Ethanol whole plant extract of *Ochna afzelii Theales (Ochnaceae)* ameliorates anaphylactic inflammation in wistar rats

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Abstract

Research has shown that *Ochna afzelii* commonly known as African Plane or African Ochna or Afzel's ochna is a rich source of complex flavonoids known to possess anti-inflammatory activity. This study aimed to investigate the immune-mediated anti-inflammatory activity of the ethanol whole plant extract of *O. afzelii* in rats. The immune-mediated anti-inflammatory activity was evaluated using an egg albumin-induced active paw anaphylaxis model in rats; and a histamine-induced paw edema model in Wistar rats For each of the models, thirty (30) Wistar rats weighing 150 - 200 g were divided into five groups containing six (6) rats each and were orally administered with single doses of the following treatments: a negative control group (distilled water, 10 ml/kg), a positive control group (dexamethasone, 0.27 mg/kg), and three test groups receiving 250, 500, and 1000 mg/kg of ethanol whole plant extract of *Ochna afzelii* respectively. The extract at the dose of 250mg/kg significantly ($p<0.05$) inhibited egg albumin-induced paw inflammation at 2 hrs post antigen exposure. Also, the extract at 500 and 1000 mg/kg also significantly ($p<0.05$) reduced paw edema induced by histamine, with the 1000 mg/kg dose showing effects

comparable to dexamethasone. Ethanol whole plant extract of *O. afzelii* possesses immune-mediated anti-inflammatory activity in Wistar rats and this justifies the traditional use of the plant in the management of anaphylactic disorders.

Keywords: Anaphylaxis, anti-inflammatory, egg albumin, histamine, immune-mediated, ochna afzelii, paw oedema

Introduction

Immune-mediated inflammatory diseases continue to cause significant health problems, reduced quality of life, and premature death (Giovanni *et al*., 2023). Chronic inflammatory diseases and persistent pain are significant medical, social, and economic problems, and their treatment remains challenging (Mills *et al*., 2019). The first examples of effective anti-inflammatory drugs, like non-steroidal anti-inflammatory drugs, glucocorticoids, and opioids, were introduced many years ago. Since then, very few new drugs with novel mechanisms of action have been successfully developed, despite significant research efforts (Lindsay and Rod, 1990). Some biological treatments targeting specific molecules have been successful for certain autoimmune/inflammatory diseases. However, they are costly, require injections, and can have long-term side effects that limit their use (Bennett *et al.,* 2018). Therefore, there's a pressing need for new immune-mediated anti-inflammatory medications with novel mechanisms of action and better safety profiles. A wide range of phyto-constituents have demonstrated a role in the modulation of inflammatory responses Bellik *et al*., 2012). Several natural product-derived medications of plant origin are in clinical use and some are undergoing Phase II and III clinical trials (Butler 2008). The understanding of the cellular and molecular mechanisms involved in the inflammatory process has increased considerably in recent decades and this has permitted the discovery of many promising targets for the development of new drugs to treat inflammatory diseases (Pushpangada *et al.,* 2015). *Ochna* species typically thrive in diverse habitats, including forests, woodlands, and savannas. The plant is locally known as "African plane or African *ochna*'' in English, "Biri mai tafarwa'' in Hausa, and "Aluko'' in Yoruba (Bandi *et al*., 2012). Research has shown that *Ochna* plants such as *Ochna afzelii* are a rich source of complex flavonoids and related phenolic compounds (Pegnyemb *et al*., 2003). Some of these compounds and their

extracts have various biological activities, including pain-relieving, anti-inflammatory, anti-HIV-1, anti-malarial, anti-microbial, and cell-killing properties. (Bandi *et al.,* 2012). Ethnomedicinal uses of the small tree *O. afzelii*, commonly found in the forests of Central Africa include local treatment of lumbago, dysentery, toothaches, jaundice, and infertility in women (Bouquet, 1969). Traditional Cameroonian medicine has utilized *Ochna afzelii* to treat liver infections, dysentery, toothaches, jaundice, and infertility in women (Bandi *et al.,* 2012). The fruits are used as food as well as medicines (Bandi *et al.,* 2012). *O. afzelii* is believed to possess natural antibacterial activity - the leaves have a mild antioxidant effect (Adeyemi *et al*., 2023), while methanol bark extract has strong inhibitory activity against beta-lactamases (Bandi *et al.,* 2012). Therefore, there's a pressing need for new anti-inflammatory and pain-relieving medications with innovative ways of working. This research aimed to investigate the ameliorative effect of the ethanol whole plant extract of *O. afzelii* on anaphylactic inflammation in rats.

Materials and methods

Chemicals

Dexamethasone (MeCure Industries PLC, Nigeria), Histamine (Sigma, USA), Egg albumin (Merck, Darmstadt, Germany), Normal saline (Dana Pharmaceuticals, Nigeria).

Plant material

The whole plant *Ochna afzelii* was collected in the month of March 2024 from Giwa local government area of Kaduna State in Nigeria. They were taxonomically identified and authenticated with the voucher specimen number (KASU/BSH/1631) in the Herbarium Unit of the Department of Botany, Ahmadu Bello University Zaria, Nigeria. The powdered plant material was stored at room temperature in airtight containers under dark conditions.

Preparation of plant extract

Using a Maceration Tank, 700 g of the coarsely powdered material was extracted using 70 %v/v ethanol in water over the course of 72 hours, agitating it every 12 hours. The extract was filtered using muslin cloth. The filtrate was concentrated by evaporating over a water bath maintained at 40 – 60℃ to yield 69g (9.68%) of the ethanol whole plant extract. The crude extract was then weighed, stored in a tightly

sealed container, and labeled as ethanol *O. Afzelii* extract (EOAE).

Experimental animals

Wistar rats (150 - 200 g) of either sex bred at Animal House Facility, Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria, were used in the study. The animals were maintained in a well-ventilated room, fed on standard rodent feed, and granted access to water *ad libitum*. They were kept in clean cages under normal light/dark cycles. All experimental procedures followed the ethical guidelines for the care and use of laboratory animals as provided by Ahmadu Bello University Research Policy (Revised, 2010) and accepted internationally (NIH 1985, Revised 1996).

Preliminary phytochemical screening

The preliminary phytochemical analysis of the extracts was carried out according to standard methods described by Evans, (2009) and/or Sofowora, (1993).

Acute toxicity studies

The acute toxicity study was conducted following the OECD 423 guidelines (OECD, 2001) where the limit test dose of 5000 mg/kg was used. Three (3) animals were used for the procedure. Following an overnight fasting period, the body weights of the rats were determined and the administered dose was calculated with reference to the body weight. The volume of the extract solution given orally to the rats was 10 ml/kg. Body weight, signs of toxicity, and mortality were observed after the administration for 14 days.

Effect of Ethanol Whole Plant Extract of Ochna afzelii on Egg albumin-induced active paw anaphylaxis in rats.

Thirty (30) rats were sensitized by administering subcutaneously three doses of 100 mg of egg albumin on days 1, 3, and 5. On day 10 of sensitization, they were divided into five (5) groups each containing six (6) rats. Group 1 (Control) received distilled water at a dose of 1 ml/kg orally. Group 2 was administered with Dexamethasone at a dose of 0.27 mg/kg orally. Groups 3, 4, and 5 were administered with ethanol extracts of the plant at graded doses of 250, 500, and 1000 mg/kg orally respectively. One (1) hour past drug treatment, animals were again challenged with 10 mg egg albumin subcutaneously on the right hind paw, and edema inhibition was calculated at 0, 1, 2, 3, and 4 hours by measuring the paw

diameter using a Vernier caliper (Patil, 2010, Aliyu *et al*., 2017).

Effect of Ethanol Whole Plant Extract of Ochna afzelii on Histamine-induced Active Paw Anaphylaxis in Rats

Thirty (30) Wistar rats were divided into five groups of six (6) rats each. Group 1 was given distilled water (10 ml/kg) which served as the negative control. Group 2 received dexamethasone (0.27 mg/kg) served as the positive control. Groups 3, 4, and 5 received single doses of the ethanol extract of *Ochna afzelii* (EEOA) at doses of 250, 500, and 1000 mg/kg *p.o.* respectively. After one hour of the above pre-treatment, all the rats were injected with 0.1 ml of 1% solution of histamine in their left hind paw. Size/volume of the left hind paw was measured using a Vernier caliper at 0 hours, 30 minutes, 1, 2, 3, 4, and 5 hours.

Statistical analysis

The results were expressed as mean ± Standard Error of the Mean (SEM). Analysis was carried out using Statistical Package for the Social Sciences (SPSS) Version 27. Repeated measures Analysis of Variance (ANOVA) followed by Bonferroni *post hoc* test was used for multiple comparisons in anti-anaphylactic studies. Mean differences were considered to be statistically significant at $p \leq 0.05$.

Results

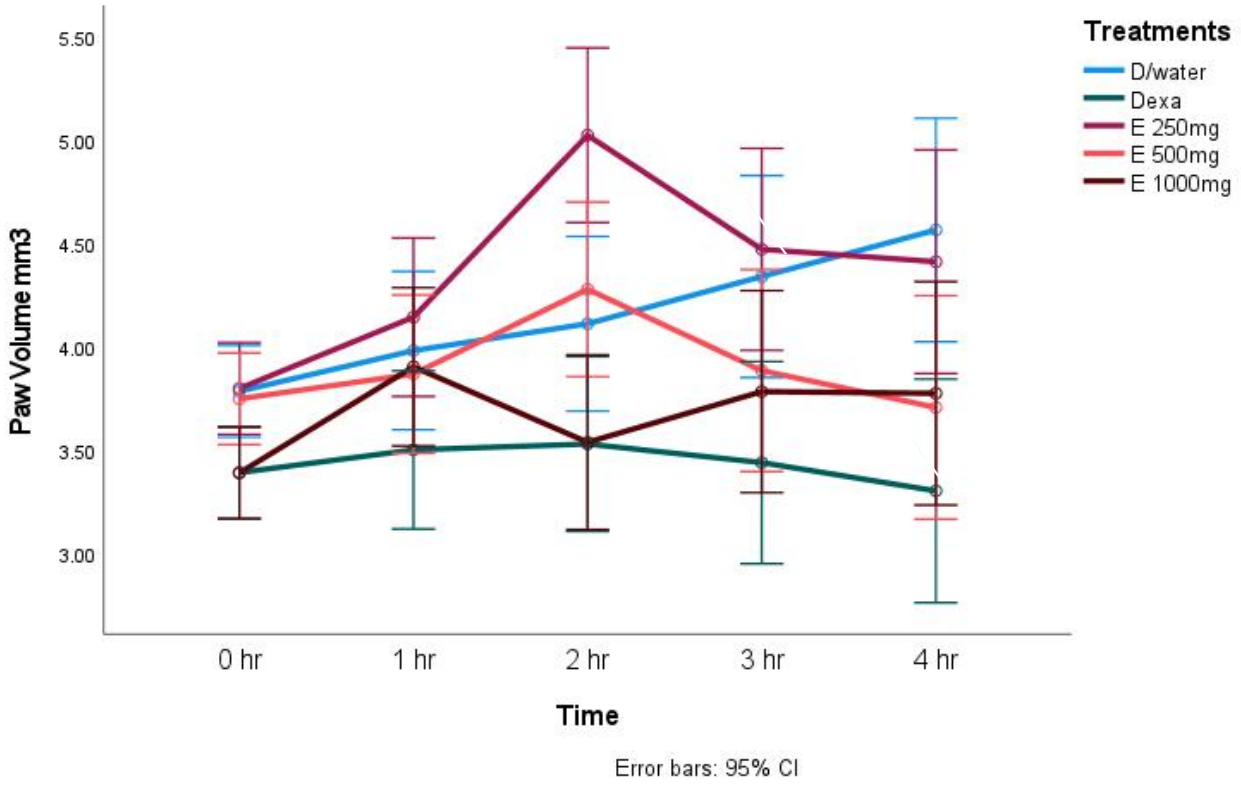
Preliminary Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, and steroids, while saponins were found absent. The oral, median lethal dose of the whole plant ethanol extract of *Ochna afzelii* was estimated to be greater than 5,000 mg/kg in rats.

In egg albumin-induced active paw anaphylaxis, egg albumin (10mg/mL) increased the paw volume in the sensitized animals, which was measurable up to the time period of 4 hours. The inhibitory effect of the extract started after 1 h for the extract at 1000 mg/kg, and 2 h for all of the 250 mg/kg, 500 mg/kg, and dexamethasone. The effect of the extract at 250 mg/kg was significant ($^{a}p<0.05$) after 3 hr of ovalbumin administration. However, the extract at 250 mg/kg dose was less effective in inhibiting paw inflammation compared to the positive control (Dexa at 0.27mg/kg) at the same time. Dexamethasone (Dexa, 0.27 mg/kg), significantly reduced ($^{b}p<0.05$) paw volume at 4 hours of antigen exposure (Table 1).

Table 1: Effect of Ethanol Whole Plant Extract of *Ochna afzelii* on Egg Albumin Induced Paw Anaphylaxis in Wistar Rats

Treatments	Dose (mg/kg)	Paw Volume (mm3)				
		0 h	1 h	2 h	3 h	4 h
D/water	1ml/kg	3.79 ± 0.11	3.98 ± 0.19	4.11 ± 0.21	4.34 ± 0.24	4.57 ± 0.26
Dexa	0.27	3.40 ± 0.11	3.50 ± 0.19	3.53 ± 0.21	3.44 ± 0.24	3.30 ± 0.26[b]
EOAE	250	3.80 ± 0.11	4.14 ± 0.19	5.02 ± 0.21	4.47 ± 0.24[a]	4.41 ± 0.26
EOAE	500	3.75 ± 0.11	3.87 ± 0.19	4.28 ± 0.21	3.89 ± 0.24	3.71 ± 0.26
EOAE	1000	3.39 ± 0.11	3.90 ± 0.19	3.54 ± 0.21	3.78 ± 0.24	3.78 ± 0.26

Values are Mean ± SEM, n=5. "a" is significantly different at $p<0.05$ which was compared to the other times in the same group, while "b" is significantly different at $p<0.05$ which was compared to negative control (Normal saline) Repeated Measure ANOVA followed by Bonferroni Post-hoc test. Dexa = Dexamethasone, D/water = Distilled water, EOAE = Ethanol Ochna afzelii Extract, mm = Millimeter



Values are Mean ± SEM, n=5. "a" and is significantly different at p<0.05 which was compared to the other times in the same group. Repeated Measure ANOVA followed by Bonferroni Post-hoc test. Dexa = Dexamethasone, D/water = Distilled water, EOAE = Ethanolic Ochna afzelii Extract

Figure 1: Effect of *Ochna afzelii* on Egg Albumin-Induced Paw Anaphylaxis in Wistar Rats.

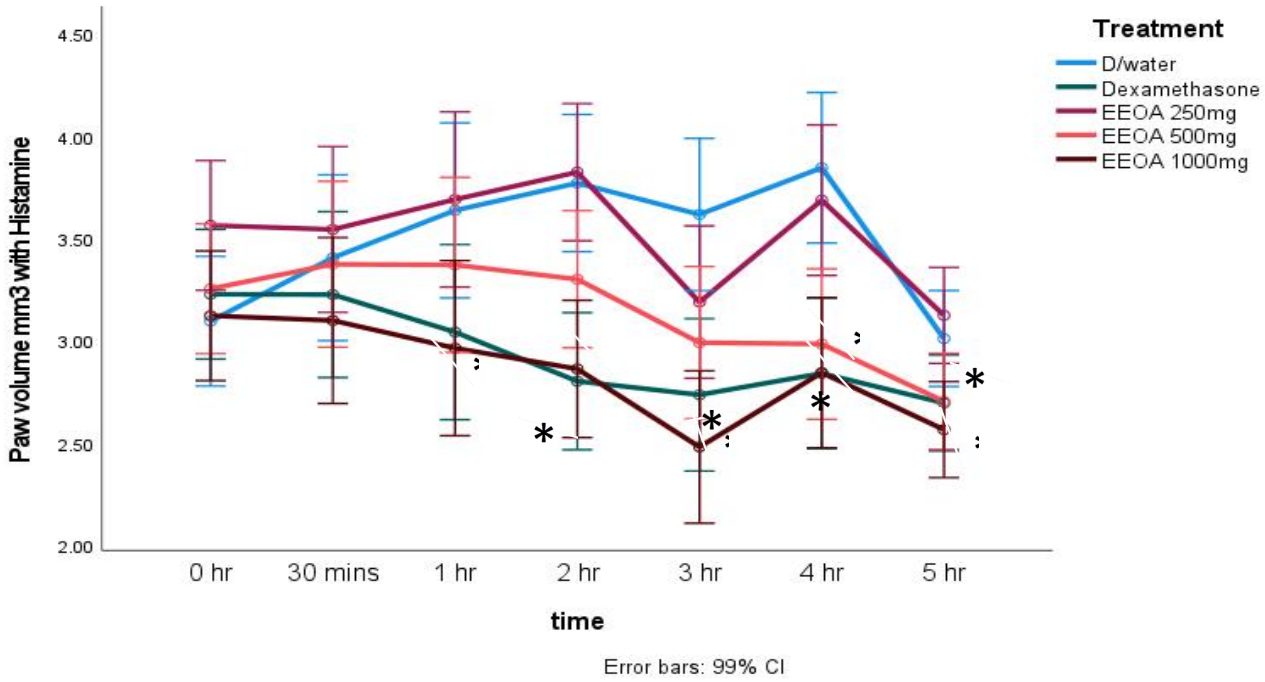
In Histamine-Induced paw inflammation in rats, histamine (10mg/mL) increased the paw volume in the animals, which was measurable up to the time period of 4hr. The extract had no effect on the histamine-induced inflammation at 250 mg/mg. However, at 500 mg/kg, the extract showed observable and significant ($^{a}p<0.05$) inhibition of inflammation after 2 hrs of histamine exposure compared to the negative control, while 1000 mg/kg of the extract demonstrated significant ($^{a}p<0.05$) inhibition 1hr after histamine exposure (Table 2). Dexamethasone (0.27 mg/kg) also, significantly reduced ($^{a}p<0.05$) paw volume after 2 hr of histamine exposure. EEOA at 1000 mg/kg dose

was more effective than dexamethasone at 0.27 mg/kg (Table 2).

Table 2: Effect of Whole Plant Extract of *Ochna afzelii* on Histamine-Induced Paw Edema in Wistar Rats

Treatment	Dose (mg/kg)	Paw Volume (mm3)						
		0 hr	0.5 hr	1 hr	2 hr	3 hr	4 hr	5 hr
D/water	10ml/kg	3.09±0.11	3.40±0.15	3.64±0.15	3.77±0.12	3.61±0.13	3.84±0.13	3.01±0.01
Dexa	0.27	3.23±0.11	3.22±0.15	3.04±0.15	2.80±0.12[a]	2.73±0.13[a]	2.83±0.13[a]	2.69±0.01
EEOA	250	3.56±0.11	3.54±0.15	3.69±0.15	3.82±0.12	3.19±0.13	3.69±0.13	3.12±0.01
EEOA	500	3.25±0.11	3.37±0.15	3.36±0.15	3.30±0.12	2.99±0.13	2.98±0.13[a]	2.70±0.01
EEOA	1000	3.12±0.11	3.10±0.15	2.96±0.15[a]	2.86±0.12[a]	2.48±0.13[a]	2.84±0.13[a]	2.56±0.01[a]

Values are Mean ± SEM, n=5. "a" is significantly different at $p<0.05$ to the standard Dexamethasone control. Repeated Measure ANOVA followed by Bonferroni test. EEOA = Ethanol Extract of Ochna Afzelii, Dexa = Dexamethasone, mm = Millimeter, D/ water = Distilled water



Values are Mean ± SEM, n=5. "" is significantly different at p<0.05 which was compared to the standard Dexamethasone control. Repeated Measure ANOVA followed by Bonferroni test. EEOA = Ethanol Extract of Ochna Afzelii, Dexa = Dexamethasone, D/ water = Distilled water*

Figure 2: Effect of *Ochna afzelii* Extract on Histamine-Induced Paw Edema in Wistar Rat

Discussion

The ethanol extract of *O. afzelii* exhibited significant inhibition of ovalbumin-induced paw edema in a time-dependent manner compared within the same group of the extract. The edema-inhibitory effect of the extract may be due to the regulation of IgE-mediated Type 1 hypersensitivity (Krishnakumar, 2023). Active paw anaphylaxis is an *in vivo* model to evaluate the modulatory effect on IgE antibody-mediated immune hyperactivity using ovalbumin as an antigen (Krisnakumar, 2017). Subcutaneous administration of egg albumin to rats raises the anti-serum of egg albumin in the plasma and subplanter, leading to anaphylaxis in rats (Pungle *et al*, 2003; Aliyu *et al*., 2017). In this experiment, there was a significant increase in paw edema in the ovalbumin control group indicating that the injection elicited the desired inflammation. The extract of *O. afzelii* showed significant inhibition of active paw anaphylaxis in Wistar rats. This may be achieved through the mechanism of inhibition of the release of anaphylactic and inflammatory mediators such as histamine from sensitized mast cells.

Chronic inflammatory conditions such as allergic asthma occur due to exposure to allergen resulting in the activation of T-lymphocytes with subsequent release of inflammatory mediators (Tadech *et al*., 2019). Immuno-modulating agents are useful in the management of asthma, they inhibit the antigen-antibody (AG-AB) reaction thereby inhibiting the release of inflammatory mediators (Dhawan *et al*, 2003; Agrawal and Mehta, 2007; Aliyu *et al*., 2017). Significant inhibition of histamine-induced inflammation by ethanol extract of *Ochna afzelii* implies that it has activity against anaphylactic inflammation. The protective effects of the extract in reducing paw volume in active paw anaphylaxis may be due to the inhibition of antigen-antibody reaction thereby inhibiting the release of inflammatory mediators including histamine (Pandit, 2008). Histamine was the first mediator implicated in mechanisms of allergy, asthma, and anaphylactic shock because it has been discovered to mimic several features of these conditions (Cezmi and Kurt, 2003). In addition to its well-characterized effects in acute inflammatory and allergic responses, it was recently demonstrated that histamine regulates several essential events in the immune response. Histamine affects the maturation of immune system cells and alters

their activation, polarization, chemotaxis, and effector functions (Cezmi and Kurt, 2003). Histamine also regulates antigen-specific Th1 and Th2 cells, as well as related antibody isotype responses (Cezmi and Kurt, 2003). It has been reported that flavonoids such as quercetin, apigenin, and luteolin can inhibit the release of histamine and neutrophil β-glucuronidase. Furthermore, Nagore and co-workers (2009) found that flavonoids are found to be active at both phases of allergic response (Aliyu *et al*., 2017). Therefore, extracts that are rich in these flavonoids can exert in vivo antihistaminic and by inference anti-allergic and anti-inflammatory effects (Krishnakumar, 2023). The presence of flavonoids identified in this extract could be responsible for the observed activity of *O. afzelii*.

Conclusion

In conclusion, the immune-mediated anti-inflammatory activity of *Ochna afzelii* whole plant extracts in both egg-albumin and histamine-induced inflammation can be attributed to the presence of flavonoids and phenolic compounds present in it and the findings substantiate the traditional medicinal use of the plant. Thus *O. afzelii* can be considered as a good candidate for the management of anaphylactic diseases.

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