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In vivo inhibitory effect of hydro-ethanol extract of *Xylopia aethiopica* fruits on mediators of acute inflammation

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ABSTRACT

Xylopia aethiopica is traditionally employed, as fruit decoction, in the management of bronchitis, asthma and arthritis in Ghana. This study is to evaluate the involvement of the hydro-ethanol extract of the dried fruit of *X. aethiopica* on some inflammation pathways by employing *in vivo* murine models. *X. aethiopica* (30, 100, 300 mg kg^{-1}) suppressed the mean maximal swelling attained at 60-90 min and also decreased the total paw swelling induced over 2.5-3 h significantly ($p < 0.05$). The extract suppressed mean maximal swelling attained at 60-90 min in the respective histamine, serotonin, bradykinin and prostaglandin E_2-induced oedema models when compared with their respective mean inflamed control responses. Total paw swellings induced over the 2.5-3 h were also significantly suppressed in all mediator-challenged mice. The current study establishes that *X. aethiopica* extract has inhibitory effect on histamine, serotonin, bradykinin and prostaglandin E_2 in acute inflammation.

Key words: Acute inflammation, anti-inflammatory, oedema, *Xylopia aethiopica* extract

INTRODUCTION

The inflammatory process is a protective response involving host immune cells, blood vessels, specialized proteins and lipid mediators, that aims at eliminating the offending agent or noxious stimuli such as tissue necrosis and infection [1]. Although beneficial, the inflammatory response can become destructive if overexaggerated or unable to resolve, such as in septic shock [2,3].

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Upon initiation of the inflammatory response by various inducers such as infection or signals from necrotic tissues, various specialized molecules are activated with subsequent stimulation of the release of inflammatory mediators such as histamine, serotonin, platelet activating factor (PAF), arachidonic acid metabolites, reactive oxygen species (ROS), cytokines and chemokines [4]. These endogenous compounds can activate or inhibit inflammation, stimulate or impair tissue repair, and also activate the effectors, which are the tissues and cells [5].

The inflammatory response is a chain of organized, dynamic responses and can be categorized into acute and chronic responses with each mediated by a different mechanism with different mediator-release profiles [6]. During acute inflammation, responses in the microvasculature occurs rapidly, usually within few minutes following microbial invasion or tissue insult leading to vasodilation and increased capillary permeability [7,8]. Increased capillary permeability facilitates interstitial oedema formation and recruitment of neutrophils to the site of injury [9]. Leukocyte infiltration during acute inflammation is stimulated by chemokines and basophil-derived histamine, PAF and leukotriene B [10,11]. Chronic inflammation on the other hand, is characterized by monocyte and lymphocyte infiltration, fibroblasts proliferation, connective tissue formation, release of reactive oxygen and nitrogen species, and protease secretion [12]. Together, these mediators give rise to diverse pathways in the inflammatory process, offering an array of targets for pharmacological intervention.

Xylopia aethiopica (Dunal) A. Rich. (Annonaceae) is a tropical evergreen plant with green fruits and aromatic seeds [13]. Traditionally, it is used in the form of the dried fruit decoction to treat bronchitis, asthma, arthritis and rheumatism in Ghana, Nigeria and Cameroon [14]. Our earlier findings have established its anti-inflammatory activity in both acute and chronic inflammation in murine subjects [15,16]. Phytochemical analysis and high-performance liquid chromatography (HPLC) fingerprint of *Xylopia aethiopica* fruit extract revealed the presence of kaurenoic acid and xylopic acid which are diterpenes known as kauranes [17]. These kauranes play a major role in the observed biologic effect of the plant [18,19]. The current study therefore aims at expounding some of the mechanisms involved in the anti-inflammatory action of the fruit extract of *Xylopia aethiopica* by investigating the activity of the extract on some prominent mediators of inflammation.

METHODOLOGY

Preparation of Plant Extract

Hydro-ethanol extraction was carried out as described by Obiri et al. [15]. *Xylopia aethiopica* fruits were obtained from the university botanical garden (6°41'7" N 1°33'48" W) in Kumasi between September and November of 2019. Identification of the fruit was made at the Department of Herbal Medicine, KNUST, voucher specimen number assigned (No. FP/09/77) and sample deposited at the herbarium of the department. The *Xylopia aethiopica* fruits were dried in open air and 3 kg of the dried material milled employing a heavy-duty blender. The pulverized material was macerated with 5 L, 70% w/v ethanol for 24 h. The filtrate was concentrated using a rotary evaporator and additionally dried in an oven to produce a 167 g solid extract. The obtained extract from the dried fruit was emulsified employing Tween-80 and normal saline and referred to as XAE.

Experimental Animals

ICR mice (25 – 30 g) were procured from the Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana. The animals were housed in a temperature-controlled room in the Department of Pharmacology, KNUST animal house facility and had free access to chow and water. Animals were randomly group after a period of acclimatization. Animals were humanely handled throughout the study with ethical approval granted by the Department's ethics committee for the study.

Chemicals and reagents

Granisetron was purchased from Roche, Basel, Switzerland; diclofenac from Novartis Int AG, Basel, Switzerland; chlorpheniramine was procured from DWD Pharmaceuticals Ltd, Mumbai, India; histamine, serotonin hydrochloride, bradykinin acetate salt and prostaglandin E_2 were obtained from Sigma-Aldrich (St Louis, USA).

Histamine-induced paw oedema

Oedema was induced as described by Mazumder and colleagues [20]. Briefly, induction of paw oedema in mice was made by subplantar injection of 0.1 mg histamine after prophylactic or therapeutic administration of normal saline (1 ml kg^{-1} p.o.), chlorpheniramine (10 mg kg^{-1} p.o.) or XAE (30, 100, 300 mg kg^{-1} p.o.). Induced oedema (as paw thickness) was measured every 30 min for 3 h. The maximal oedema and total oedema were then calculated using the equation:

$$\% \text{ change in paw thickness} = 100 \times \frac{T_t - T_i}{T_i}$$

Where, Ti is paw thickness before phlogenic agent injection

Tt is paw thickness at time T.

The recorded paw thicknesses were individually normalized as percentage change from paw thickness before subplantar injection and averages computed. The total oedema was obtained in arbitrary determination as the area under the time course curve (AUC) and percentage oedema inhibition was established using the relation:

$$\% \text{ 100 inhibition of oedema} = \frac{AUC_{(control)} - AUC_{(treatment)}}{AUC_{(control)}} \times 100$$

Serotonin-induced paw oedema

Briefly, subplantar administration of 0.1 mg of serotonin was made as the phlogenic agent. Prophylactic or therapeutic administration of normal saline (1 ml kg^{-1} p.o.), granisetron (100 μg kg^{-1} p.o.) or XAE (30, 100, 300 mg kg^{-1} p.o.) were made to respective groups and paw thickness measured with electronic calipers every 30 min for 3 h. The maximal oedema and total paw oedema responses were determined as explained under histamine-induced paw oedema [20].

Bradykinin-induced paw oedema

Mice in respective groups were pre-treated with 5 mg kg^{-1} captopril subcutaneously. The subsequent process is as portrayed in histamine-induced paw oedema section. Briefly, subplantar administration of 1 μg of bradykinin was made. Prophylactic or therapeutic administration of normal saline (1 ml kg^{-1} p.o.) or XAE (30, 100, 300 mg kg^{-1} p.o.) were made to respective groups and paw thickness measured with calipers every 30 min for 3 h. The maximal oedema and total paw oedema responses were determined as described under histamine-induced paw oedema [20].

Prostaglandin E_2-induced paw oedema

The process is as described in section on histamine-induced paw oedema. Briefly, subplantar injection of 1 nM of prostaglandin E_2 was made. Prophylactic or therapeutic administration of normal saline (1 ml kg^{-1} p.o.), diclofenac (0.93 mg kg^{-1} p.o.) or XAE (30, 100, 300 mg kg^{-1} p.o.) were made to respective groups and paw thickness measured with calipers every 30 min for 2.5 h. The maximal oedema and total paw oedema responses were determined as explained above [20].

Statistical analysis

All obtained data are presented as the mean ± SEM (n = 5). Two-way ANOVA followed by Bonferroni's test were used to analysed the time-course curves while one-way ANOVA followed by Dunnett's post hoc test was employed in analysing the AUCs. Graphs were plotted with GraphPad Prism version 8.00 for MacBook (GraphPad, San Diego, CA)

RESULTS AND DISCUSSION

During infection or tissue damage, the immune system reacts through a sequence of organized events involving molecular, cellular and physiological alterations, in an attempt to eliminate the noxious stimuli and restore homeostasis [4]. This chain of immune responses is coordinated by inflammatory mediators, produced and released by blood, resident inflammatory cells and damaged tissue in response to the noxious stimulus [21,22]. The release of these mediators from mast cells, neutrophils, monocytes/macrophages, platelets, fibroblasts, smooth muscle cells and endothelial cells makes the acute inflammatory response immediate, adaptive and specific [4]. However, the process is sometimes dysregulated resulting in detrimental effects such as observed in septic shock [2]. These mediators can act together and give rise to diverse pathways in the inflammatory process, and can offer a variety of targets for pharmacological intervention.

Histamine–induced paw oedema

Mast cell activation in response to noxious stimuli increases synthesis and release of vasoactive amines such as histamine, which promotes vasodilation and increases vascular permeability, vascular hydrostatic pressure and efflux of intravascular fluid into interstitial space. Thus, resulting in the development of oedema [23,24]. It was observed that XAE (30, 100, 300 mg kg^{-1}) when administered before the induction of the histamine-induced paw oedema caused the mean maximal swelling attained at 60 min to be reduced to 76.80 ± 11.44%, 79.36 ± 10.95% and 49.57 ± 2.29% respectively relative to the control response of 92.14 ± 6.44% (Fig 1A). The total paw swellings induced over the 3 h (measured as the area under the time course curve, AUC) were also significantly suppressed to 352.38 ± 47.72%, 328.42 ± 51.55% and 204.44 ± 6.70% respectively from 490.10 ± 28.75% (Fig 1B).

In the therapeutic protocol, XAE (30 – 300 mg kg^{-1}) suppressed the mean maximal swelling attained at 60 min to 38.37 ± 3.41%, 40.88 ± 4.94% and 28.46 ± 5.56% respectively relative to the inflamed control response of 61.63 ± 4.55% (Fig 1C). The total paw swellings induced over the 3 h were also significantly suppressed to 156.66 ± 14.80%, 155.70 ± 15.33% and 143.94 ± 20.00% respectively from 283.54 ± 17.73% (Fig 1D).

The inhibition of paw oedema by XAE is believed to be mediated via the inhibition of histamine release, and/or interference of histamine's activity on H_1 and H_4 receptors. Antagonism of the former leads to an inhibition of histamine-mediated vasodilation, while H_4 antagonism results in inhibition of propagation of the inflammatory response [24,25].The extract thus inhibits acute inflammatory response mediated by this vasoactive amines, that is mainly characterized by the development of interstitial oedema owing to exudation of fluid and plasma proteins [26].

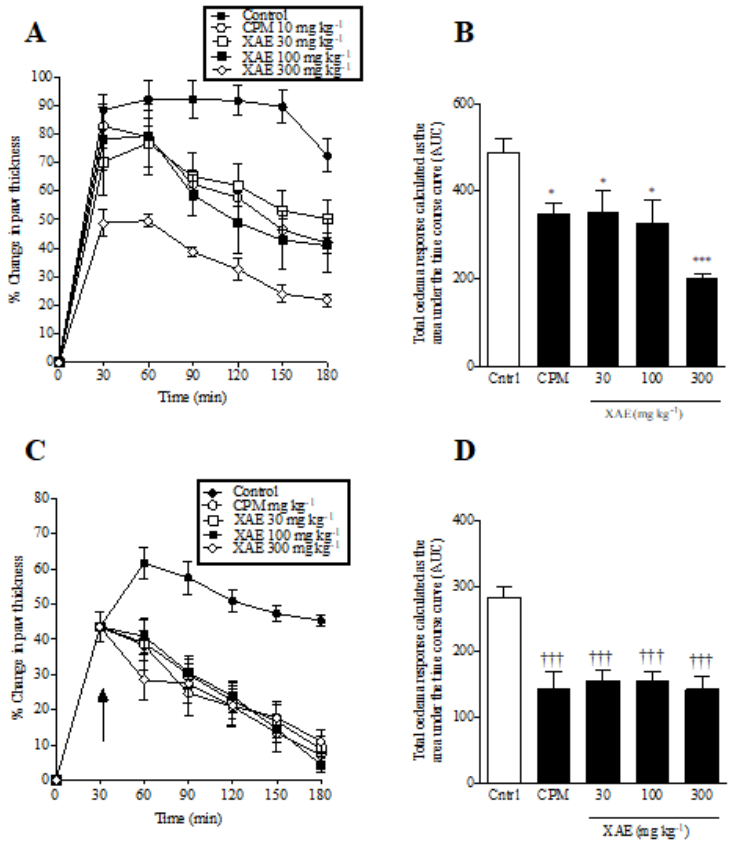

Figure 1. Effect of *Xylopia aethiopica* on histamine-induced paw oedema in mice. Oedema was monitored at 30 min intervals over 3 h as percentage increase in paw thickness (A and C). Total oedema induced during the 3 h was calculated as area under the time course curves, AUC (B and D). *P < 0.04, ***P < 0.0001, †††P< 0.0004 when compared with vehicle-treated control mice. Arrow indicates point of Chlorpheniramine or XAE administration

Serotonin - induced paw oedema

The increased synthesis and release of vasoactive amines such as serotonin upon mast cell activation promotes vasodilation, capillary permeability and plasma exudation with subsequent development of oedema [23,24]. XAE (30, 100, 300 mg kg^{-1}) when administered before the induction of the serotonin-induced paw oedema caused the mean maximal swelling attained at 90 min to be reduced to 50.74 ± 5.30%, 39.81 ± 3.16% and 34.66 ± 3.35% respectively relative to the inflamed control response of 71.38 ± 5.61% (Fig 2A). The total oedema response induced over the 3 h were also significantly suppressed to 230.58 ± 21.08% and 190.92 ± 9.96% in the 100 mg kg^{-1} and 300 mg kg^{-1} XAE-treated groups respectively from 363.62 ± 34.54% (Fig 2B).

XAE (30, 100, 300 mg kg^{-1}) when given therapeutically suppressed the mean maximal swelling attained at 90 min to 44.00 ± 6.60%, 36.34 ± 3.47% and 30.31 ± 8.94% respectively relative to the inflamed control response of 71.38 ± 5.61% (Fig 2C). The total paw swellings induced over the 3 h were also dose-dependently and significantly suppressed to 233.48 ± 35.49%, 195.44 ± 18.58% and 153.22 ± 24.10% respectively from 344.94 ± 28.30% (Fig 2D).

Inhibition of serotonin-induced paw oedema indicates that XAE mitigates the inflammatory process, possibly via inhibition of peripheral 5-hydroxytryptamine 2 ($5\text{-}HT_2$) receptor family-mediated decrease in vascular resistance and increase in intravascular hydrostatic pressure [27]. These observed effects on histamine and serotonin are supported by the findings that, xylopic acid, isolated from XAE inhibits paw oedema induced by these mediators [19].

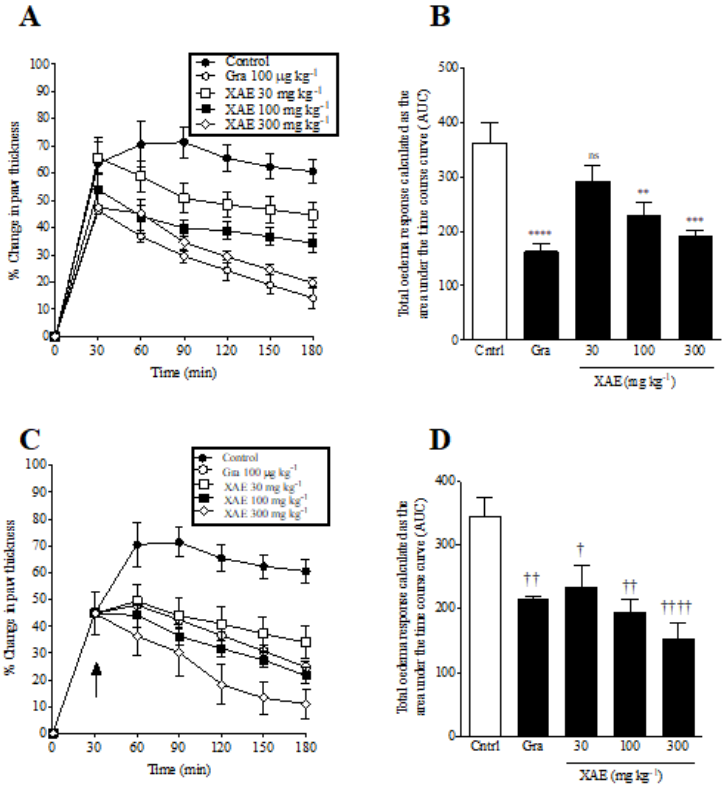

Figure 2. *Effect of Xylopia aethiopica* on serotonin-induced paw oedema in mice. Oedema was monitored at 30 min intervals over 3 h as percentage increase in paw thickness (A and C). Total oedema induced during the 3 h was calculated as area under the time course curves, AUC (B and D). **P = 0.0023, ***P = 0.002, ****P < 0.0001, nsP > 0.05, †P = 0.015, ††P < 0.0045, ††††P < 0.0001 when compared with vehicle-treated control mice. Arrow indicates point of Granisetron or XAE administration.

Bradykinin–induced paw oedema

Plasma mediators such as kinins, following their activation by selective proteolytic enzymes, initiate and amplify the inflammatory response via modulation of specific receptors and signaling pathways. Their activities on these targets control the nature and duration of tissue response to noxious stimuli [5]. One such mediator is the nonapeptide, bradykinin, released during the early stages of tissue damage by action of kallikreins on kininogens, the glycoprotein precursors [28]. In bradykinin-induced paw oedema, bradykinin acts on B_2 receptors which increases vascular permeability via nitric oxide-induced relaxation of perivascular smooth muscles [4]. In addition, kinins upon activation, up-regulate immune activation to enhance the inflammatory response. Kinins

also induce pain either directly or indirectly through the enhancement of the expression of PGE_2 and PGI_2 by endothelial cells and fibroblasts, and the production of tachykinins, ultimately aiding inflammation and subsequent tissue damage [29,30].

Results from the study shows that XAE (30, 100, 300 mg kg^{-1}) when administered before the induction of the bradykinin-induced paw oedema caused the mean maximal swelling attained at 90 min to be reduced to 18.13 ± 5.84%, 24.26 ± 2.88% and 14.31 ± 5.85% respectively relative to the mean inflamed control response of 45.22 ± 5.38% (Fig 3A). The total oedema induced over the 3 h were also significantly suppressed to 96.45 ± 25.66%, 106.11 ± 16.30% and 72.74 ± 20.35% respectively from 186.20 ± 23.58% (Fig 3B). Therapeutically, XAE (30, 100, 300 mg kg^{-1}) suppressed the mean maximal swelling attained at 90 min to 42.53 ± 6.60%, 36.42 ± 3.19% and 30.94 ± 9.03% respectively relative to the mean inflamed control response of 70.40 ± 4.74% (Fig 3C). The total oedema induced over the 2.5 h were also dose-dependently and significantly suppressed to 186.38 ± 24.84%, 170.06 ± 14.07% and 137.82 ± 20.91% from 279.40 ± 24.50% respectively (Fig 3D). Thus, *X. aethiopica* extract inhibits bradykinin-mediated inflammatory response possibly through inhibition of bradykinin B_2 receptor activity.

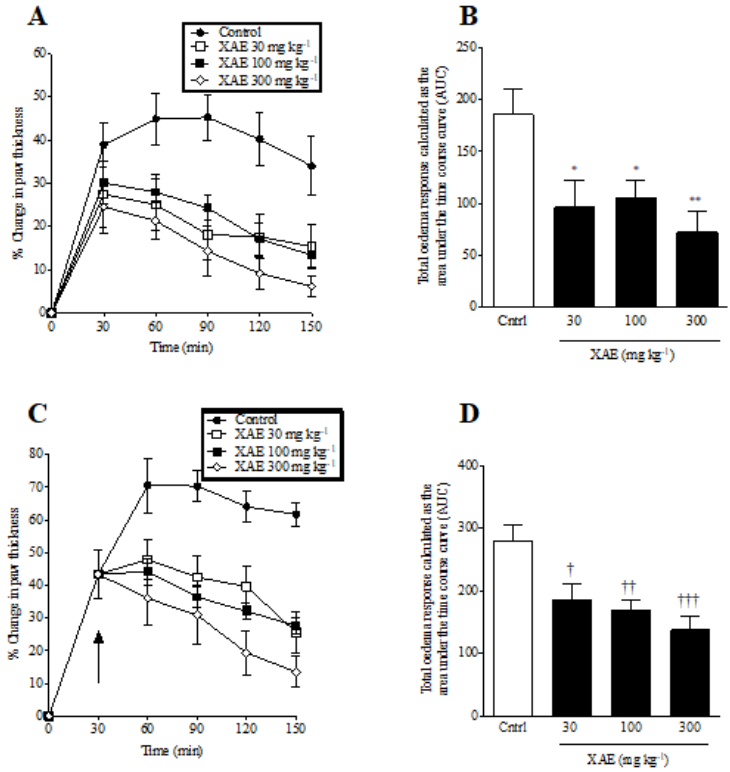

Figure 3. *Effect of Xylopia aethiopica* on bradykinin-induced paw oedema in mice. Oedema was monitored at 30 min intervals over 3 h as percentage increase in paw thickness (A and C). Total oedema induced during the 2.5 h was calculated as area under the time course curves, AUC (B and D). *P < 0.049, **P < 0.0054, †P = 0.02, ††P = 0.007, †††P = 0.007 when compared with vehicle-treated control mice. Arrow indicates point of XAE administration.

Prostaglandin E_2-induced paw oedema

During tissue injury, phospholipids and fatty acids from plasma membranes are metabolized by inflammatory cells and injured tissues into mediators and homeostatic regulators [31]. Unlike majority of inflammatory mediators, eicosanoids are not stored in granules after transcription and mRNA translation, but are produced from arachidonic acid metabolism following the inflammatory stimulus prior to enrollment of leukocytes and the infiltration of immune cells [4]. One such eicosanoid is Prostaglandin E_2 (PGE_2) which is very abundant and also plays critical role in mediating the cascade of events that culminates in classic inflammation signs, notably, arterial dilatation and increased vascular permeability and subsequent oedema [32].

Prophylactically, XAE (30, 100, 300 mg kg^{-1}) caused the mean maximal swelling attained at 60 min to be reduced to 34.53 ± 8.91%, 20.61 ± 7.29% and 18.32 ± 1.00% respectively compared with inflamed control response of 48.60 ± 3.68% (Fig 4A). The total oedema induced over the 2.5 h were also dose-dependently and significantly suppressed by 92.54 ± 35.49%, 77.10 ± 21.52% and 40.57 ± 3.01% respectively from 179.60 ± 21.87% (Fig 4B). In the therapeutic model, XAE (30, 100, 300 mg kg^{-1}) treatment suppressed the mean maximal swelling attained at 60 min to 39.66 ± 5.06%, 39.95 ± 3.51% and 27.93 ± 5.18% respectively compared with the inflamed control response of 61.63 ± 4.55% (Fig 4C) with the total oedema induced over the 2.5 h were also significantly suppressed to 149.92 ± 17.58%, 150.23 ± 15.25% and 131.71 ± 19.92% respectively from 241.82 ± 15.26% (Fig 4D).

The ability of XAE to inhibit paw oedema was partly mediated via interference of PGE_2 receptor activity due to the presence of xylopic acid [33]. As observed in this study, XAE inhibits the action of PGE_2 on E prostanoid receptors 1–4 (EP1–4) which may subsequently inhibit the activation of the rhodopsin-like G protein-coupled receptors [31,34]. In addition to the presence of xylopic acid, these findings may also be attributed to the presence of kaurenoic acid, another kaurane diterpene found in XAE. This kaurene diterpene is known from earlier findings to inhibit the inflammatory response via suppression of PGE_2 synthesis [35].

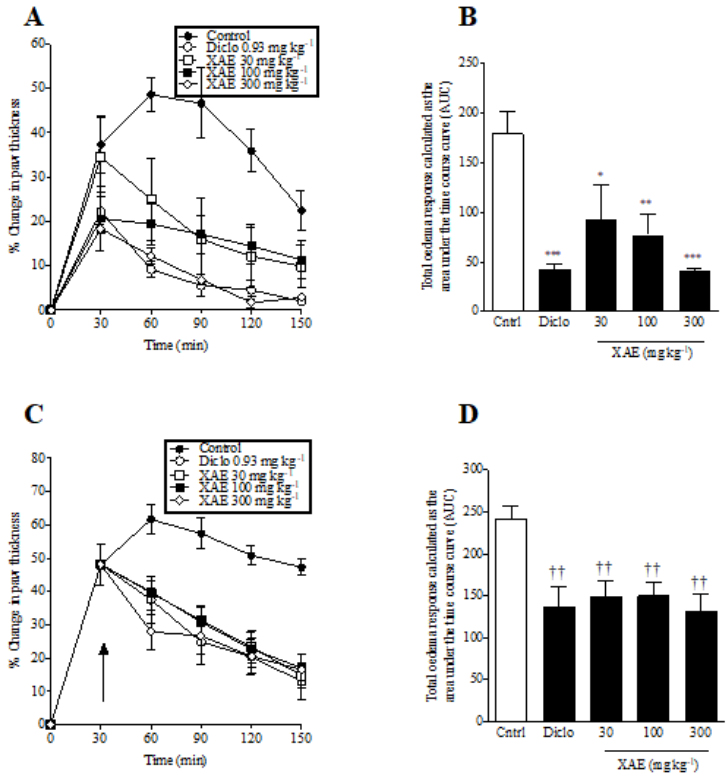

Figure 4. *Effect of Xylopia aethiopica* on prostaglandin E2-induced paw oedema in mice. Oedema was monitored at 30 min intervals over 2.5 h as percentage increase in paw thickness (A and C). Total oedema induced during the 2.5 h was calculated as area under the time course curves, AUC (B and D). *P = 0.03, **P = 0.01, ***P < 0.0007, ††P < 0.008 when compared with vehicle-treated control mice. Arrow indicates point of Diclofenac or XAE administration.

STATEMENT OF ETHICS

In accordance with internationally accepted principles for laboratory animal use and care (EEC Directive of 1986: 86/609 EEC), the animals were considerately handled throughout the experiment. Additionally, all animal experiments were approved by the Department Ethics Committee [Approval No. DPEC/FPPS/18/009. Valid from 1st June 2018 to 31st May 2019].

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTION

NO, Conceived the idea of the experimental design and analysed the obtained the data; OKY, Performed the experiment and drafted the manuscript.

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