

Albizia zygia (DC.) Macbr. Hydroethanol Root Extract Exerts Anti-Oedemic and *In Vivo* Antioxidant Activities in Animal Models

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ABSTRACT

Various parts of *Albizia zygia* (DC.) Macbr. (Family: Leguminosae-Mimosoideae) are used traditionally in the management of arthritis and pain. The present study evaluated the anti-inflammatory and *in vivo* antioxidant effects of the hydroethanolic root extract of *Albizia zygia* in animal models. Oedema was induced by the injection of carrageenan (2% suspension) into the right footpads of 7-day old chicks. The footpad thickness was measured hourly for 5 h. Carrageenan was also administered into rat paw subcutaneous tissues, which subsequently induced oxidative stress and inflammation at the injected site. After 5 h the rats were sacrificed and inflamed paw tissues isolated for antioxidant enzyme analysis. The extract (30-300 mg/kg, *p.o.*) and the positive control, diclofenac (3-30 mg/kg, *i.p.*), significantly attenuated pedal oedema when given either pre-emptively or curatively. The extract also augmented the endogenous expression of superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) as well as reduced the activity of myeloperoxidase (MPO) and malondialdehyde (MDA) level at the inflamed site. The results suggest that the hydroethanolic extract of *Albizia zygia* exerts *in vivo* anti-inflammatory activity after oral administration and also has antioxidant properties which may contribute to its activity.

INTRODUCTION

Inflammation is a biological response of an organism's immune system to cellular and vascularized tissue damage (Nathan, 2002). Inflammation when gone awry, initiates an innocent bystander attack on the host cells within hours after trauma. The local release of toxic metabolites and enzymes destroy uninjured tissue (Stahel *et al.*, 2007). Tissue injury can generate reactive oxygen species (ROS) by releasing transition metal ions from damaged cells, activating phagocytes or generating NO[•] (Aruoma, 1998). The phagocytes also generate O₂⁻, H₂O₂ and HOCl to kill pathogens. The overproduction of these ROS can further worsen tissue damage (Aruoma, 1998).

Many medicinal plant sources have been investigated as alternate medications for managing injury-induced inflammation. In this study the roots of *Albizia zygia* (DC.) Macbr. (Family: Leguminosae-Mimosoideae), was investigated for its activity in acute inflammation. Its potential antioxidant effect was also evaluated *in vivo*. *Albizia zygia*, also known as West African Walnut, *Okuro* (Ghana), *Nyie avu* (Igbo), *Ayinre were* (Yoruba), *Red Nongo* (Uganda) or *Nongo* (Swahili). It is indigenous to West and East Africa, India and Australia (Anim-Kwapong *et al.*, 1995; Orwa *et al.*, 2009). The bark and leaf decoctions are used traditionally in the management of waist pain, fever and venereal diseases (Arbonnier, 2004; Ndjakou Lenta *et al.*, 2007). Root bark formulations are used to treat venereal diseases (Ndjakou Lenta *et al.*, 2007). *A. zygia* exhibits cytotoxicity against human T-lymphoblast-like leukaemia, prostate and breast cancer cell lines (Appiah-Opong *et al.*, 2016).

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The methanol stem bark extract is very active in malaria and sleeping sickness (Arbonnier, 2004; Abdalla *et al.*, 2012). Since there is scanty scientific documentation on the efficacy of *A. zygia* roots in the traditional management of inflammation, the present study validates such folkloric use of the plant.

MATERIAL AND METHODS

Plant material collection

Albizia zygia roots were collected from the campus of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana (6°40'31.8"N; 1°34'44.1"W) in the month of January, 2015. The root sample was authenticated by Dr. George Henry Sam in the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST. A voucher specimen (no: KNUST/H/M/2016/R001) has been preserved in the Faculty herbarium.

Preparation of extract

The roots were room-dried for 2 weeks, and then pulverized into fine powder. The powder was cold macerated with 70 % (v/v) ethanol. The hydro-alcoholic supernatant was filtered and then concentrated to a brown syrupy mass under reduced pressure at 50°C in a rotary evaporator (R-210, BUCHI, Switzerland). The extract was further dried in a hot air oven at 50°C and stored at 4°C until use. The final product, of yield 9.03 % (w/w), is subsequently referred to as AZE or extract in this study.

Phytochemical screening

Preliminary phytochemical tests were carried out on AZE according to methods described by Trease and Evans (Trease *et al.*, 1989) and Sofowora (Sofowora, 1993).

Animals

Cockerels (*Gallus gallus*; strain Shaver 579, Akropong Farms, Kumasi, Ghana) were obtained one-day post-hatch and housed in stainless steel cages (34 cm × 57cm × 40 cm) at a population density of 12-13 chicks per cage. Feed (Agricare Ltd, Kumasi, Ghana) and water were available *ad libitum* through 1-qt gravity-fed feeders and waterers. Overhead incandescent illumination was provided with room temperature at 29°C. Chicks were tested at 7 days of age. Group sample sizes of six were utilized throughout the study.

Sprague-Dawley rats (100-200 g) of both sexes acquired from Noguchi Memorial Institute of Medical Research (NMIMR), University of Ghana, Accra were grouped (n=6) in stainless steel colony cages. Animals had free access to chow (Agricare Ltd, Kumasi, Ghana), and were provided water *ad lib*. The animals were humanely handled in all experiments according to the internationally accepted principles concerning animal protection in experimental protocols (EU Directive of 2010; 2010/63/EU). In addition, ethical approval was granted by the Ethics Committee of the Department (FPPS/PCOL/010/2015).

Drugs and chemicals

The enlisted drugs and chemicals were used: λ-carrageenan, trichloroacetic acid (TCA), thiobarbituric acid (TBA), potassium dichromate, Tris(hydroxymethyl) aminomethane, Triton X-100, EDTA, sodium bicarbonate, 5,5-dithio bis-2-nitrobenzoic acid (DTNB) (Sigma-Aldrich Inc., St. Louis, MO, USA); diclofenac sodium (Troge, Hamburg, Germany); sodium dihydrogen orthophosphate monohydrate (Hopkins & Williams Ltd, Swansea, Wales); analytical grade glacial acetic acid, ethanol, hydrogen peroxide, chloroform and disodium hydrogen phosphate (BDH, Poole, England); Complete Protease Inhibitor Cocktail Tablet, EDTA-free (Santa Cruz Biotechnology, Dallas, TX, USA).

Carrageenan-induced pedal oedema test

The acute anti-oedemic effect of AZE was investigated in the carrageenan-induced oedema model in chicks (Roach and Sufka, 2003; Abotsi *et al.*, 2012). A volume of 10 µl carrageenan suspension (2 % w/v) was administered into the chicks' right footpads. Dorso-ventral ipsilateral pedal thickness was measured initially (0 h) and at every hour post-carrageenan administration for 5 h (using electronic callipers Z22855, Milomex Ltd, Bedfordshire, UK) (Murayama *et al.*, 1991). Oedema was calculated as the normalized percentage change in foot thickness from time 0 h using the equation: % change in pedal thickness = $[(V_t - V_0)/V_0 \times 100]$; where, V_0 is carrageenan pre-administration pedal thickness (0 h) and V_t is carrageenan post-administration pedal thickness (at time t).

Two sets of experiments, prophylactic and curative, were performed. Drug administration were made 30 min (intraperitoneal) or 1 h (oral) pre-carrageenan challenge in the prophylactic experiment; or 1 h post-carrageenan in the curative protocol. Chicks were grouped according to their drug treatments (n=6-7). The treatments groups were: vehicle control (10 ml/kg of 2% tragacanth, *p.o.*), AZE suspended in 2% tragacanth (30-300 mg/kg, *p.o.*) or diclofenac (3-30 mg/kg, *i.p.*).

In vivo antioxidant assay

The assay of endogenous antioxidants was carried out according to the protocol prescribed by Halici *et al.* (2007). Intraplantar carrageenan administration was made into the subcutaneous paw tissues of rats, which subsequently induced oxidative stress at the inflamed site. Rats were assigned into eight groups (n=3) with respective drug treatments as follows: experimental naïve control (normal saline; 10 ml/kg; *i.p.*), vehicle control (2% w/v tragacanth, 10 ml/kg; *p.o.*), AZE suspended in 2% tragacanth (30-300 mg/kg, *p.o.*) or diclofenac (3-30 mg/kg, *i.p.*). General intraplantar introduction of 100 µl carrageenan (1% w/v), either 30 min (*i.p.*) or 1 h (*p.o.*) post-drug administration, induced oedema in the right hind paw. Five hours after carrageenan administration, rats were sacrificed by cervical dislocation. Oedematous paw tissues were harvested and immediately stored at -80°C for subsequent enzyme analysis. A volume of 4.5 ml of TNG buffer at pH 7.4 (150 mM Tris HCl, 150 mM NaCl, 1%

Triton X 100, 10% glycerol and protease inhibitor cocktail) was added to each sample, and homogenized on ice using a homogenizer (Ultra-Turrax T-25; IKA-Labor Technik, Staufen, Germany) at 24000 $\times g$ for 15 min. Each sample was centrifuged at 4000 $\times g$ for 20 min, decanted and preserved for enzyme analysis. Sample protein content was calculated using the Bradford method (Bradford, 1976). All subsequent microtiter plate readings were carried out in triplicates.

Superoxide dismutase (SOD) assay

The activity of superoxide dismutase (SOD) was measured according to the method of Misra and Fridovich (1972). A volume of 500 μl homogenate was centrifuged in 150 μl of ice-cold chloroform and 750 μl ethanol (96 % v/v) at 2000 $\times g$ for 20 min. Successive additions of 1 ml carbonate buffer (0.1 M; pH 10.2) and 0.5 ml EDTA (0.6 mM) were made to 500 μl of supernatant aliquot. A 1.3 mM adrenaline solution of volume 0.05 ml was then added to initiate adrenochrome formation. A blank solution containing all reagents except tissue homogenate was processed in a similar manner. A volume of 150 μl was then dispensed into a 96-well plate. Absorbance was read spectrophotometrically at 480 nm using Synergy H1 Multi-mode Reader (BioTek Technologies, Winooski, VT, USA). The percentage inhibition of adrenaline autoxidation was calculated as:

$$\% \text{ inhibition} = \left(\frac{\text{Absorbance}_{\text{test}} - \text{Absorbance}_{\text{blank}}}{\text{Absorbance}_{\text{test}}} \right) \times 100$$

Specific SOD activity was expressed in units per mg protein, where 1 unit is the enzyme quantity needed to prevent 50 % of the autoxidation of adrenaline at 25 °C; calculated using the formula:

$$\text{Unit of SOD activity/mg protein} = \left(\frac{\% \text{ inhibition}}{50 \times \text{wt of protein}} \right)$$

Catalase (CAT) assay

The test principle is based on the propensity of CAT to hydrolyse H_2O_2 , thereby inhibiting the dichromate in acetic acid reduction to chromic acetate by H_2O_2 (Sinha, 1972). Successive additions of 0.4 ml H_2O_2 (1.18 M) and 1 ml phosphate buffer (0.01 M; pH 7.0) were made to 0.1 ml of homogenate and incubated for 5 min (25°C). A 2 ml dichromate-acetic acid mixture (containing 3 parts glacial acetic acid and 1 part 5% potassium dichromate) was then added to terminate the reaction. A volume of 150 μl of the mixture was pipetted into a 96-well plate. Chromogenate absorbance was calorimetrically read at 620 nm.

Specific CAT activity was expressed as units per mg protein based on the molar extinction coefficient of H_2O_2 , 39.4 M⁻¹ cm⁻¹ at 620 nm. One unit is the enzyme quantity needed to hydrolyse 1 mmol of H_2O_2 /min in a neutral pH (25 °C), i.e.

$$\text{mUnit of CAT activity/mg protein} = \left(\frac{\text{Absorbance}_{620 \text{ nm}}}{39.4 \times \text{wt of protein}} \right) \times 1000$$

Reduced glutathione (GSH) assay

The concentration of GSH in inflamed paw was measured by the procedure stated by Ellman (1959). To 100 μl of the homogenate was added 2.4 ml 0.02 M EDTA; solution was then cooled at 4 °C for 10 min. Additions of 2 ml H_2O and 0.5 ml of 50 % w/v TCA were made to the mixture and centrifuged at 3000 $\times g$ for 5 min. About 50 μl of 10 mM DTNB solution and 2 ml of Tris buffer (0.4 M; pH 8.9) were then thoroughly mixed with 1 ml of the supernatant and the reaction incubated for 5 min (25°C). A reaction mixture was repeated also for the blank. The wells of 96-well plate were filled with 150 μl of mixture and absorbance was spectrophotometrically read at 412 nm. GSH concentration was expressed in μmol per mg protein, and determined using the curve $y = 0.0004x + 0.0026$.

Myeloperoxidase (MPO) assay

Enzyme concentration was determined spectrophotometrically by a modified *o*-dianisidine method (Şenoğlu *et al.*, 2009). An assay mixture—consisting of 5 ml 0.02 M *o*-dianisidine + 3 ml H_2O_2 (0.01 M) + 3 ml phosphate buffer (0.1 M; pH 6.0) and made up to 30 ml—was freshly prepared. About 125 μl aliquot of it was dispensed into a 96-well microtiter plate and topped up with 0.01 ml of tissue homogenate. The absorbance increase was straightway read at 460 nm in 60 s cycles for 600 s. MPO specific activity was expressed in units per mg protein, where 1 unit increases absorbance by 0.001 per 60 s.

$$\text{Unit of MPO/mg protein} = \left(\frac{\text{Absorbance}_{460 \text{ nm/min}}}{0.001 \times (\text{total protein}/10 \mu l \text{ aliquot})} \right)$$

Lipid peroxidation assay

MDA levels were determined by a modified protocol (Heath *et al.*, 1968). To a 1 ml volume of homogenate was mixed 3 ml of the mixture (3 ml of 20 % TCA containing 0.5% TBA) in a test tube. It was heated at 95°C for 30 min, cooled immediately and then centrifuged at 5000 $\times g$ for 10 min. Absorbance was initially read at 532 nm and then read again at 600 nm to correct for nonspecific absorbance. The molar extinction coefficient of MDA-TBA adduct, 155 mM⁻¹cm⁻¹, was used to determine the levels of MDA from the equation:

$$\text{nmol MDA/mg protein} = \frac{\text{Absorbance}_{532 \text{ nm}} - \text{Absorbance}_{600 \text{ nm}}}{155 \times \text{total protein}} \times 10^6$$

Analysis of data

Statistical analyses and ED₅₀ determinations were done using GraphPad Prism for Windows version 6 (GraphPad Software, San Diego, CA, USA). The time course curves were analyzed by two-way repeated measures analysis of variance (ANOVA). The mean treatment differences at each time period were compared by Tukey's *post hoc* test. Subsequent area under the curves (AUCs) were then calculated (arbitrary units) to

determine the total treatment effect. One-way ANOVA was used to analyse the AUC mean differences; pair-wise comparisons were done using Tukey's *post hoc* test. $P < 0.05$ between treatments was considered statistically significant.

RESULTS

Phytochemical screening

Alkaloids, tannins, flavonoids, saponins, glycosides and terpenoids were found to be present in AZE.

Carrageenan-induced pedal oedema test

The introduction of 10 μ l of 2% carrageenan suspension into pedal tissues of chicks effectuated an acute local oedema. Oedema peaked at 2-3 h in all paws (Figures 1 and 2), correlating with the findings of treatments across the time courses of AZE (30-300 mg/kg; *p.o.*) and diclofenac (3-30 mg/kg, *i.p.*) (Roach and Sufka, 2003). Figures 1(a, c) and 2(a, c) represent the time course

of effects of the respective treatments. The column graphs of AZE and diclofenac are represented in Figure 1(b, d) for preemptive and Figure 2(b, d) for curative treatments respectively.

Analysis of the curves with respect to the vehicle control group shows a significant reduction in the mean maximal paw oedema at 2-3 h by AZE (prophylaxis: $F_{3, 20}=10.87$, $P < 0.0001$; therapeutic: $F_{3, 24}=6.107$, $P=0.0031$) and diclofenac (prophylaxis: $F_{3, 99}=97.64$, $P < 0.0001$; therapeutic: $F_{3, 24}=20.32$, $P < 0.0001$). Total oedema was significantly reduced by the treatments. AZE demonstrated maximal inhibition of mean total oedema by 57.65 ± 6.70 % (pre-emptive) and 55.41 ± 7.37 % (curative) at dose 300 mg/kg. Diclofenac also similarly inhibited the mean total oedema by 77.08 ± 3.80 % (pre-emptive) and 75.87 ± 4.00 % (curative) at 30 mg/kg. Figure 3 compares the dose-response relationships of AZE and diclofenac. The ED_{50} values of AZE and diclofenac (obtained by non-linear regression) were 195.2 ± 63.98 mg/kg (pre-emptive); 1650 ± 6.63 mg/kg (curative) and 1.43 ± 0.69 mg/kg (pre-emptive); 38.05 ± 1.49 mg/kg (curative) respectively.

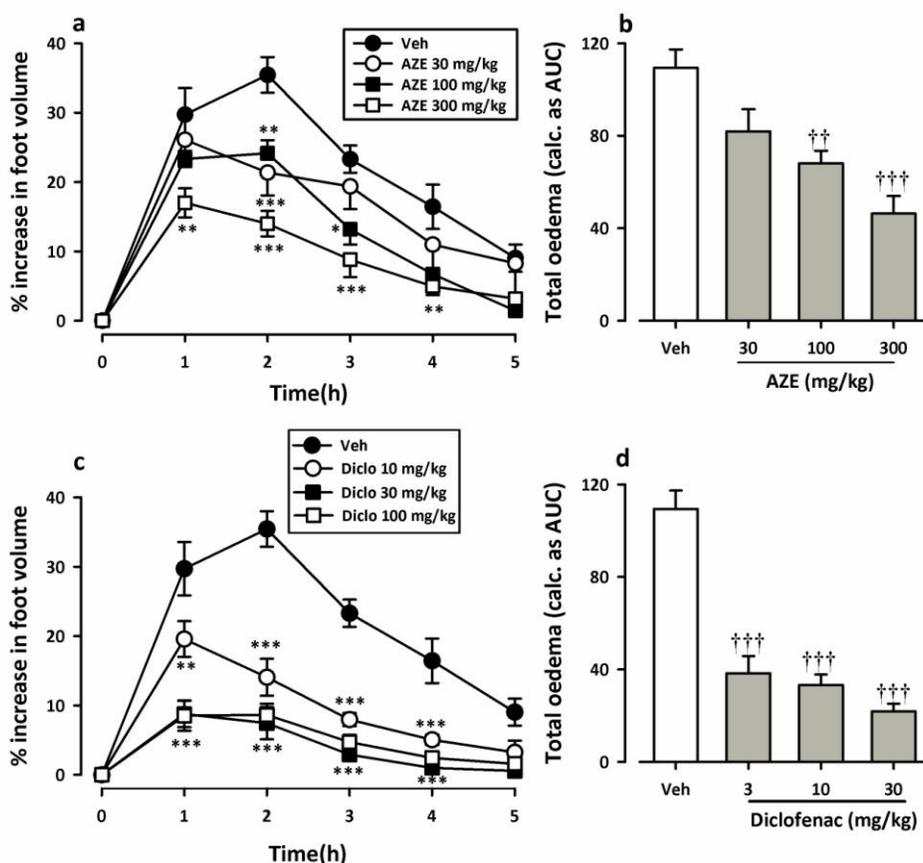


Fig. 1: Effect of AZE (30-300 mg/kg; *p.o.*) and diclofenac (3-30 mg/kg, *i.p.*) on time-course curves (a and c respectively) and the total oedema response (b and d respectively) in the prophylactic protocol of the carrageenan-induced foot oedema in chicks.

Values are mean \pm S.E.M. (n=6). $****P < 0.05 / < 0.01 / < 0.001$ versus vehicle control (Two-way ANOVA; Tukey's *post hoc* test).
 $††††P < 0.01 / < 0.001$ versus vehicle control (One-way ANOVA; Tukey's *post hoc* test).

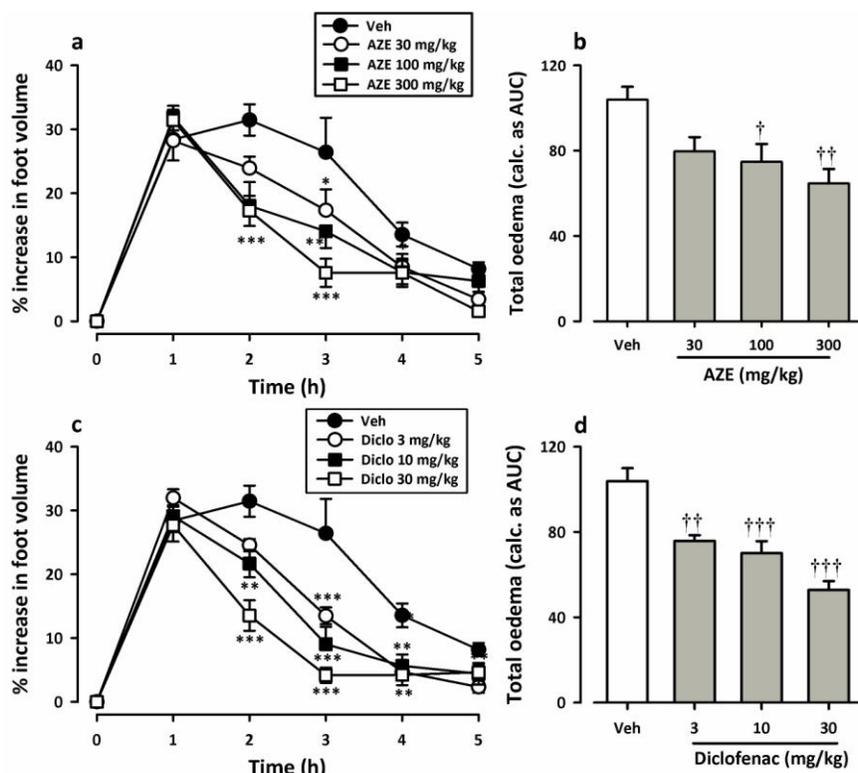


Fig. 2: Effect of AZE (30-300 mg/kg, p.o.) and diclofenac (3-30 mg/kg, i.p.) on time-course curves (a and c respectively) and the total oedema response (b and d respectively) in the curative protocol of the carrageenan-induced foot oedema in chicks. Values are mean±S.E.M (n=6).
 /*P<0.05/<0.01/<0.001 versus vehicle control (Two-way ANOVA; Tukey post hoc test).
 †††††P<0.05/<0.01/<0.001 versus vehicle control (One-way ANOVA; Tukey post hoc test).

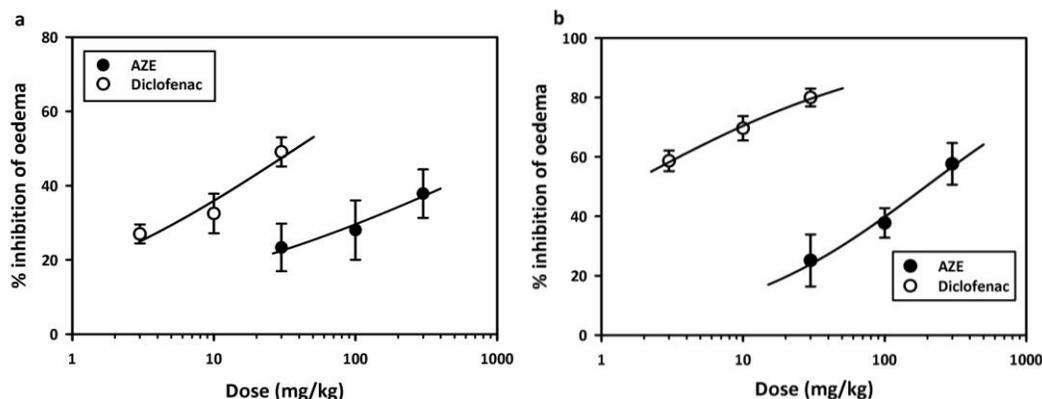


Fig. 3: Dose-response curves for AZE (10-300 mg/kg, p.o.) and diclofenac (3-30 mg/kg, i.p.) in the prophylactic (a) and curative (b) protocols of carrageenan-induced foot oedema in the chick.

Table 1 Effect of AZE and diclofenac on the levels of SOD, CAT, MPO, GSH and MDA.

Group	SOD (mU/mg prot.)	CAT (mU/mg prot.)	MPO (U/mg prot.)	GSH (µmol/mg prot.)	MDA (nmol/mg prot.)
Control	398.37±35.95 [†]	43.44±3.59	279.84±53.89	353.16±23.64 ^{†††}	151.87±15.04 ^{†††}
Vehicle	192.47±18.19	26.22±3.43	499.18±43.11	135.37±21.60	504.11±49.97
AZE 30 mg/kg	253.15±16.14	40.04±1.77	382.11±74.74	80.04±23.26	263.15±61.32 ^{**}
AZE 100 mg/kg	370.56±25.61 [*]	55.48±0.81 ^{**}	184.20±99.96 [*]	246.65±19.31	188.22±20.20 ^{***}
AZE 300 mg/kg	431.08±32.28 ^{**}	59.45±1.55 ^{**}	207.87±67.81	308.48±20.69 ^{**}	168.33±25.86 ^{***}
Diclo 3 mg/kg	371.69±44.63 [*]	57.85±4.50 ^{**}	-	185.20±36.67	250.86±38.71 ^{**}
Diclo 10 mg/kg	521.95±23.21 ^{***}	74.36±5.38 ^{***}	-	305.19±18.57 ^{**}	177.00±42.28 ^{***}
Diclo 30 mg/kg	661.79±54.12 ^{***}	85.91±9.94 ^{***}	211.49±57.47 [*]	360.57±32.82 ^{***}	135.64±8.71 ^{***}

Values are mean±SEM (n=3). ^{*}P<0.05, ^{**}P<0.01, ^{†††}P<0.001 compared to vehicle-treated group (One-way ANOVA followed by Tukey's post hoc test). SOD: Superoxide dismutase; CAT: Catalase; MPO: Myeloperoxidase; GSH: reduced glutathione; MDA: Malondialdehyde; Prot: protein

***In vivo* antioxidant assay**

Superoxide dismutase assay

The vehicle-treated group showed a marked reduction in SOD activity at the inflamed site compared to that in the naïve subjects (Table 1). AZE (100 and 300 mg/kg; *p.o.*)-treated rats had a significant increase in SOD activity ($F_{4,10}=13.91$, $P=0.0004$). A dose-dependent effect was obtained for diclofenac ($F_{4,10}=21.71$, $P<0.0001$).

Catalase assay

The intraplantar carrageenan administration induced an acute local inflammation in the rat paw with resultant oxidative stress. The oxidative stress caused a marked reduction in CAT levels in negative control group compared to the naïve group (Table 1). On the other hand, AZE (100 and 300 mg/kg; *p.o.*) and diclofenac (3-30 mg/kg; *i.p.*) increased CAT activity ($F_{7,16}=16.24$, $P<0.0001$). The lowest dose of AZE produced no significant change in CAT activity but a dose-dependent effect was seen with diclofenac.

Reduced glutathione assay

Increased oxidative imbalance significantly reduced GSH concentration (Table 1). Preemptive administration of AZE (300 mg/kg, *p.o.*) and diclofenac (10 and 30 mg/kg, *i.p.*), however, significantly increased GSH expression in the rat paw ($F_{7,16}=16.81$, $P<0.0001$).

Myeloperoxidase assay

MPO activity increased in the negative control group relative to naïve group (Table 1). AZE and diclofenac pre-treatments significantly reversed the increased MPO concentration in their respective groups ($F_{5,11}=3.221$, $P=0.0493$). The significant effects were seen in only AZE 100 mg/kg (*p.o.*) and diclofenac 30 mg/kg (*i.p.*)

Lipid peroxidation assay

Oxidative stress increases lipid peroxidation with a resultant increase in malondialdehyde generated from the decomposition of lipid hydroperoxides. The measurement of MDA levels revealed an increased concentration in negative control group relative to the naïve group (Table 1). The administration of AZE (30-300 mg/kg, *p.o.*) and diclofenac (3-30 mg/kg, *i.p.*) significantly and dose-dependently reduced MDA levels ($F_{7,16}=10.47$, $P<0.0001$).

DISCUSSION

This present study has established that oral administration of the hydroethanolic root extract of *Albizia zygia* has anti-inflammatory and antioxidant properties in animals.

Carrageenan-induced pedal oedema in chicks is simple to conduct, reproducible and highly reliable. Overdependence on rodent models is curtailed by replacing rodents with a less sentient species, chicks (Fereidoni *et al.*, 2000; Roach and Sufka, 2003).

Carrageenan administration produces a local inflammation with no systemic effects and no antigenicity. Pedal oedema in chicks lasts approximately 6 h, peaking 2 h post-carrageenan injection. Subcutaneous injection of carrageenan induces a biphasic inflammation characterized by oedema, erythema and pain. Pro-inflammatory mediators- histamine, serotonin, bradykinin, tachykinins, complement and ROS are responsible for inducing the inflammation (Morris, 2003). Prostaglandins then induce the last phase of the oedema; as a result of the action of inducible forms of nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) (Posadas *et al.*, 2004). Preemptive and curative administration of AZE to chicks significantly suppressed the acute local oedema in both treatment protocols. The anti-oedemic effects of AZE may possibly be attributable to the inhibition of synthesis, release and/or activity of the inflammatory mediators. The precise mechanism by which AZE suppresses inflammation however needs to be established. AZE was compared to the standard anti-inflammatory analgesic, diclofenac. It is a non-selective COX inhibitor which prevents the synthesis of the arachidonic acid metabolites such as prostaglandins, thromboxanes and leukotrienes (Gan, 2010). The body's generation of ROS helps maintain homeostasis in the cells of a healthy tissue. They also serve as signaling molecules (Devasagayam *et al.*, 2004). Oxidative imbalance abounds in inflamed tissues after trauma. The relative diminution of endogenous antioxidants increase cellular susceptibility to oxidative damage (Wang *et al.*, 2004; Valerio *et al.*, 2009). Free radicals possess short half-lives and are less concentrated; as such oxidative damage is best assessed by measuring the amount of products and enzymes produced (Hovatta *et al.*, 2010). SOD converts O_2^- to H_2O_2 , and then either CAT or GPx converts the H_2O_2 to oxygen and water. GSH and its congeners detoxify peroxy nitrates, hydroperoxides and electrophiles such as reactive aldehydes (malondialdehyde, MDA). MPO is used by neutrophils to synthesize HOCl during phagocytic lysis of foreign bodies (Zhu *et al.*, 2008; Şenoğlu *et al.*, 2009; Halliwell, 2012). Histamine, serotonin and eicosanoids mediate the early phase of the carrageenan-induced paw oedema whilst polymorphonuclear infiltration is responsible for the delayed inflammatory phase. Neutrophil-derived OH, H_2O_2 and O_2^- , MPO and MDA concentrations are augmented whilst antioxidant levels are reduced in the paw tissue (Bilici *et al.*, 2002; Halici *et al.*, 2007). Pretreatment of the rats with AZE significantly elevated the activities/levels of the preventive enzyme antioxidants (SOD and CAT) and the scavenger, chain breaking or repair antioxidant, GSH, at the inflamed site. AZE also reduced the formation of MDA and the pro-inflammatory enzyme MPO. Similar results were obtained for diclofenac. The anti-inflammatory effect of AZE may at least partly be as the result of the prevention of oxidative damage.

CONCLUSION

In conclusion, the hydroethanol extract of the roots of *Albizia zygia* demonstrated anti-oedemic and *in vivo* antioxidant

activity in animals. The results validate the folkloric use of *A. zygia* in the treatment of inflammatory disorders.

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