

Original article

ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF LEAF EXTRACTS OF *LANDOLPHIA OWARIENSIS*

OWOYELE B.V,¹ OLALEYE S.B^{1*}, ²OKE J.M. AND ELEGBE R.A¹.

¹Departments of Physiology and ²Pharmaceutical Chemistry, University of Ibadan, Nigeria

The aqueous, methanol and chloroform extracts of *Landolphia owariensis* leaves (AELO, MELO & CELO respectively) was investigated for anti-inflammatory and analgesic activities. All the extracts (100mg/kg each) were found to significantly ($P < 0.05$) inhibit paw edema induced by carrageenan in rats and the nociception induced by Tail immersion in hot water ($50.0 \pm 1.0^{\circ}\text{C}$) and acetic acid. The methanol extract produced the highest paw edema inhibition while in thermally induced nociception both the MELO and CELO show high and comparable analgesic activity with acetylsalicylic acid (150mg/kg). However in chemically induced pain (acetic acid) MELO produced the highest and comparable analgesic activity to acetylsalicylic acid (150mg/kg). We therefore conclude, that apart from the folklore uses of *L. Owariensis* leaves as antimalarial agents, the various extracts of the plant also possess anti-inflammatory and analgesic activities. Phytochemical analysis showed that the methanolic extract of *L. owariensis* contain some secondary metabolites namely: alkaloids and some polyphenolic compounds. Also, this extract exhibits some anti-oxidative activities.

Keywords: *Landolphia owariensis* – analgesic - anti-inflammatory-rats

*Author for correspondence

INTRODUCTION

Landolphia owariensis P. beauv. (family Apocynaceae) commonly called vine rubber and known locally by various names (Ibo-nwalikali, mba Yoruba, *Ibo alaitipa*, Hausa ciwo) is widely used for the treatment of many ailments. The decoction of leaves is used as a purgative and to cure malaria. The root is soaked in local gin for about a week and the extract given two full wine glass a day to cure gonorrhoea. (Gill, 1992). Lewis and Lewis (1977) also reported the use of stem bark as vermifuge. The latex is drunk or used in french Equatorial Africa as an enema for intestinal worms and in parts of Ivory Coast the latex forms an ingredient arrow poison (Irvine 1961). It is used to make native beer and beverage in senegal and upper Nile land respectively (Dalziel, 1937).

Very few reports are available in literature to establish the anecdotal uses of this plant. Ebi and ofoefule (1997) have validated the folkloric use of *Landolphia Owariensis* as an antimicrobial agent. This study was aimed at investigating the effects of various extracts (aqueous, methanol and chloroform extracts) of the leaves of *Landolphia owariensis* P. beauv on pain (Thermally and chemically induced) and inflammation in mice and rats respectively.

MATERIALS AND METHODS

Plant Material: *L. Owariensis* leaves was collected from their natural habitat at Gambari forest reserve, Oyo State, Nigeria in January, 2000 and authenticated by Mr. T.K. Odewo, a Taxonomist of the forestry research Institute of Nigeria (FRIN) Ibadan. Identification of the plant took place at FRIN by the same Taxonomist. A voucher specimen (FHI 105678) has been deposited in the Herbarium of the same institution.

Extract Preparation: Air-dried and powdered leaves of *L. Owariensis* P. beauv were extracted successively with H_2O , Me-OH and CHCl_3 at 80°C , 40°C and room temperature respectively. The dried extract was stored

at 4°C until use. The extract yields of the plant were 1.2g, 3.0g and 2.0g from 20.0g, 30.0g and 20.0g of powdered leaves in 150ml water, 300ml methanol and 250ml of chloroform respectively. The aqueous extract (AELO) was dissolved in 0.9% saline while the methanol extracts (MELO) and chloroform extract (CELO) were each dissolved in 2.5% Tween 80 and subsequently in normal saline.

Animals: Adult male and female Swiss mice (20-28g) and albino rats (120 - 150g) obtained from the animal house, College of Medicine, University of Ibadan, Nigeria were used. They were housed in cages at room temperature with free access to mice cubes (Ladokun Feeds Nig. Limited, Ibadan, Ibadan Nigeria).

Phyto-Chemical Investigation: The crude extracts (methanolic and dichloromethane) were investigated for secondary metabolites by standard methods (Persino and Quimby, 1967; Odebiyi and Sofowora, 1979). The dichloromethane extract gave positive results for alkaloids. Methanol extract gave positive results when developed in a predetermined system and further sprayed with Goddin's reagent to show the presence of polyphenolic compounds (Persino and Quimby, 1967; Odebiyi and Sofowora, 1979).

The crude methanolic extract was further spotted on Aluminium Foil TLC plates F254, developed in ethylacetate formic acid -; H_2O - (15; 3;1) and latter sprayed with 2, 2-diphenyl - picryl hydrazine (DPPH) spray to give positive test for anti-oxidants. Hence further separation through the vacuum liquid and size exclusion. Chromatographs, thus producing slightly pure extracts. This pure extracts were further investigated with the DPPH spray. Clear yellow spots over purple backgrounds confirmed the presence of flavonoids (Potteract, 1997).

Free Radical Scavenging Activity: DPPH, a stable free radical was dissolved in ethanol give a 100 μM

solution. To 3.0ml of the ethanol solution of DPPH was added 0.5ml of the methanol extract of *L. owariensis* in ethanol. The decrease in DPPH absorption at 517nm was measured 10min. later. The actual decrease in absorption induced by the investigated extract was calculated by subtracting that of the control (Mellors and Tappel A.C. 1966; Poteract, 1997).

Anti-Inflammatory Activity: The effect of oral administration of 100mg/kg of the extracts of *Landolphia owariensis* (AELO, MELO & CELO). 150mg/kg Acetylsalicylic acid (Dysprin^R - Reckitt & Coleman) or vehicle (Saline, 10ml/kg) on the hind-paw oedema induced by subplantar injection of 0.1ml carrageenan (1% w/v) was evaluated according to the method described by Winter *et al* (1962). Paw oedema was measured by wrapping a piece of cotton thread round the paw and measuring the circumference with a metre rule (Hess and Miloning, 1972; Bamgbose and Noamesi, 1981). Measurement was carried out immediately before and 3 hours following carrageenan injection. The inhibitory activity was calculated according to the following formula.

$$\text{Percentage inhibition} = \frac{(\text{Ct} - \text{Co})_{\text{control}} - (\text{Ct} - \text{Co})_{\text{treated}}}{(\text{Ct} - \text{Co})_{\text{control}}} \times 100$$

Inhibitory activity at 3 hours was taken as a measure of oedema.

ANALGESIC ACTIVITY: *L. Owariensis* leaf extracts (AELO, MELO & MELO) was evaluated for analgesic activity in mice using Tail immersion (Jansen & Jagenav, 1959) and acetic acid induced writhing (Koster *et al*, 1959) tests.

a. Tail immersion: Mice were treated orally with 100 mg/kg of the leaf extracts (AELO, MELO & CELO), reference drug (150mg/kg Acetylsalicylic acid) and vehicle (Saline, 10ml/kg). 1 hour before the measurement of extract effect. Water was heated to 50.0 + 1.0oC in a water bath. The time taken for the animal to remove it tail out of the water was recorded.

b. Acetic acid induced writhing: Mice were injected in traperitoneally with 0.6% aqueous acetic acid (10ml/kg), 1 hour after oral administration of 100mg/kg of AELO, MELO and CELO or vehicle (saline, 10ml/kg). Acetylsalicylic acid (150mg/kg, p.o) treated group was induced in the study as a positive control. The number of writhing movement of each mouse was counted for 10min, commencing 5min after injection of acetic acid.

Statistical Analysis

All values were expressed as mean + S.E.M. Statistical significance was determined using the students t-test. Values with P<0.05 were considered significant.

RESULTS

Phytochemical screening: The phytochemical analysis showed that the extract of *L. owariensis* contained alkaloids (in the dichloromethane extracts) and flavonoids (in the methanolic extract). Investigation on free radical scavenging activity with 2, 2-diphenyl -1-picryl hydrazine showed that the methanol extract of *L.*

owariensis possesses free radical scavenging (anti-oxidative) activity

Anti-Inflammatory activity: The results obtained with 100mg/kg of the aqueous, methanol and chloroform extracts of *Landolphia owariensis* on carrageenan-induced rat hind paw oedema are shown in Table 1. The extracts significantly (P<0.05) inhibited the inflammatory oedema, though the inhibition was highest in MELO. The effect of MELO was the same as that of 150mg/kg of Aspirin.

Table 1.
Effect of the various extract of *Landolphia owariensis* leaves on carrageenan-induced paw oedema in rats.

Treatment	Dose (mg/kg)	Paw size (Mean ± S.E.M)	Inhibition
Control (saline)		3.14 ± 0.07	-
<i>L. Owariensis</i>			
Aqueous extract	100	2.58 ± 0.07*	52.27
Methanol extract	100	2.50 ± 0.07*	61.36
Chloroform extract	100	2.72 ± 0.05*	43.18
Acetylsalicylic	150	2.62 ± 0.04*	61.40
Acid			

*P<0.05 (c.f; Vehicle), n = 5, student's t-test.

Table 2:
Effect of the various extracts of *Landolphia Owariensis* leaves on Tail immersion in 50 ± 1°C hot water (mice).

Treatment	Tolerance ^a	Pre-treatment 0 min	After-treatment	
			60 min	90 min
Control (saline)	Latency (sec)	8.25 ± 1.05	11.25 ± 1.05	11.88 ± 0.44
	% Protection		-	-
AELO (100mg/kg)	Latency (Sec)	10.13 ± 0.52	16.75 ± 1.61*	17.50 ± 0.44*
	% Protection		48.88	47.31
MELO (100mg/kg)	Latency (Sec)	8.25 ± 0.37	22.88 ± 1.11*	23.0 ± 0.71*
	% Protection		103.38	93.60
CELO (100mg/kg)	Latency (Sec)	7.13 ± 0.30	20.13 ± 0.70*	20.50 ± 0.60*
	% Protection		78.93	72.26
ASA (150mg/kg)	Latency (Sec)	11.0 ± 0.32	17.38 ± 0.87*	20.75 ± 1.36*
	% Protection		54.48	74.66

^aPercentage protection = $\frac{\text{Latency (test)} - \text{Latency (control)}}{\text{Latency (control)}} \times 100$

*P<0.05 (c.f. Vehicle), n = 8; ASA = Acetylsalicylic acid

Analgesic activity: Table 2 shows the responses of mice to Tail immersion. Treatment with 100mg/kg of aqueous, methanol and chloroform extracts of *Landolphia owariensis* significantly (P<0.05) protected the animals from the thermal stimuli. The percentage

protection of the animal by the extracts from the thermal stimuli were comparable to that of 150mg/kg of Aspirin.

Table 3 shows the response of mice to acetic acid-induced writhing. Treatment with 100mg/kg of aqueous, methanol and chloroform extracts of *Landolphia owariensis* significantly ($P < 0.05$) reduced the number of writhes. The inhibitions were 52.3%, 59.2% and 74.9% respectively for aqueous, methanol and chloroform extracts. At the dose of 100mg/kg, the chloroform extract inhibited the writhing response almost to the same degree as aspirin (79.4% inhibition) at 150mg/kg.

DISCUSSION

The results of the present study have shown that the crude extract of the investigated plant exhibited very high anti-inflammatory and analgesic activities. These activities may be linked with the presence of polyphenolic compounds present in the extract. The phytochemical tests showed that the extract of *L. owariensis* contained anti-oxidative constituents, which includes flavonoids or flavonoidal compounds. Many plants containing flavonoids have been shown to have diuretic, laxative, antispasmodic, anti-hypertensive and anti-inflammatory actions (Okuda, 1962).

The test with DPPH is very confirmatory for anti-oxidative activity of compounds. This test gives information on the reactivity of the extract with a stable free radical: thus the odd electrons of DPPH radical give a strong absorption band at 517nm in visible spectroscopy (deep violet colour). As the electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes and the resulting decolourisation is stoichiometric with the number of electrons taken up. Flavonoids have also been reported to possess antioxidant and antiradical properties (Robaki, 1988; Birs et al, 1991)

Table 3: Effect of the various extracts of *Landolphia Owariensis* leaves on acetic acid-induced writhing test in mice.

Treatment	Dose (mg/kg)	No of Writhing (Mean \pm S.E.M.)	Inhibition (%)
Control (saline)	-	35.88 \pm 1.86*	-
<i>L. Owariensis</i>			
Aqueous extract	100	17.13 \pm 0.81*	52.3
Methanol extract	100	14.63 \pm 0.62*	59.2
Chloroform extract	100	9.0 \pm 0.42*	74.9
ASA	150	7.38 \pm 0.32*	79.4

* $P < 0.001$, n = 8

The highest anti-oxidant activity was observed in the methanol extract. This may explain the finding that

the highest anti-inflammatory activity was observed in the methanol extract.

The present study has shown that the aqueous methanol and chloroform extracts of *L. owariensis* P. Beauv leaf have moderate analgesic activity and high anti-inflammatory activity. We have shown that at 100mg/kg these various extracts protected the animals from pain produced by thermal and chemical stimuli. This is a part confirmation for the anecdotal use of *L. Owariensis* leaves decoction as cure for malaria (Gill, 1992) since pain mostly accompany malaria fever.

REFERENCES

- Bangbose, S.O.A, Noamesi, B.K., (1981):** Studies on cryptolepine inhibition of carrageenan-induced oedema. *Planta medica* 42, 392 – 396.
- Birs, W; Heller W. Micheal C. Satan M. (1991)** Flavonoids as antioxidant. Determination of antiradical efficiencies. *Methods litcymal* 186: 343-353.
- Dalziel J.M. (1937):** Useful plants of West Tropical Africa. Published by the crown Agents for the colonies, London Pp 374.
- Dirosa, M., Giroud, J.P., Willoughby, D.A., (1971)** Studies of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. *J. pathol.* 104, 15 – 29.
- Ebi, G.C & Ofoefule, S.I. (1997).** Investigations into the folkloric anti-microbial activities of *landolphia owariensis*. *Phytother. Res.* 11(2): 149 – 151.
- Gill, L.S. (1992):** Ethnomedical uses of plants in Nigeria pp 145 Uniben press.
- Hess, S.M. Miloning, R.C. (1992).** Assay for anti-inflammatory drugs. In Lepow, I.H. Ward, P.A. (Eds), *Inflammation, Mechanisms and control.* Academic press, New York, pp 1-2.
- Irvine, F.R., (1961).** The Woody plants of Ghana. Oxford University Press, London, P.
- Iyer, P.R., Brown, J.K, Chaubal, M.G and Malone, M.H. (1997).** *Brufelsia hopeana* I. Hippocratic screening and anti-inflammatory evaluation. *Lloydia*, 40 (4): 356-360.
- Lewis, W.H., Lewis, M.P.E., (1977):** *Medical Botany.* John Wiley and Sons New York.
- Mellors A. Tappel A.C. (1966).** The inhibition of mitochondrial peroxidation by libiquinone and abiquinol. *J. Biol. Chem.* 241 : 4353 – 4356.
- Odebiyi O.O. and Sofowora E.A. (1979)** Phytochemical screening of Nigerian Medicinal plants – Part I 2nd Ona/STRC Inter African Symposium and Traditional Pharmacopea Pub. No. 115 Lagos.
- Persino G.J. and Quimby M.W. (1967)** Nigerian Plants III. Phytochemical screening of alkaloids, saponins, Tannis, J. *Pharm. Sci.* 56 (2) 1512.
- Poteract C. (1997).** Anti-oxidants and free radical scavenging of Natural origin. *Current orgnic chemistry* 1. 415 – 440.
- Recio, M.C., Giner, R.M., Manez, S., Gueho, J., Julien, J. Hosttmann and Rios J.L. (1995):** Investigations on the steroidal anti-inflammatory activity of iriterpenoids from *Diospyros leucomelas*. *Planta medica* 61:9-12.
- Robaki. Glyglewski R.J. (1988).** Flavonoids are scavengers of supeoxide anim. *Biochem. Pharmacol.* 37. 837-841.
- Winter, C.A., Risley, E.A. Nuss, C.W., (1962).** Carrageenan-induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proceedings of the society for Experimental Biol. and Med.* 111, 544-547.

Received: January 2001

Accepted in final form: September 2002