

Preliminary Evaluation of the Acute and Sub-Acute Anti-Inflammatory Activities of Aqueous and Butanol Leaf Fractions of *Olox subscorpioidea* Oliv. (Olacaceae).

Odoma Saidi,^{1,2*} Zezi A. Umar,³ Danjuma N. Muhammed,³ Ahmed Abubakar⁴ Magaji, M. Garba,³ and Munir A. Sani.⁵

¹Department of Pharmacology and Therapeutics, Kogi State University, Anyigba, Nigeria.

²Department of Pharmacology and Toxicology, Kampala International University, Uganda.

³Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria.

⁴Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria.

⁵Department of Veterinary Surgery and Radiology, Ahmadu Bello University, Zaria, Nigeria.

Abstract

Olox subscorpioidea (Oliv.) leaf is widely used as a traditional remedy for pain, reduction of small tumors, edema, painful swellings, and other inflammatory conditions. Its anti-inflammatory potential in experimental animals has been documented. Furthermore, an anti-inflammatory assay guided fractionation showed that the aqueous and butanol leaf fractions were the most active. This study is aimed at investigating further, the acute and sub-acute anti-inflammatory potentials of the aqueous and butanol leaf fractions in mice and rats.

Carrageenan induced paw edema in rats, xylene induced ear edema in mice, and cotton pellet induced granuloma in rats were the models employed for the studies. For each of the methods, four randomly-selected groups of animals (n=5/6) were orally administered with distilled water (1 ml/kg), aqueous or butanol fraction (1,000 mg/kg) and standard drugs (acetylsalicylic acid [ASA] 300 mg/kg or dexamethasone, 1 mg/kg).

The aqueous and butanol fractions each showed significant ($p < 0.01$) inhibition of ear swelling, the fractions also significantly ($p < 0.05$, $p < 0.01$) decreased the paw edema, and significantly ($p < 0.01$) inhibited the granuloma formation.

The result of the study suggests that aqueous and butanol leaf fractions of *Olox subscorpioidea* are effective against acute and sub-acute inflammation.

Keywords: Carrageenan, Cotton-pellet granuloma, Inflammation, *Olox subscorpioidea*, Xylene.

*Corresponding Author, (email: odoma.s@ksu.edu.ng, Tel: +2348027547778)

INTRODUCTION

Inflammation is the body's immune system's response to infection and injury [1]. It helps to repair and heal damaged tissues, and to defend the body against external invaders in the form of viruses and bacteria [2]. Acute inflammation is a short term response, characterized by redness, swelling, heat, pain, and loss of function; as it returns the body to a stable system. But, when an abnormal condition is sustained, it leads to chronic inflammation [1]; and this has been implicated in almost all chronic diseases, such as cancer, cardiovascular diseases, arthritis, inflammatory bowel syndrome, atherosclerosis and autoimmune diseases [3-5]

Steroidal and non-steroidal anti-inflammatory drugs are among the most commonly prescribed drugs in the management of both acute and chronic inflammatory conditions [6]. However, the currently used agents are prone to evoking serious adverse reactions [7]. Side effects such as gastrointestinal tract (GIT) irritation, blood disorders, liver damage, renal damage, tinnitus, and hypersensitivity reactions, have significantly limited their use [8]. This justifies the search for new anti-inflammatory substances from the array of available natural

products; with fewer side effects and better efficacy.

Medicinal plants and their by-products have been used for ages to treat different kinds of acute and chronic inflammatory diseases. *Olex subscorpioidea* is one of such medicinal plants and it has wide patronage. It is a shrubby plant, a member of the family Olacaceae, and distributed widely in African tropics with numerous uses. Traditionally, the root has been used for the management of cancer, pregnancy-associated fat, rheumatism, and constipation [9, 10]. The leaf has also been used in the management of swelling, pain, and yellow fever [10, 11]. The crude methanol extract, aqueous, butanol, and hexane leaf fractions were reported to possess phytochemicals such as alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, steroids, and tannins [11, 12].

Previously, the anti-inflammatory activity of the methanol crude leaf extract of *O. subscorpioidea* has been reported by our group [11]. We also reported that the aqueous and butanol leaf fractions were more efficacious than other fractions in a bioactivity guided fractionation study [12]. The current study is aimed at assessing the acute and sub-acute anti-inflammatory

activities of the aqueous and butanol leaf fractions. Acute anti-inflammatory activity was studied in carrageenan-induced paw edema in rats and xylene induced ear edema models in mice. The cotton pellet induced granuloma in rats model was used to study the sub-acute anti-inflammatory activity.

MATERIALS AND METHODS

Collection and Identification of Plant.

O. subscorpioidea leaves were collected from a farm in Anyigba, Kogi State, North Central Nigeria. The identification was done by Dr. E. I. Aigbokhan, a plant taxonomic with the Department of Biological Sciences, Kogi State University, Anyigba, Kogi State, Nigeria. A voucher specimen (KSUH-277-2013-01) was deposited in the herbarium for future reference.

Extraction and Fractionation.

The extraction and fractionation were carried out according to a standard method [13]. The leaves of *O. subscorpioidea* were shade dried for several days till constant weight was achieved. The dried leaves were pulverized with mortar and pestle to achieve a fine powder. About one thousand grams (1kg) of the powder was extracted exhaustively with aqueous-methanol (20-80, v/v) using a soxhlet apparatus, the extract was then concentrated on a water bath (50°C). About 100g of the methanol extract was suspended

in water and successively partitioned with hexane, ethyl-acetate, and butanol to afford the corresponding fractions. The residual aqueous and the butanol fractions were removed by placing fractions, individually, on a water bath (50°C). Each fraction was sealed in a bottle container and stored in a desiccator before use. Solutions of each of the fractions were freshly prepared with distilled water before each study.

Laboratory Animals

Adult Wistar Rats (140-170g) and Balb/c Mice (20-30g) were obtained from the Animal House facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University (ABU), Nigeria. The animals were kept under standard environmental conditions and fed with standard diet and water ad libitum. The approval for the experiments was given by the Ethical Committee of the Department of Pharmacology and Therapeutics, ABU, Nigeria (protocol number: DAC/IW-OT/137/14). The experiments were carried out following the criteria outlined in the Guide for the Care and Use of Laboratory Animals [14]

Carrageenan-induced edema in rats

Randomly-selected four groups of rats (n=6) were orally administered with distilled water (1 ml/kg), aqueous or butanol fraction (1,000

mg/kg) and acetylsalicylic acid (ASA, 300 mg/kg). Sixty minutes post-treatment, each rat was injected with 0.1 ml of 1% carrageenan into the plantar surface of rat's right hind paw [15]. The paw diameter was measured using the cotton thread method [16] at times 0, 1, 2, 3, and 4 hours after the injection of carrageenan. The increase in paw diameter (paw edema volume) for each rat was calculated as the difference in paw diameter before carrageenan injection and after carrageenan injection at each time interval, while the percent inhibition of edema was calculated for each group with respect to its vehicle-treated control group by the following relationship:

$$\frac{\text{Mean increase in paw volume of control} - \text{Mean increase in paw volume of treated}}{\text{Mean increase in paw volume of control}} \times 100$$

Xylene induced ear edema

Randomly selected four groups of mice (n=6) were orally administered with distilled water (10 ml/kg), aqueous or butanol fraction (1,000 mg/kg), and dexamethasone (1 mg/kg). One hour post-treatment, 30 µL of pure xylene was applied to the inner surface of the mouse's right ear to induce edema [17]. Sixty minutes post edema induction, each mouse was euthanized under chloroform anesthesia, and both ears were immediately cut off and weighed on an analytic balance.

The difference between the intact (left) ear and the xylene-insulted (right) ear was recorded. Percentage inhibition of the ear edema was calculated relative to the left ear

Cotton pellet-induced granulation formation in rats (sub-acute inflammation)

Male rats were divided into four groups (n=5). Cotton pellet granuloma was produced by the method previously described by Winter and Porter [18]. The groin region of the rat was shaved under aseptic conditions, then, through a single needle incision, a sterile pre-weighed cotton pellet (50 mg) soaked in distilled water (0.2 mL) containing streptomycin (0.13 mg) and penicillin (0.1 mg), was implanted subcutaneously in the groin under ketamine (15 mg/kg) anesthesia.

Distilled water (1 ml/kg), aqueous or butanol fraction (1,000 mg/kg), or ASA (300 mg/kg) were administered orally for nine (9) consecutive days from the day of cotton pellet implantation. On day 10, the cotton pellet was dissected out, weighed, and dried at 60 °C until the weight remained constant. The difference in the weight of the cotton pellet before and after implantation was recorded and considered as granuloma tissue deposit. The difference in the body weights of the rats for the first and the last day of the experiments was also recorded

Statistical Analysis

Values were expressed as mean \pm standard error of the mean (mean \pm SEM). The difference in mean was analyzed using one way and repeated measure ANOVA followed by Tukey's post-hoc test for Multiple Comparison using the Graph Pad Prism (statistical) software, version 7. The differences between means were considered significant when $p \leq 0.05$.

RESULTS

Carrageenan-induced edema in rats

The fractions and standard drug significantly ($p < 0.05$, $p < 0.01$, $p < 0.001$) decreased paw edema due to carrageenan (Figure 1a). The standard drug had a better anti-inflammatory activity compared to the fractions, with a maximum inhibition of 60.04%, at the 4th hour. This is followed by the aqueous fraction with a maximum inhibition of 44.16% at the 4th hour. The butanol fraction possesses the least anti-inflammatory activity with a maximum inhibition of 41.55% at the 4th hour (Figure 1b).

Xylene induced ear edema

A significant ($p < 0.01$) inhibition of ear edema was observed in all the treated animals (Figure 2). The butanol fraction had the highest inhibition (34.9%) of edema, followed by the standard drug (32.66%) then the aqueous fraction (27.96%).

Sub-acute inflammation

The results of the sub-acute inflammatory test are shown in Table 1. The plant fractions and ASA demonstrated significant inhibitory activity on the weight of granuloma. The butanol fraction and ASA had significant ($p < 0.001$) inhibitory effects on the granuloma formation compared to the aqueous fraction ($p < 0.01$). The butanol fraction had the highest inhibitory effect with 42.49% and 61.39%, followed by ASA with 41.35% and 50.63% then the aqueous fraction with 23.64% and 35.44% on the wet and dried granuloma respectively.

Effects of the extracts on rats' body weight

The effects of the test drugs and the standard drug are presented in Table 2. There is no statistically significant difference in weight gain in the groups treated with the fractions when compared with the negative control group. But, the group treated with the standard drug had a significant ($p < 0.05$) reduction in the weight gain when compared with the negative control.

DISCUSSION

Previously we reported that the crude methanol leaf extract, aqueous, butanol, and hexane leaf fractions of *Olox subscorpioidea* possess anti-inflammatory activities [11, 12]. Currently, the aqueous and butanol leaf fractions are being investigated for acute and sub-acute anti-inflammatory activities using

the dose with the best anti-inflammatory response (1,000 mg/kg) from our previous study.

Cotton pellet granuloma study is a sub-acute inflammation model that is widely used to determine the proliferative constituents of chronic inflammation [18]. The weight of the wet cotton pellets is related to proliferative material and the weight of the dry pellets is related to the amount of granulomatous tissue [19]. Cotton pellet granuloma tends to mimic the chronic inflammation caused by non-microbial and non-degradable pathogens. In this model, a non-immunological type of inflammation is observed and it is mediated by the activation of inflammatory mediators such as kinins [20]. Kinins cause both vasodilatation and increase vascular permeability in the early stages of inflammation [21]. NSAIDs exact their antiproliferative activity by decreasing granuloma tissue, preventing the occurrence of the collagen fiber, and suppressing mucopolysaccharides [19]. Thus, the results of the present study suggest the fractions' ability to inhibit sub-acute inflammation.

The reduction in weight gain caused by acetylsalicylic acid may be due to the ability of salicylates to rapidly reduce circulating triglycerides and or free fatty acids in obese rats and humans with type 2 diabetes by the

activation of AMP-activated protein kinase (AMPK) [22].

The xylene ear edema model distinguishes non-steroidal anti-inflammatory drugs (NSAIDs) from steroidal anti-inflammatory agents [7]. It is sensitive to steroids and less sensitive to NSAIDs [23]. After the topical application of xylene, signs of acute inflammation are detected by severe vasodilation, edema, changes of the skin, and infiltration of inflammatory cells which causes the increased thickness of the ear tissues [23, 24]. Steroids exert their anti-inflammatory effect by inducing the synthesis of a group of proteins (lipocortins). Lipocortins suppress the activation of phospholipase A2, thereby decreasing the release of arachidonic acid and the production of pro-inflammatory eicosanoids [25]. Thus, the results of the present study suggest that the plant might have inhibited the acute inflammation induced by xylene by decreasing the release of arachidonic acid and the production of pro-inflammatory eicosanoids.

Carrageenan induced rat's paw edema is the most widely used animal model for evaluation of anti-inflammatory property of medicinal plants [15], as it is not known to be antigenic and is devoid of apparent systemic effects [23]. Carrageenan induced edema

gives a biphasic response. The first phase response is believed to be due to the release of serotonin, histamine, and bradykinin on vascular permeability and the second phase response is due to the overproduction of prostaglandin in tissues [26]. The ability of the fractions to inhibit rats' paw edema may be due to inhibition of inflammatory mediators such as prostaglandins, histamine, and serotonin.

Based on these findings, it may be inferred that the aqueous and butanol leaf fractions of *O. subscorpioidea* both possess acute and sub-acute anti-inflammatory activities. These results further confirm the folkloric use of the plant in the management of inflammatory diseases. Further investigations are anticipated to identify the active components responsible for its observed pharmacological activities.

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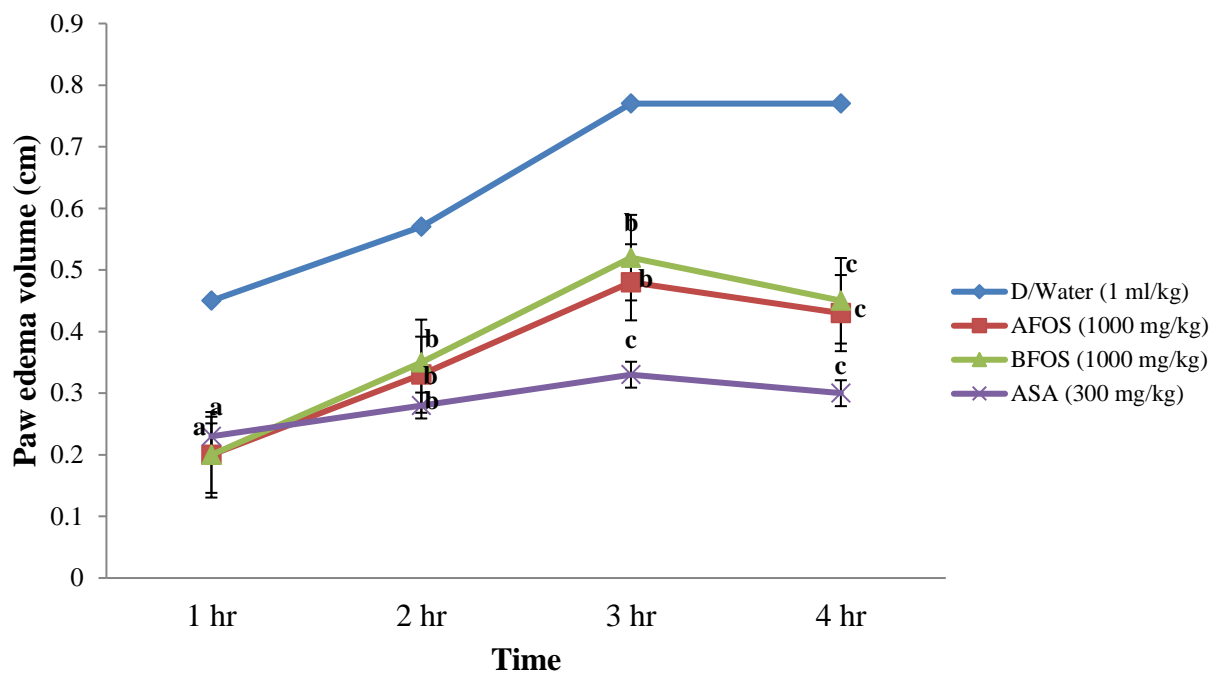


Figure 1a: Effect of aqueous and butanol leaf fractions of *Olax subscorpioidea* on carrageenan induced inflammation. a = $p < 0.05$, b = $p < 0.01$, c = $p < 0.001$ versus distilled water. AFOS (aqueous fraction), BFOS (butanol fraction) and ASA (acetylsalicylic acid), n=6.

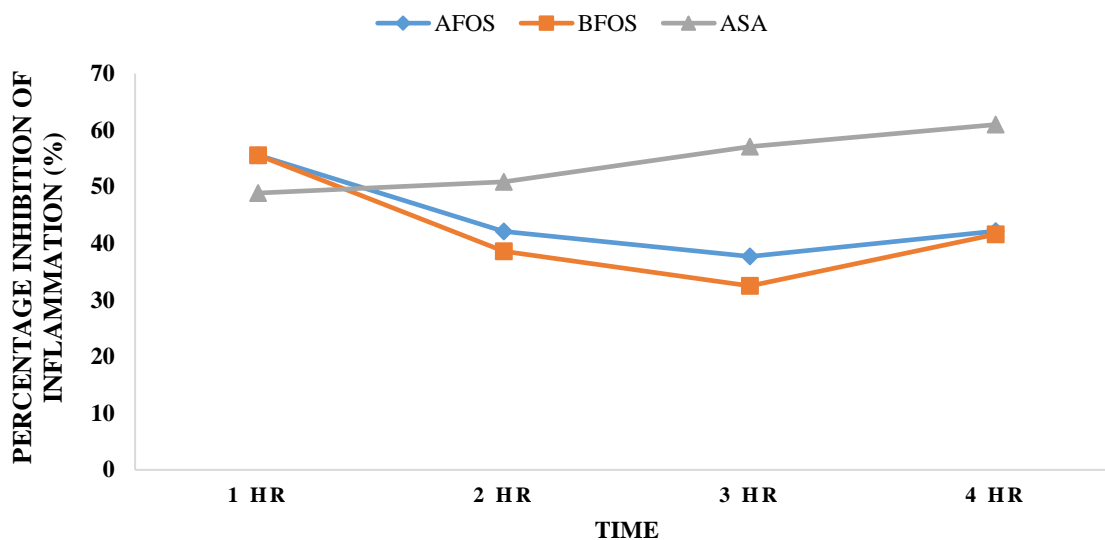


Figure 1b: Percentage inhibition of inflammation by the leaf fractions of *Olox subscorpioidea* and the standard drug. AFOS (aqueous fraction), BFOS (butanol fraction) and ASA (acetylsalicylic acid), n=6.

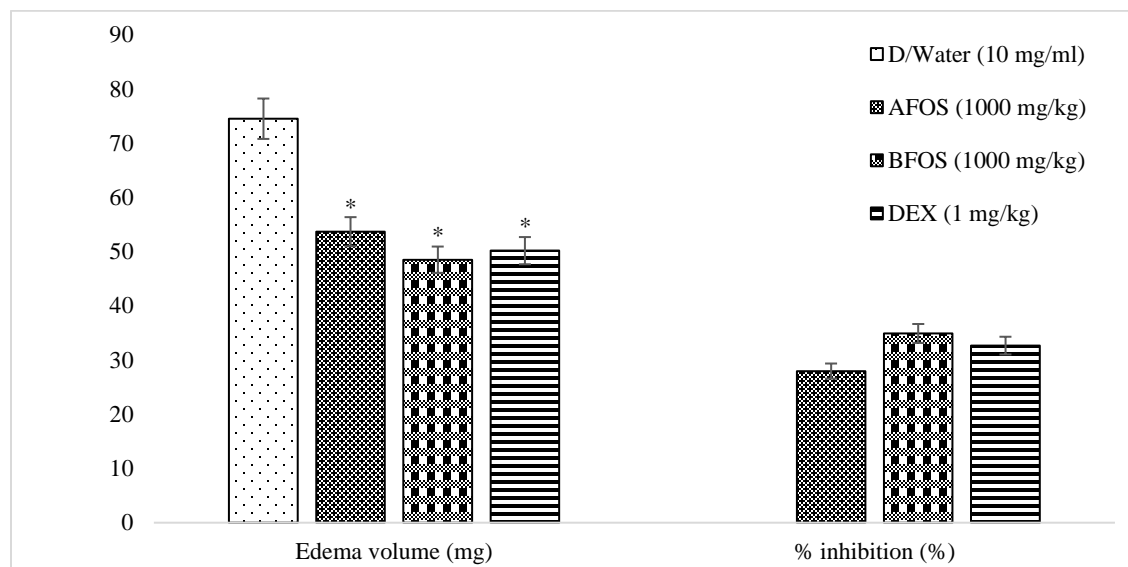


Figure 2: Effect of aqueous and butanol leaf fractions of *Olox subscorpioidea* on xylene-induced ear edema in mice. * $p < 0.01$ versus distilled water. AFOS (aqueous fraction), BFOS (butanol fraction) and Dex (dexamethasone) n=6.

Table 1: Effect of aqueous and butanol leaf fractions of *O. subscorpioidea* on sub-acute inflammatory model in rats

	Wet granuloma weight (mg)	Percentage inhibition of granuloma (%)	Dry granuloma weight (mg)	Percentage inhibition of granuloma (%)
Distilled water	438.20 ± 14.87		63.20 ± 2.46	
AFOS 1000 mg/kg	334.60 ± 18.42*	23.64	40.80 ± 2.18*	35.44
BFOS 1000 mg/kg	252.00 ± 16.30**	42.49	24.40 ± 4.62**	61.39
ASA 300 mg/kg	257.00 ± 16.9**	41.35	31.20 ± 4.32**	50.63

* $p < 0.01$, ** $p < 0.001$ versus distilled water. AFOS (aqueous fraction), BFOS (butanol fraction) and ASA (acetylsalicylic acid), n=5.

Table 2: Effect of aqueous and butanol leaf fractions of *O. subscorpioidea* on rats' body weight

Group	Dose (mg/kg)	Body Weight (g)		
		Initial	Final	Gain
Distilled water		143.4 ± 5.33	167.2 ± 4.66	23.8 ± 3.68
AFOS	1,000	149.4 ± 8.90	166.0 ± 9.72	16.6 ± 2.11
BFOS	1,000	146.8 ± 10.15	165.4 ± 10.26	18.6 ± 4.46
ASA	300	145.2 ± 14.47	155.2 ± 5.40	10.0 ± 2.59*

* $p < 0.05$ versus distilled water. AFOS (aqueous fraction), BFOS (butanol fraction) and ASA (acetylsalicylic acid), n=5.