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Anti-inflammatory Effect of the Alkaloid-rich Fraction of *Landolphia owarensis*

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract
Background: Plants have several chemical compounds acclaimed to be responsible for the pharmacological actions produced when herbal products are administered to biological systems.
Objectives: This study was designed to investigate the anti-inflammatory effect of the alkaloid-rich fraction of the ethanol leaf extract of *Landolphia owariensis*.
Methods: Qualitative phytochemical analyses were carried on the crude extract using standard methods. The alkaloid-rich fraction was obtained from the crude ethanol extract, using the classical acid/base shake-up method and the obtained fraction tested positive to Dragendorf's reagent. Oral acute toxicity was evaluated by OECD method (No 423). Anti-inflammatory effect of the fraction was evaluated using xylene-induce ear oedema and carrageenan-induced paw inflammation in mice at doses of 100, 200 and 400 mg/kg.
Results: Phytochemical screening revealed presence of alkaloids, flavonoids, tannins, saponins, steroids/terpenes and glycosides. Acute toxicity studies showed no adverse symptoms of toxicity during the 14-day observation period and no mortality was recorded, thus the LD_{50} was estimated to be greater than 2000 mg/kg. The alkaloid-rich fraction dose-dependently inhibited inflammation induced by xylene and carrageenan. In the xylene test, the fraction produced significant inhibition of 41.70 % at 400 mg/kg ($p \leq 0.05$) while in the carrageenan test 55.69 % significant inhibition ($p \leq 0.001$) was recorded with 400 mg/kg at 60 mins after induction of inflammation.
Conclusion: This study showed the anti-inflammatory potentials of the alkaloid-rich fraction of *Landophia owariensis*.
Keywords: *Landolphia owariensis*; Phytochemicals; Alkaloid; Oedema

INTRODUCTION

Alkaloids are an important class of nitrogen-containing plant natural products featuring a diverse array of intriguing structural frameworks and interesting biological and pharmacological properties. The anti-inflammatory activity of alkaloids, involving inhibition or regulation of important inflammation mediators such as NF-κB, COX-2, and iNOS are documented (Yang *et al*, 2006; Zhao *et al*, 2015; Pacheco de Oliveira *et al*, 2015). A review by Souto *et al*, (2011) to evaluate the anti-inflammatory activity of alkaloids reported 40 compounds with significant activity.

Landolphia owariensis P. Beauv (Apocynaceae) commonly known as vine rubber, is a shrub or climbing plant widely distributed in the tropical, subtropical and coastal lowlands of West Africa. It is an important ethnomedicinal plant and extensively used among the rural societies in the region. Different parts of the plant are used as a purgative and vermifuge, for the treatment of fever pains, malaria, venereal infections and as an ingredient of arrow poison (Irvine 1961; Bouquet 1969; Burkill 1985; Gill 1992). Pharmacological activities reported for the plant include analgesic, anti-inflammatory, anti-ulcer and gastric anti-secretory effects (Owoyele *et al*, 2001; Olaleye *et al*, 2008). The plant was also found to have

anti-microbial effects (Ebi and Ofoefule, 1997). The phytochemistry of *L. owariensis* recently described the isolation of flavonoids, saponin and terpenoid compounds (Ibekwe *et al*, 2019).

Based on the aforementioned and with the knowledge that species of the Apocynaceae family are rich in alkaloids, we targeted our investigation on the anti-inflammatory potentials of the alkaloid-rich fraction of the leaves of *L. owariensis*

METHODOLOGY

Plant material

The fresh leaves of *L. owariensis* were collected at Chaza, Suleja LGA of Niger State, Nigeria in September 2018 by Mr. Muazzam Ibrahim. The plant was identified and authenticated by Mr. Akeem Lateef at the herbarium of the National Institute for Pharmaceutical Research and development, Abuja, where a voucher specimen (NIPRD/7030) was deposited.

Phytochemical analysis

Preliminary qualitative screening for phytochemical constituents of *L. owariensis* leaves was carried out using standard methods (Harbone, 1998; Sofowora 2008).

Extraction procedure

The extraction of alkaloids was carried out using the method of Guo *et al*, 2012. The air-dried powder of *L. owariensis* leaves (300 g) was extracted three times with EtOH–H_2O (95:5, v/v). The combined extracts were concentrated under reduced pressure, followed by partitioning between EtOAc and 3% tartaric acid. The aqueous phase was adjusted to pH 9–10 with saturated Na_2CO_3 and then extracted with successive portions of $CHCl_3$. The pooled organic layers were concentrated *in vacuo* to obtain the alkaloid-rich fraction (0.73g).

Detection of alkaloids

Thin Layer Chromatography was employed in the preliminary detection of alkaloids. The obtained alkaloid solution in chloroform was applied as a spot on the TLC plate by using a capillary tube. The TLC plate was placed in a chromatographic tank saturated with chloroform: methanol (9:1) as the solvent system for development of the plate. The developed plate was sprayed with Dragendroff's reagent.

Animals

Adult Swiss albino mice (30–34g) of either sex obtained from the Animal Facility Centre (AFC) of the National Institute for Pharmaceutical Research and Development (NIPRD) Idu, Abuja were used for the tests. The animals were kept under ambient conditions and maintained on standard rodent feed with free access to clean drinking water from the Municipal water system (NIPRD SOP No 05-03-003). All drugs were freshly prepared and administered orally using an oro-gastric cannula.

Acute toxicity tests

The OECD (No 423) method of the limit test was employed to determine the acute toxicity of the alkaloid-rich fraction (ALO). Five non-gravid, nulliparous female mice were each given a single oral dose of 2000 mg/kg of ALO. The animals were observed for signs of toxicity and mortality for the first 30 min, then periodically over the following 24 h, after which the animals were monitored daily for signs of toxicity for a duration of 14 days. Animals were weighed weekly (OECD, 2001).

Studies on xylene induced ear oedema

Mice were randomly placed into groups of 6 animals each. Group 1 received distilled water 10 ml/kg, group 2 received 1 mg/kg of dexamethasone, while groups 3-5 were treated with ALO at 100–400 mg/kg respectively. Sixty minutes after treatment one drop of about 0.02 ml of xylene was applied on the inner surface of the right ear of each mouse. After 2 h, the animals were sacrificed by inhalation of diethyl ether. The entire ear was removed and round pieces of 6 mm were cut out from both ears and the weight taken using a digital weighing balance. The difference between the weight of both ears was calculated and the inhibition ratio determined using the formula reported by Tiwari *et al*, 2013.

% Inhibition = $\{1 - Vt/Vc\}\,100$

Where Vc = Difference between right and left ear in untreated group and

Vt = Difference between right and left ear in groups treated with Alkaloid-rich fraction of *Landolphia owariensis* or Aspirin

Studies on carrageenan induced inflammation

This study was carried out following the method described by Winter *et al*, (1962). Swiss albino mice (30) were randomly assigned into five groups of 6 animals each and treated as follows: animals in group 1 served as negative control and were given distilled water. Mice in group 2 were treated with Aspirin

(ASA, 150 mg/kg) and served as the positive control. While, animals in groups 3 – 5 were treated with the alkaloid-rich fraction at doses of 100, 200 and 400 mg/kg respectively. After sixty minutes, oedema was induced by injection of 0.05 ml of 1.5 % carrageenan into the right sub-plantar region of the right hind paw of each mouse. The paw volume was measured using a digital plethysmometer (Ugo Basile model:7141, No. 0235U13) at 0, 30, 60, 90, 120 minutes and 24 h after carrageenan administration. The actual inflammation was calculated as the change in paw volume after subtraction of basal paw volume. The inhibitory effect was determined by using following formula:

$$\% \text{ Inhibition} = \frac{(Ct-Co)control - (Ct-Co)treated}{(Ct-Co)control} \times 100$$

Where $(C_t - C_0)_{control}$ is the difference in the size of paw in control mice, and

$(C_t - C_0)_{treated}$ is the difference in the size of paw in mice treated either with Alkaloid-rich fraction of *Landolphia owariensis* or Aspirin (Gupta *et al*, 2015).

STATISTICAL ANALYSIS

The Graphpad PRISM® 6.0 was used for statistical analysis. Results were presented as Mean ± SEM. One-way ANOVA was used to evaluated the change in weight between the mean of the right and left mouse ear followed by a post hoc Dunnett's test for multiple comparison. While Two-way ANOVA was used to compare the change in paw volume in carrageenan treated rats followed by a post hoc Tukey test. The level for statistical significance was set at $p<0.05$.

RESULTS

Phytochemical screening

The phytochemical tests carried out on the leaf extracts of *L. owariensis* indicated the presence of saponins, alkaloids, steroids/terpenes, tannins, glycosides and flavonoids. The results are presented in Table 1. The alkaloid-rich fraction which was obtained by the extraction procedure, eluted as orange spots on the TLC plate when sprayed with Dragendorff's reagent, confirming the extraction of alkaloids.

Table 1: Phytochemical screening for metabolites of leaf of *L. owariensis*

Test	Plant Extract
Flavonoids	+
Tannins	+
Saponins	+
Steroids/Terpenes	+
Glycosides	+
Alkaloids	+

+=positive

Acute toxicity studies

The oral LD_{50} of the alkaloid-rich fraction of *L. owariensis* was estimated to be greater than 2000 mg/kg because no sign of toxicity or mortality was recorded in treated mice during the 14 days of observation.

Effects on xylene induced ear oedema

Administration of 0.02 ml of xylene to each ear produced ear swelling in all mice indicating that inflammation was produced on administration of xylene. The results obtained (Table 2) showed decrease in weight of the mice ear with inhibition rates of 11.10 %, 16.70 % and 41.70 % for ALO at 100 – 400 mg/kg respectively. Aspirin produced an inhibition of 47.25 % in this study.

Table 2: Effect of alkaloid rich fraction of *L. owariensis* on xylene induced ear oedema in mice

Treatment	Dose (mg/kg)	Difference in weight of ear	% Inhibition
Normal saline	10 ml/kg	12.00 ± 1.71	-
ALO	100	10.67 ± 0.42	11.10
ALO	200	10.00 ± 0.89	16.70
ALO	400	7.00 ± 1.13[a]	41.70
Aspirin	150	6.33 ± 0.95[a]	47.25

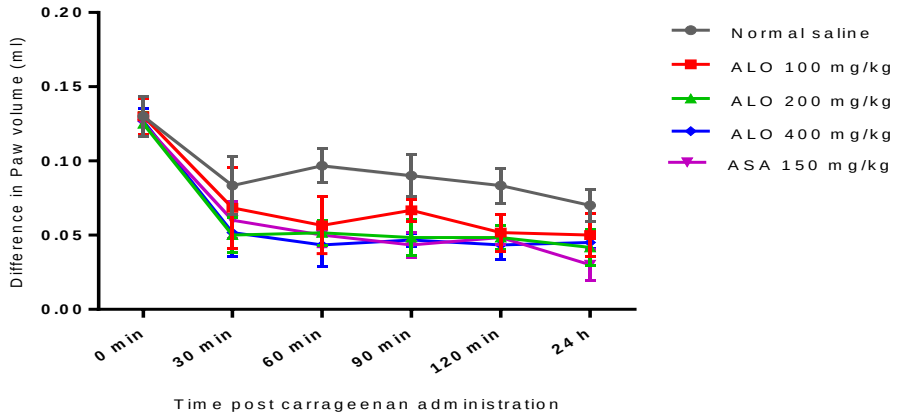
ALO = Alkaloid rich fraction of *L. owariensis*

Values are presented as mean ± SEM (n = 6), Significance: compared to control

[a]$p < 0.05$, groups (Two-way ANOVA, Post Hoc – Dunnett's)

Effects on carrageenan induced inflammation

The results obtained in this study showed decrease in paw volume in the groups treated with 100 to 400 mg/kg of ALO when compared with the control groups. The change in volume was significant ($p < 0.05$) from 60 minutes for the group treated with 100 - 400 mg/kg of ALO (Fig 1). The inflammation was inhibited in dose-dependent manner as reflected in Table 3.



ALO = **alkaloid-rich fraction of *Landolphia owariensis***
Values are presented as mean ± SEM (n = 6), Significance: compared to control
[a]p < 0.05, [b] p < 0.01, groups (Two-way ANOVA, Post Hoc - Tukey)

Figure 1: Effect of an alkaloid-rich fraction of *Landolphia owariensis* on carrageenan induced inflammation in mice

Table 3: Effect of an alkaloid rich fraction of *Landolphia owariensis* on carrageenan induced paw oedema in mice
NS = Normal saline, ALO = Alkaloid rich fraction of *L. owariensis*

Difference in paw volume (ml)	Treatment (mg/kg)	NS (10 mL/kg)	ALO 100	ALO 200	ALO 400	Aspirin 150
	30 min	0.083 ± 0.019	0.068 ± 0.027	0.050 ± 0.012	0.052 ± 0.016	0.060 ± 0.012
	% Inhibition	-	18.07	39.76	37.35	27.71
	60 min	0.097 ± 0.011	0.057 ± 0.019[a]	0.052 ± 0.008[b]	0.043 ± 0.008[c]	0.050 ± 0.008[b]
	% Inhibition	-	41.24	46.39	55.69	48.45
	90 min	0.090 ± 0.014	0.067 ± 0.008	0.043 ± 0.012[a]	0.047 ± 0.004[a]	0.043 ± 0.008[b]
	% Inhibition	-	25.56	46.67	47.78	52.22
	120 min	0.083 ± 0.012	0.052 ± 0.012	0.048 ± 0.008	0.043 ± 0.010[a]	0.0043 ± 0.005
	% Inhibition	-	37.35	42.17	48.19	42.17
	24 h	0.075 ± 0.013	0.050 ± 0.015	0.042 ± 0.012	0.045 ± 0.004	0.030 ± 0.010
	% Inhibition	-	33.33	44.00	40.00	60.00[b]

Values are presented as mean ± SEM (n = 6), Significance: compared to control
[a]p < 0.05, [b] p < 0.01, [c]p<0.001 groups (Two-way ANOVA, Post Hoc - Tukey)

DISCUSSION

The presence of some classes of secondary metabolites as indicated in the preliminary qualitative phytochemical studies was confirmed in a recent study by Ibekwe *et al*, 2019 where two flavonoids, a terpene and a saponin were isolated and identified. The effects of the alkaloid-rich fraction of *L. owariensis* were evaluated in mice using *in-vivo* models of carrageenan and xylene induced oedema. The limit test is usually employed whenever a test substance is suspected to be non-toxic based on historical information about the test substance (Erhirhie *et al*, 2018). Loomis and Hayes (1996), classified substances that are in 500 – 5000 mg/kg as being slightly toxic while 5000 – 15,000 mg/kg are classed as practically non-toxic. The Globally Harmonized System (GSH) of classification and labeling of chemicals placed substances with LD_{50} within the range of 2000 to 5000 mg/kg as agents which are of relatively low acute toxicity, while substances with LD_{50} greater than 5000 mg/kg were unclassified (OECD, 2001). Although there are no previous reports on the isolation of the alkaloid of this plant, the existing data on the LD_{50} of the crude extract (2000 ≤ 5000 mg/kg) formed the basis for the selection of the limit tests for acute toxicity studies (Nwogu *et*

al, 2008, Ezike *et al*, 2016). Results obtained from this study showed ALO with an estimated LD_{50} greater than 2000 mg/kg, thus indicating that ALO has low potential to cause toxicity on acute oral administration. Carrageenan and xylene are substances commonly used to induce inflammation in laboratory animals. Carrageenan causes inflammation by release of inflammatory mediators that include histamine, 5-hydroxytryptamine, leukotrienes, kinins and cyclooxygenases in the early phase while the late phase is characterized by production of prostaglandins, bradykinin, neutrophil infiltration (Gupta *et al*, 2015). Xylene induced ear oedema reflects the oedematization that occurred during the early stages of acute inflammation which is probably related with the release of inflammatory mediators (Ravelo-Calzado *et al*, 2011). The data obtained in this study suggested anti-inflammatory action by ALO. These results correspond with the report of Owoyele *et al*, 2001 on the analgesic and anti-inflammatory effects of the leaf extracts of *Landolphia owariensis*. The study also reported the leaf extracts of *L. owariensis* contains the phyto-chemicals alkaloids and flavonoids. Pharmacological activities of plants have been linked to the effects of the plants' constituents acting synergistically or by means of an active compound (Yuan *et al*, 2016). Alkaloids have been shown to possess anti-inflammatory actions (Souto *et al*, 2011), thus the presence of the alkaloids (ALO) obtained from the plant may have contributed to the anti-inflammatory effects of *L. owariensis* as demonstrated by the results shown in this study. This action is in a similar manner to the NSAID Aspirin. Although the mechanism of action of ALO in alleviating inflammation is yet to be determined, it can be postulated to be through the inhibition of the precursors of inflammatory mediators such as the cyclooxygenases in a manner similar to aspirin (Vane and Botting, 2003).

CONCLUSION

The results obtained in this study lend credence to the reported anti-inflammatory properties of alkaloids and may also explain some of the ethnomedicinal uses of the plant in traditional medicine.

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