Nigerian Journal of Pharmaceutical Sciences
Vol. 14, No.1, March, 2015, ISSN: 0189-823X

ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES GUIDED-FRACTIONATION OF *OLAX SUSCORPIOIDEA* LEAF EXTRACT IN MICE AND RATS

Odoma S[1, 2] *, Zezi AU[2], Danjuma NM[2] and Ahmed A[3]

[1]*Department of Pharmacology and Therapeutics, Faculty of Medicine, Kogi State University, Anyigba, Nigeria.*
[2]*Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria.*
[3]*Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria.*

***Author for correspondence:** odoma.s@ksu.edu.ng, +2348027547778.

ABSTRACT

The crude methanol leaf extract of *Olax subscorpioidea* has been reported to possess analgesic and anti-inflammatory activity. The present study therefore aims to investigate the analgesic and anti-inflammatory activities of the liquid-liquid partitioned leaf fractions. The oral median lethal dose of the fractions was determined using method of Lorke. The analgesic activity was evaluated using acetic acid-induced writhing, formalin induced pain and hot plate tests in mice, while the anti-inflammatory activity was evaluated using carrageenan-induced hind paw oedema model in rats. Preliminary phytochemical screening of the entire fractions was also conducted using standard method. The oral median lethal dose values of the aqueous and butanol fractions was found to be greater than 5,000 mg/kg in mice and rats while that of the hexane fraction was estimated to be 2,154mg/kg in mice and 3,808mg/kg in rats respectively. The acetic acid induced writhes and the formalin induced pain leaking effect were significantly ($p<0.01$, $p<0.001$) reduced by the fractions. The hot plate latency was significantly ($p<0.01$, $p<0.001$) increased in the fractions (except hexane fraction). The paw oedema was also significantly ($p<0.05$, $p<0.01$, $p<0.001$) reduced by the fractions as compared to that of the control. Preliminary phytochemical screening revealed the presence of saponins, tannins, steroids, cardiac glycosides, flavonoids, alkaloids and carbohydrates in all the fractions except for the ethyl-acetate fraction which is devoid of saponins. The results suggest that *O. subscorpioidea* leaf fractions possess effective analgesic and anti-inflammatory activities mediated via peripheral and central mechanisms.

Keywords: Acetic acid, analgesics, anti-inflammatory, formalin, hotplate, *Olax subscorpioidea*.

INTRODUCTION

The British Pain Society (BPS) has defined pain as an emotion experienced in the brain; it is not like touch, taste, sight, smell or hearing (BPS, 2014). Pain can be perceived as a warning of potential damage, but can also be present when no actual harm is being done to the body (Moffat and Rae, 2010). It is the most common symptom of injuries and diseases (Haddad, 2007). It triggers various responses in the spinal cord and the brain, including reflexes, conscious perception, cognitive learning and memory

processes, emotional reactions such as depression, and drug addiction (Gu *et al.,* 2005). Inflammatory reaction, typically characterized by redness, swelling, heat, and pain, is one of the most important host defense mechanisms against invading pathogens. However, persistent or over inflammation leads to tissue damage and possibly failure of organs (Chi-ren *et al*., 2012). It has been implicated in the pathogeneses of arthritis, cancer, and stroke, as well as in neurodegenerative and cardiovascular disease (Ricciotti and FitzGerald, 2011). A disturbance that is successfully cleared results in a return to basal homeostatic set points. When conditions that induce inflammation are persistent, or resolution mechanisms fail, a state of chronic inflammation ensues that can lead to loss of normal physiological functions (Hotamisligil and Erbay, 2008).

Opioids and non steroidal anti-inflammatory drugs (NSAIDs) have been the mainstay of pain management for a very long time. Their adverse effects and insufficient effectiveness in many types of pain and inflammatory conditions were the main driving forces in the development of new analgesics and anti-inflammatory drugs (Kissin, 2010). Over the past 50 years, many new drugs have been introduced for the relief and prevention of pain and inflammation. Yet, success in the development of new analgesic and anti-inflammatory drugs is quite limited (Kissin, 2010). There is therefore, an intensification of search for newer analgesic and anti-inflammatory agents from the huge array of medicinal plant resources with better efficacy and fewer side effect profiles (Bellik *et al*., 2013).

Plants have been the basis of many traditional medicine systems throughout the World for thousands of years and still remains as the main new source of structurally important chemical substances that lead to the development of innovative drugs (Bellik *et al*., 2013). *Olax subscorpioidea* Oliv. is a multipurpose tree which belongs to the family Olacaceae (Burkill, 1997). It is a shrubby plant commonly found in tropics, especially Africa. Traditionally, it is widely employed in the treatment of various ailments (Burkill, 1997). The roots has been used for pregnancy associated fat, constipation (Okoli *et al*., 2007), management of cancer (Soladoye *et al*., 2010) and rheumatism (Ogunmefun and Gbile 2012). The leaf has been used for yellow fever, jaundice, venereal diseases and guinea worm infestation (Okoli *et al*., 2007). The leaf decoction is used by the Igala people of North Central Nigeria in the management of swelling and pains (T/Dr Bamidele Dada, Herbalist; personal communication, 2012). Experimentally, the root has been reported to posses' anti-ulcer properties (Ukwe *et al*., 2010), anthelmintic (Koné *et al*., 2012) and the stem posses' antimicrobial activities (Ayandele and Adebiyi, 2006).
Preliminary phytochemical studies show that the stem of *O. subscorpioidea* contains alkaloids, flavonoids, and steroids (Ayandele and Adebiyi, 2007), and the root contains glycosides, alkaloids, steroids and terpenoids (Ukwe *et al*., 2010). Previously, the antinociceptive (Adeoluwa *et al*., 2014), analgesic and anti-inflammatory effects of the crude extract have been reported (Odoma *et al*., 2014). In our attempt to isolate and characterize bioactive principles with analgesic and anti-inflammatory effect from the leaf of the plant, the present study is designed to evaluate the analgesic and anti-inflammatory activity of different leaf fractions of *O. subscorpioidea*.

MATERIALS AND METHODS

Collection and Identification of Plant

The leaf of *O. subscorpioidea* was collected from a farm in Anyigba, Kogi State, North Central Nigeria, in March 2013. It was

identified by a taxonomist, Dr Emmanuel I. Aigbokhan, of the Department of Biological Sciences, Faculty of Natural Sciences, Kogi State University, Anyigba, Kogi State, Nigeria, where a voucher specimen number (KSUH-277-2013-01) was deposited for future references.

Extraction and Fractionation

The plant material was shade dried for several days until constant weight was obtained and then reduced into fine powder with the aid of a mortar and pestle. One kilo grams (1kg) of the powdered material was extracted exhaustively with aqueous-methanol (20-80%) (2500 ml) (Sultana *et al*., 2009), using continuous soxhlet apparatus. The extract was concentrated under reduced pressure to yield a dark brown mass weighing 311.4 g (31.14% w/w). One hundred grams (100g) of the crude methanol extract was suspended in water (500ml) and successively partitioned with hexane (500 ml) five times, ethyl-acetate (500 ml) five times and Butanol (500 ml) five times to afford the corresponding fractions. The hexane and ethyl-acetate fractions were evaporated to dryness while the butanol and residual aqueous fractions were concentrated using water bath set at 50°C. Each of the fractions was placed in a bottle container and stored in a desiccator prior to use. Solutions of the fractions were prepared freshly with distilled water for each study.

Phytochemical Screening

The phytochemical screening was carried out in accordance with the standard protocol as described by Evans (2009).

Laboratory Animals

Adult Wistar Rats (120-170g) and Swiss albino Mice (20-30g) were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. The animals were maintained under standard environmental conditions (12 h/12 h light/dark cycle) and fed with standard rodent pellet diet and water *ad libitum*. The experiments were carried out in the central laboratory of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University in accordance with the criteria outlined in the Guide *for the Care and Use of Laboratory Animals* by the National Institutes of Health (Publication No. 80-23, revised 1996).

Acute Toxicity Study

The oral Lethal Dose (LD_{50}) of the fractions in rats and mice was conducted according to the method of Lorke (1983). In the first phase 3 groups of 3 animals (mice/rats) were administered the fractions (hexane, aqueous or butanol) 10, 100 and 1000 mg/kg. The animals were observed for signs of toxicity and death for the first 4 hours and intermittently for 24 hours. In the second phase, 4 groups of 1 animal each were administered graded doses of the fractions based on the result from the first phase and were also observed for signs of toxicity and death for the first 4 hours and intermittently for 24 hours. The LD_{50} value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived.

Acetic Acid-induced Writhing in Mice

The method previously described by Koster *et al.,* (1959) was adopted. Randomly-selected groups of mice (n=6) were orally administered with normal saline (10 ml/kg), hexane fraction (150, 300 and 600mg/kg), aqueous/butanol fraction (250, 500 and 1000mg/kg) or acetylsalicylic acid (ASA) (300 mg/kg). 60 min after oral

administration, acetic acid 0.6% v/v (10ml/kg) was administered to each mouse (Donkor *et al.,* 2013). Five minutes after acetic acid injection, mice were placed in individual cage and the number of writhes was counted for each mouse for a period of 10 minutes. A reduction in the number of writhes as compared to the control animals was considered as evidence for the presence of analgesia and expressed as percent inhibition of writhes.

Percentage Inhibition (%) =

$$\frac{\text{Mean No.of vrithes (Normal Saline)} - \text{Mean No.of vrithes (Test)}}{\text{Mean No.of writhes (Normal Saline)}} \times 100$$

Formalin-induced Pain in Mice

The method previously Tjølsen *et al*., (1992) was adopted in this study. Randomly-selected groups of mice (n=6) were orally administered with normal saline (10 ml/kg), hexane fraction (150, 300 and 600mg/kg), aqueous/ butanol fraction (250, 500 and 1000mg/kg) or morphine (10mg/kg). 60min post treatment, 20µl of freshly prepared 2.5% formalin in saline was injected subcutaneously into the right hind paw of each mouse. The mice were placed individually in an observation chamber and monitored for 40mins. The time (in sec) spent in licking or biting the injected paw, indicative of pain, was recorded for each animal. The responses of the mice were observed for the first 5 min (neurogenic phase) and 15–40 min (inflammatory phase) post formalin injection (Khanavi *et al*., 2012).

Hot plate test in mice.

The method of Eddy and Leimbach (1953) was adopted to determine the central analgesic activity. Randomly-selected groups of mice (n=6) were orally administered with normal saline (10 ml/kg), hexane fraction (150, 300 and 600mg/kg), aqueous/ butanol fraction (250, 500 and 1000mg/kg) or morphine (10mg/kg). Mice were individually placed on a hot plate (45±1°C), before drug treatment so that each animal serves as its own control. The time until the animal either licked the paw, fluttered any of the paws or jumped was taken as reaction time and recorded. A cut-off time of 20s was used to avoid paw tissue damage. The latency was observed and recorded after 60, 90, 120 and 150 min. The prolongation of the latency times was taken as an analgesic response (per cent maximum possible effect {%MPE}).

$$\%MPE = \frac{\text{Test} - \text{Baseline}}{\text{Cutoff} - \text{Baseline}} \times 100$$

Test = latency to respond after treatment

Baseline = latency to respond prior to treatment and

Cut-off (20 s) = preset time at which the test was ended in the absence of a response.

Carrageenan-induced oedema in Rats

Randomly-selected groups of rats (n=6) were orally administered with normal saline (1 ml/kg), hexane (150, 300 and 600mg/kg), aqueous, butanol fraction (250, 500 and 1000mg/kg) or morphine (10mg/kg). 60min post treatment, each rat was injected with 0.1 ml of 1% carrageenan into plantar surface of rat right hind paw (Winter *et al*., 1962). The hind paw oedema was measured and recorded at times 0, 1, 2, 3, 4, 5 and 24 hours (Ishola *et al*., 2012) using vernier caliper to determine the diameter of the oedema. The percent inhibition of oedema was calculated for each group with respect to its vehicle-treated control group by the following relationship:

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

Statistical Analysis

All values were expressed as Mean ± Standard Error of the Mean (SEM). The data were analyzed by one way/repeated measures analysis of variance (ANOVA) followed by Tukey's Post hoc test for Multiple Comparison using the Graph Pad Prism (statistical) software. The differences between means were considered significant when $P<0.05$.

RESULTS

Percentage Yield and Phytochemical Analysis

The methanol leaf extract of *O. subscorpioidea* was fractionated sequentially with solvents of different polarity (hexane, ethyl acetate, butanol and water) using sequential procedure to afford the corresponding fractions. The ethyl-acetate fraction had the least yield which was not sufficient enough for pharmacological studies. However, phytochemical analysis was carried out on it. The percentage yield of the sequential fractions of *O. subscorpioidea* is presented in Table 1.

Table 1: Percentage yields of *O. subscorpioidea* leaf fractions

Extractant	Mass extracted (from methanol extract, 100g) (g)	Percentage yield (%)
N-Hexane fraction	28	28
Ethyl-acetate fraction	2	2
N-Butanol fraction	26	26
Residual Aqueous fraction	22	22

Table 2: Phytochemical constituents of leaf fractions of *Olax subscorpioidea.*

Chemical constituents	Aqueous Fraction	Butanol Fraction	Ethyl-acetate Fraction	Hexane Fraction
Carbohydrates	++	++	++	+
Anthraquinone glycosides	-	-	-	-
Steroid and triterpenoid	+	+++	++	++
cardiac glycosides	+	+	++	++
Saponins	++	+++	-	+
Tannins	++	++	+	+++
Flavonoids	++	+++	+	++
Alkaloids	+	++	+	+++

+ = slightly present; ++ = moderately present; +++ = highly present; and - = absent.

Preliminary phytochemical screening of the fractions indicated the presence of carbohydrates, cardiac glycosides, tannins, flavonoids, alkaloids, saponins, steroid and triterpenoid. The ethyl-acetate fraction was devoid of saponins (Table 2).

Acute Toxicity Study

In the acute toxicity test, the oral median lethal dose (LD_{50}) of the aqueous and butanol fractions was estimated to be greater than 5,000 mg/kg in both rats and mice. The hexane fraction was estimated to be 2,154mg/kg in mice and 3,808mg/kg in rats.

Acetic Acid-induced Writhing Test

All the fractions evoked significant ($p< 0.01$, $p< 0.001$) and dose-dependent reduction in the number of acetic acid-induced writhes in mice when compared to control (Table 3). The highest percentage inhibition of abdominal constriction was observed in aqueous fraction (38.37, 51.74 and 69.76%) followed by the butanol fraction (31.97, 55.81 and 66.86%) then the hexane (37.79%) which was effective only at 600mg/kg. The standard drug, ASA, produced the highest effect with 81.97% pain inhibition.

Table 3: Effects of leaf fractions of *Olax subscorpioidea* on acetic acid induced writhing in mice.

Treatments	Mean no of Writhes ± SEM	Percentage Inhibition (%)
Normal Saline	34.40 ± 2.84	
AFOS 250mg/kg	21.20 ± 2.80**	38.37
AFOS 500mg/kg	16.60 ± 1.44**	51.74
AFOS 1000mg/kg	10.40 ± 1.03**	69.76
BFOS 250mg/kg	23.40 ± 1.86*	31.97
BFOS 500mg/kg	15.20 ± 1.24**	55.81
BFOS 1000mg/kg	11.40 ± 1.63**	66.86
HFOS 150mg/kg	31.00 ± 1.58	9.88
HFOS 300mg/kg	26.40 ± 2.20	23.26
HFOS 600mg/kg	21.4 ± 1.03**	37.79
ASA 300mg/kg	6.20 ± 1.83**	81.97

Values represent mean ± SEM,* P<0.01, ** P<0.001 versus normal saline (one-way ANOVA followed by Tukey's post hoc multiple-comparison test). AFOS (aqueous fraction), BFOS (butanol fraction), HFOS (hexane fraction) and ASA (acetylsalicylic acid) n=6.

Formalin-Induced Pain

The fractions showed significant ($p<0.01$, $p<0.001$) reduction in the paw licking effect (Table 4). The butanol fraction showed the

highest pain inhibition of 37.77, 54.42 and 71.09% inhibition in the early phase and 38.89, 50.12 and 72.56% inhibition in the late phase at the doses of 250, 500 and 1000mg/kg respectively. This is followed by the aqueous fraction with percentage inhibition of 31.77, 46.61 and 58.86% in the early phase and 35.80, 52.50 and 62.77% in the late phase at the doses of 250, 500 and 1000mg/kg respectively. However the hexane fraction showed percentage inhibition of 11.45 and 35.42% in the early phase and 28.84 and 41.04% in the late phase at the doses of 300 and 600mg/kg respectively. Morphine (10mg/kg) showed the highest inhibition in both phases with 85.94 and 82.57% inhibition in the early and late phases respectively.

Table 4: Effect of leaf fractions of *Olax subscorpioidea* on formalin-induced pain in mice.

Treatment	Duration of paw licking (sec)		Duration of paw licking (sec)	
	0-5 min	% inhibition	15-30 min	% inhibition
Normal saline	64.00 ± 2.77		69.83 ± 2.8	
AFOS 250mg/kg	43.67 ± 1.23***	**31.77**	44.83 ± 0.48***	**35.80**
AFOS 500mg/kg	26.33 ± 0.87***	**46.61**	33.17 ± 0.65***	**52.50**
AFOS 1000mg/kg	26.33 ± 0.87***	**58.86**	26.00 ± 1.32***	**62.77**
BFOS 250mg/kg	39.83 ± 0.31***	**37.77**	42.67 ± 0.95***	**38.89**
BFOS 500mg/kg	29.17 ± 1.30***	**54.42**	34.83 ± 1.47***	**50.12**
BFOS 1000mg/kg	18.50 ± 0.99***	**71.09**	19.16 ± 2.10***	**72.56**
HFOS 150mg/kg	60.50 ± 2.08	**5.47**	63.50 ± 1.36	**9.06**
HFOS 300mg/kg	56.67 ± 1.15*	**11.45**	55.00 ± 0.63***	**28.40**
HFOS 600mg/kg	41.33 ± 0.80***	**35.42**	41.17 ± 0.91***	**41.04**
Morphine 10mg/kg	9.00 ± 0.73***	**85.94**	12.17 ± 1.25***	**82.57**

Values represent mean ± SEM,* P<0.01, ** P<0.001 versus normal saline (one-way ANOVA followed by Tukey's post hoc multiple-comparison test). AFOS (aqueous fraction), BFOS (butanol fraction) and HFOS (hexane fraction). n=6.

Hot Plate Test in Mice

The effects of the fractions and morphine on the latency times of mice in the hot plate test (calculated as %MPE) are presented in Table 5. Aqueous and butanol fractions produced significant ($p<0.01$, $p<0.001$) time and dose-dependent increase in pain latency. The butanol fraction (1000mg/kg) produced a peak effect (52.99% MPE) at 150 min post treatment in comparison to the control. Similarly, aqueous fraction (1000mg/kg) produced a peak effect (39.98% MPE) at 150min. Morphine produced the highest effect (74.00% MPE) at 150min post-treatment. However, hexane fraction did not produce significant ($p>0.05$) central analgesic effect.

Table 5: Effect of methanol leaf extract of *Olax subscorpioidea* on hotplate test in mice

Treatments	0 min	60 min	90 min	120 min	150 min
Normal Saline	2.32 ± 0.15	1.38 ± 0.23	1.81 ± 0.26	1.80 ± 0.42	1.60 ± 0.20
AFOS 250mg/kg	1.65 ± 0.15	2.02 ± 0.42 **(2.02)**	3.19 ± 0.56 **(8.39)**	4.01 ± 0.65 **(12.86)**	4.34 ± 1.34 **(14.66)**
AFOS 500mg/kg	1.38 ± 0.09	2.21 ± 0.38 **(4.46)**	4.21 ± 0.37 **(15.20)**	6.76 ± 0.41 **(28.89) ***	7.21 ± 1.43 **(31.31) ***
AFOS 1000mg/kg	1.64 ± 0.24	2.19 ± 0.16 **(3.00)**	3.91 ± 0.33 **(12.36)**	6.98 ± 0.86 **(29.08) ****	8.98 ± 0.54 **(39.98) ****
BFOS 250mg/kg	1.62 ± 0.14	1.98 ± 0.26 **(1.96)**	3.25 ± 0.26 **(8.87)**	3.35 ± 0.19 **(9.41)**	4.18 ± 0.77 **(13.93)**
BFOS 500mg/kg	1.51 ± 0.14	2.63 ± 0.46 **(6.06)**	3.96 ± 0.50 **(13.25)**	4.88 ± 1.02 **(18.23)**	6.51 ± 1.24 **(27.04)**
BFOS 1000mg/kg	1.58 ± 0.21	2.63 ± 0.39 **(5.70)**	5.08 ± 0.84 **(19.00) ***	6.65 ± 1.51 **(27.52) ***	11.33 ± 2.23 **(52.99) ****
HFOS 150mg/kg	1.39 ± 0.16	2.26 ± 0.23 **(4.67)**	2.38 ± 0.22 **(5.32)**	3.60 ± 0.43 **(11.88)**	3.36 ± 0.35 **(10.59)**
HFOS 300mg/kg	1.88 ± 0.27	2.25 ± 0.24 **(2.04)**	2.66 ± 0.17 **(4.30)**	3.15 ± 0.24 **(7.01)**	3.43 ± 0.34 **(8.06)**
HFOS 600mg/kg	1.62 ± 0.11	2.43 ± 0.08 **(4.41)**	2.88 ± 0.21 **(6.86)**	3.25 ± 0.18 **(8.87)**	3.62 ± 0.16 **(10.88)**
Morphine 10mg/kg	2.08 ± 0.29	8.07 ± 1.26 **(33.42) ****	11.84 ± 1.77 **(54.46) ****	15.11 ± 1.29 **(72.71) ****	15.34 ± 0.87 **(74.00) ****

Values represent mean ± SEM, * $P<0.01$, ** $P<0.001$ versus normal saline (repeated measures ANOVA followed by Tukey's post hoc multiple-comparison test). Figures in parentheses are percent maximum possible effect (%MPE).AFOS (aqueous fraction), BFOS (butanol fraction) and HFOS (hexane fraction) n=6.

Carrageenan-induced Oedema in Rats

All the fractions significantly ($p<0.05$, $p<0.01$, $p<0.001$) decreased paw oedema in all the administered doses (Table 6). The aqueous and butanol fractions had better anti-inflammatory activities in all the doses compared to the standard anti-inflammatory drug, ASA. The aqueous fraction had maximum inhibition of 70.54%, 81.86% and 87.87% (250, 500 and 1000mg/kg respectively) in the 5th hour. This is

followed by the butanol fraction with maximum inhibition of 71.04%, 82.12% and 82.43% (250, 500 and 1000mg/kg respectively) in the 5th hour. The hexane fraction possesses the least anti-inflammatory activity with maximum inhibition of 61.39%, 64.36% and 67.33% (150, 300 and 600mg/kg respectively) at the 5th hour. The standard drug, ASA has maximum inhibition of 67.55% at the 5th hour.

Table 6: Effects of leaf fractions of *Olax subscorpioidea* on carrageenan-induced rat paw oedema

Treatment	Mean Increase in rats' paw diameter					
	1 hr	2 hr	3 hr	4 hr	5 hr	24 hr
N/Saline	1.99 ± 0.26	3.55 ± 0.43	3.58 ± 0.29	3.77 ± 0.22	4.04 ± 0.29	0.57 ± 0.20
AFOS 250mg/kg	1.64 ± 0.16 **(17.58)**	2.48 ± 0.22 **(30.14)***	2.12 ± 0.24 **(40.78)*****	1.43 ± 0.14 **(62.07)*****	1.19 ± 0.11 **(70.54)*****	0.28 ± 0.08 **(50.88)**
AFOS 500mg/kg	1.38 ± 0.08 **(30.65)**	1.84 ± 0.20 **(48.17)*****	1.62 ± 0.19 **(54.75)*****	1.15 ± 0.26 **(69.50)*****	0.74 ± 0.19 **(81.68)*****	0.19 ± 0.09 **(66.67)**
AFOS 1000mg/kg	0.83 ± 0.13 **(58.29)*****	1.81 ± 0.07 **(49.10)*****	1.31 ± 0.19 **(63.41)*****	1.01 ± 0.14 **(73.21)*****	0.50 ± 0.16 **(87.87)*****	0.09 ± 0.03 **(84.21)**
BFOS 250mg/kg	1.30 ± 0.12 **(34.67)**	1.89 ± 0.25 **(46.76)*****	1.50 ± 0.18 **(58.10)*****	1.13 ± 0.04 **(70.03)*****	1.17 ± 0.15 **(71.04)*****	0.40 ± 0.07 **(29.82)**
BFOS 500mg/kg	1.09 ± 0.08 **(45.23)****	1.44 ± 0.15 **(59.44)*****	1.51 ± 0.11 **(57.82)*****	1.11 ± 0.15 **(70.56)*****	0.72 ± 0.11 **(82.18)*****	0.31 ± 0.07 **(45.61)**
BFOS 1000mg/kg	0.88 ± 0.14 **(55.78)*****	1.16 ± 0.22 **(67.32)*****	1.25 ± 0.11 **(65.08)*****	0.75 ± 0.16 **(80.11)*****	0.71 ± 0.19 **(82.43)*****	0.21 ± 0.1 **(63.16)**
HFOS 150mg/kg	1.56 ± 0.14 **(21.61)**	2.10 ± 0.09 **(40.85)*****	2.36 ± 0.13 **(34.08)****	1.92 ± 0.12 **(49.07)*****	1.56 ± 0.19 **(61.39)*****	0.45 ± 0.10 **(21.05)**
HFOS 300mg/kg	1.10 ± 0.21 **(44.72)****	1.87 ± 0.19 **(47.32)*****	2.25 ± 0.19 **(37.15)*****	1.91 ± 0.17 **(49.34)*****	1.44 ± 0.17 **(64.36)*****	0.45 ± 0.11 **(21.05)**
HFOS 600mg/kg	0.88 ± 0.11 **(55.78%)*****	1.75 ± 0.12 **(50.70%)*****	2.24 ± 0.19 **(37.43%)*****	1.78 ± 0.12 **(52.79%)*****	1.32 ± 0.12 **(67.33%)*****	0.33 ± 0.09 **(42.11%)**
ASA 300mg	1.44 ± 0.21 **(27.64%)*****	1.96 ± 0.11 **(44.79%)*****	1.52 ± 0.16 **(57.54%)*****	1.23 ± 0.16 **(67.37%)*****	1.23 ± 0.21 **(69.55%)*****	0.35 ± 0.19 **(38.60%)**

Values represent mean ± SEM, * P<0.05, ** P<0.01, *** P<0.001 versus normal saline (repeated measures ANOVA followed by Tukey post hoc multiple-comparison test). Figures in parentheses are percentage inhibition of inflammation. AFOS (aqueous fraction), BFOS (butanol fraction), HFOS (hexane fraction) and ASA (acetylsalicylic acid), n=6.

DISCUSSION

The present study attempt to investigate the analgesic and anti-inflammatory activity of fractions obtained from the crude methanol leaf extract of *O. subscorpioidea*. The administration of the aqueous and butanol fractions up to 5,000mg/kg caused no death and also no physical sign of toxicity was observed. These suggest that these two fractions may be relatively safe (Matsumara, 1975; Loomis and Hayes 1996). However, the hexane fraction is slightly toxic in mice (LD_{50}: 2154 mg/kg) and rats (LD_{50}: 3808 mg/kg) according to the classification of toxicity (Matsumara, 1975; Loomis and Hayes, 1996).

Acetic acid is the most commonly used substances for the stimulation of visceral pain in mice (Ma and Zhang, 2011) by injecting it into the peritoneal cavity. After injection, it may activate nociceptors directly and/or produce pain through inflammation of visceral (subdiaphragmatic organs) and subcutaneous (muscle wall) tissues (Siegmund *et al*., 1957). The animal reacts with a characteristic stretching behaviour called writhing (Milind and Monu, 2013). In this test both central and peripheral analgesics are detected (Vogel, 2008). ASA and other NSAIDs reduce writhes induced by acetic acid by inhibiting cyclooxygenase (COX) in peripheral tissues by blocking the release and/or synthesis of inflammatory mediators (Donkor *et al*., 2013). The leaf fractions of *O. subscorpioidea* exhibit high level of analgesic activities by effectively inhibiting pain induced by the acetic acid. The ability of the fractions to inhibit the acetic acid induced pain suggest that the fractions may be eliciting its analgesic effect by inhibiting COX in the peripheral tissues by blocking the release and/or synthesis of inflammatory mediators.

The formalin test is used to test peripheral and central acting analgesics (Vogel, 2008). It differs from most other nociceptive tests, such as the hot plate, tail flick and tail pinch tests, in that it is a chemical, little or no restraining of experimental animals is needed during testing and the nociceptive stimulus and response are persistent rather than transient (Current Protocols in Neuroscience (CPN), 2002). Also the formalin test enables evaluation of analgesic activity towards moderate, continuous pain generated by injured tissue. The nociceptive response produced by formalin is biphasic, and the two phases can be distinguished pharmacologically, non steroidal anti-inflammatory drugs (NSAIDs) are ineffective in attenuating the phase 1 response, but are effective in attenuating phase 2 (CPN, 2002; Vogel, 2008). Central acting analgesics are effective in attenuating both phases (Vogel, 2008). The early phase starts immediately following injection of formalin, only lasts for approximately 5 min and is probably due to direct chemical stimulation of nociceptors (acute pain) (Meunier, 1998) and the second phase lasts 15 to 40 min following formalin injection (Khanavi *et al*., 2012). Experimental data suggest that peripheral, inflammatory processes are involved in the second phase (Meunier, 1998). Nociceptive behavior in the formalin test can be quantified in several ways; the scaling method (in rat and mouse) (Dubuisson and Dennis, 1977), counting number of flinches in the formalin-injected paw (in rats) (Dickenson and Sullivan, 1987) (formalin induces a characteristic flinching behavior in rats that can be counted easily, CPN, 2002) and counting the time spent licking the paw (in mouse). Counting the time spent licking the paw is the most commonly used to quantify formalin-induced nociception in mouse (CPN, 2002). The antinociceptive activity of the fractions in the two phases of formalin

induced pain suggests that they might relieve pain by both peripheral and central mechanism.

The hot plate thermal model is used to assess central antinociceptive effect of drugs (Bhalke and Pal, 2012). It is only the centrally acting drugs that are capable of affecting this test (Hosseinzadeh and Younessi, 2002). The centrally acting agents like morphine activate the release of endogenous peptide by periaqueductal gray matter (PAG), which are carried to the spinal cord to inhibit the pain muscle transmission within the dorsal horn (Katzung, 2005). The ability of the aqueous and butanol fractions to increase the latency in the hot plate model indicates that the fractions may possess central analgesic activity. The inability of the hexane fraction to significantly increase the latency suggests that it may lack or have limited central analgesic activity.

Following the carrageenan injection, the diameter of rats' paw increased as oedema developed, indicating inflammatory activities. Carrageenan induced inflammation is most commonly used as an experimental model for evaluating the anti-inflammatory potency of compounds or natural products (Winter *et al*., 1962). After the carrageenan injection, oedema develops mainly in two phases: the first 30 min after the injection, the second beginning at the end of the first hour and lasting until the third hour after injection. The oedema peaks 3 to 5 hours after injection (Ma and Zhang, 2011). The first phase has been attributed to the release of histamine, serotonin and bradykinin on vascular permeability and the later phase has been due to over production of prostaglandin in tissues (Bhalke and Pal, 2012). The mean increases in the paw diameter of the rats were measured to determine the extent of protection of the leaf fractions. In the results it was observed that the fractions significantly decrease the paw oedema at all the time except 24 hour after carrageenan injection. This may be due to the fact that inflammatory response in this model is peaked between 3 to 5 hours after carrageenan injection. The fractions anti-inflammatory activity may be due to inhibition of inflammatory mediators such as histamine, serotonin and prostaglandins.

The results of the present study also showed that the aqueous and butanol fractions exhibit comparable magnitude of analgesic and anti-inflammatory activities in all models which show that the phytochemical constituents responsible for the analgesic and anti-inflammatory effects might concentrate mainly within these two fractions.

Analgesic and anti-inflammatory effects have been observed in flavonoids, saponins alkaloids as well as tannins (Anilkumar, 2010; Bellik *et al*., 2013). Flavonoids from plants have been reported to inhibit COX-1 and COX-2 (Jang *et al*., 2002; Likhiwitayawuid *et al*., 2002) and reduce prostaglandin E2 and nitric oxide (Takahashi *et al*., 2004). Saponins (saikosaponins) have been reported to inhibit inflammatory mediators (Bellik *et al*., 2013) and inhibit activation of nuclear factor-kappaβ (Haridas *et al*., 2001). The analgesic and anti-inflammatory effect of the fractions may therefore, be due to the presence of flavonoids and saponins, due to their abundance in the aqueous and butanol fractions. However, further studies are in progress to isolate the active constituents responsible for the observed effect, and to elucidate the possible mechanisms of action responsible for the analgesic and anti-inflammatory activities of the leaf fractions.

CONCLUSION

In conclusion, this study demonstrated that the aqueous, butanol and hexane leaf fractions of *O. subscorpioidea* have significant analgesic and anti inflammatory activity mediated via peripheral and central mechanisms. Further studies will be necessary to establish the probable mechanism of action of their analgesic and anti-inflammatory activities and isolate the bioactive compound(s).

ACKNOWLEDGEMENTS

We are thankful to T/Dr. Michael Bamidele Dada (General Secretary, National Association of Nigeria Traditional Medicine Practitioners (NANTMP), Kogi State Chapter), for vital information on the traditional use of the plant and Dr Emmanuel I. Aigbokhan, for the identification of the plant. We are also thankful to the technical staffs of the Department of Pharmacology and Therapeutics and the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, for their technical support during the study.

REFERENCES

Adeoluwa, O. A., Aderibigbe, A. O. and Olonode E. T. (2014). Antinociceptive property of Olax subscorpioidea Oliv (Olacaceae) extract in mice. *Journal of Ethnopharmacology*, 28(156): 353-357. doi: 10.1016/j.jep.2014.08.040.

Anilkumar, M. (2010). Ethnomedicine: A Source of Complementary Therapeutics. *Research Signpost*, India, Pp 267-293.

Ayandele A. and Adebiyi A. (2007). The phytochemical analysis and anti-microbial screening of extract of *Olax subscorpioidea*. *African Journal of Biotechnology*, 6: 868–870.

Bellik Y., Laïd B., Hasan, A. A., Balkees, A. B., Fatiha, A., Hammoudi, S. M. and Mokrane, I. (2013). Molecular Mechanism Underlying Anti-Inflammatory and Anti-Allergic Activities of Phytochemicals: An Update. *Molecules*, 18: 322-353.

Bhalke, R. D. and Pal, S. C. (2012). Anti-Inflammatory and Antinociceptive Activity of *Pterospermum Acerifolium* Leaves. *Asian Journal of Pharmaceutical and Clinical Research*, 5(2): 23-26.

British Pain Society. Useful definitions & glossary (online), 2014; http://www.britishpainsociety.org/ patient_def_glossary.htm.

Burkill H. M. (1997). Useful Plants of West Tropical Africa. *Royal Botanic Gardens, Kew*, 4: 969.

Chi-Ren L., Chun-Pin K., Wen-Huang P, Yuan-Shiun C., Shang-Chih L. and Yu-Ling H. (2012). Analgesic and Anti-Inflammatory Activities of Methanol Extract of *Ficus pumila* L. inMice. *Evidence-Based Complementary and Alternative Medicine*, Article ID 340141, 9 pages. doi:10.1155/2012/340141.

Current Protocols in Neuroscience (online) (2002). John Wiley & Sons, Inc. http://www.mrw2.interscience.wiley.com/cponline/ts...yId=0&matchNum=0&getSearchResults=0-0&numMatches=0 (3 / 3) [02-4-25 14:51:59]

Dickenson, A.H. and Sullivan, A.F. (1987). Peripheral origins and central modulation of subcutaneous formalin-induced activity of rat dorsal horn neurons. *Neurosci. Lett.* 83:207-211.

Donkor K., Stephen A., Jerry A., Nutifafa T., Nii O. M. and Laud K. O. (2013). Analgesic and anti-inflammatory activities of *Asena*, a herbal preparation for treatment of arthritis, using rodent models. *Medicinal and Aromatic Plant Research Journal,* 1(2): 20-29.

Dubuisson, D. and Dennis, S.G. (1977). The formalin test: A quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. *Pain* 4:161-174.

Eddy N.B. and Leimbach D. (1953). Systemic analgesics. II. Dithienylbutenyl and dithienylbutylamines. *Journal of Pharmacology and Experimental Therapeutics*, 107: 385–393.

Evans, W.C., (2009). Trease and Evans Pharmacognosy. Elselviers, 16th edition.

Gu, J., Zhuo, M., Caterina, M., MacDermott, A.M., Malmberg, A., Neugebauer, V. and Yoshimura, M. (2005). Molecular pain, a new era of pain research and medicine. *Molecular Pain*, 1: 1-2.

Haddad J. J. (*2007).* Molecular Regulation of Inflammatory Pain and Hyperalgesia - Is NF-κB the Lynchpin? Focus Article and Critical Review. *EXCLI Journal, 6:68-92.*

Haridas, V., Arntzen, C.J. and Gutterman, J.U. (2001). Avicins, a family of triterpenoid saponins from *Acacia victoriae* (Bentham), inhibit activation of nuclear factor-kappa-B by inhibiting both its nuclear localization and ability to bind DNA. *Proc. Natl. Acad. Sci. USA*, 98: 11557–11562.

Hosseinzadeh, H. and Younessi, H.M. (2002). Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice. *BMC Pharmacology* 2, 7.

Hotamisligil GS, Erbay E (2008). Nutrient sensing and inflammation in metabolic diseases. *Nat Rev Immunol*., 8(12):923-34.

Ishola I. O., Agbaje O. E., Narender T., Adeyemi O. O. and Shukla R. (2012). Bioactivity guided isolation of analgesic and anti-inflammatory constituents of *Cnestis ferruginea* Vahl ex DC (Connaraceae) root. *Journal of Ethnopharmacology, 142(2):* 383–389.

Jang, D.S.; Cuendet, M.; Hawthorne, M.E. (2002). Prenylated flavonoids of the leaves of *Macaranga conifera* with inhibitory activity against cyclooxygenase-2. *Phytochemistry*, 61: 867–872.

Katzung, B.G. (2005). Basic and Clinical Pharmacology, 6th ed. *Appleton and Lange*, Connecticut,; pp. 297–302.

Khanavi, M., Delnavazi, M., Ni-Koui, V., Ostadhadi S. and Bakhtiarian A. (2012). Evaluation of analgesic effect of hydroalcohol extract of *Murrubium parviflorum* by formalin test in mice. *Asian Journal of Plant Sciences,* 11 (2): 96-99.

Kissin I. (2010). The Development of New Analgesics over the Past 50 Years: A Lack of Real Breakthrough Drugs. *Anesthesia & Analgesia*, 110 (3): 780-789.

Koné W. M., Vargas M. and Keiser J. (2012). Anthelmintic activity of medicinal plants used in Côted'Ivoire for treating parasitic diseases. *Parasitol Res*.110:2351–2362.

Koster R, Anderson M. and De Bear E.J. (1959). Acetic acid for analgesic screening. *Federation Proceedings*, 18: 412- 416.

Likhiwitayawuid, K.; Sawasdee, K.; Kirtikara, K. (2002). Flavonoids and stilbenoids with COX-1 and COX-2 inhibitory activity from *Dracaena loureiri*. *Planta Medica,* 68: 841–843.

Loomis, T. A. and Hayes, A.W. (1996). Loomis's essentials of toxicology. 4th ed., California, *Academic press*: 208- 245.

Lorke, D. (1983). A new approach to acute toxicity testing. *Archives of toxicology*, 54: 275-287.

Ma C. and Zhang J. (eds.), (2011) *Animal Models of Pain*, Neuromethods, vol. 49, DOI 10.1007/978-1-60761-880-5_1, Springer Science Business Media, LLC.

Matsumara, F. (1975). Toxicology of Insecticides., New York and London. *Plenum Press* Pp 24-26.

Meunier C. J., Burton J., Cumps J., Verbeeck R. K. (1998). Evaluation of the *formalin test to assess the analgesic activity of diflunisal* in the rat. *European Journal of Pharmaceutical Sciences,* 6: 307–312.

Milind P. and Monu Y. (2013). Laboratory models for screening analgesics. *International research journal of pharmacy,* 4 (1): 15-19.

Moffat R. and Rae C. P. (2010). Anatomy, physiology and pharmacology of pain. *Anaesthesia and Intensive Care Medicine* 12(1): 12-15.

Odoma S., Zezi A. U., Danjuma N. M and Ahmed A. (2014). Analgesic, anti-inflammatory and Phytochemical Properties of Methanol Leaf Extract of *Olax subscorpioides* Oliv (Olacaceae) in Mice and Rats. *Journal of Pharmacology and Tropical Therapeutics,* 4 (1): 29 – 37.

Ogunmefun, O. T. and Gbile, Z. O. (2012). An Ethnobotanical Study of Anti-Rheumatic Plants in South-Western States of Nigeria. *Asian Journal of Science and Technology.* 4 (11): 063-066.

Okoli R. I., Aigbe O., Ohaju-Obodo J. O. and Mensah J. K. (2007). Medicinal Herbs Used for Managing Some Common Ailments among Esan People of Edo State, Nigeria. *Pakistan Journal of Nutrition,* 6 (5): 490-496.

Ricciotti, E. and FitzGerald, G.A. (2011). Prostaglandins and inflammation. *Arteriosclerosis, Thrombosis, Vascular Biol*., 31, 986-1000.

Siegmund E, Cadmus R, Lu G. (1957). A method for evaluating both non-narcotic and narcotic analgesics. *Proceedings of the Society for Experimental Biology and Medicine,* 95(4):729–31.

Soladoye M. O., Amusa N. A, Raji-Esan S.O., Chukwuma E. C. and Taiwo A. A. (2010). Ethnobotanical Survey of Anti-Cancer Plants in Ogun State, Nigeria. *Annals of Biological Research*, 1 (4): 261-273.

Sultana B., Anwar F. and Ashraf M. (2009). Effect of Extraction Solvent/Technique on the .

Antioxidant Activity of Selected Medicinal Plant Extracts. *Molecules.* 14: 2167-2180.

Takahashi, T.; Takasuka, N.; Ligo, M.; Baba, M.; Nishino, H.; Tsuda, H. (2004). Isoliquilitigenin, a flavonoid from licorice, reduces prostaglandin E2 and nitric oxide, causes apoptosis, and suppresses aberrant crypt foci development. *Cancer Sci.*, 95: 448–453.

Tjølsen, A., Berge, O., Hunskaar, S., Rosland, J. H. and Hole, K. (1992). The formalin test: An evaluation of the method. *Pain,* 52: 5-17.

Ukwe, C. V., Ubaka, C. M. and Madusque, U. J. (2010). Evaluation of the antiulcer activity of *Olax subscorpioidea* Oliv. roots in rats. *Asian Pacific Journal of Tropical Medicine,* 3 (1): 13-16.

Vogel H. G. (2008). Drug Discovery and Evaluation: Pharmacological Assays. *Springer-Verlag,* Berlin, 3rd edition.

Winter, C. A., Risley, E. A. and Nuss, G. W. (1962). Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proceedings of the Society for Experimental Biology and Medicine*, 111: 544-547