Vol. 3 no. 2, pp 111-115 (September 2006)
http://www.ajol.info/journals/jpb

Journal of
PHARMACY AND
BIORESOURCES

Acute toxicity studies, anti-inflammatory and analgesic activities of the methanolic extract of the stem bark of *Enantia chlorantha* and *Nauclea latifolia*

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Received 20th June 2005; Accepted 25th August 2006

Abstract

Methanolic extracts of the stem bark of *Enantia chlorantha* and *Nauclea latifolia* were investigated in rats and mice for anti-inflammatory and analgesic activities. The activities of the extracts were tested on egg white-induced oedema, acetic acid-induced writhing and hot plate models. Methanolic extract of *Nauclea latifolia* was found to be very toxic with an LD_{50} of 0.852 g/kg. The LD_{50} *Enantia chlorantha* was 2.588 g/kg. At low concentrations, *Nauclea latifolia* (150 mg and 500 mg) extract was found to have weak analgesic and anti-inflammatory effects. *Enantia chlorantha* extract was found to possess anti-inflammatory and analgesic activities, at higher doses (400 mg and 1100 mg/Kg). Higher dose was not used for *Nauclea latifolia* because of its toxicity in experimental animals. Phytochemical tests revealed that both extracts contain mainly flavonoids, tannins and low concentration of glycosides. The results from this study support the claims by traditional healers that these plants have analgesic and anti-inflammatory properties.

Keywords: *Enantia chlorantha; Nauclea latifolia;* Analgesic activity, Anti-inflammatory activity; Phytoconstituents

Introduction

Enantia chlorantha and *Nauclea latifolia* are used in West Africa as medicine for the management of diseases in both man and animals (Keharo and Adam 1974). They are used particularly for the management of fever, malaria and general body pains by traditional healers in Northern Nigeria (Dalziel 1937; Oliver 1960; Irvine 1961). *E. chlorantha* has been reported to contain alkaloids known as berberine, protoberberine (Virtanen., *et al* 1988), while *N. latifolia* contain nauclefolonine (Ngnokam *et al.* 2003; Shigemori *et al.*, 2003). *E. chlorantha* and *N. latifolia* stem bark is often mixed with the stem bark of other plants for the treatment of Malaria and general body pain.

Some alkaloid from the extract of *N. latifolia* has been reported to interact in vitro with DNA of bacteria and mammalian cells, leading to G2-M cell cycle arrest and heritable DNA-damage, as well as inducing in vivo single-strand breaks in liver, kidney and blood cells (Traore *et al.*, 2000). This plant also have anti-malarial activity (Azas *et al.*, 2002; Traore-Keita *et al.*, 2000; Benoit-Vical *et al.*, 1998), anthelmintic activity (Onyeyili *et al.*, 2001; Fakae *et al.*, 2000), anti-amoebic

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ISSN 0189-8442

activities, spasmolytic activities (Tona *et al.*, 2000), and molluscicidal activity (Kela *et al.*, 1989).

The use of *Enantia chlorantha* for its Prophylactic and healing properties on ulcers (Tan *et al.*, 2000), effect on chloroquine resistant malaria (Kimbi *et al.*, 1996; Agbaje *et al.*, 1991), and anti-microbial (Moody *et al.*, 1995) has been confirmed by researcher. These plants are therefore valuable tools in the hands of Traditional healers for the management of different disease states. Patients often regard any traditional healer with knowledge of "good medicinal plants". The first conviction to show that the Traditional healer is good in his field is when the drug he administers relieves pain resulting from the ailment promptly. This therefore means that most of the medicinal plants may actually have analgesic activity.

In this study, the methanolic extracts of the two plants were screened for analgesic and anti-inflammatory activities.

Experimental

N. latifolia and *E. chlorantha* stem bark were collected from Babale, Jos, Plateau State, Nigeria in July 2003. They were authenticated by a taxonomist (Mr. Kareem) of the Federal College of Forestry and Department of Botany, University of Jos, Jos. Plateau State. Powdered stem bark of *E. chlorantha* and *N. latifolia* were respectively subjected to soxhlet extraction using methanol for 72 hours. The resultant mixture was vaporized at low temperature and the resultant residue refrigerated at -4^0C until use. The percentage yields were 12.3% and 14.7% for *E. chlorantha* and *N. latifolia* respectively.

Animals. Albino mice (both sexes and weighing 20- 30g) and Wistar rats (both sexes and weighing 140-170g) were used. The animals were bred in the Animal House, Nigerian Institute of Trypanosomiasis Research, Jos, Nigeria, under standard environmental conditions, and fed with standard diet (NITR) and water *ad libitum*.

Acute toxicity. The LD_{50} values of the extracts were determined in mice following intra-peritoneally administration as described by Lorke (1983).

Test for analgesia in mice.

Writhing reflex test. Mice of either sex were randomly divided into groups of six each. One group received the distilled water (control) and the other two groups received two doses of *E. chlorantha* extract (400 mg and 1100 mg/Kg) intra-peritoneally. Thirty minutes later, 0.01% acetic acid injected intra-peritoneally. The number of abdominal contractions (writhing movement) observed were recorded for 15minutes, starting from 5 minutes after injection of acetic acid. The percentage inhibition of writhing movement (relative to the control animals) was then calculated. The same procedure was repeated for *Nauclea latifolia* extracts (150 mg and 500 mg/kg, i.p.) according to the methods reported by Oriowo (1982) and Otimenyin (2004).

Hot plate test. Rats were kept in a glass cylinder (open at both ends) on a hot plate, such that the rats have direct contact with the hot plate maintained at constant temperature of 55 ± 1^0C. Time taken for responses like paw licking or jumping was recorded. Rats were divided into three groups; one group (control) received distilled water, while the other groups received *E. chlorantha* extract (400 mg and 1100 mg/kg, i.p.), 30 minutes before placement on the hot plate. This procedure was repeated for *N. latifolia* extract (150 mg and 500 mg/Kg, i.p.). This procedure is similar to that reported by Williamson *et al.* (1996) and Otimenyin (2004).

Anti-inflammatory test. Acute inflammation was induced by injecting egg-white into the sub-plantar surface of the rat's hind paw. Acute inflammation was measured by increase in the rat's right hind paw linear circumference. Adult Wistar rats of either sex

(140-170g) were divided into five groups of six each. Animals used for this experiment were food-fasted for 12h and deprived of water only during the experiment. *E. chlorantha* extract (400mg and 1000mg/kg, i.p.) was administered intra-peritoneally, 30 minutes before the induction of inflammation. Paw circumference was measured with the air of vernier caliper at 1hour, 2hour and 3hours from the time of induction of inflammation. To obtain a measure of edema, the measurement at zero time was subtracted from the measurement at specific time intervals. The above procedure was repeated for *N. latifolia* (150mg and 500mg/kg, i.p.). Control rats received an equal volume of water 30 minutes before the administration of egg white in accordance with the method of Hess and Milong (1972); Oriowo, (1982); and Otimenyin (2004)

Statistical analysis. Differences between control and treatment groups were determined using the Student t-test (Snedecor and Cochran, 1967).

Results and Discussion

The LD_{50} values of the methanolic extract of *E. chlorantha* was 2.588 g/kg, which implies that it is safe for consumption at safe doses, while the LD_{50} for *N. latifolia* was 0.852 g/kg, this show that this plant is very toxic if administered intra-peritoneally. This result suggests that *N. latifolia* is very toxic in mice when administered intra-peritoneally. This results supports the report by Traore and his colleges (2000) that *N. latifolia* interacts in vitro with DNA of mammalian cells, leading to G2-M cell cycle arrest and heritable DNA-damage, as well as inducing in vivo single-strand breaks in liver, kidney and blood cells. The plant extract should be taken with care, and traditional healers educated on the inherent danger of administering the ethanolic preparations of the *N. latifolia.* More studies into the mechanism of toxicity of *N. latifolia* may provide a useful biologic agent. Plants with toxic effect have been reported to be of pharmacologic importance. For example, eserine from Calabar bean, which was once used as an ordeal poison to assess the guilt or innocence of suspected criminals and heretics has been found to be of pharmacological importance. The substance physiostigmine, which was discovered to be responsible for its toxicity, is now an effective drug for the management of glaucoma (Rang and Dale, 1991). It has however been reported that the aqueous extract of *N. latifolia* is safe for consumption and effective as an analgesic (Ngnokam *et al.*, 2003). Methanol may have extracted more of the toxic principles than water. Traditional healers should therefore be advised to use aqueous preparations rather than the ethanolic preparation of *N. latifolia.*

The extracts of *N. latifolia* and *E. chlorantha* from the results obtained showed anti-inflammatory activity (Table 1). They showed dose and time–dependent inhibition of egg-white induced paw edema (Table 1). At time interval of 2 hours both extracts produced significant ($P<0.05$) inhibition of egg-albumin induced rat paw edema. Both extracts have anti-inflammatory activity.

Results obtained from this study further showed that the Methanolic extracts of *N. latifolia* (150 mg and 500 mg/kg ip) did not ($P>0.05$) affect the stay time of rats on the hot plate, but it significantly inhibited ($P<0.05$) acetic acid induced writhing in mice. *E. chlorantha* extract significantly ($P<0.05$) inhibited acetic acid induced writhing in mice and prolonged the stay time of rats on the hot plate. Non-narcotic analgesics (peripherally active) are differentiated from narcotic (centrally active) analgesics by the ineffectiveness of non-narcotics in the prolongation of the stay time of rats on the hot plate (Turner, 1965). *N. latifolia* can be assumed to be effective on peripherally induce pain, but not centrally induced pain. *E. chlorantha* was effective in the protection of

experimental animals (in both models use) again peripherally and centrally induce pain.

Table 1: Anti-inflammatory effects of methanolic extracts of *E. chlorantha* and *N. latifolia*

Treatment	Ave. Inf. (1h)	Inh. (1h)	% Inh	Ave. Inf. (2h)	Inh. (2h)	% Inh	Ave. Inf. (3h)	Inh. (3h)	% Inh
Enantia chlorantha									
Control	3.55±0.75			3.47±0.35			3.35±0.65		
150mg/kg	3.18±0.033	0.37	10	2.69±0.076	0.72	22	2.49±0.046	0.89	26
500mg/kg	1.77±0.011	1.78	50	1.99±0.019	1.53	43	1.77±0.012	1.58	47
Nauclea latifolia									
Control	3.55±0.75			3.49±0.35			3.35±0.65		
150mg/kg	3.19±0.034	0.36	10	2.99±0.066	0.52	14	2.18±0.036	1.17	35
500mg/kg	1.38±0.014	2.17	61	2.11±0.009	1.36	39	1.89±0.022	1.46	44

Ave. Inf. = Average inflammation (mm±SEM; n = 6); Inh = Inhibition

Table 2: Effect of Methanolic extract of *Enantia chlorantha* and *Nauclea latifolia* on acetic acid- induced writhing in mice.

Treatment	N	Dose (mg/kg, i.p)	No. of writhes	Inhibition (%)
Control	8	-	49.23 ± 5.1	-
Enantia chlorantha	6	400	25.70 ± 3.2*	47.80
Enantia chlorantha	6	1,100	14.12 ±3.7*	71.13
Nauclea latifolia	6	150	39.98 ± 6.9	18.79
Nauclea latifolia	6	500	30.71 ± 3.7*	37.62

No. of writhes are mean ± S. E. M., * P<0.05 vs. control, student's t-test. N= No. of animals

Table 3: Antinociceptive effect of Methanolic extracts of *Enantia chlorantha* and *Nauclea latifolia* in the hot-plate test.

Treatment	N	Dose (mg/kg, i.p)	Latency (S)
Control	8	-	1.79 ± 0.67
Enantia chlorantha	6	400	3.00± 1.46*
Enantia chlorantha	6	1,100	3.83 ± 1.93*
Nauclea latifolia	6	150	1.99± 0.54^{+}
Nauclea latifolia	6	500	1.78 ± 0.48^{+}

Latency values are mean ± S.E.M., *P<0.05, $^{+}$P>0.05 vs. control, student's t-test. N = no. of animals.

E. chlorantha and *N. latifolia* were shown by this study to have inflammatory and analgesic properties. These findings may not be unrelated to the presence of flavonoids (Hotellier *et al.*, 1979 and as suggested by the phytochemical studies carried out) which are known to be responsible for the anti-inflammatory properties of many medicinal plants (Manthey 2000). These results support the claim of traditional healers that *E. chlorantha* and *N. latifolia* have anti-inflammatory and analgesic activity.

Conclusion

The present study has shown that the methanolic extract of the stem bark of *E. chlorantha* (400 mg and 1100 mg/Kg, ip) and *N. latifolia* (150 mg and 500 mg/Kg, ip) possess anti-inflammatory and analgesic activity in experimental animals.

References

Agbaje EO, Onabanjo AO. (1991). The effects of extracts of Enantia chlorantha in malaria. *Ann Trop Med Parasitol.* 85(6):585-90.

Azas N, Laurencin N, Delmas F, Di GC, Gasquet M, Laget M, Timon-David P. (2002). Synergistic in vitro antimalarial activity of plant extracts used as traditional herbal remedies in Mali. *Parasitol Res.* 88(2):165-71.

Benoit-Vical F, Valentin A, Cournac V, Pelissier Y, Mallie M, Bastide JM. (1998). In vitro antiplasmodial activity of stem and root extracts of Nauclea latifolia S.M. (Rubiaceae). *J Ethnopharmacol.* 61(3):173-8.

Dalziel, J.M. (1937). The useful plants of West Tropical Africa. London Crown Agents, London, Great Britain. An Appendix to Dalziel, J.M. and Hutchinson, J. 1927-1936. The flora of tropical West Africa. London Crown Agents, London, Great Britain.

Fakae BB, Campbell AM, Barrett J, Scott IM, Teesdale-Spittle PH, Liebau E, Brophy PM. (2000). Inhibition of glutathione S-transferases (GSTs) from parasitic nematodes by extracts from traditional Nigerian medicinal plants. *Phytother. Res.* 14(8):630-4.

Hess, S.M. and Milong, R.C. (1972); In: Inflammation Mechanism and Control. Lepow, I.H. and Ward, P.A. (1st Eds), academic Press, New York, p.1-2.

Hotellier F, Delaveau P, Pousset JL. (1979). [Alkaloids and glyco-alkaloids from leaves of Nauclea latifolia SM (author's transl)]. *Planta Med.* 1979 Mar;35(3):242-6.

Irvine, F.R. (1961). Woody plants of Ghana with special references to their uses. London, Oxford University Press. Pp. 868.

Kela SL, Ogunsusi RA, Ogbogu VC, Nwude N. (1989). Screening of some Nigerian plants for molluscicidal activity. *Rev Elev Med Vet Pays Trop.* 42(2):195-202

Kerharo, J. and Adam, J. C. (1974). La Pharmacopee senegalaise traditionnalle: Plantes medicinales et toxiques. 1st Ed. Vigot Freres, Paris, 101-107.

Kimbi HK, Fagbenro-Beyioku AF. (1996). Efficacy of Cymbopogon giganteus and Enantia chlorantha against chloroquine resistant *Plasmodium yoelii* nigeriensis *East Afr Med J.* 73(10):636-7.

Lorke, D.(1983). A new approach to practical acute toxicity testing. *Arch. Toxicol.* 54(4): 275-287.

Manthey J.A. (2000). Biological properties of flavonoids pertaining to inflammation. *Microcirculation*, 7 (6Pt 2): S29-34.

Moody JO, Bloomfield SF, Hylands PJ. (1995). In-vitro evaluation of the antimicrobial activities of Enantia chlorantha Oliv. extractives. *Afr J Med Sci.* 24(3):269-73.

Ngnokam D., Ayafor, F.J, Connolly, D.J. and Nuzillard, M.J. (2003). Nauclefolininine: A new alkaloid from the roots of *Nauclea latifolia, Bulletin of the chemical Society of Ethiopia,* 17(2), Pp 173-176

Oliver, B. (1960). Medicinal plants in Nigeria. College of Arts, Sciences and Technology. Ibadan. Pp 24.

Onyeyili PA, Nwosu CO, Amin JD, Jibike JI. (2001). Anthelmintic activity of crude aqueous extract of Nauclea latifolia stem bark against ovine nematodes. *Fitoterapia.* 72(1):12-21

Oriowo, M.A. (1982). Anti-inflammatory activity of piperonyl-4-acrylic isobutyl amide, an extract from *Zanthoxylum zanthoxyloides. Planta Med.* 44(1): 54-56.

Otimenyin O.S: (2004). Anti-inflammatory and analgesic activity of *Ficus thonningii* and *Pseudocedrela kotschyi* extracts. *Nig. J. of Pharm. Res.;* 3(1) Pp 82-85.

Rang, H.P. and Dale, M.M. (1991). Pharmacology. Churchill Livingstone, 2nd Ed. Pp 174.

Shigemori H, Kagata T, Ishiyama H, Morah F, Ohsaki A, Kobayashi J. (2003). Naucleamides A-E, new monoterpene indole alkaloids from Nauclea latifolia. *Chem Pharm Bull* (Tokyo). 51(1):58-61.

Snedecor G.W. and Cochran, W.C. (1967). Statistical Methods. 6th Ed. Ames, Iowa: The Iowa state university Press, Pp 423.

Tan PV, Nyasse B, Enow-Orock GE, Wafo P, Forcha EA. (2000). Prophylactic and healing properties of a new anti-ulcer compound from Enantia chlorantha in rats. *Phytomedicine.* 7(4): 291-6.

Tona L, Kambu K, Ngimbi N, Mesia K, Penge O, Lusakibanza M, Cimanga K, De Bruyne T, Apers S, Totte J, Pieters L, Vlietinck AJ. (2000). Antiamoebic and spasmolytic activities of extracts from some antidiarrhoeal traditional preparations used in Kinshasa, Congo. *Phytomedicine.* 7(1):31-8

Traore F, Gasquet M, Laget M, Guiraud H, Di Giorgio C, Azas N, Doumbo O, Timon-David P. (2000). Toxicity and genotoxicity of antimalarial alkaloid rich extracts derived from *Mitragyna inermis* O. Kuntze and *Nauclea latifolia. Phytother Res.* 14(8):608-11.

Traore-Keita F, Gasquet M, Di Giorgio C, Ollivier E, Delmas F, Keita A, Doumbo O, Balansard G, Timon-David P.(2000). Antimalarial activity of four plants used in traditional medicine in Mali. *Phytother Res.* 14(1):45-7

Turner, R.A. (1965): Screening methods in Pharmacology. Academic Press, New York: P 106.

Virtanen P, Lassila V, Njimi T, Ekotto Mengata D. (1988). Regeneration of D-galactosamine-traumatized rat liver with natural protoberberine alkaloids from Enantia chlorantha. *Acta Anat* (Basel). 32(2):159-63.

Williamson, E.M, Okpako, T.D. and Evans, J.F. (1996). "Pharmacological methods in phytotherapy research".Vol. I, John Wiley & Sons pp 4-5.