherally. In acetic acid-induced abdominal writhing which is a visceral pain model, causes the release of prostaglandin via lipooxygenase pathway and prostaglandin biosynthesis plays a role in the nociceptive mechanism (Meade *et al*., 1986). Acetic acid has been reported to cause hyperalgesia by liberating endogenous substances such as prostaglandins, leukotrienes, serotonin, histamine and kinins which have been implicated in the mediation of pain perception (Chan *et al*., 1995; Rang et al., 1999). The ability of the crude extract to suppress acetic acid-induced nociceptive behaviours suggests a peripheral analgesic effect similar to that of aspirin (acetylsalicylic acid); a nonsteroidal anti-inflammatory drugs (NSAIDs) (Pini *et al*., 1997). Acetylsalicylic acid produces analgesic effect mainly by blocking the synthesis of prostaglandins E and F (Parma and Gosh, 1978). It is possible that the crude extract may be acting like acetylsalicylic acid peripherally by reducing the synthesis of prostaglandins as this suggestion was further confirmed by the findings that *Albizia altissima* inhibited the pain responses associated with the second phase of the formalin test. This investigation revealed that the crude extract of *Albizia altissima* reduced nociceptive responses and inflammatory conditions in animals, thus providing experimental evidence that may support its potential applications in the treatment of pain and inflammatory disorders.

Phytochemical screenings showed that the crude saponin fraction from the root of *Albizia adianthifolia* contains two new triterpenoidal prosapogenins 1 and 2 (Haddad *et al*., 2002). Anti-inflammatory activities of many plants have been attributed to their triterpenoids, hence the analgesic and anti-inflammatory activities of the plant may be attributed to the presence of triterpenoidal prosapogenins.

In conclusion, The present study reveals that *Albizia altissima* significantly reduced carrageenan and histamine-induced paw oedema thus showing the anti-inflammatory properties of the plant. The plant also possessed analgesic effect centrally and peripherally. However, further studies are necessary to elucidate the possible mechanisms through which *Albizia altissima* mediates its analgesic and anti-inflammatory activities.

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