



PROTECTIVE AND CURATIVE EFFECTS OF ETHANOL EXTRACT OF *NAUCLEA LATIFOLIA* ON ADJUVANT-INDUCED ARTHRITIS IN RATS

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ABSTRACT

Nauclea latifolia Smith (Rubiaceae) is traditionally used in Nigeria in the management of various ailments such as abdominal pains, dysentery, diabetes, hepatitis, hypertension, rheumatism, yellow fever, jaundice and malaria. Its local use in the treatment of rheumatism has prompted the investigation into its anti-arthritic efficacy. The current study was therefore designed to evaluate the prophylactic and curative effects of ethanol root extract of *Nauclea latifolia* (ERENL) on development of adjuvant arthritis in rats. Arthritis was induced in Sprague-Dawley rats by a single injection of 0.1 ml complete Freund's adjuvant (CFA) into the sub-plantar aponeurosis of the left hind paw. Rats were treated by once daily oral administration of ERENL (25, 50 and 100 mg/kg) on the same day of arthritis induction (prophylactic treatment) and day 12 post arthritis induction (curative treatment) till the 28th day. Indomethacin (2 mg/kg) and methotrexate (1 mg/kg) were used as the standard drugs and administered twice weekly. The effects of the extract and standard drugs were evaluated by changes in paw volume, ankle diameter, arthritis score, body weight, erythrocyte sedimentation rate (ESR), haemoglobin and leucocyte counts. Prophylactic and curative treatment with ERENL caused a dose-dependent and significant suppression of paw and ankle swelling, arthritis score, ESR and body weight loss but had no significant effects on spleen or liver weight indices, haemoglobin and leucocyte count compared to arthritic control rats. The ethanol root extract of *Nauclea latifolia* possesses anti-arthritic activity and was effective irrespective of whether it was administered alongside arthritis induction or after arthritis had already begun. Suppression of arthritis following administration of the extract before and after induction of arthritis demonstrates its ability to protect against development of arthritis as well as suppress an already established one.

KEYWORDS: *Arthritis, Nauclea latifolia, Complete Freund's adjuvant, Inflammation*

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease which typically involves the joints of the hands and feet. Characteristically, peripheral joints are symmetrically inflamed, leading to progressive destruction of articular structures, usually accompanied by systemic symptoms [1]. Drugs used in the treatment of arthritis, including disease-modifying anti-rheumatic drugs (DMARDs), tissue necrosis factor (TNF)- α antagonists, and interleukin 1 (IL-1) receptor antagonists among a host of others, all seem to slow

progression of disease. However, none of these medications is ideal due to long term side effects, toxicity, high cost and inability to modify the fundamental pathological processes responsible for, or to address all the problems associated with, this chronic inflammatory disease [2]. These limitations have led to the search for new compounds from medicinal plants with potential better efficacy, clinical and cost effectiveness and safety against RA. Hence many herbal medicines have come into use around

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the world for the treatment of rheumatic and arthritic diseases [3].

Nauclea latifolia Smith (Rubiaceae), commonly called pin cushion tree or African peach in English is a straggling, evergreen, multi-stemmed shrub or small tree native to tropical Africa. The leaves, stem, stem bark, root and fruits have been found useful, in folk medicine. Extracts from various parts of the plant have been reported to possess antibacterial [4], antimalarial [5, 6], antihypertensive [7], hypoglycaemic [8] and wound healing [9] activities.

Although the plant has been shown to possess anti-nociceptive and anti-inflammatory activities [10, 11] which justify its use in pain and inflammation locally, there is no scientific study to show its anti-arthritic activities. The present study was, therefore, designed to investigate the prophylactic and curative effects of ethanol root extract of *Nauclea latifolia* (ERENL) on adjuvant-induced arthritis in rat.

MATERIALS AND METHODS

Collection and extraction of plant material

Roots of *Nauclea latifolia* were collected around University of Benin, Benin City, Nigeria in the month of May 2013. The plant was identified and authenticated by Mr. O. A. Ugbogu at the Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria, where a herbarium specimen number FHI 106687 was deposited for future reference.

The roots were washed, cleaned and sliced thinly to aid drying. The sliced roots were dried at room temperature and pulverized into coarse powder. The powdered plant material (300 g) was extracted with 2 L of 70 % ethanol by cold maceration, with intermittent agitations for 5 days and filtered. The crude extract was concentrated in a rotary evaporator and further dried in an oven set at 40°C for 24 hours to give a yield of 34.59 g (11.53 %).

Animals

In-bred male Sprague-Dawley rats (190 ± 40 g) were housed in cages under standard laboratory conditions (12:12 h light/dark cycle at a temperature of 28±1 °C) and had free access to standard pellets and water. The study was approved by the Committee on the Use of Laboratory Animals, Faculty of Pharmacy, University of Benin, Benin City, Nigeria and rats were handled according to standard protocols (National

Institute of Health USA: Public Health Service Policy on Humane Care and Use of Laboratory Animals, 2002).

Acute toxicity study

Groups of rats were administered ethanol root extract of *Nauclea latifolia* (suspended in 3 % acacia gum, 0.5, 1, 2.5 and 5 g/kg body weight) by oral intubation and the control group received acacia gum (3 % suspended in distilled water) by the same route. The mortality rate within a 24 hour period was recorded. All animals were observed for a further 2 weeks for any latent signs of delayed toxicity.

Pharmacological studies

Rats were randomly divided into 6 groups of six animals each. Group 1 (arthritic control) was given gum acacia (0.1 ml, 3 % in distilled water) by oral intubation for 28 days. Rats in groups 2, 3 and 4 received orally 25, 50 and 100 mg/kg of ERENL, respectively, for 28 days. Indomethacin (2 mg/kg) and methotrexate (1 mg/kg) were used as the standard drugs and were administered orally twice weekly for the duration of the study.

Arthritis was induced by a single injection of 0.1ml Complete Freund's Adjuvant (Sigma) into the sub-plantar aponeurosis of the left hind paw of the rat.

In the curative study, the ethanol extract (25, 50 and 100 mg/kg, daily), indomethacin (2 mg/kg) and methotrexate (1 mg/kg) were administered twice weekly 12 days post arthritis induction till the 28th day.

Arthritis and other associated indicators were evaluated as follows:

Hind paw swelling

Twice weekly measurement of both paw volumes using a plethysmometer (Ugo Basile, Italy).

Ankle diameter

Twice weekly measurement of left and right ankle diameters using vernier calipers.

Arthritis scoring

Rats were assessed for signs of arthritis on days 4, 8, 11, 15, 18, 22, 25 and 28 using a combination of two scoring methods [12, 13].

Body weight

Body weights of the rats were taken on days 0, 4, 8, 11, 15, 18, 22, 25 and 28.

Haematological indices

These were evaluated using routine laboratory methods. Erythrocyte sedimentation rate (ESR) was estimated by the Sediplast Westergren method [14].

Paw, spleen and liver weights: The relative wet weights of the isolated spleen and liver were expressed as the ratio (mg/g) based on the body weight of the rat. The hind paws were transected at the level of the knee, weighed and expressed relative to the body weight.

Area under the curve (AUC) in the plots of body weight, paw/ankle oedema and arthritic score was calculated using the trapezoid method [15].

Statistical analysis

Results are presented as mean \pm standard error of mean (SEM) and n represents the number of animals

per group. Data were analyzed using one-way ANOVA followed by Dunnett's post-hoc test for multiple comparisons (GraphPad Prism 6). Values were considered significant at $p < 0.05$.

RESULTS

Acute toxicity studies

ERENL up to an oral dose of 5 g/kg caused neither death nor any observable symptoms of toxicity in the rats and no delayed toxic effects were observed after two weeks.

Effect of *N. latifolia* on body weight changes in adjuvant-induced arthritis

Treatment with ERENL (prophylactic and curative) markedly and significantly increased the total percentage change in body weight of all the treatment groups relative to control (Figure 1). These effects were comparable to those of methotrexate and indomethacin.

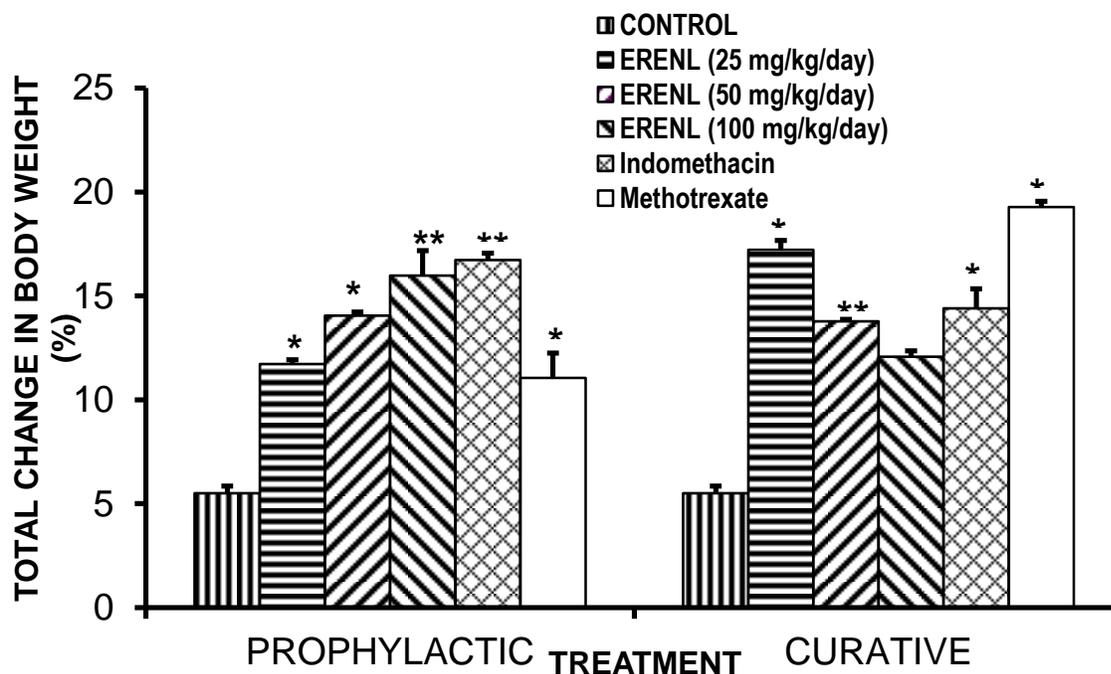


Figure 1: Effect of ERENL on body weight in adjuvant-induced arthritis in rats. Data are shown as mean \pm SEM of n = 6 animals. * $p < 0.05$, ** $p < 0.01$ (one-way ANOVA). ERENL = Ethanol root extract of *Nauclea latifolia*

Effect of *N. latifolia* on paw volume in adjuvant-induced arthritis

Prophylactic (Figure 2) and curative (Figure 3) treatments with ethanol root extract of *Nauclea latifolia* caused significant, but non dose-dependent, reductions in the left paw volume at all doses of the extract relative to the control.

Both treatments also exhibited similar patterns of reduction in right paw volume (secondary inflammation associated with adjuvant arthritis) but these were not significant. Indomethacin and methotrexate also showed same trend.

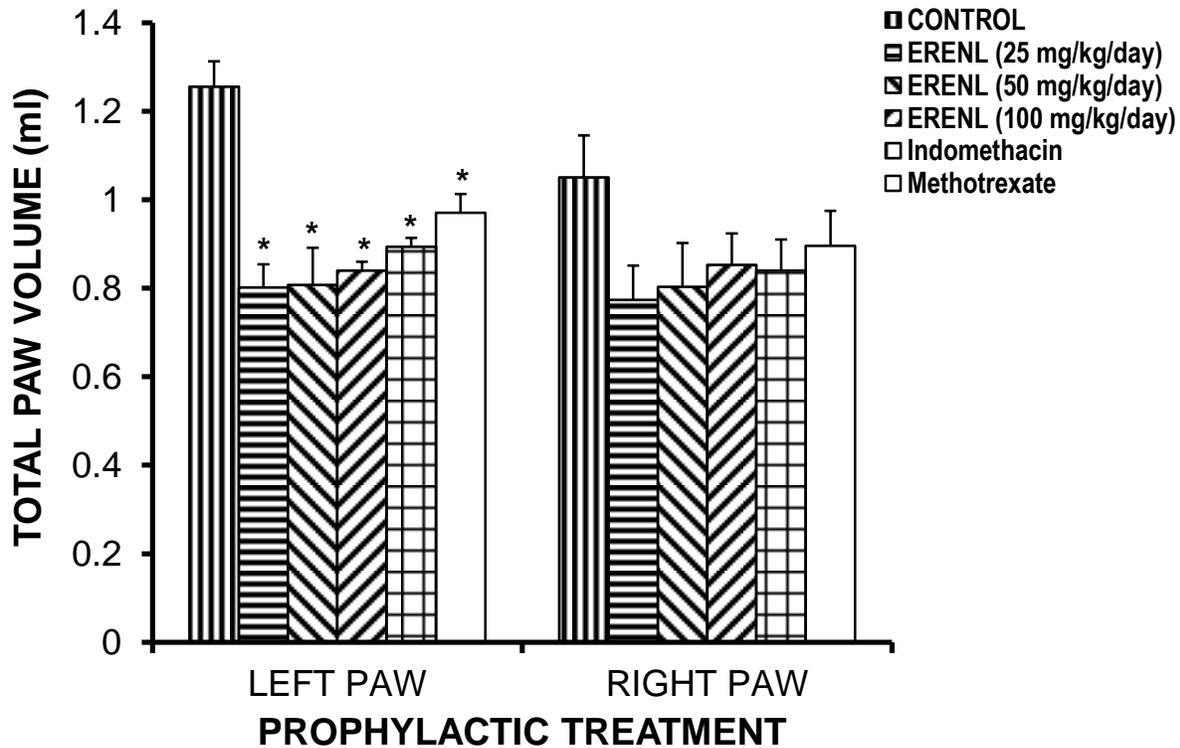


Figure 2: Effect of prophylactic administration of ERENL on paw volume in adjuvant-induced arthritis in rats
 Data are shown as mean ± SEM of n = 6 animals; *p < 0.05, (one-way ANOVA). ERENL = Ethanol root extract of *Nauclea latifolia*.

Effect of *N. latifolia* on changes in ankle diameter in adjuvant-induced arthritis

Only the highest dose (100 mg/kg/day) of ERENL showed significant reduction in left ankle diameter (Figure 4) when give prophylactically, but significant inhibitions of right ankle oedema was observed with all

the doses. On the other hand, curative administration of ERENL caused significant inhibition (p<0.05) of the left ankle swelling only at the dose of 50 mg/kg/day (Figure 5) while right ankle oedema was inhibited by all doses, similar to the effect observed with prophylactic administration.

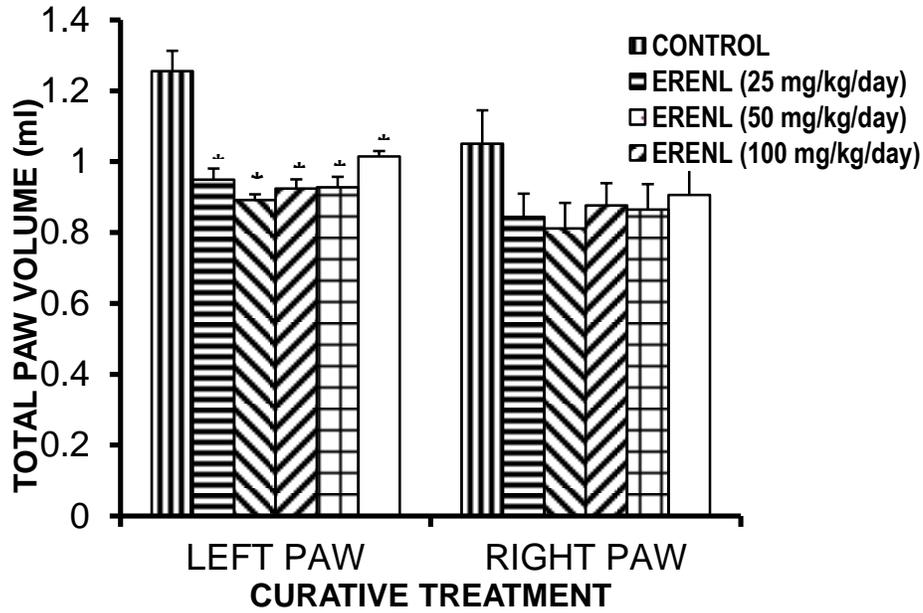


Figure 3: Effect of curative administration of ERENL on paw volume in adjuvant-induced arthritis in rats; Data are shown as mean \pm SEM of n = 6 animals. *p < 0.05, (one-way ANOVA); ERENL = Ethanol root extract of *Nauclea latifolia*.

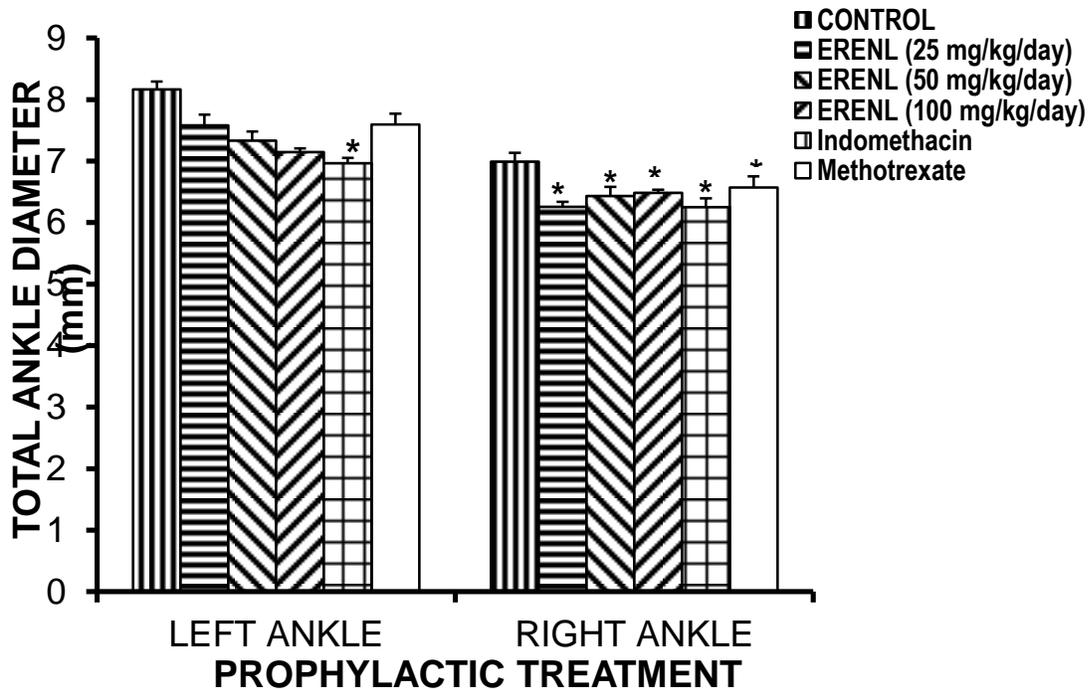


Figure 4: Effect of prophylactic administration of ERENL on ankle diameter in adjuvant-induced arthritis in rats; Data are shown as mean \pm SEM of n = 6 animals; *p < 0.05, p < 0.01 relative to control; ERENL = Ethanol root extract of *Nauclea latifolia*.

Effect of *N. latifolia* on arthritis score

Prophylactic administration of ERENL for 28 days caused significant reduction in the arthritis score only at the highest dose (Figure 6). However, curative treatment produced a dose-dependent and significant decrease in arthritic score, at the two lower doses. Indomethacin and methotrexate were both effective when given curatively.

Effect of *N. latifolia* on organ and paw weights in adjuvant-induced arthritis

ERENL given curatively or prophylactically did not significantly alter the liver and spleen wet weights or the paw weights compared to control.

Effect of *N. latifolia* on haematological parameters in adjuvant-induced arthritis

Table 2 shows the effect of *N. latifolia* root extract on some haematological indices. Prophylactic or curative administration of the extract, at all doses, significantly decreased the electrolyte sedimentation rate and caused slight but non-significant increases in RBC count and haemoglobin concentration relative to control. The high WBC count observed in control rats was mildly decreased at all doses of the extract and different modes of administration. Indomethacin given curatively caused a non-significant increase in WBC count.

Table 1: Effect of *N. latifolia* on paw and organ weight indices in adjuvant-induced arthritic rats

Treatment	Dose (mg/kg)	Prophylactic				Curative			
		Organ and paw weight indices				Organ and paw weight indices			
		Liver	Spleen	Left paw	Right paw	Liver	Spleen	Left paw	Right paw
Control	-	32.53±1.23	3.10±0.61	0.84±0.07	0.57±0.02	32.53±1.23	3.10±0.61	0.84±0.07	0.57±0.02
ERENL	25	31.78±1.16	3.31±0.45	0.85±0.05	0.58±0.02	36.52±1.75	4.39±0.41	0.77±0.03	0.57±0.02
	50	35.79±0.80	4.56±0.51	0.76±0.03	0.54±0.02	34.09±0.97	4.31±0.47	0.75±0.02	0.54±0.01
	100	34.16±0.80	4.93±1.80	0.81±0.01	0.56±0.04	35.59±1.06	5.27±0.62	0.76±0.03	0.55±0.02
Methotrexate	1	32.04±1.13	2.97±0.32	0.81±0.05	0.54±0.02	34.08±2.13	4.71±0.63	0.73±0.03	0.54±0.02
Indomethacin	2	35.96±0.87	4.81±0.68	0.78±0.03	0.49±0.03	40.69±3.34	4.54±0.63	0.83±0.04	0.59±0.02

Values are mean ± SEM (n = 6 animals); **ERENL** - Ethanolic root extract of *Nauclea latifolia*

Table 2: Effect of *N. latifolia* on haematological parameters in adjuvant-induced arthritic rats

Treatment	Dose (mg/kg)	RBC (x 10 ⁶ /μL)	WBC (x 10 ³ /μL)	Hb conc (g/dL)	ESR (mm/hr)
Control		7.27±0.39	13.53±2.10	13.57±0.95	3.67±0.49
<u>Prophylactic</u>					
ERENL	25	7.98±0.46	9.30±0.61	15.14±0.93	2.60±0.60
	50	7.72±0.23	12.94±3.30	14.34±0.48	1.20±0.20**
	100	7.83±0.25	11.85±0.05	14.40±0.20	1.00±0.00**
Methotrexate	1	7.29±0.28	9.75±1.62	13.28±0.43	1.67±0.33*
Indomethacin	2	7.19±0.56	10.30±4.11	12.50±0.63	2.33±0.88
<u>Therapeutic</u>					
ERENL	25	7.50±0.09	10.24±1.78	14.48±0.23	1.80±0.37
	50	7.51±0.33	11.02±1.61	14.20±0.52	1.40±0.22**
	100	7.58±0.29	11.92±1.71	14.05±0.66	1.17±0.17**
Methotrexate	1	7.13±0.29	13.15±1.78	13.27±0.67	1.20±0.16**
Indomethacin	2	6.93±0.78	15.68±2.48	12.40±1.29	1.50±0.22*

Values are mean ± SEM (n=6/group). *p<0.05, **p < 0.01, ***p < 0.001, compared to control; **ERENL** - Ethanol root extract of *Nauclea latifolia*; **CFA** – Complete Freund's Adjuvant; **WBC**- White Blood Cell, **ESR**- Erythrocyte Sedimentation Rate; **RBC**- Red Blood Cell; **Hb Conc**- Haemoglobin Concentration

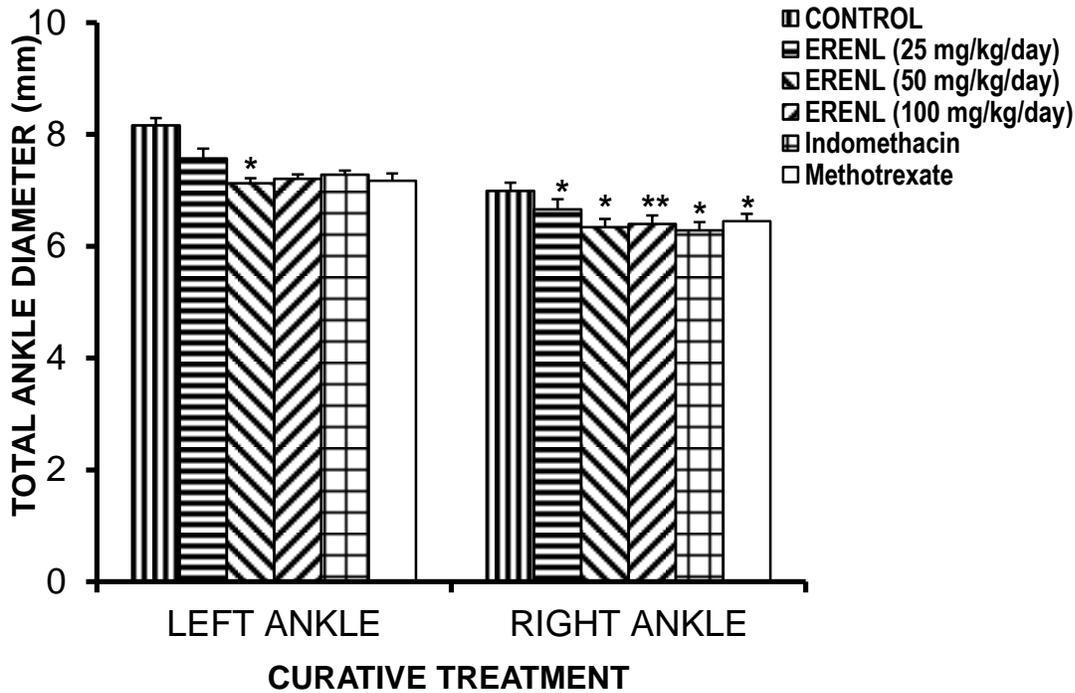


Figure 5: Effect of curative administration of ERENL on ankle diameter in adjuvant-induced arthritis in rats; Data are shown as mean \pm SEM of n = 6 animals; *p < 0.05, **p < 0.01 (one-way ANOVA); **ERENL** = Ethanol root extract of *Nauclea latifolia*.

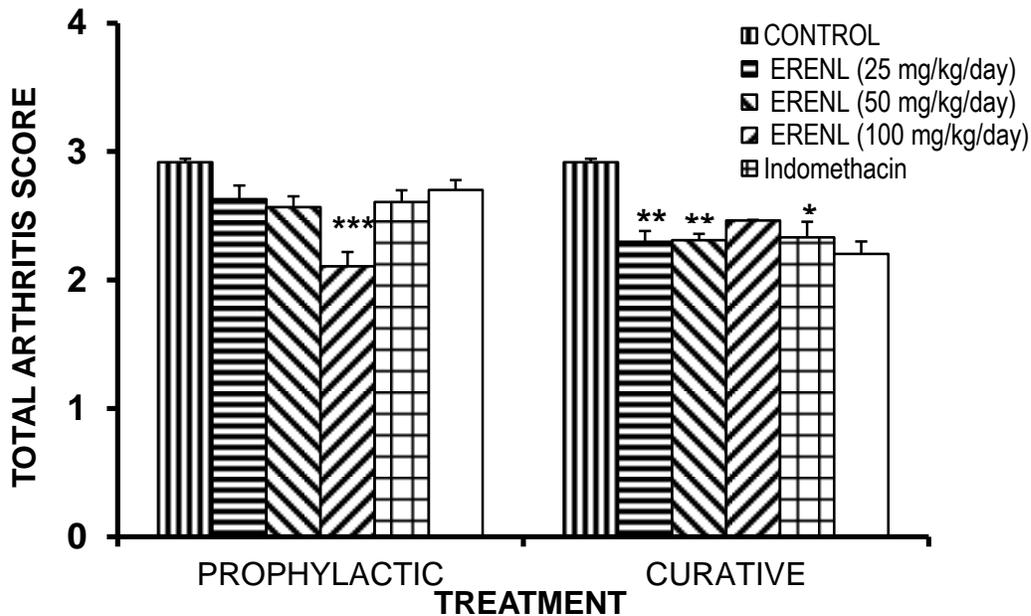


Figure 6: Effect of ERENL on arthritic score in adjuvant-induced arthritis in rats; Data are shown as mean \pm SEM of n = 6 animals; *p < 0.05, **p < 0.01, ***p < 0.001 (one-way ANOVA); **ERENL** = Ethanol root extract of *Nauclea latifolia*

DISCUSSION

Adjuvant-induced arthritis shares some features with human RA such as swelling, cartilage degradation and loss of function, including the involvement of inflammatory mediators in the arthritic aetiology [16].

The prophylactic and curative potential of ethanol root extract of *Nauclea latifolia* for the treatment of adjuvant-induced arthritis in rats was evaluated. The results obtained show that the extract possesses anti-arthritic effects. It was observed that the extract, after either prophylactic or curative administration, markedly suppressed the indices for measuring anti-arthritic activity in rats. In effect, it significantly reduced the arthritis index (score), loss in body weight, increase in ankle joint diameter, increase in hind paw volume and increase in erythrocyte sedimentation rate, in a dose-dependent manner.

Results of acute toxicity study showed no visible changes in autonomic and behavioural patterns of animals with oral administration of the extract at the highest dose of 5 g/kg. No mortality was recorded and no signs of delayed toxic effects were observed after two weeks. ERENL can, therefore be said to be safe up to a dose of 5 g/kg.

A significant loss in body weight of untreated arthritic rats was observed in the present study. Body weight loss is an indication of an abnormal condition in adjuvant-induced arthritis [17, 18] and may arise from decreased food intake due to the immobility accompanying hyperalgesia in such animals [19], or reduced absorption of glucose and leucine in rat intestine [20]. Body weight was significantly increased in arthritic rats given the extract and standard drugs compared to untreated arthritic rats. The extract may therefore inhibit loss in body weight in rats by increasing food intake via attenuation of hyperalgesia and inflammation or improving the absorption capacity of the intestine.

The progression of arthritis is characterized by increase of the paw footpad and tibiotarsal joint diameters. Rats with adjuvant-induced arthritis are often relatively immobile due to the severity of their paws swelling [21]. Paw swelling (determined by paw volume and ankle diameter) is an index for assessing the degree of inflammation and therapeutic efficacy of drugs. Injection of adjuvant into the plantar aponeurosis of the rat induces inflammation that reaches a maximum after 3-5 days. This initial reaction of edema and soft-tissue thickening at the site of injection is caused by the irritant effect of the

adjuvant and is believed to be sensitive to the NSAIDs, whereas the late-phase arthritis and flare in the injected foot are presumed to be immunologic events [22]. Inflammation or lesions outside the injected limb is regarded as secondary inflammation and is a manifestation of cell-mediated immunity. The suppression of such secondary lesions by a drug shows its immunosuppressive activity [23, 24]. The increase in the left hind paw volume of arthritic rats was significantly ($p < 0.05$) inhibited by the prophylactic, as well as the curative, administration of ERENL, in a non-dose-dependent manner. The more pronounced effect of the extract on right ankle swelling compared to the left ankle in both modes of administration is a further indication of its immunomodulatory activity.

Arthritic score is an index of evaluating the anti-arthritic effect of various drugs and was also employed to determine the activity of ERENL. Reduction in total arthritic score was significant with 100 mg/kg in the prophylactic group and at 25 and 50 mg/kg in the curative group.

Hepatomegaly and splenomegaly are usually seen in adjuvant-induced arthritic rats due to hypertrophy of hepatocytes and profound induction of extramedullary hematopoiesis in the red pulp along with mild to marked lymphoid atrophy [25]. Extract treated rats in both prophylactic and therapeutic groups exhibited lower liver and higher spleen weight indices compared to control but these were not significant, showing the activity and beneficial effects of *N. latifolia* on arthritis.

Adjuvant-induced arthritic rats generally exhibit reduced RBCs and haemoglobin concentration which represents an anaemic condition probably due to bone marrow changes and prevention of iron release for incorporation into RBCs [26, 27]. The elevation of these parameters by the extract, though not significant, shows its ability to reverse the anaemia associated with rheumatoid arthritis. In contrast, the non-significant reduction in RBC count and haemoglobin concentration produced by indomethacin and methotrexate may be a consequence of the ulcerogenic effect of the former and myelosuppressive effect of the latter.

Elevated levels of ESR have been found in the blood during virtually all diseases associated with active inflammation or tissue destruction, particularly in patients with rheumatoid arthritis [28]. Therefore, ESR is one of the parameters measured clinically to assist in establishing the presence of RA and other

inflammatory disorders and in assessing the activity of disease and its response to treatment. The high ESR count in arthritic control group was significantly inhibited by *N. latifolia* as well as the standard drugs, indomethacin and methotrexate, thus justifying their significant roles in arthritic conditions.

The significant increase in WBC count in adjuvant-induced arthritic rats may be due to production of immune complexes and antibodies, macrophage migration as well as lymphocytes infiltration [1]. The decrease in extract-treated groups shows the immunomodulatory effect of the extract.

In conclusion, ERENL possesses anti-arthritic activity, as evidenced by its effects on the various indices for measuring arthritis, and was effective irrespective of whether it was administered alongside arthritis induction or after onset of arthritis. This demonstrates its ability to protect against development of arthritis as well as suppress an already established one.

COMPETING INTERESTS

The authors declare no competing interests.

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