Antinociceptive and anti-inflammatory effects of *Olax subscorpioidea* Oliv. (Olacaceae) leaf extract in rodents: possible mechanisms of antinociceptive action

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ABSTRACT

Background: *Olax subscorpioidea* Oliv. (Olacaceae) is a medicinal plant commonly used in traditional medicine to relieve pain in inflammatory processes.

Objective: In the present study, the *in vivo* anti-inflammatory and antinociceptive effects of aqueous leaf extract of *Olax subscorpioidea* (OLS) and its possible mechanisms of action were investigated.

Methods: *OLS* (50, 100, 200, and 400 mg/kg, p.o.) was administered 1 h before injection of 0.6%v/v acetic acid (10 ml/kg, i.p.), 1%$^{v}/_{v}$ formalin (20 µl) or tail flick tests to evaluate antinociceptive effect while acute anti-inflammatory effect was investigated using xylene-induced ear and carrageenan-induced paw edema models, chronic anti-inflammatory effect was investigated using the complete Freund's adjuvant (CFA) test in rats.

Results: *OLS* (50-400 mg/kg) significantly (P<0.001) reduced acetic acid-induced writhes (68.28%, 50 mg/kg), duration of paw licking/biting by 73.10% (early phase) and 70.50% (late phase), 50 mg/kg) in formalin-test, while also increasing reaction latency by 79.73 and 92.47% at 150 and 180 min, respectively, in tail-flick test, at 400 mg/kg, in comparison to vehicle-treated, control. The antinociceptive effect elicited by the extract was prevented by pretreatment of mice with metergoline (5-HT_2 receptor antagonist), sulpiride (dopamine D_2 receptor antagonist) and glibenclamide (K^-_{ATP} sensitive channels blocker). The extract (400 mg/kg) or diclofenac (20 mg/kg) inhibited carrageenan-induced oedema by 73.08% and 80.77%, respectively, 5 h post-carrageenan injection. Moreover, OLS (400 mg/kg) significantly reduced CFAinduced chronic inflammation by 85.30%, on day 12 post-CFA injection which was similar to the effect of celecoxib (76.50% inhibition). Conversely, OLS failed to inhibit xylene-induced ear edema.

Conclusion: *Olax subscorpioidea* possesses antinociceptive effect through interaction with 5-HT_2, dopamine D_2 and sensitive potassium ATP channels as well anti-inflammatory effect. Thus confirm its folkloric uses in the treatment of painful and inflammatory conditions.

Key words: *Olax subscorpioidea;* serotonergic; dopaminergic; potassium-ATP sensitive channel; complete Freund's adjuvant

Les effets anti-nociceptifs and anti-inflammatoires de l'extrait de la feuille *Olax subscorpioidea* Oliv. (Olacaceae) chez les rongeurs: les mécanismes possibles d'une action anti-nociceptive

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RÉSUMÉ

Objectif: Etudier le mécanisme des effets anti-nociceptifs et anti-inflammatoires de l'extrait de la feuille aqueuse *Olax subscorpioidea* Oliv. (Olacaceae) (OLS) chez les rongeurs.

Méthodes: *OLS* (50, 100, 200, et 400 mg/kg, p.o.) a été administré 1 h avant l'injection de 0,6%v/v d'acide acétique (10 ml/kg, i.p.), 1%$^{v}/_{v}$ de formol (20 µl) ou des tests de coup de queue pour évaluer l'effet antinociceptif tandis que l'effet anti-inflammatoire aigu était étudié en utilisant l'oreille induite par le xylène et les modèles d'œdème de la patte induite par la carraghénane, l'effet anti-inflammatoire chronique a été étudié à l'aide du test de adjuvant de Freund complet (CFA) chez les rats.

Résultats: *OLS* (50-400 mg/kg) a réduit considérablement ($P<0.001$) les contorsions induites par l'acide acétique (68,28%, 50 mg/kg), la durée de léchage/morsure de la patte de (73,10% (phase initiale), and 70,50% (phase finale), 50 mg/kg) en test de formol, alors qu'également la latence de réaction croissante de 79,73 et 92,47% à 150 et 180 min, respectivement, en test de coup de queue, à 400 mg/kg, par rapport au contrôle traité avec le véhicule. L'effet anti-nociceptif élicité par l'extrait était empêché par le prétraitement des souris à la métergoline (antagoniste récepteur 5-HT_2), sulpiride (antagoniste récepteur de dopamine D_2) et glibenclamide ($K\text{-}_{ATP}$ bloqueur de canaux sensibles). L'extrait (400 mg/kg) ou l'œdème induit de carraghénane inhibé de diclofénac (20 mg/kg) de 73,08% et 80,77%, respectivement, 5 h d'injection post-carraghénane. En outre, OLS (400 mg/kg) a réduit considérablement l'inflammation chronique induite par le CFA de 85,30%, le jour 12 d'injection post-CFA qui était similaire à l'effet du célécoxib (inhibition de 76,50%). Inversement, OLS a manqué d'inhiber l'œdème de l'oreille induite de xylène.

Conclusion: *Olax subscorpioidea* possède un effet anti-nociceptive à travers l'interaction avec 5-HT_2, dopamine D_2 et les canaux ATP sensibles au potassium de même que l'effet anti-inflammatoire. Ceci confirme ainsi ses usages folkloriques dans le traitement des conditions douloureuses et inflammatoires.

Mots-clés: *Olax subscorpioidea;* sérotonergique; dopaminergique; canal potassium-ATP sensible; l'adjuvant complet de Freund

INTRODUCTION

Traditional medicine is undoubtedly a reliable alternative approach to health care delivery in the metropolis because it is cheap, easily accessible, and efficacious. In spite of the millions of chemical compounds currently synthesized in the laboratories. Natural products, particularly of plants origin remain the most important sources of new drugs.[1] *Olax subscorpioidea* (Oliv), family Olacaceae is commonly known as "Ewe Ifon'' (Yoruba, Southwest, Nigeria). It is widely distributed in Nigeria, Zaire, across the region from Senegal to West Cameroons.

O. subscorpioidea (Oliv) is used in traditional African medicine in the treatment of pain, jaundice, yellow fever, rheumatoid arthritis, depression, constipation and as genital stimulant/depressants and constipation.[2] Studies have shown possible antioxidant,[3] antimicrobial[4] and antiulcer[5] effects of O. subscorpioidea in rodents. In this study, the antinociceptive and anti-inflammatory properties of starch water extract of O. subscorpioidea was evaluated using validated standardized models of pain and inflammation. Moreover, Adeoluwa *et al.*[6] had previously reported antinociceptive effect of this plant. *Hence, in t*his study, the involvement of opioidergic, serotonergic, dopaminergic and adrenergic systems in the antinociceptive action of *O. subscorpioidea* extract was evaluated *in vivo.* Opioids have been the mainstay of pain treatment for thousands of years, and they remain so today.[7] Similarly, studies have reported that the spinal serotonergic system may suppress incoming noxious input to the spinal cord and inhibit pain transmission.[8] Thus, suggesting its role in the modulation of pain and nociception. In addition, the dopaminergic, cholinergic and adrenergic systems have also been implicated in pain transmission[8].

METHODS Plant material

Fresh leaves of *Olax subscorpioidea* were obtained from Abatadu village, Osun State, South-west, Nigeria. It was identified and authenticated by Mr. T. K. Odewo (a forestry expert) of the Department of Botany, University of Lagos, Nigeria. The herbarium voucher specimen with number LUH 5577 was deposited at the herbarium center for reference.

Preparation of extract

Personal information obtained from the traditional healers' shows that the leaves of O. subscorpioidea are soaked in starch water for the treatment of pain and inflammatory conditions. The freshly harvested leaves of *O. subscorpioidea* were gently but thoroughly washed under a running tap water and air-dried for one week under shade. The dried leaves were then pulverized into fine powder using laboratory hammer mill. The powdered material (150.98 g) was soaked in 2.1 L of corn-starch water for three days after which the preparation was filtered using Whatmann's filter paper size ~ 9 cm. The filtrate was oven dried at 40°C to obtain a dark-brown residue of the crude extract. Hence, the starch water effect would not be linked to observed effect. The dried extract was stored in the refrigerator at 4ºC until required for experimentation.

Preliminary phytochemical screening

The Preliminary phytochemical screening of *Olax subscorpioidea* was carried out using the method of Trease and Evans.[9]

Drugs and chemicals

Atropine, sulpiride, glacial acetic acid, complete Freund's adjuvant, xylene, carrageenan, naloxone, metergoline, prazosin, glibenclamide, diclofenac, yohimbine (Sigma Aldrich, St. Louis Mo, USA), celecoxib, dexamethasone (Xasten, Jiangsu Pengyao Pharmaceutical Inc, China), normal saline (Unique Pharmaceutical, Lagos, Nigeria), morphine sulphate (Martindale Medical Limited, UK) and ibuprofen (Ranbaxy Pharmaceutical, Mumbai, India) were used in the study.

Experimental animals

Male and female albino rats (130 – 150 g) and Swiss albino mice (15 – 20 g) were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Nigeria. The animals were maintained under standard environmental conditions and had free access to rodent pellet diet (Livestock Feed Plc, Lagos, Nigeria) and water. The animals were acclimatized in the laboratory conditions for a week before the commencement of the study. The experimental procedures adopted in this study were in compliance with the ethical standards approved by the Research Grant and Animal Experimentation Committee of the College of Medicine, University of Lagos, Nigeria and in accordance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (1985).

Acute toxicity study

Female Swiss albino mice were selected and fasted 12 h before the test. Four groups of mice (n =5) received 10

ml/kg normal saline and *O. subscorpioidea* (500, 2500 and 5000 mg/kg p.o.). The animals were observed for toxic symptoms and behavioral changes (sedation, hyperactivity, diarrheal, writhing, piloerection, restlessness) continuously for 2 h, then occasionally for further 4 h and 24 h for possible mortality. Animals were kept under observation for up to 14 days for signs of delayed toxicity. All doses following oral administration did not cause any mortality in mice.

Pharmacological investigations Antinociceptive models Mouse writhing test

Mice (15-20 g, n=5) were treated with normal saline (10 ml/kg, p.o.), ibuprofen (100 mg/kg, p.o) or *OLS* (50, 100, 200 and 400 mg/kg, p.o.), 60 minutes prior to the intraperitoneal injection of acetic acid (10 ml/kg; 0.6% (v/v)) to induce writhes. (i.e., constriction of the abdominal muscle with simultaneous stretching of the hind limbs), as described by Ishola *et al.*[10] The number of writhes was counted cumulatively over a period of 30 minutes by an observer unaware of the treatment groups. The percentage inhibition of writhing reflex was calculated using the formula:

% inhibition = mean number of writhes (control)- mean number of writhes (treatment)x100/mean number of writhes (control)

Formalin-induced nociception

Mice fasted overnight were divided into six groups (n =5). The different groups of animals were treated with normal saline (10 mL/kg, p.o.), *OLS* (50, 100, 200 or 400 mg/kg p.o.), or morphine (10 mg/kg, s.c.). One hour after oral administration or 30 min after subcutaneous injection of drugs, formalin (20 µl of 1%v/v in saline) was injected subcutaneously into the right hand paw of each mouse. The time (in seconds) spent in licking or biting the injected paw, indicative of pain, was recorded for each animal.[10] The responses of the mice were observed for the first 5 min (neurogenic phase) and 15–30 min (inflammatory phase) post-formalin injection by an observer unaware of the treatment groups.

% inhibition = reaction time (control)-reaction time (treatment) x 100/reaction time (control)

Tail Immersion model

Rats (130-150 g, n=5) were treated with normal saline (10 ml/kg, *p.o*), morphine (10 mg/kg, *s.c*) or *OLS* (50, 100, 200 and 400 mg/kg). The lower 5 cm portions of the rats' tail were marked. This part of the tail was immersed in a water bath maintained at 55°±0.5°C. The reaction time was determined before and periodically after administration of drugs for 0, 30, 60, 90, 120, 150 and 180 min.[11] The cut off time of the tail immersion was kept at 60 s to prevent tissue damage.

Anti-inflammatory models Xylene-induced ear edema

Mice were allotted to six groups (n =5). Thirty minutes after oral treatment of mice with normal saline (10 ml/kg), dexamethasone (1 mg/kg, p.o), or OLS (50, 100, 200 and 400 mg/kg), oedema was induced in each mouse by instilling 30 µl of xylene to the inner surface of the right ear. Fifteen minutes after xylene application, the animals were euthanized under ether anesthesia and both ears were cut off, sized, and weighed. The mean of the difference between the right and left ears was determined for each group and percentage

inhibition was calculated.[10,12]

Carrageenan-induced paw oedema

Sprague–Dawley rats (130–150g, n=5) were randomly divided into groups and were used after a 12 h fast but allowed free access to water except during the experiment. Oedema was induced by injection of 100 µl of carrageenan (1%w/v in normal saline) into the plantar surface (i.pl.) of the right hind paw. The animals were treated with normal saline 10 mL/kg, *OLS* (50, 100, 200 and 400 mg/kg p.o.), or diclofenac (20 mg/kg, p.o.), 1 h before injection of carrageenan. Paw diameter was measured using the cotton thread method of Bamgbose and Noamesi[13] before and 1, 2, 3, 4, 5, and 6 h after injection of carrageenan. Anti-inflammatory activity was expressed as the percentage reduction in oedema in treated rats in comparison to controls.

% inhibition = mean change in paw size (control)- mean change in paw size (treatment)x100/mean change in paw size (control)

Complete Freund's adjuvant-induced arthritis in rats

This is frequently used as an animal model to study the chronic inflammation. Rats (130-150g, n=5) were treated with normal saline (10 ml/kg, *p.o*), celecoxib (2 mg/kg, *p.o*) or *OLS* (50, 100, 200 and 400 mg/kg p.o.) once a day for a period of 12 days. Sixty minutes after oral drug or extract administration, 100 µl of CFA was

injected into the left hind paw of the animals. The paw volume of the left and right hind limbs of each animal were measured separately, using white thread method of Bamgbose *et al*[13] before and after the injection of CFA on 1st, 3rd, 6th, 9th and 12th day from the day of adjuvant injection to assess the degree of inflammation including the body weight of each animal.[14]

Statistical analysis

Results obtained were expressed as Mean ± SEM. The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiplecomparison test for Anti-nociceptive study while twoway ANOVA followed by Bonferroni post hoc multiplecomparison test was used for anti-inflammatory study. Statistical analysis were carried out using Graphpad prism version 6 (GraphPad Software, Inc. CA, USA).

RESULTS Preliminary phytochemical screening

Preliminary phytochemical screening on Olax subscorpioidea extract revealed the presence of tannins, reducing sugars, deoxysugar (and cardenoides), cardiac glycosides, flavonoids and phlobatannins.

Acute toxicity study

No death was recorded for all doses administered orally. The extract caused abdominal writhes, behavioral and sensory changes e.g. sedation, reduced motor activity and diarrhoea.

Mouse writhing test

As shown in Table 1, oral administration of OLS reduced the number of writhes at all doses of treatment compared to control group, with ~68.30% inhibition at 50 mg/kg. This reduction was similar to that observed with ibuprofen (67.70%).

Table 1: Effect of *O. subscorpioidea* on acetic acid-induced mouse writhing in mice

Treatment	Dose (mg/kg)	No of Writhes	% Inhibition
Vehicle	10ml/kg	55.80±6.93	
OLS	50	17.70±2.91*	68.28
OLS	100	27.75±2.46*	50.27
OLS	200	28.00±1.47*	49.82
OLS	400	32.60±2.02*	41.58
Ibuprofen	100	18.00±1.78*	67.74

Results expressed as mean ± SEM (n=5). *p< 0.05 compared to control; statistical level of significance analysis by one way ANOVA followed by Tukey *post hoc* multiple comparison test

Formalin-induced nociception

Intra-plantar injection of formalin into the right hand paw of mice produced nociceptive response of biting and licking within a duration of 105.00 ±14.20s in the first phase (0 – 5mins) and 124 ±16.40s in the second phase (15-30mins) in control group. However, subcutaneous injection of morphine (10 mg/kg) significantly reduced the duration of biting and licking behavior in the first phase (31.00 ± 6.47s; 73.1%) and second phase (33.00 ± 6.49s; 70.50%). Similarly, oral administration of OLS (200 mg/kg) significantly reduced the duration of licking in the first phase (47.70 ±10.10s; 54.60%) and second phase (83.60 ±12.9s; 70.50%) when compared to the vehicle-treated control group (Figure 1).

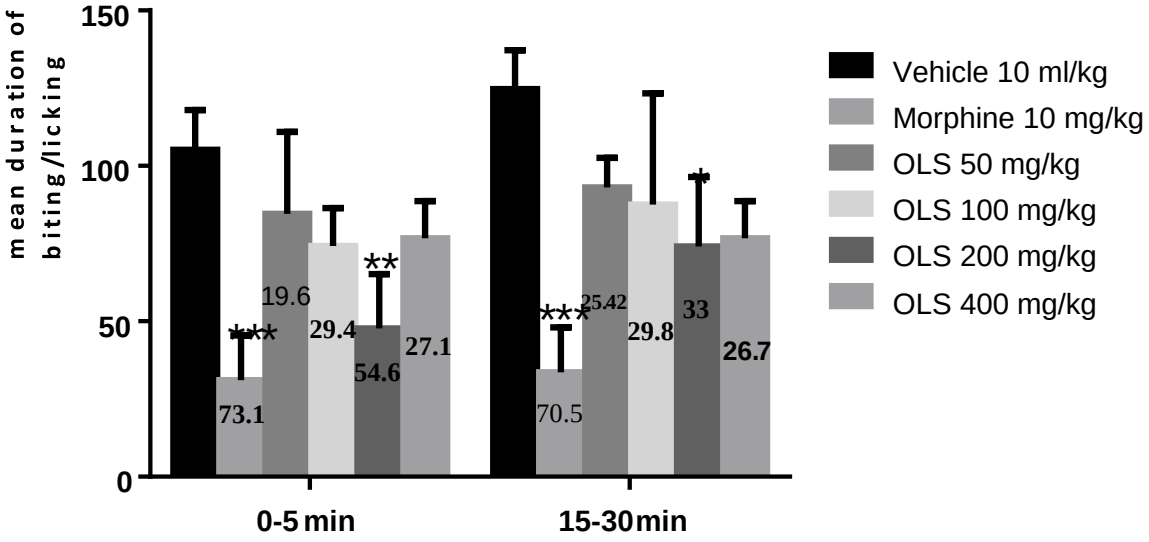

Fig 1: Effect of O. subscorpioidea leaf extract on formalin-induced nociception test. Values are presented as mean ± SEM (n =5). Values on bar chart represent % inhibition. *p< 0.05;**p< 0.01; ***p< 0.001 as compared to vehicle-treated control group. Statistical level of significance analysis by one way ANOVA followed by Tukey post hoc multiple comparison test

Tail immersion model

As shown in Table 2, pretreatment of rats with OLS Similarly, morphine treatment increased reaction significantly increased reaction latencies from latency to 5.60±0.31s. 2.54±0.19s to 4.90±0.43s 60 min post-treatment.

Table 2: Effect of *O. subscorpioidea* on Tail immersion test in rats

	Dose	Reaction Latency (sec)						
Treatment	**(mg/kg)**	**0 min**	**30 min**	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**

Vehicle								
OLS	10 ml/kg	2.00 ± 0.33	2.4±0.19	2.54±0.19	2.60±0.19	2.16±0.15	2.60±0.40	2.16±0.12
OLS	50	2.80±0.66	2.60±0.58 (2.63)	3.00±0.71 (6.17)	3.60±0.60 (13.51)	4.20±1.16 (26.02)	5.00±1.92 (32.43)	6.50±1.50 (55.36)
OLS	100	2.40±0.25	2.80±0.23 (5.26)	3.70±0.24 (15.55)	4.60±0.20 (27.61)	5.70±0.54 (45.15)	7.26±0.49 (62.97)	7.00±1.16 (61.73)
OLS	200	2.18±0.15	3.58±0. 19 (15.53)	4.60±0.21 (27.61)	6.10±0.23 (47.29)	7.20±0.57 (64.29)	7.60±0.56 (67.57)	8.50±0.87** (80.87)
Morphine	400	2.62±0.36	3.84±0.28 (18.95)	4.90±0.43** (31.64)	6.60±0.23 (54.05)	7.33±1.45 (65.94)	8.26±0.59** (76.49)	9.00±1.00*** (87.24)
	10	2.43±0.19	4.10±0.41 (22.37)	5.60±0.31** (41.02)	7.00±0.23 (41.02)	7.80±0.45*** (71.94)	8.50±0.21 (79.73)	9.41±0.65*** (92.47)

Values are expressed as Mean ± SEM (n =5). *p< 0.05;**p < 0.01; ***p < 0.001 as compared to control; Values in parenthesis are % MPE. OLS, *Olax subscorpioidea*; MPE, maximum possible effect

Elucidation of possible mechanism of the antinociceptive effect of *O. subscorpioidea*

Intraperitoneal injection of metergoline (4 mg/kg, i.p., 5-HT_2 receptor antagonist), glibenclamide (10 mg/kg, i.p., a K_{ATP} channel blocker) and sulpiride (50 mg/kg, i.p., dopamine D_2 antagonist) reversed the anti-nociceptive effect elicited by O. subscorpioidea in mice. However, pretreatment of mice with prazosin (α_1-adrenoceptor antagonist), yohimbine (α_2-adrenoceptor antagonist), atropine (muscarinic cholinergic receptor antagonist) and naloxone (opioid receptor antagonist) failed to prevent the anti-nociceptive effect of O. subscorpioidea (Table 3).

Participation of Opioid pathway in the antinociceptive effect of O. Subscorpioidea in tail immersion test As shown in Table 4, oral administration of O. Subscorpioidea (50 mg/kg) produced time course increase in reaction latency with maximum possible effect of 90.88%, 90 min post-treatment which was similar to the increase in pain threshold produced by morphine (10 mg/kg). However, naloxone pretreatment failed to prevent the increase in reaction time produced by the extract but was able to reverse the time course increase in reaction latencies elicited by morphine. Ruling out involvement of opioidergic pathway in its mechanisms of antinociceptive effect in mice.

Table 3: Mechanisms of antinociceptive effect of O. subscorpioidea in mice

Treatment	Dose	No of writhes (in 30mins)	% inhibition
Control	10 ml/kg	89.00±5.86	
OLS	50 mg/kg	18.40±2.17	79.33
Prazosin + OLS	1 + 50 mg/kg	1.20±0.65	98.65
Metergoline + OLS	4+ 50 mg/kg	33.40±1.33*	62.72
Yohimbine + OLS	1 + 50 mg/kg	27.20±2.10	69.44
Glibenclamide + OLS	10 +50 mg/kg	71.80±5.14**	19.33
Atropine + OLS	1 + 50 mg/kg	29.20±2.37	67.19
Naloxone + OLS	5 + 50mg/kg	22.80±2.06	74.38
Sulpiride + OLS	50 + 50mg/kg	33.60±4.89*	62.25

Values are expressed as mean ± SEM (n =5). *p< 0.05;**p < 0.01 as compared to OLS 50 mg/kg treated group; Statistical level of significance analysis by one way ANOVA followed by Tukey post hoc multiple comparison test

Table 4: Opioid involvement in the antinociceptive effect of O. Subscorpioidea

		Reaction time (sec)						
Treatment	Dose (mg/kg)	0 min	30 min	60 min	90 min	120 min	150 min	180 min
Vehicle	10ml/kg	4.20±0.95	4.80±0.95	6.20±0.95	5.20±0.79	3.00±0.73	2.60±0.33	2.60±0.55
OLS	50	5.00±0.52	41.50±6.53**	52.67±1.89***	55.00±1.88***	45.30±5.79***	45.00±5.95***	21.17±2.17***
			-66.49	-86.38	-90.88	-74.21	-73.87	-32.35
NAL 5 + OLS		5.00±0.84	50.00±2.92	57.60±1.91	57.00±1.89	44.20±6.98	41.00±6.36	24.40±4.12
			-81.88	-93.12	-94.53	-72.28	-66.89	-37.98

Morphine	10	4.20±0.80	33.60±11.0**	56.80±1.28	54.20±5.98	43.80±8.90	32.40±6.52	24.00±12.8
			-52.17	-91.67	-98.91	-71.58	-51.92	-37.28
NAL(5)+Morphine		4.20±0.58	15.60±6.59*** 23.60±4.92***		26.40±6.71**	20.40±5.58**	27.20±4.99	20.40±3.71
			-19.57	-32.34	-38.69	-30.53	-42.86	-31.01

Values are expressed as mean ± SEM (n=5). **p < 0.01; ***p <0.001 compared to OLS 50mg/kg treated group. Values in parenthesis are % MPE. OLS (50 mg/kg); MPE, maximum possible effect. Statistical level of significance analysis by one way ANOVA followed by Tukey post hoc multiple comparison test.

Xylene-induced ear oedema by xylene in mice when compared with vehicle-treated As depicted in Figure 2, pretreatment of mice with O. control group. However, the standard drug, subscorpioidea (50,100, 200 and 400 mg/kg) produced dexamethasone (1 mg/kg) produced 73.10% inhibition no significant (p>0.05) reduction of ear edema induced of edema in comparison to control.

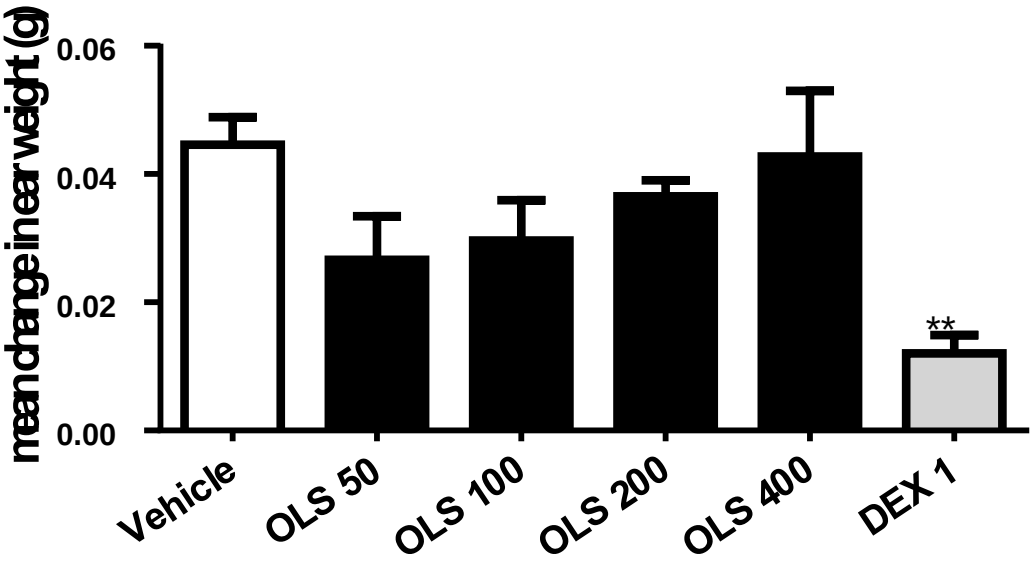

Fig 2: Effect of aqueous leaf extract of Olax subscorpioidea on xylene-induced ear oedema in rats. Values are expressed as Mean ± SEM (n =5). **p< 0.01, compared to control; Statistical level of significance analysis by one way ANOVA followed by Tukey post hoc multiple comparison test.

Carrageenan induced paw oedema (75.00%). This effect was similar to the anti-
Pretreatment of rats with O. subscorpioidea produced oedematogenic effect of standard drug, diclofenac time course inhibition of carrageenan-induced paw (77.27% inhibition), 5 h post carrageenan injection oedema with peak effect recorded at 400 mg/kg when compared to control treated group (Table 5).

Table 5: Effect of *O. subscorpioidea* on Carrageenan-induced paw edema in rats

		Time course of paw edema (cm)					
Treatment	**Dose (mg/kg)**	**1 h**	**2h**	**3 h**	**4 h**	**5 h**	**6 h**
Vehicle	10 ml/kg	0.42±0.08	0.62±0.09	0.74±0.06	0.52±0.07	0.44±0.09	0.32±0.07
OLS	50	0.18±0.03*	0.22±0.07**	0.30±0.12*	0.20±0.03*	0.18±0.05*	0.18±0.05*
		(57.14)	(64.52)	(59.46)	(61.54)	(59.10)	(43.75)
OLS	100	0.20±0.13*	0.40±0.08	0.50±0.05	0.20±0.05*	0.18±0.07*	0.18±0.07
		(52.38)	(34.38)	(45.95)	(61.54)	(59.10)	(43.75)
OLS	200	0.18±0.07	0.22±0.04*	0.34±0.02	0.19±0.02*	0.12±0.02**	0.11±0.03**
		(57.14)	(64.52)	(54.05)	(65.38)	(72.73)	(65.63)
OLS	400	0.17±0.07	0.21±0.10*	0.29±0.19**	0.14±0.04**	0.11±0.03***	0.10±0.03***
		(59.52)	(66.13)	(60.82)	(73.08)	(75.0)	(68.75)
Diclofenac	10	0.34±0.05	0.42±0.04	0.32±0.02*	0.10±0.00**	0.10±0.00***	0.10±0.02***
		(23.81)	(32.26)	(58.33)	(80.77)	(77.27)	(68.75)

Results expressed as Mean ± SEM (n=5). $p < 0.05$; *** $p < 0.01$; *** $p < 0.001$, compared to control; Values in parenthesis are % MPE. OLS, Olax subscorpioidea; MPE, maximum possible effect

Complete Freund's adjuvant-induced arthritis in rats

As shown in Table 6, one way ANOVA revealed that intra-plantar injection of CFA caused a significant increase in rat paw volume in control group on day 1 to day 6. However, subchronic treatment of rats with O. subscorpioidea reduced the paw edema induced by CFA injection when compared with the vehicle-treated control group. Moreover, on day 12, maximum inhibition of paw oedema was recorded in O. subscorpioidea (400 mg/kg) treated group with 85.30% inhibition of paw which was comparatively similar to that of COX-selective inhibitor, celecoxib, (76.50%).

DISCUSSION

pathways.[16] The results reported here indicated that

Table 6: Effect of *O. subscorpioidea* on Complete Freund's Adjuvant-induced Arthritis in rats

		Paw thickness (cm)			
Treatment	**Dose (mg/kg)**	**Day 1**	**Day 3**	**Day 6**	**Day 12**
Vehicle + CFA		0.68±0.06	0.76±0.06	0.76±0.06	0.68±0.04
CFA + OLS	50	0.72±0.05 (-5.88)	0.62±0.05 (18.40)	0.24±0.05 (68.40)	0.18±0.02 (73.50)
CFA + OLS	100	0.72±0.06 (-5.88)	0.60±0.05 (21.00)	0.36±0.07 (52.6)*	0.16±0.33 (76.50)**
CFA + OLS	200	0.66±0.03 (2.94)	0.46±0.04 (39.50)*	0.32±0.02 (57.90)*	0.16±0.02 (76.50)**
CFA + OLS	400	0.54±0.06 (20.59)	0.50±0.03 (34.20)*	0.28±0.03 (63.20)**	0.10±0.03 (85.30)***
CFA + Celecoxib	2	0.68±0.08 (0.00)	0.66±0.08 (13.20)	0.44±0.07 (42.10)	0.16±0.06 (76.50)**

Results expressed as Mean ± SEM (n=5). *p <0.05; **p < 0.01; ***p < 0.001, compared to control; Values in parenthesis are % MPE. OLS, Olax subscorpioidea; MPE, maximum possible effect

The results of the present study showed that the aqueous leaf extract of O. subscorpioidea possesses antinociceptive action possibly through interaction with serotonergic, dopaminergic and ATP-sensitive potassium channels receptors as well as antiinflammatory effect. The antinociceptive effect of the extract was evaluated using the chemical and thermalinduced nociception such as the acetic acid-induced mouse writhing, formalin-induced pain, and tail immersion tests, respectively.

The acetic acid model is a convenient stimulus assay for screening of substances that might have antinociceptive and/or anti-inflammatory actions. Its nociceptive effects are attributed indirectly to the production and release of endogenous mediators i.e. PGE_2 and $PGF_{2\alpha}$,[15] which stimulate the nociceptive neurons, and directly by activation of non-selective cation channels located in the primary sensory oral administration of O. subscorpioidea reduced the number of abdominal constrictions. However, acetic acid-induced nociception test has been shown to be non-specific due to its inability to differentiate between the involvements of peripheral or central mechanism in observed analgesic effects.[17] Due to poor specificity of the acetic acid- induced nociception (e.g., muscle relaxant can also reduce number of writhes), and to differentiate between the central and peripheral antinociceptive effect of O. subscorpioidea, the formalin test was carried out. Formalin-induced pain as an experimental model of analgesic is useful for elucidating mechanism of pain and analgesia since it measures the response to a long-lasting nociceptive stimulus and therefore, resembles clinical pain.[18] Subcutaneous injection of 1% formalin into mice hindpaw produces biphasic nociceptive response.[19] The first transient phase caused by the direct effect of

formalin on sensory C-fibers,[20] and the second prolonged phase associated with the development of the injury induced spinal sensitization, responsible for facilitated pain processing, a central sensitization of the dorsal horn neuron which occurs during inflammatory pain. It is associated with generation and release of inflammatory mediators, such as histamine, serotonin, prostaglandin E (PGE), and bradykinin.[21] Drugs that act centrally, such as the narcotics inhibit both phases of formalin-induced pain, while peripherally acting drugs such as aspirin only inhibit the late phases.[22] Findings from this study showed that O. subscorpioidea at dose of 200 mg/kg significantly ($p<0.05$) attenuates both the early and late phases of formalin-induced pain, thus suggesting its central and peripheral anti-nociceptive actions. The tail immersion model is used to differentiate central opioidlike analgesics from peripheral analgesics. The result from this study showed that O. subscorpioidea produced time course significant increase in reaction latency in mice suggesting possible opioidergic involvement.

Further experiments were undertaken to elucidate the molecular mechanism by which O. subscorpioidea exerted its antinociceptive activity. Previous studies by Alves et al[23] showed that the pretreatment of mice with glibenclamide (K_{ATP} channel blocker) completely reversed the anti-nociceptive effect of diclofenac on inflammatory pain in the formalin test. Moreover, K_{ATP} channel opening produces anti-nociceptive effects by reducing the neuronal excitability and inhibiting the release of different neurotransmitters including substance P in the spinal cord.[24] In this study, pretreatment of mice with glibenclamide (10 mg/kg, i.p.) reversed the anti-nociceptive effect of O. subscorpioidea, suggesting possible involvement of ATP-sensitive K^+ channels in the anti- nociceptive effect of O. subscorpioidea.

Moreover, the central monoaminergic system mediates the inhibition of pain, particularly chronic pain.[25] There are evidences showing involvement of noradrenergic neurotransmission in the mechanism of the phenomena accompanying chronic pain, such as anxiety and depression.[26] Also, central serotonergic and dopaminergic systems have been implicated in pain transmission in the CNS.[27, 28] Furthermore, the results of the present study provide consistent evidence that antinociception elicited by the O. subscorpioidea is independent of the activation of important endogenous analgesic systems, namely cholinergic and noradrenergic systems. In fact, the treatment of animals with atropine, a nonselective muscarinic antagonist, failed to interfere with O. subscorpioideainduced antinociception when assessed in the formalin model of pain. Moreover, the $\alpha_1$and α_2-adrenoceptors appear unlikely to be involved in the antinociceptive action of O. subscorpioidea, evidenced by the fact that selective antagonists of these receptors failed to alter the antinociception caused by treatment with O. subscorpioidea, in conditions where they produce significant inhibition of the antinociception caused by the respective selective agonists.

It is believed that when a harmful stimulation occurs, there is an increase in dopamine "turnover" in specific nervous system regions, suggesting an augmentation in the activity of descending dopaminergic pathways[8]. Even though some studies confirm the involvement of this system in the antinociception[29]. In this study, pretreatment of mice with sulpiride (50 mg/kg, i.p., dopamine D_2 receptor antagonist) reversed the antinociceptive effect elicited by O. subscorpioidea in mice. Thus suggest possible interaction of the extract with dopamine D_2 receptors.[29]

It is well known that serotonin (5-HT) pathways within the CNS arise from a series of nuclei situated in the midline of the brain stem, the raphe nuclei, which represent the richest source of neuronal 5-HT synthesised in the mammalian brain.[8,30] In addition, several studies have shown that the bulbospinal serotonin system may suppress incoming noxious input to the spinal cord and inhibit pain transmission[31]. Descending serotonergic pathways modulate the activity of projection neurons directly, as well as via interneurones.[30] The multiple 5-HT receptor types within the spinal cord appear to fulfil different roles in the control of nociception, reflecting their contrasting patterns of coupling to intracellular transduction mechanisms.[8] The activities of 5-HT receptors are complex and sometimes even contrasting, and can depend on (1) the receptor subtype being activated, (2) the relative contributions of pre-versus postsynaptic actions of receptors, and (3) the nociceptive paradigm in terms of quality and intensity of stimulus[8], and (4) the dose-related effect, which can be pro- or antinociceptive, of agonists and antagonists of serotonergic receptor subtypes.[31] In this study, pretreatment of mice with metergoline reversed the antinociceptive effect of O. subscorpioidea. Thus suggest possible interaction of the extract with 5-HT_2.

Contrary, to the results obtained from the formalin induced pain and tail immersion models suggesting possible involvement or interaction of O. subscorpioidea with opioidergic receptor, pretreatment of mice with naloxone (a non-selective opioid receptor antagonist) prevented the antinociceptive effect elicited by morphine. The present results lead to the conclusion that the opioid system is unlikely to be involved. This is drawn from the fact that pre-treatment of animals with naloxone, a non-selective opioid receptor antagonist, completely inhibited the antinociceptive effect of morphine but not the action of O. subscorpioidea.

The xylene-induced ear edema model is useful for the evaluation of anti-inflammatory effect of topical steroids or for non- steroidal antiphlogistic agents, especially those inhibiting phospholipase A_2.[32] However, in this study oral administration of the extract produced no significant reduction in ear edema in comparison to control whereas standard drug (dexamethasone 1 mg/kg) elicited 73.10% significant inhibition of ear edema. Carrageenan-induced rat paw edema is a suitable experimental animal model commonly used for the study of acute inflammation and is believed to be biphasic.[33] In general, the first phase (1-2 h) involves inflammation mediated by the release of serotonin and histamine and increased synthesis of prostaglandins in the surroundings of the damaged tissues. The second phase (3-5 h) is the result of the release of kinins mainly prostaglandins.[33] The extract produced a time course significant inhibition of carrageenan-induced paw edema in rats starting from the first hour after administration. The maximum inhibitory effect (75%) was recorded 5 h post-carrageenan injection. This observed effect of the extract was similar to that of diclofenac treated (NSAID). The ability of the extract to inhibit the early and late phase inflammation in the carrageenan model suggest that the anti-inflammatory effect of O. subscorpioidea may involve inhibition of the release of inflammatory mediators including histamine, serotonin, bradykinin and prostaglandins.[34] The effect of the extract on chronic inflammation was investigated in complete Freund's adjuvant (CFA) model. CFA induces an inflammatory cascade causing swelling and pain lasting 24 h to 12 days. The classical symptoms of this model are swelling of the CFA-injected paw (edema), redness, allodynia and hyperalgesia. In this study, injection of CFA into the right hind limb was effective in stimulating cell mediated immunity after 12 days. It was found that O. subscorpioidea produced significant ($p<0.001$) dose dependent inhibition of edema. Maximal inhibition (85.30%) was observed at 400 mg/kg, comparatively similar to the effect of the reference drug, celecoxib, which produced significant ($p<0.01$) (76.50%) inhibition of edema. Another important observation from this study was the fact that the peak antinociceptive effect was obtained at the lowest dose of 50 mg/kg while the peak antiinflammatory effect was observed at the highest dose of 400 mg/kg but not dose dependent. Phytochemical analysis of O. subscorpioidea revealed the presence of flavonoids, anthraquinones, saponins, tannins, phlobatannnins, steroids, cardiac glycoside, and polyphenols. Saponins have previously been reported to display anti-nociceptive, anti-inflammatory and antipyretic activities while flavonoids possess antiinflammatory activities.[35,36] The antinociceptive and anti-inflammatory effects of O. subscorpioidea could be attributed to the presence of these phyto-active components. However, further study is required to isolate, characterize and elucidate the active principles in O. subscorpioidea responsible for the pharmacologic effect.

CONCLUSION

The data from the present study show that O. subscorpioidea exerts pronounced antinociception in chemical (acetic acid-, and formalin-induced pain) and thermal (tail-flick test) models of nociception in rodent. In addition, antinociceptive effect of O. subscorpioidea involves an interaction with potassium sensitive ATP channels, serotonergic (specifically 5-HT_2) and dopaminergic systems, but not with opioidergic nor noradrenergic systems nor the cholinergic pathway. Furthermore, this study confirm anti-inflammatory action demonstrated in the present study supports the ethnomedical uses of this plant in the treatment of painful and inflammatory conditions.

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REFERENCES

1. Odugbemi T. (2006). Outlines and pictures of medicinal plants from Nigeria. University of Lagos press, Lagos, Nigera, Pp: 1-85.
2. Burkill HM (1995). The useful plants of West Tropical Africa.2nd ed. vol. 3, Families J-L. Royal Botanic Gardens, Kew, Richmond, United Kingdom, p. 857.
3. Womeni HM, Djikeng FT, Tiencheu B. Linder M (2013). Antioxidant potential of methanolic extracts and powders of some Cameroonian spices during accelerated storage of soybean oil. *Advances in Biological Chemistry*, 3:304-313.
4. Ayandele AA, Adebiyi AO. (2007). The phytochemical analysis and antimicrobial screening of extracts of *Olax subscorpioidea*. *African Journal of Biotechnology*, 6:868-870.
5. Ukwe CV, Ubaka CM, Madusque UJ. (2010). Evaluation of the antiulcer activity of *Olax subscorpioidea* Oliv. roots in rats. *Asian Pacific Journal of Tropical Medcine* 3:13-16.
6. Adeoluwa OA, Aderibigbe AO, Olonode ET. (2014). *A*ntinociceptive property of *Olax subscorpioidea* Oliv (Olacaceae) extract in mice. *Journal of Ethnopharmacology*, 156:353-7.
7. Gustein, H.B, Akil H. (2006). Opioid analgesic. Goodman and Gilman's: The Pharmacological Basis of therapeutics.12th Edn., McGraw–Hill Medical Publishing, New York 12th edn. pp 547-590.
8. Millan MJ. (2002). Descending control of pain. *Progress in Neurobiology* 66:355–474.
9. Trease GE, Evans WC. (1989). Pharmacognosy 11th edition. Brailliar Tiridel Canada. Macmillian publishers.
10. Ishola IO, Akindele AJ, Adeyemi OO. (2011). Analgesic and anti-inflammatory activities of *Cnestis ferruginea* Vahl ex DC (Connaraceae) methanolic root extract. *Journal of Ethnopharmacology* 135(1): 55-62.
11. Ishola IO, Awodele O, Olusayero AM, Ochieng CO. (2014). Mechanisms of Analgesic and Anti-Inflammatory Properties of *Annona muricata* Linn. (Annonaceae) Fruit Extract in Rodents. *Journal of Medicinal Food* 17(12):1375-82.
12. Nunez Guillen ME, Emim JA, Souccar C, Lapa AJ. (1997). Analgesic and anti-inflammatory activities of the aqueous extract of *Plantago major* L. *International Journal of Pharmacognosy* 35:99–104.
13. Bamgbose SO, Noamesi BK. (1981). Studies on cryptolepine inhibition of carrageenan - induced oedema. *Planta Medica* 42:392-396.
14. Laird JMA, Carter AJ, Grauert M, Cervero F. (2001). Analgesic activity of a novel use-dependent sodium channel blocker, crobenetine, in mono-arthritic rats. *British Journal of Pharmacology*, 134(8): 1742–1748.
15. Deraedt R, Jouquey S, Delevallée F, Flahaut M. (1980). Release of prostaglandins E and F in an algogenic reaction and its inhibition. *European Journal of Pharmacology*, 61(1):17-24.
16. Bentley GA, Newton SH, Starr J. (1983). Studies on the anti-nociceptive action of a-agonist drugs and their interactions with opioid mechanisms. *British Journal of Pharmacology*, 79:125–134.
17. Ribeiro RA, Vale ML, Thomazzi SM, *et al*. (2000). Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. *European Journal of Pharmacology*, 387:111–118
18. Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. (1992). The formalin test: an evaluation of the method. *Pain* 51,5-7.
19. Shibata M, Ohkubo T, Takahashi H, Inoki R (1989). Modified formalin test: characteristic biphasic pain response. *Pain* 38: 347–352.
20. Heapy CG, Jamieson A, Russell NJW (1987). Afferent C-fiber and A- bAU10 delta activity in models of inflammation. *British Journal of Pharmacology* 90:164.
21. Hunskaar S, Hole K (1987). The formalin test in mice: dissociation between inflammatory and non- inflammatory pain. *Pain* 30:103–114. 22. Santos ARS, Filho VC, Neiro R, Viana AM, Morenof N, Carpos MM *et al*. (1994). Analgesic effect of callus culture extract from selected species of *Phyllanthus* in mice. *Journal of Pharmacy and Pharmacology* 46:755.

23. Alves DP, Tatsuo MA, Leite R, Duarte ID (2004). Diclofenac-induced peripheral antinociception is associated with ATP-sensitive K^+ channels activation. *Life Science*, 74:2577–2591. 24. Soares AC, Leite R, Tatsuo MA, Duarte ID. (2000). Activation of ATP-sensitive K^+ channels: mechanism of peripheral anti-

nociceptive action of the nitric oxide donor sodium nitroprusside. *European Journal of Pharmacology* 400:67–71.

25. Pertovaara A (2006). Noradrenergic pain modulation. *Progress in Neurobiology* 80: 53–83. 26. Roczniak W, Wróbel J, Dolczak L, Nowak P. (2013). Influence of central noradrenergic system lesion on the serotoninergic 5-HT_3 receptor mediated analgesia in rats. *Advances in Clinical and Experimental Medicine* 22(5):629-38.
27. Jeong HJ, Lam K, Mitchell VA, Vaughan CW. (2013). Serotonergic modulation of neuronal activity in rat midbrain periaqueductal gray. *Journal of Neurophysiology* 109(11):2712-9.
28. Meyer PJ, Morgan MM, Kozell LB, Ingram SL. (2009). Contribution of dopamine receptors to periaqueductal gray-mediated antinociception. *Psychopharmacology* (Berl) 204(3):531-40.
29. Tamae A, Nakatsuka T, Koga K, Kato G, Furue H, Katafuchi T *et al.* (2005). Direct inhibition of substantia gelatinosa neurones in the rat spinal cord by activation of dopamine D_2-like receptors. *Journal of Physiology* 568 (Pt 1):243-53.
30. Sasaki M, Ishizaki K, Obata H, Goto F. (2001). Effects of 5-HT_2 and 5-HT_3 receptors on the modulation of nociceptive transmission in rat spinal cord according to the formalin test. *European Journal of Pharmacology* 424(1):45-52.
31. Munro G. (2007). Dopamine D(1) and D(2) receptor agonism enhances antinociception mediated by the serotonin and noradrenaline reuptake inhibitor duloxetine in the rat formalin test. *European Journal of Pharmacology* 575(1-3):66-74.
32. Zaninir JC, Medeiros YS, Cruz AB, Yunes RRA, Calixto JB. (1992). Action of compounds from Mandevilla velutina on croton oil induced ear edema in mice; A comparative study with steroidal and non-steroidal anti-inflammatory drugs. *Phytotherapy Research* 6:1–5
33. Di Rosa M, Giroud JP, Willoughby DA (1971). Studies on the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. *Journal of Pathology* 104:15-29.
34. Crunkhorn P, Meacock SCR. (1971). Mediators of the inflammation induced in the rat paw by carrageenan. *British Journal of Pharmacology* 42:392- 402.
35. Mohammad S., Naghmeh H., Mohammad K. (2004). Analgesic and anti- inflammatory activity of *Lactuca sativa* seed extract in rat. *Journal of Ethnopharmacology* 92, 325-329.
36. Aquila S, Giner RM, Recio MC, Spegazzini ED, Rios JL. (2009). Anti- inflammatory activity of flavonoids from *Cayaponia tayuya* roots. *Journal of Ethnopharmacology* 121, 333-337.