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Evaluation of the anti-inflammatory properties of the aqueous extract of *Albizia zygia* stem bark

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Abstract
Albizia zygia is used in folk medicine for treatment of a wide variety of ailments including wounds, toothache and inflammation. Its acclaimed local use in the treatment of inflammation prompted the investigation into its anti-inflammatory activity. The effect of the aqueous stem bark extract of *Albizia zygia* on inflammation was investigated using acute (carrageenan induced-, dextran induced-, histamine induced-paw oedema, carrageenan-induced exudate formation and croton oil induced ear oedema) and chronic (formalin induced arthritis) models of inflammation in rats and mice. The extract caused significant ($p<0.05$), inhibitions of croton oil-induced ear oedema, carrageenan- and dextran-induced paw oedema without any appreciable effect on histamine induced oedema. The extract also significantly inhibited leucocyte migration without affecting the volume of exudate formation. The anti-inflammatory effects of the extract on these acute models of inflammation were not dose-dependent as greatest activity was seen at the dose of 200mg/kg. This same dose also caused a significant reduction in ankle, but not paw, oedema in the formaldehyde-induced arthritis model. The aqueous stem bark extract of *Albizia zygia* possesses anti-inflammatory activity on acute and chronic inflammatory models in rats and mice.

Keywords: *Albizia zygia*; Arthritis; Carrageenan; Formaldehyde; Oedema

INTRODUCTION

Inflammation is the local response of living mammalian tissues to injury due to noxious stimuli. It is a body defense reaction to prevent the spread of injurious agent and to remove the necrosed cells and tissues. Inflammation may be caused by bacteria, viruses, fungi, parasites, antigen-antibody reaction, mechanical trauma, organic and inorganic poisons and foreign bodies. The currently available therapies for treatment of inflammation and pain are associated with unwanted side effects and low efficacy, hence the great demand for more effective anti-inflammatory drugs (Newman and Cragg, 2007). The management of inflammatory diseases is a serious issue in the rural community; the population in these areas use many alternative drugs such as substances obtained from medicinal plants.

Albizia zygia DC (Fabaceae), known as *Nyieavu* by the Igbos of Southeast Nigeria is a medium-sized deciduous tree that is widespread in tropical Africa. The plant is generally used as a shade tree, feed for goats and cattle, etc. Different parts of the plant are traditionally used in the treatment of malaria, ophthalmia, bronchial disease, female

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sterility, inflammation, sores, wounds and toothache (Burkill, 1995). Scientific studies on *Albizia zygia* have demonstrated molluscicidal (Ayoub and Yankov, 1986) antiprotozoal (Ndjakou *et al*., 2007), anti-oxidant and anti-microbial (Oloyede and Ogunlade, 2013) activities. Essential oils from *Albizia zygia* have also been demonstrated to possess *in vitro* free radical scavenging activity (Oloyede and Ogunlade, 2015).

Although other Albizia species have been shown to have moderate anti-inflammatory activity, (Kokila *et al*., 2013), no studies have been carried out to validate the traditional use of this plant in inflammatory diseases. The present study was, therefore, planned to evaluate the effects of aqueous stem bark extract of *Albizia zygia* on inflammation.

EXPERIMENTAL

Collection of plant material. The fresh stem bark of *Albizia zygia* was collected in August, 2014 along Technical Road, Ugbowo, Benin City, Edo state. The plant was duly identified and authenticated by Messrs. O.A. Ugbogu and O.S. Shasanya at the Forestry Research Institute of Nigeria (FRIN), Ibadan where an herbarium specimen (FHI 109704) was deposited for future reference.

Preparation of extract. The fresh barks were air-dried for 7 days and reduced to powder form with a mortar and pestle. The dried powdered plant material g (270g) was extracted with distilled water (1.5L) by simple maceration process for 48 hours. The solution was filtered using a muslin bag and successively with filter paper. The filtrate obtained was dried in an oven set at 40^{o}C to give a yield of 22.3g (8.26% $^{w}/_{w}$). A stock solution of the extract (200mg/ml) was prepared from which other concentrations were made as required.

Laboratory animals. In-bred Sprague-Dawley rats (170 ± 25g) of either sex obtained from the Animal House Unit of Department of Pharmacology and Toxicology, University of Benin, Benin City Nigeria were used. They were acclimatized to standard laboratory conditions and water *ad libitum*. All the animals were handled according to standard protocols for the use of laboratory animals (National Institute of health USA; Public health service policy on humane care and use of laboratory animals, 2002).

Anti-inflammatory studies.

Carrageenan-induced paw oedema. Rats were randomly allotted to different treatment groups of six animals per group. Group 1 (control) was pretreated orally with distilled water (5ml/kg) while groups 2, 3 and 4 were given 100, 200 and 500 mg/kg of the aqueous extract, respectively. Indomethacin (10mg/kg, dissolved in 10% sodium carbonate) was used as the standard drug. Oedema was induced in rats by injection of freshly prepared carrageenan (0.1 ml, 1% in normal saline) into the sub plantar region of the right hind paw (Winter *et al*., 1962). Measurements of paw diameter were made, using a Vernier caliper (Marry *et al*., 1998), immediately before and after injection of carrageenan at hourly intervals for six hours. The paw swelling at each time was calculated as the difference between the paw diameter at time t (D_t) and that at zero hour (D_o).

Dextran-induced paw oedema. Rats were randomly selected into different treatment groups of six animals per group. Control group was given distilled water (5ml/kg, oral). Groups 2, 3 and 4 animals were pretreated orally with the aqueous extracts (100, 200 and 500 mg/kg) respectively, while the standard group received cyproheptadine (10mg/kg). Oedema was induced in the rats by injection of freshly prepared dextran (0.1 ml, 1% w/v in normal saline) into the sub-plantar aponeurosis of the right hind paw (Nishida *et* al., 1979). Measurement of paw diameter was made before and at one hour

intervals after injection of dextran, for 3 hours. Oedema was monitored as the percentage increase in paw thickness in the dextran injected paw

Histamine-induced paw oedema. Rats were divided into groups of at least five animals per group. Groups 1, 2 and 3 were pretreated **o**rally with the aqueous extracts (100, 200 and 500 mg/kg) respectively, while the standard group (group 4) received chlorpheniramine (10mg/kg). Distilled water (5ml/kg) was used as the **c**ontrol. Oedema was induced one hour after administration of the extract and standard drug by injection of histamine (0.1 ml, 1% w/v in normal saline) into the sub-plantar tissue of the right hind paw. The paw diameter was measured using a Vernier caliper and measurements were made before injection of histamine (i.e. basal reading) and at 30 minute intervals for 3 hours.

Carrageenan-induced exudate formation. The method used was similar to that described previously (Ribeiro *et al.,* 1976). Rats were divided into 5 groups of at least, five animals each. The aqueous extract (100, 200 and 500 mg/kg), indomethacin (10mg/kg) or distilled water (5 ml/kg) was administered, orally, one hour before the intraperitoneal injection of carrageenan (0.15ml, 1% w/v in normal saline). The animals were sacrificed three hours later and the peritoneal cavity washed with 3.5ml phosphate buffered saline (containing 5 iu/ml heparin and 3% egg albumin). The exudate volume (minus the wash fluid, 3.5ml) was measured, using a measuring cylinder. Total leucocyte counts were performed using Neubauer WBC counting chamber. The potassium and sodium contents of the wash fluid were determined using a flame photometer (FP 640). The percentage inhibition of leukocyte migration was calculated as: $100 \times (1\text{-}T/C)$ where, T represents the WBC counts of the treated groups and C represents the WBC counts of the control groups (Umapathy *et al*., 2010)

Croton oil-induced ear oedema. Mice were selected into six groups of six animals each. Groups 1, 2 and 3 received 100, 200 and 500 mg/kg of the aqueous stem bark extract of *Albizia zygia*, respectively. Group 4 was given dexamethasone (2mg/kg) while group 5 served as the control and was given distilled water (5 ml/kg). All administrations were via the oral route. Thirty minutes after drug administrations, inflammation was induced by application of 20µL croton oil (1% *v/v* in acetone solution) to the inner surface of the right ear while the left served as the control (Tubaro *et al*., 1985). Four hours later, the animals were sacrificed by cervical dislocation, and the left and right ears were cut off. The difference between the weights of the two ears was recorded as the result of the oedema induced by the croton oil.

Formaldehyde-induced arthritis. Rats were divided into four treatment groups of six animals per group**.** Arthritis was induced in the rats by injection of formalin (0.1ml, 2% w/v in normal saline) into the sub-plantar region of the right hind paw on days 1 and 3 (Selye, 1949). The paw and ankle diameters were measured using Vernier calipers before induction of oedema (basal reading) and daily for 10 days and on day 21. Distilled water (2ml/kg/day), aqueous extract of *Albizia zygia* (100 and 200 mg/kg/day) and diclofenac sodium (2mg/kg/day) were, respectively, given orally to animals in all the groups for 10 days.

Statistical analysis. Results are presented as mean ± standard error of mean (SEM) and n represents the number of animals per group. The statistical significance between the groups was assessed by one-way analysis of variance (ANOVA) followed by the Dunnette's multiple post hoc test. Values were considered statistically significant at $p<0.05$. Results were analysed using GraphPad Prism 6 (GraphPad Software, Inc. San Diego, California, U.S.A).

RESULTS

Carrageenan-induced paw oedema. Peak oedema response was achieved at 4 hours in the control group. Administration of the extract caused reduction in the area under the curve. However, the doses of 100 and 200mg/kg of aqueous extracts of *Albizia zygia* used in this study showed significant reduction ($p<0.05$) in total paw oedema compared to control (Fig. 7). The dose of 200mg/kg has 37.29% inhibition of total paw oedema compared to the control group.

Dextran induced paw oedema. Dextran caused a rapid swelling of the rat paw after injection, with peak swelling occurring at the 2nd hour, in control rats, and gradually faded at the 3rd hour. The aqueous extract of *Albizia zygia* at a dose of 200 mg/kg caused a significant inhibition of the peak oedema response. Cyproheptadine showed significant oedema inhibition in the area under the curve (Fig 2).

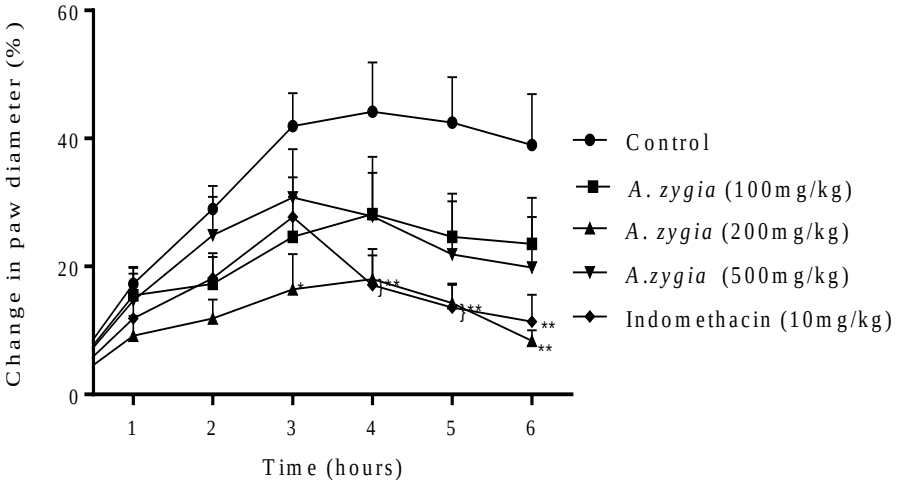

Fig. 1: Effect of aqueous bark extract of *A. zygia* on carrageenan-induced paw oedema in rats. *p<0.05, **p<0.01, one-way ANOVA compared to control.

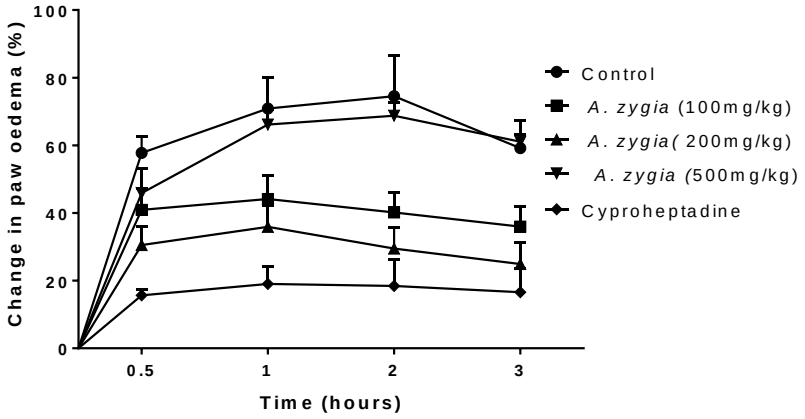

Fig. 2: Effect of aqueous extract of *A. zygia* bark on dextran-induced paw oedema in rats. *p<0.05, one-way ANOVA compared to control

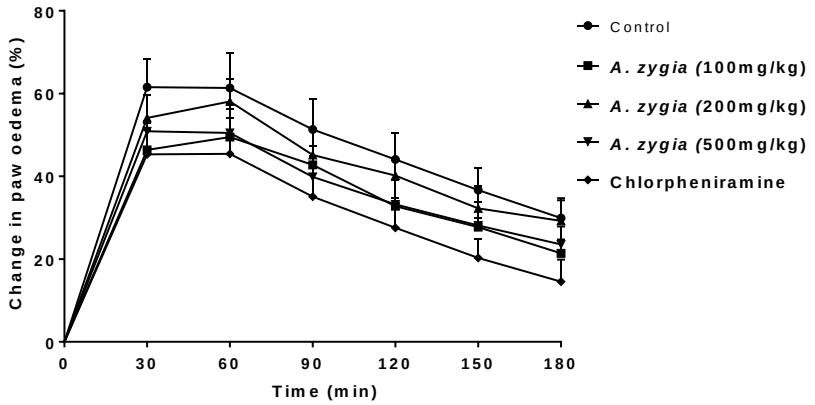

Fig 3: Effect of *A. zygia* on histamine-induced paw oedema.

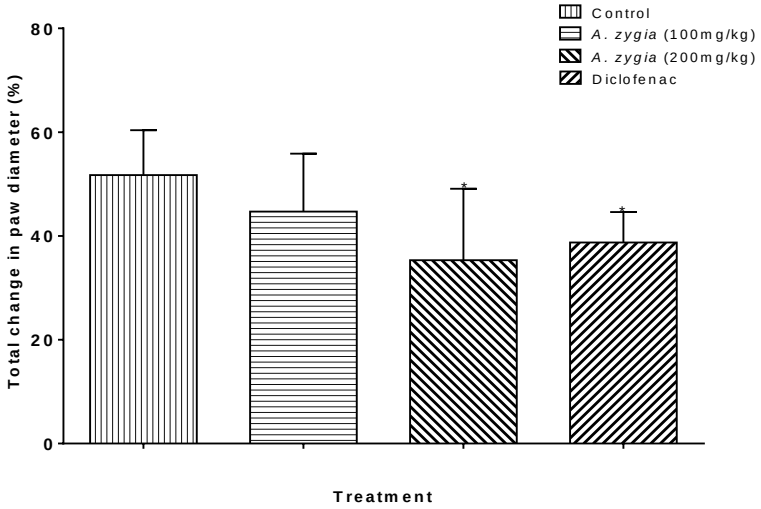

Fig 4: The effect of *A. zygia* on total paw oedema response to formaldehyde-induced arthritis. Data presented as Mean ± SEM (n=6 per group). *p<0.05, compared to control.

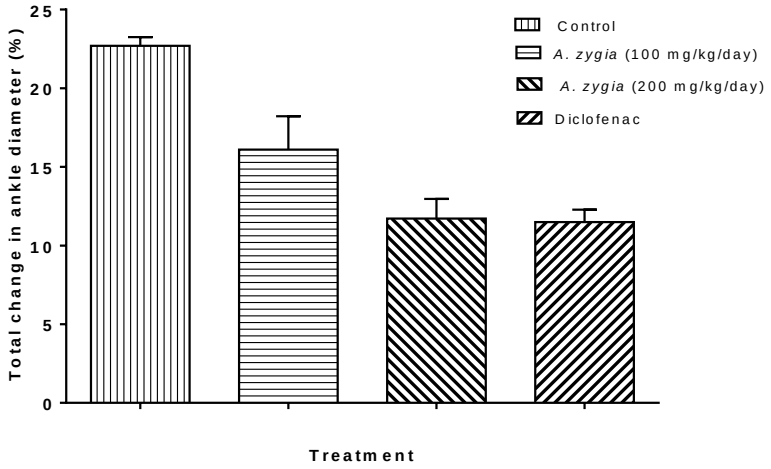

Fig 5: The effect of *A. zygia* on total ankle oedema response to formaldehyde-induced arthritis. Data presented as mean ± SEM (n=6 per group)**.** *p<0.0001, compared to control.

Table 1: Effect of *A. zygia* on carrageenan-induced exudate formation.

Treatment	Dose (mg/kg)	Exudate volume (ml)	WBC migration ($10^3/\mu L$)	Inhibition of migration (%)	K^+ (mM/L)	Na^+ (mM/L)
Control (DW)	5 ml/kg	1.52±0.11	19.11±1.83	-	8.04±0.68	129.40±0.87
A. zygia	100	1.18±0.15	10.44±4.37*	45.37	6.30±0.51	129.80±1.50
A. zygia	200	0.84±0.15	9.49±1.32*	50.34	7.68±0.54	130.20±1.11
A. zygia	500	0.86±0.12	12.37±3.72	35.27	7.14±0.94	134.20±4.96
Indomethacin	10	0.72±0.08*	6.09±1.34*	68.13	7.54±0.47	130.20±1.11

Data are Mean±SEM, n=6 per group. *p<0.05, one-way ANOVA compared to control; DW = Distilled water

Table 2: Effect of *A. zygia* on croton oil-induced ear oedema in mice.

Treatment	Dose (mg/kg)	Change in weight (mg)	% inhibition
Control (DW)	5 ml/kg	46.67±4.94	-
A. zygia	100	26.67±4.22*	42.85
A. zygia	200	21.67±1.67**	53.57
A. zygia	500	26.67±5.58*	42.85
Dexamethasone	2	23.33±4.22**	50.01

Data presented as Mean±SEM, n=5 per group. *p<0.05, **p<0.01, vs control. DW = Distilled water

Histamine-induced paw oedema. The aqueous extract of *Albizia zygia* at various doses reduced the oedema produced by injection of histamine, but these were not significant compared to the standard control (Fig 3).

Carrageenan induced exudate formation. Oral treatment with *A. zygia* significantly reduced the level of leucocyte migration to the peritoneal cavity at the lower doses (100 and 200 mg/kg). However, none of the doses significantly reduced the exudate volume (Table 1). The animals treated with the standard drug, indomethacin (5 mg/kg), exhibited significant reductions of inflammatory exudate volume as well as leukocyte migration. No significant changes were elicited by the extract or indomethacin on sodium ion (Na^+) and potassium ion (K^+) concentrations.

Croton oil-induced ear oedema. Table 2 shows the results of the inhibition caused by oral administration of the extracts on croton oil-induced mouse ear oedema. All doses of the extract caused significant ($p<0.05$) decreases in the ear oedema, with the greatest effect at 200mg/kg of the aqueous extract, lower than that of the positive control – dexamethasone.

Formalin induced arthritis. Administration of the extract caused dose dependent reductions in the total oedema response of both paw (Fig. 4) and ankle (Fig. 5), which were significant ($p<0.05$) at the higher dose (200 mg/kg/day). This was also similar to the effect of the standard drug – Diclofenac.

DISCUSSION

Inflammation is characterized by marked vascular changes, including vasodilation, increased permeability and increased blood flow, which are induced by the actions of various inflammatory mediators. The present study evaluated the anti-inflammatory effect of aqueous stem bark extract of *Albizia zygia* using in vivo models of inflammation. The results obtained indicate that the extract possesses acute and chronic anti-inflammatory activity against various phlogistic agents.

Carrageenan-induced paw oedema is a widely used model for acute experimental animal inflammation and is believed to be triphasic. The first phase (0-1.5hr) of the carrageenan model is mediated by histamine

and serotonin, the 2nd phase (1.5-2.5hr) mediated by kinin and the last phase (2.5-6hr) which begins after the kinin phase is responsible for the release of prostaglandins (Lin and Lin, 1995; Suba *et al*., 2005). The release of prostaglandins is closely associated with migration of leucocytes into the inflamed site (Brito and Antonio, 1998) and has been reported to be sensitive to most clinically effective anti-inflammatory agents. The results from this study indicate that the extract significantly inhibited rat paw oedema development between the 3rd and 6th hour, suggesting a possible inhibition of prostaglandins release.

Dextran-induced oedema results from liberation of histamine and serotonin from mast cells (Chawla et al., 1987; Aziz *et al*., 2011). The extract at a dose of 200mg/kg caused a significant decrease ($p<0.05$) in the oedema produced by sub-plantar injection of dextran, suggesting that it may inhibit inflammation by blocking the release of histamine and 5-HT.

Histamine is a potent vasodilator and increases vascular permeability (Okhawa *et al*., 1979). The peak oedema response to histamine occurred thirty minutes after injection and gradually faded over the next three hours. The extract at all the tested doses did not significantly attenuate the effect of histamine.

Carrageenan-induced exudate formation is an acute inflammatory response, characterized by increased exudate accumulation in the peritoneal cavity and leukocyte migration. It is considered a screening tool for evaluation of anti-inflammatory activity of drugs (Iwata *et al*., 2009; Melo *et al*., 2009). Although the extract reduced exudate formation but this was not significant. The degree of inhibition of leukocyte migration to the peritoneal cavity exerted by the extract in this study was similar to that of indomethacin, suggesting that the extract may interfere with some molecular pathways of the inflammatory process.

Reduction in paw oedema induced by carrageenan, dextran and decreased leucocyte count are indicators of decreased vascular permeability and cellular migration to the injured site (Cuman *et al*., 2001; Linardi *et al*., 2002). These effects are known to be mediated by the release of histamine, serotonin, cytokines, leukotrienes and prostaglandins (Hwang *et al*., 1996).

The systemic effect of *A. zygia* on ear oedema induced by croton oil in mice was demonstrated in the present study. Ear edema induced by croton oil is a useful model for evaluating natural products with purported anti-inflammatory activity (Vinegar, 1979; Veras *et al*., 2013). Croton oil is a highly irritant agent which, on topical application, provokes an intense dermatitis characterized by vasodilatation, oedema and leukocytic migration, in addition to local liberation of the inflammatory mediators; histamine, serotonin, bradykinin and prostaglandins (Tubaro *et al*., 1985). These changes are triggered by protein kinase C (PKC) activation, thus promoting an increase in the activity of phospholipase A_2 (PLA_2) with consequent increased levels of arachidonic acid and its metabolites, such as prostaglandins and leukotrienes (Otuki *et al*., 2005). The pronounced effect observed with *A. zygia* in this model may be related to its ability to inhibit the formation of inflammatory mediators stimulated by the PKC pathway or adjacent pathways involved in the inflammatory response.

Formaldehyde-induced arthritis is a chronic animal model of inflammation which results from cell damage and provokes the production of endogenous mediators. Its inhibition is one the most suitable test procedures to screen anti-inflammatory and anti-arthritic agents as it closely resembles human arthritis (Qin *et al*., 2008). In this model, 200 mg/kg/day of *Albizia zygia* extract showed significant inhibitory activity on

oedema development in both paw and ankle indicating the potential usefulness of the extract in the chronic proliferative type of inflammation.

The anti-inflammatory activity of the extract in all the models used in this study was not dose dependent as greater activity was consistently seen at the dose of 200mg/kg compared to other doses.

The results of the present study indicate an anti-inflammatory effect of aqueous stem bark extract of *A. zygia* on paw oedema and leukocyte migration in peritoneal exudate induced by carrageenan, croton oil-induced ear oedema and an anti-arthritic effect in the formaldehyde-induced arthritis test. The effectiveness of the extract in suppressing oedema may be due to its ability to either inhibit the synthesis, release or action of autacoids like histamine, serotonin, prostaglandins and other mediators involved in inflammation. However, its effect may not include direct antagonism of the histamine receptors, since it had no effect on histamine induced oedema.

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