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Full Length Research Paper

Anti-arthritic and antioxidant properties of the ethanolic stem bark extract of *Newbouldia laevis* (P. Beauv.) Seaman ex Bureau (Bignoniaceae)

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Newbouldia laevis **(fam. Bignoniaceae) is used in traditional medicine for the treatment of various conditions. The stem bark is used for pain, ulcer, arthritis, rheumatism and other inflammatory conditions. The purpose of this study is to pharmacologically validate the use of *N. laevis* stem bark extracts in African traditional medicine for the management of inflammatory conditions such as arthritis. Ethanolic stem-bark extract of *N. laevis* (10 - 100 mg kg^{-1}) exhibited anti-arthritic activity by inhibiting the polyarthritic phase edema by 25.30 ± 2.53 - 28.11 ± 2.02 % with no significant inhibition of the acute phase response of the rat adjuvant-induced arthritis. Phenols were detected in the total ethanolic extract. Though both the extract and α-tocopherol produced a concentration-dependent increase in reducing power properties, the extract (EC_{50} 2.8 ± 1.59) was six-fold more potent than α-tocopherol (EC_{50} 18.18 ± 4.29). In contrast the ability of the extract to de-colorize DPPH free radical solution (EC_{50} 39.86 ± 3.33 X 10^{-2}) and inhibit lipid peroxidation in rat brain homogenate (IC_{50} 1499.00 ± 408. 22 X 10^{-3}) was less than α-tocopherol and *n*-propyl gallate. Overall, our results indicate that *N. leavis* has anti-arthritic and antioxidant properties**.

Key words: *Newbouldia laevis*, anti-inflammatory, lipid peroxidation, total phenol, DPPH scavenging, reducing power.

INTRODUCTION

Several medicinal plants are used by traditional medicine practitioners in Ghana for the treatment of various ailments. *Newbouldia laevis* (P. Beauv.) Seeman ex Bureau. fam. Bignoniaceae commonly called the fence tree or the African border tree is locally known in Ghana as *sasanemasa, (*Twi), *aviati,*(Ewe), *hiatso* (Ga), and *esisimansa* (Fante) (Mshana et al., 2000). It is a fast-growing hairless perennial, normally a small tree that grows up to 15m high but commonly bushy and shrubby around settlements and secondary forests (Irvine, 1961). The roots and leaves are used in treating elephantiasis and convulsion. The roots and stem are used to treat malaria, while the bark and twigs are used to treat pelvic pain in females. The stem bark is used to treat peptic ulcer, otalgia, skin ulcer, epilepsy, hemorrhoids and constipation. The leaves and stem bark are used for treating cough, while the leaves are used to treat orchitis (gonococcal) (Mshana et al., 2000). Antimicrobial activity of various isolates against a wide range of micro-organisms (Eyong et al., 2006) and anthelmintic pro-perties have been reported (Hounzangbe-Adote et al.,

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Table 1. Grouping of animals for induction of arthritis.

Group	Treatment
Group I	Arthritic control/CFA (intraplantar injection of 0.1 ml CFA)
Group I	Non-arthritic control/IFA (intraplantar injection of 0.1 ml of IFA)
Groups III-V	Pre-treated with dexamethasone 0.3, 1.0 and 3 mg kg^{-1} i.p. respectively, 30 min before intraplantar injection of CFA.
Groups VI-VIII	Pre-treated with diclofenac 10, 30 and 100 mg kg^{-1} i.p. respectively,. 30 min before intraplantar injection of CFA.30 min before intraplantar injection of CFA.
Groups IX-XI	Pre-treated with extract 10, 30 and 100 mg kg^{-1} *p.o.* respectively, 1 h before intraplantar injection of CFA.

2005). Pharmacological studies by Olajide et al. (1997) reported that the methanolic stem bark extracts of *N. laevis* inhibited carrageenan-induced oedema in the rat hind paw, in a dose-dependent fashion. The methanolic extract of the stem bark was also found to produce a reduction of yeast-induced pyrexia and significant analgesic activity in acetic acid-induced "writhings" in mice (Olajide et al., 1997). Although the carrageenan-induced oedema model supports the use of *N. laevis* in acute inflammation, the method does not establish the effecttiveness of the extract in chronic inflammation, particularly the use of the stem bark extracts from *N. laevis* in African traditional medicine for chronic inflammatory conditions such as arthritis. Further, the possible mechanism involved in the reported anti-inflammatory activity of *N. laevis* is yet to be elucidated. Antioxidants are now known to play a major role in the resolution of inflamematory conditions (Sakai et al., 1999) and several anti-inflammatory agents from plant sources have been found to exhibit antioxidants properties (Narendhirakannan et al., 2005; Vijayalakshmi et al., 1997). The antioxidant activities of some of these plants have been ascribed to their phenolic constituents (Ozgova et al., 2003). Phenolic compounds have been identified in methanolic extract of *N. laevis* (Gormann et al., 2006). Here, we determined the phenolic content in the ethanolic stem-bark of *N. laevis*, investigated the effect of the extract in Freund's adjuvant-induced arthritis in rats and assessed its possible anti-oxidant activity. In this study dexamethasone, a steroidal anti-inflammatory agent and diclofenac, a non-steroidal anti-inflammatory drug (NSAID) were used as reference antiarthritic drugs.

MATERIALS AND METHODS

Plant material

The stem bark of *N. laevis* was collected from the Botanic Gardens, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana in June 2006 and authenticated at the Department of Pharmacognosy, College of Health Sciences, KNUST.

Phytochemistry

Phytochemical screening was carried out on powdered bark of *N. laevis* to determine the qualitative composition of secondary metabolites like glycosides, alkaloids, anthraquinones, flavonoids, tannins, saponins and steroids. Glycosides, unsaturated steroids, and anthraquinones were present, but tannins, alkaloids, saponins and flavonoids were absent

Preparation of extract

The bark was sun-dried for two weeks and powdered by a Hammer mill. The powdered bark was then Soxhlet-extracted with 70 % v/v ethanol at 60^{o}C. The hydro-alcoholic extract was then evaporated to a syrupy mass under reduced pressure in a rotary evaporator, air-dried and kept in a desiccators. The brownish final product (yield: 9.7%w/w) obtained is subsequently referred to as NLE or extract.

Animals

Sprague-Dawley rats (200 - 250 g) of both sexes were purchased from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, and maintained in the Animal House of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. The animals were housed in groups of 6 in stainless steel cages (34 × 47 × 18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (GAFCO, Tema), given water *ad libitum* and maintained under laboratory conditions (temperature 24 - 28°C, relative humidity 60 - 70%, and 12 h light- dark cycle). All procedures and techniques used in these studies were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health and Human Services publication no. 85 - 23, revised 1985) and were approved by the Departmental Ethics Committee.

Induction of arthritis

Adjuvant arthritis was induced as previously described (Pearson, 1956). Briefly, animals were injected intraplantar with 0.1 ml Complete Freund's Adjuvant (CFA) into the right hind paw of each rat. CFA was prepared by triturating heat-killed *Mycobacterium tuberculosis* [strains C, DT and PN (mixed) obtained from the Ministry of Agriculture, Fisheries and Food, U.K]) in paraffin oil to make a 3 mg ml^{-1} suspension. The animals were grouped as shown in Table 1 for the induction of arthritis.

Foot volume was measured by water displacement plethysmography (Fereidoni et al., 2000) for both the ipsilateral (injected paw) and the contralateral paw (non-injected paw) before intraplantar injection of CFA (day 0) and every other day (day 2, 4, 6, 8,….28).

The edema component of inflammation was quantified by measuring the difference in foot volume between day 0 and the various time points.

The extract was suspended in 2% tragacanth mucilage, whilst the reference drugs (diclofenac and dexamethasone) were dis-solved in normal saline. Test drugs were prepared such that doses were administered in volumes not exceeding 100 ml kg^{-1} . All drugs were freshly prepared. Inflamed control animals were given 2% tragacanth mucilage (100 ml kg^{-1}, *p.o.*).

Raw scores for ipsilateral and contralateral paw volumes were individually normalized as percentage of change from their values at day 0, and then averaged for each treatment group.

Total phenol determination

Total phenols present in NLE were determined using the Folin-Ciocalteau reagent (Singleton et al., 1999). Various concentrations (0.3, 1, 3 mg ml^{-1}) of NLE (1 ml) were mixed with the Folin–Ciocalteau phenol reagent (1 ml; diluted 1:10 with distilled water). This mixture was incubated at room temperature (28°C) for 5 min. Na_2CO_3 (2% w/v, 1 ml) was added and incubated for 2 h. It was then centrifuged (650 *g* for 10 min) to get a clear solution, and the absorbance measured at 760 nm using spectrophotometer (Cecil CE 7200 UV/VIS spectrophotometer, Cecil Instrument Limited, Milton Technical Centre, England) . Tannic acid was used as reference, and the total phenols in NLE expressed as milligrams per milliliter of tannic acid equivalents (TAEs).

Reducing power

The reducing capacity of the extract was determined by its ability to reduce Fe^{3+} to Fe^{2+} (Oyaizu, 1986) as previously described by (Amarowicz et al., 2005) with some modifications. One millilitre (1 ml) of various concentrations of NLE (0.1, 0.3, 1, 3 mg ml^{-1}, in methanol) was mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide solution (K_3 Fe[CN]$_6$) in a test tube. The mixture was incubated at 50°C for 20 min. Trichloroacetic acid (10%; 1.5 ml) was then added to the mixture and centrifuged at 650 *g* for 10 minutes. Two and a half milliliters (2.5 ml) of the supernatant was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride solution. The absorbance was then measured at 700 nm using the spectrophotometer. Blank samples were prepared as follows: 1 ml distilled water was added to 2.5 ml sodium phosphate buffer and 2.5 ml potassium ferricyanide and the mixture processed as above. Vitamin E (-tocopherol) (0.1, 0.3, 1, 3 mg ml^{-1}, in methanol) was used as standard antioxidant. Each test was done in triplicate. The greater the reducing power, the higher the absorbance.

Data was presented as concentration-absorbance curves and the EC_{50} (concentration that gives 50% of maximal response) computed

Scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical

The free radical scavenging activity was determined as described by (Govindarajan et al., 2003) with a few modifications. 1 ml of test drug (in methanol) was added to 3 ml methanolic solution of DPPH solution (20 mg l^{-1}) in a test tube. The reaction mixture was kept at 25°C for 1 h. The absorbance of the residual DPPH was determined at 517 nm in a spectrophotometer. Methanol (99.8%) was used as blank.

The free radical scavenging activity of NLE (1, 3, 10, 30 mg ml^{-1} in methanol) was compared to the reference drugs *n*-propyl gallate (0.01, 0.03, 0.1, 0.3 mg ml^{-1} in methanol) and -tocopherol (0.01, 0.03, 0.1., 0.3 mg ml^{-1}, in methanol). One milliliter (1 ml) methanol (99.8%) was added to 3 ml DPPH solution incubated at 25°C for 1 h and used as control. The absorbance decreases with increasing free radical scavenging ability.

Results were expressed as percentages of blank (100%). The concentration required to cause a 50% decrease in the absorbance was calculated (EC_{50}). Each test was carried out using three replicates.

Lipid peroxidation

The extent of lipid peroxidation in rat brain homogenate was determined using thiobarbituric acid (TBA), as previously described (Auddy et al., 2003; Ohkawa et al., 1979), with modifications.

Preparation of rat brain homogenate

Rats (200 - 250 g) were sacrificed by decapitation and whole brain (except cerebellum) was dissected out and homogenized (100 mg ml^{-1}) in ice-cold phosphate buffer (0.1 M, pH 7.4) using an Ultra-Turrax T 25 homogenizer (IKA Labortehnic, Staufen, Germany). This was used as a source of polyunsaturated fatty acids (PUFA) for lipid peroxidation.

Assay of lipid peroxidation

Two and a half millilitres (2.5 ml) of brain homogenate was mixed with 1 ml phosphate buffer and 0.5 ml of test drug. Lipid peroxidation was then initiated by the addition of 0.5 ml of 0.1 mM ascorbic acid and 0.5 ml of 5 mM ferric chloride. The mixture was incubated in a shaking water bath at 37°C for one hour after which 0.1 ml of the reaction mixture was taken into a test tube containing 1.5 ml of 10% trichloroacetic acid (TCA) and allowed to stand for ten minutes. The tubes were then centrifuged at 1150 *g* for 10 min.

The supernatant was mixed with 1.5 ml of 0.67% thiobarbituric acid (TBA) in 20% acetic acid in a test tube. This reaction mixture was heated in a hot water bath at 85 °C for one hour, allowed to cool to room temperature (28^{o}C) and absorbance taken at 535 nm in a spectrophotometer. Phosphate buffer was used as blank.

The effects of the extract (1-30 mg ml^{-1}) was compared with the reference drugs *n*-propyl gallate (0.03 - 0.3 mg ml^{-1}) and -tocopherol (0.01, 0.03, 0.1., 0.3 mg ml^{-1}). NLE and reference drugs were dissolved in methanol. Each test was carried out using three replicates.

Percentage inhibition of lipid peroxidation by the test drugs was assessed by comparing the absorbance of the drug test with that of the control (homogenate mixture without any drug).

Data was presented as percentage inhibition of lipid peroxidation against concentration.

Analysis of data

For the arthritic experiment the time-course curves for foot volume were subjected to two-way (*treatment* × *time*) repeated measures analysis of variance with Bonferroni's *post hoc t* test. Total foot volume for each treatment was calculated in arbitrary units as the area under the curve (AUC) and to determine the percentage inhibition for each treatment, the following equation was used.

$$\%\ inhibition\ of\ edema = \frac{AUC_{control} - AUC_{treatment}}{AUC_{control}} \times 100$$

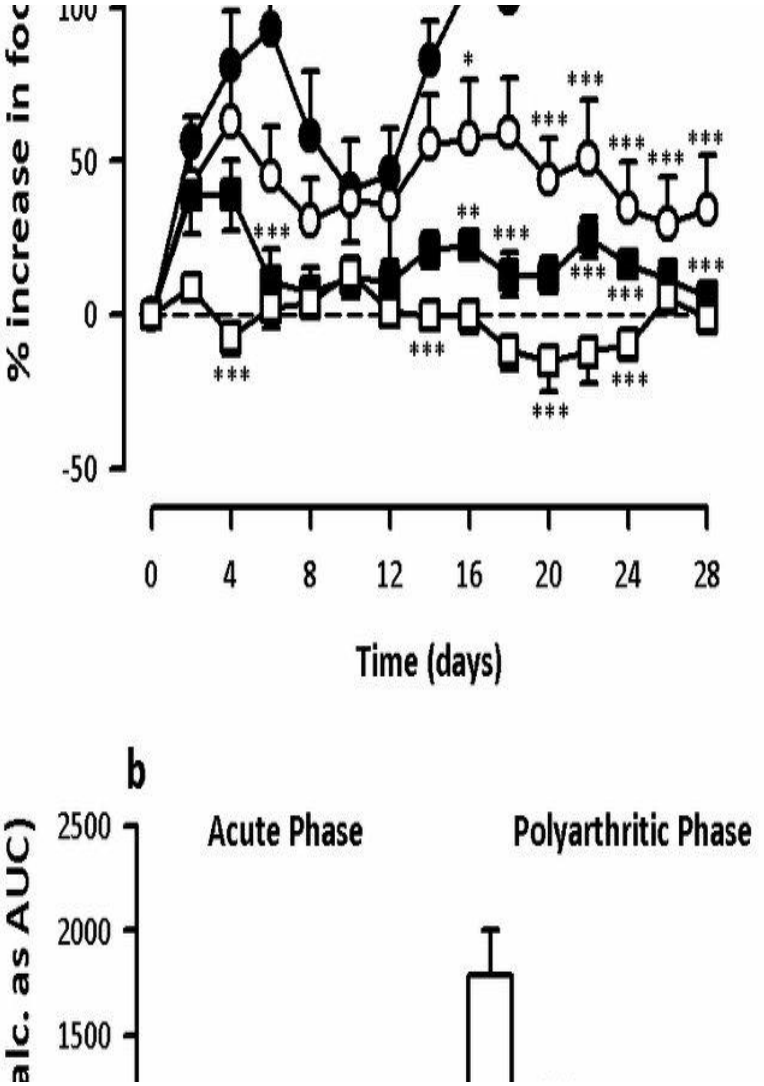

Figure 1. (a) Time course effects of NLE (10 - 100 mg kg^{-1}) on CFA-induced increase in the ipsilateral foot volume and (b) the total edema response [define as area under the time course curve (AUC)] in the acute and polyarthritic phase. Values are means ± S.E.M. (n = 6 - 8). ***P < 0.001; ** P < 0.01; *P < 0.05 compared to vehicle-treated group (Two-way ANOVA followed by Bonferroni's *post hoc* test). $^{\dagger\dagger\dagger}P$<0.001; $^{\dagger\dagger}P$<0.01 compared to vehicle-treated group (One-way ANOVA followed by Newman-Keul's *post hoc* test.

Differences in AUCs were analyzed by ANOVA followed by Student -Newman-Keuls' *post hoc* test.

Doses and concentrations responsible for 50% of the maximal effect (ED_{50}, EC_{50} and IC_{50}) for each drug were determined using an iterative computer least squares method, with the following nonlinear regression (three-parameter logistic) equation.

$$Y = \frac{a + (b - a)}{\left(1 + 10^{(LogED_{50} - X)}\right)}$$

Where, X is the logarithm of dose and Y is the response. Y starts at a (the bottom) and goes to b (the top) with a sigmoid shape.

The fitted midpoints (ED_{50}s etc) of the curves were compared statistically using F test (Miller, 2003; Motulsky et al., 2003). GraphPad Prism for Windows version 4.03 (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses and ED_{50} determinations. P < 0.05 was considered statistically significant.

RESULTS

Adjuvant-induced arthritis in rats

Intraplantar injection of CFA into the right foot pad of rats induced an inflammatory response characterized by paw swelling in both the ipsilateral and the contralateral paw. The response on the injected paw was biphasic. It consists of an acute and a polyarthritic/chronic phase corresponding to day 0 - 10 and day 10 - 28 post CFA inoculation respectively. The acute phase response was characterized by unilateral inflammatory edema of the ipsilateral paw peaking around days 4 - 6, followed by subsequent polyarthritic/chronic phase response which began around day 10 characterized by inflammatory edema of the contralateral paw.

NLE (10-100 mg kg^{-1}) had no significant effect on the acute phase response ($F_{3,120}$=2.08, P=0.1357) but inhibited the polyarthritic phase response by 25.30 ± 2.53 % - 28.11 ± 2.02 % ($F_{4,200}$=19.46, P<0.0001) (Figure 1a and b).

Dexamethasone (a steroidal anti-inflammatory drug) caused a dose-dependent inhibition of the acute phase response by 26.98 ± 12.95 - 90.00 ± 3.44 % ($F_{3,120}$ =7.43, P=0.0016) and the polyarthritic phase response by 51.87 ± 18.20 – 110.52 ± 9.81 % ($F_{3,160}$=18.11, P<0.0001) (Figure 2a and b).

Diclofenac, an NSAID (10-100 mg kg^{-1}) likewise dose-dependently and appreciably reduced the acute phase response by 38.40 ± 15.84 - 76.61 ± 9.78 % ($F_{3,120}$ =3.57, P=0.0322) and the polyarthritic phase response by 49.16 ± 7.39 – 92.99 ± 6.23 % ($F_{3,160}$=30.65, P< 0.0001) (Figure 3a and b).

The rank order of potency was; dexamethasone > diclofenac > extract for both the acute and polyarthritic phases (Table 2)

Total phenols

The phenol content of tannic acid increased with increasing concentration (r^2 = 0.99). The extract (0.3-3 mg ml^{-1}) also showed a concentration-dependent increase in phenolic content (0.009 ± 0.003 – 0.258 ± 0.003) expressed as tannic acid equivalent (Figure 4a).

Reducing power

NLE (0.1-3 mg ml^{-1}) and the standard antioxidant -tocopherol (0.1-3 mg ml^{-1}) dose- dependently reduced

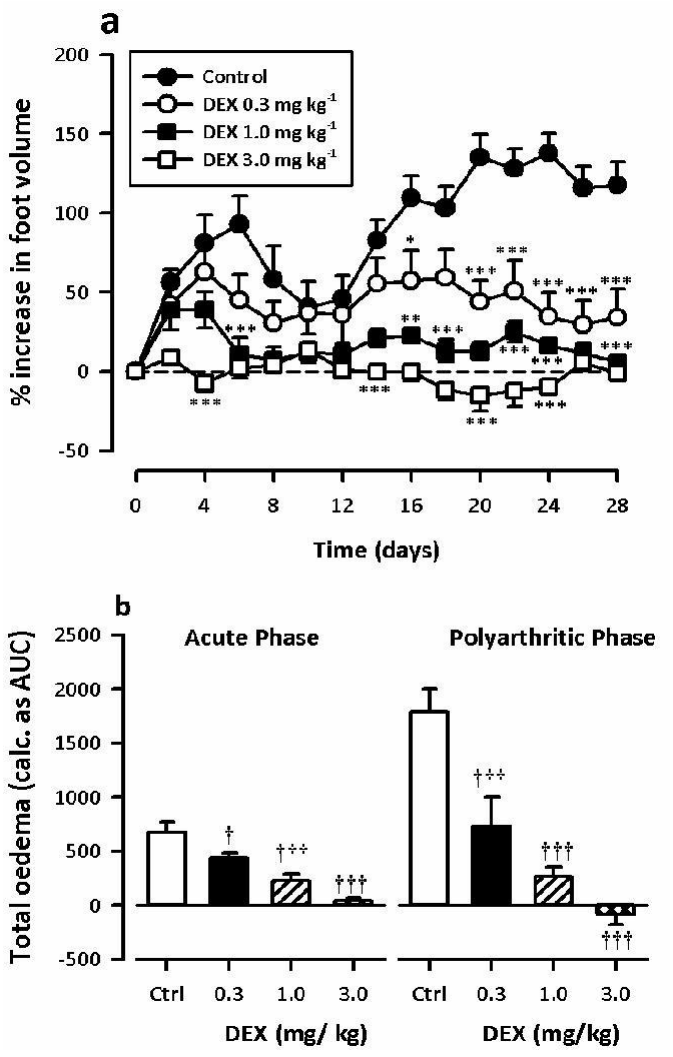

Figure 2. (a) Time course effects of dexamethasone (0.3 - 3.0 mg kg^{-1}) on CFA induced increase in the ipsilateral foot volume and (b) the total edema response [define as area under the time course curve (AUC)] in the acute and polyarthritic phase. Values are means ± S.E.M. (n = 6 - 8). ***P < 0.001; ** P < 0.01; *P < 0.05 compared to vehicle-treated group (Two-way ANOVA followed by Bonferroni's *post hoc* test). †††P<0.001; †P<0.05 compared to vehicle-treated group (One-way ANOVA followed by Newman-Keul's *post hoc* test).

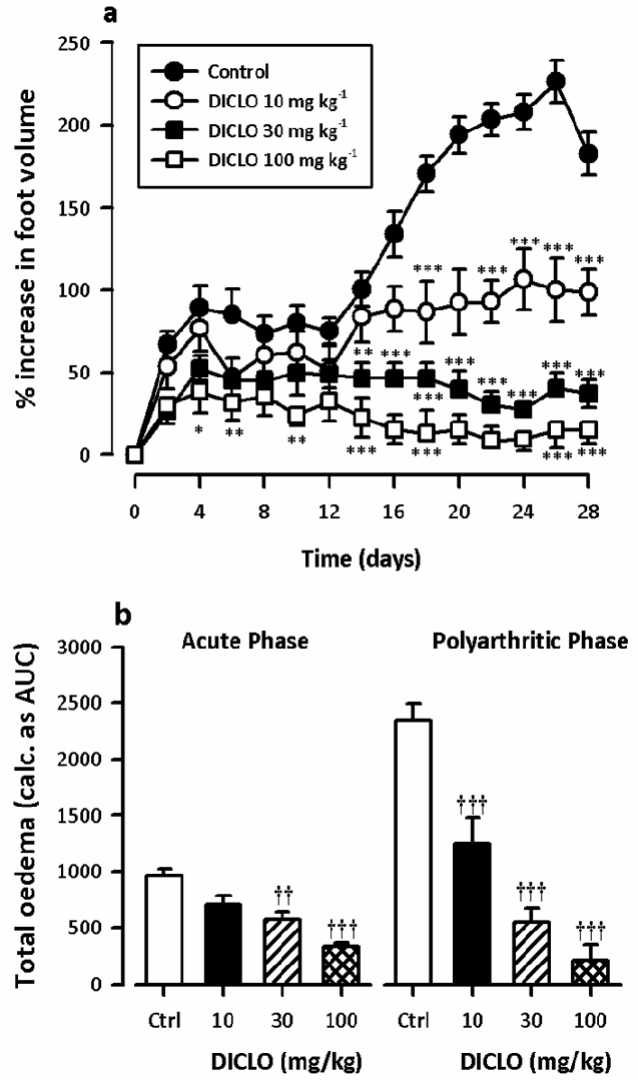

Figure 3. (a) Time course effects of diclofenac (10 - 100 mg kg^{-1}) on CFA-induced increase in the ipsilateral foot volume and (b) the total edema response [define as area under the time course curve (AUC)] in the acute and polyarthritic phase. Values are means ± S.E.M. (n = 6 - 8). ***P < 0.001; ** P < 0.01; * P < 0.05 compared to vehicle-treated group (Two-way ANOVA followed by Bonferroni's *post hoc* test). †††P<0.001; ††P<0.01 compared to vehicle-treated group (One-way ANOVA followed by Newman-Keul's *post hoc* test).

Fe^{3+} to Fe^{2+} as indicated by a dose-dependent increase in absorbance (Figure 4b).

From EC_{50} (in mg ml^{-1}) obtained for the extract (2.80 ± 1.59) and -tocopherol (18.18 ± 4.29) the extract was found to be about six-fold more potent than the reference drug α-tocopherol (Table 3).

DPPH scavenging

The reference drugs *n*-propyl gallate (0.01 - 0.3 mg ml^{-1}), α-tocopherol (0.01 - 0.3 mg ml^{-1}), and the test drug NLE (0.1- 3 mg ml^{-1}) exhibited dose-dependent free radical scavenging activity (Figure 5a). The rank order of potency (defined by EC_{50} in mg ml^{-1}) was found to be: *n*-propyl gallate (1.81 ± 0.22 × 10^{-2}) > α-tocopherol (8.64 ± 0.99 × 10^{-2}) > extract (39.86 ± 3.33 × 10^{-2}) (Table 3).

Lipid peroxidation

NLE (1-3 mg ml^{-1}) and the reference antioxidants *n*-propyl gallate (0.01 - 0.3 mg ml^{-1}) and α-tocopherol (0.01-0.3 mg ml^{-1}) showed dose dependent ability to inhibit lipid peroxi-

Table 2. ED_{50} values for the effect of the extract, dexamethasone and diclofenac in the acute and polyarthritic phase of adjuvant-induced arthritis in rats.

Drug	ED_{50} (mg kg^{-1})	
	Acute Phase	Polyarthritic Phase
Newbouldia extract	-	124.30±36.76
Dexamethasone	0.58±0.20	0.22±0.11***
Diclofenac	29.37±10.47	9.54±1.89***

***P 0.001 compared NLE (F test).

peroxidation (Figure 5b). The rank order of potency (defined by ED_{50} in mg ml^{-1}) was found to be: α-tocopherol ($2.01 \pm 0.35 \times 10^{-3}$) > *n*-propyl gallate ($4.46 \pm 1.41 \times 10^{-3}$) > extract ($1499.00 \pm 408.22 \times 10^{-3}$) (Figure 5 and Table 3)

DISCUSSION

The rat adjuvant arthritis is the most frequently used chronic inflammatory model (Aota et al., 1996; Crofford et al., 1992; Weichman, 1989) used in the screening of NSAIDs, steroids and immunosuppressive drugs, (Weichman, 1989). The major limitation of this model is its inability to identify disease modifying antirheumatic drugs (Rainsford, 1982; Weichman, 1989).

In adjuvant arthritis bacterial peptidoglycan and muramyl dipeptide are responsible for its induction (Crofford et al., 1992; Rainsford, 1982). It occurs through cell mediated-autoimmunity by structural mimicry between mycobacteria and cartilage proteoglycans in rats (Vijayalakshmi et al., 1997). The anti-inflammatory effect of diclofenac (an NSAID) is mediated chiefly through inhibition of COX and prostaglandin production (Furst and Manning, 2001). Dexamethasone (a steroidal anti-inflammatory drug) exerts anti-inflammatory effects through profound inhibitory effects on peripheral leucocytes and its suppressive effects on the inflammatory cytokines and on other lipid and glucolipid mediators of inflammation (Chrousos et al., 2001). This is achieved through interaction with specific receptor proteins in target tissues to regulate the expression of corticosteroid- responsive genes, thereby changing the levels and array of proteins synthesized by the various target tissues (Schimmer et al., 2006).

The precise effect of *N. laevis* extract on these inflamematory pathways was outside the scope of this study. However the inability of the extract to inhibit the acute phase edema response in the rat adjuvant-induced arthritis, contrary to the effective inhibition produced by diclofenac and dexamethasone in both the acute and polyarthritic phase may suggest anti-inflammatory mechanisms other than those of dexamethasone and diclofenac. The extract exhibited antioxidant properties by showing reducing properties in the reducing power test, dose-dependently decolorizing DPPH free radical solution, and effectively inhibiting lipid peroxidation in rat brain homogenate. Plants have been found to produce signify-cant amount of antioxidants (Attaguile et al., 2000; Periera da Silva et al., 2000; Scartezzini and Speroni, 2000) to prevent oxidative stress caused by photons and oxygen (Auddy et al., 2003).

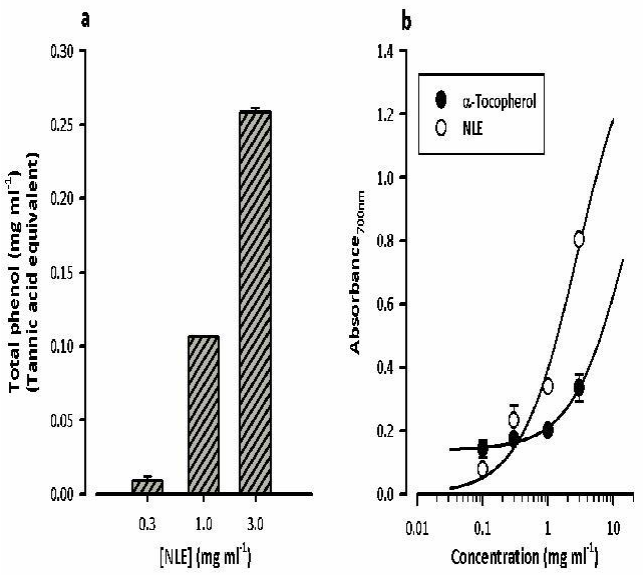

Figure 4. (a) Total phenols present in varying concentrations of NLE (0.3 - 3 mg ml^{-1}), expressed as tannic acid equivalent and (b) reducing properties of NLE (0.1-3 mg ml^{-1}) compared to α-tocopherol (0.1 - 3 mg ml^{-1}). Values are means ± S.E.M.(n = 3).

The antioxidant properties exhibited by NLE are supported by the detection of plant phenols in the phenol test. Polyphenols (electron-rich compounds) have the ability to go into electron-donation reactions with oxidizing agents and still form stable species (Kang et al., 2005) and thus inhibit or delay the oxidation of different biomolecules (Seidel et al., 2000). In addition, plant sterols such as beta-sitosterols also found in *N. laevis* stem bark (Eyong et al., 2006) have been found to inhibit lipid peroxidation under certain experimental conditions (van Rensburg et al., 2000). All the above may contribute to the antioxidant properties of *N. laevis* stem bark observed in this study.

The antioxidant properties of plants have been linked to their therapeutic and protective effects in many diseases such as Parkinson's disease, Alzheimer's disease, cancer, cardiovascular disorders, bacterial and viral infections and inflammation (Arthan et al., 2002; Auddy et al., 2003; Jung et al., 2005; Koo et al., 2006). Excessive production of reactive oxygen species (ROS) can injure cellular biomolecules such as nucleic acids, proteins, car-

Table 3. EC_{50}/IC_{50} values for the extract, *n*-propyl gallate and α- tocopherol in the reducing power, DPPH scavenging and lipid peroxidation assays.

Drug	EC_{50}/IC_{50} (mg ml^{-1})		
	Reducing Power	DPPH Scavenging×10^{-2}	Lipid Peroxidation×10^{-3}
Newbouldia extract	2.80±1,59	39.86±3.33	1499.00±408.22
n-Propyl gallate	-	1.81±0.22***	4.46±1.41***
α- Tocopherol	18.18±4.29***	8.64±0.99***	2.01±0.35***

****P* 0.001 compared NLE (*F* test)

carbohydrates, and lipids, causing cellular and tissue damage, which in turn augments the inflammatory state (Cui et al., 2004; Mitra et al., 2002; Schaller, 2005). This occurs in a number of pathophysiological conditions associated with inflammation or oxidant stress such as atherosclerosis, ischaemic heart disease, the aging process, inflammatory lesions, diabetes milletus, different immunosuppressive diseases, metabolic disorders, and neuromuscular degenerative conditions (Ames et al., 1993; Cui et al., 2004; Mitra et al., 2002). Indeed apart from histamine, 5-hydroxytryptamine, bradykinin, prostaglandins, other cycloxygenase (COX) products, and various cells involved in inflammatory changes, free radical activities have all been implicated in the development of carrageenan-induce paw oedema and the rat adjuvant arthritis (Sakai et al., 1999; Vijayalakshmi et al., 1997).

ROS have been proposed to mediate cell damage via a number of independent mechanisms including the initiation of lipid peroxidation, the inactivation of enzymes and depletion of glutathione (Ames et al., 1993; Cuzzocrea et al., 2001; Ozgova et al., 2003) . ROS generation is implicated in cytotoxicity and lipid peroxidation leading to the degradation of membrane lipids and disruption of cell membranes (Costa et al., 2004) contributing to tissue damage during inflammation (Hischi et al., 1989; Salvemini et al., 1996). Compounds that have scavenging activities toward these radicals and/or suppressive effects on lipid peroxidation have been found to exhibit therapeutic effects in inflammatory diseases (Auddy et al., 2003; Jung et al., 2005; Koo et al., 2004). Investigations have shown that the anti-inflammatory effects of several compounds are related to their ability to scavenge free radicals and to protect against lipid peroxidation (Claxson et al., 1990; Griffiths et al., 1992). Non-enzyme antioxidants such as ascorbic acid (Sakai et al., 1999) and various plant extracts with potent antioxidant properties (Jung et al., 2005; Narendhirakannan et al., 2005; Vijayalakshmi et al., 1997) have been found to exhibit anti-arthritic effect in rat adjuvant-induced arthritis. Some drugs such as probucol (hypocholesterolaemic) and salicylates (anti-inflammatory) have been reported to have free radical scavenging activity under certain experimental conditions (Cui et al., 2004). Indeed the found to directly and dose-dependently scavenge NSAIDs such as

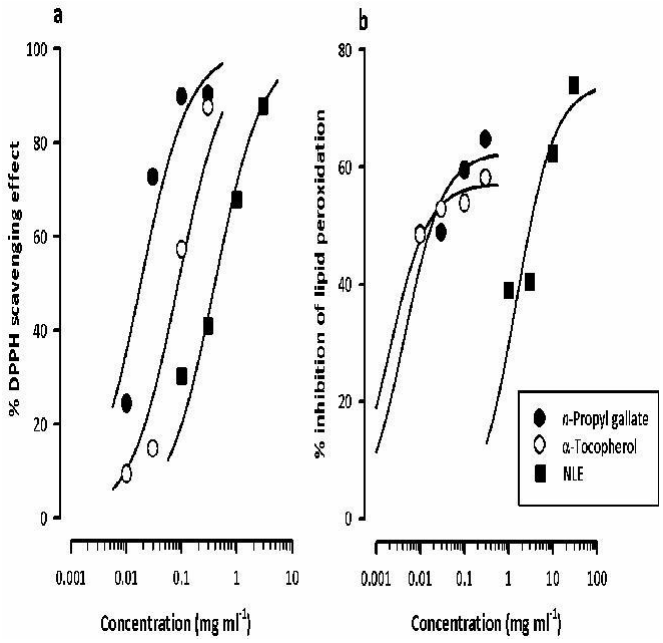

Figure 5. (a) Free radical scavenging ability of NLE (0.1 - 3 mg ml^{-1}) compared to *n*-propyl gallate (0.01-0.3 mg ml^{-1}) and α-tocopherol (0.01 - 0.3 mg ml^{-1}) in the DPPH radical assay and (b) percentage inhibition of lipid peroxidation by NLE (1-30 mg ml^{-1}) compared to *n*-propyl gallate (0.03- 0.3 mg ml^{-1}) and α-tocopherol (0.01-0.3 mg ml^{-1})**.** Values are mean ± S.E.M (n = 3)

Aspirin, mefenamic acid, indomethacin and ketoprofen, were generated nitric oxide (an oxidant) *in vitro* experiments (Asanuma et al., 2001). Therefore the antioxidant activities exhibited by NLE may play a significant role in the anti-inflammatory activities observed in the rat adjuvant arthritis. Moreover the flavonoids luteolin and apigenin also found in *N. laevis* stem bark (Gormann et al., 2006) are reported to inhibit the expression of some pro-inflammatory molecules (Hirano et al., 2006a; Hirano et al., 2006b; Xagorari et al., 2002). Therefore the various naphthoquinones (Eyong et al., 2005; Gormann et al., 2003) and flavonoids (luteolin and apigenin) in the stem bark may possibly contribute as well to the anti-inflammatory effects observed in the rat adjuvant arthritis.

Conclusion

The results of this study show that the ethanolic stem bark extract of *N. laevis* has anti-arthritic activity in the rat adjuvant-induced arthritis. It also has antioxidant properties which may be contributing to its anti-inflammatory effects.

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