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Original Article

Inhibition of leucocyte migration: A mechanism of anti-inflammatory effect of the ethanol extract of the stem bark of *Alstonia boonei* in Wistar rats

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ABSTRACT

Background and aim: The stem bark of *Alstonia boonei* is used in traditional medicine to treat or reduce inflammation and pain in Nigeria. In view of these, the effect of the ethanol extract of its stem bark on agar-induced leucocyte migration in rats as a mechanism of anti-inflammation was investigated.

Methods: The effect of the ethanol extract of the stem bark of *A. boonei* on agar-induced leucocyte migration in rats was evaluated using standard analytical method.

Results: The results showed significant ($p < 0.05$) inhibitions of leucocyte migrations in the rats administered 400, 800 and 1200 mg/kg body weight (b. w) compared to the leucocyte migration of the rats in the control group (5 ml/kg b. w of normal saline). The inhibitory effect of the extract was dose-dependent and comparable to that of the reference anti-inflammatory drug, indomethacin (0.3 mg/kg b. w).

Conclusion: The data indicate that the ethanol extract of the stem bark of *A. boonei* possesses remarkable inhibitory effect on leucocyte migration and therefore, justifies the traditional use of the stem bark of *A. boonei* as an anti-inflammatory agent.

1. Introduction

Plants form the main ingredients of medicine in traditional medical practice and have been the major source of several pharmaceutical drugs. Their uses are increasing world wide due to the persistent and sometimes expansion of traditional medicine and a growing interest in herbal treatments.[1] Inflammation is part of the complex biological response of vascular tissues to harmful stimuli including pathogens, irritants or damaged cells.[2] It is also a pathophysiological response of living tissues to injuries that leads to the local accumulation of plasmatic fluids and body cells. It is a protective attempt by an organism to remove injurious stimuli as well as initiate a healing process for tissues. The process of inflammation is necessary for healing of wounds, however, if not controlled, may lead to the onset of diseases as vasomotor rhinorrhoea, rheumatoid arthritis, atherosclerosis and cancer inter alia.[3]

Alstonia boonei de Wild (Fig. 1) (Apocynaceae) is a medicinal plant used extensively in west and central Africa. It has been found to elicit several pharmacological and therapeutic actions. It is a large deciduous tree that is up to 45 m tall and

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Fig. 1 – *Alstonia boonei* de Wild.

1.2 m in diameter; bole often deeply fluted up to 7 m; small buttresses present; bark greyish-green or grey; rough, exuding a copious milky latex and branches in whorls. It occurs from Senegal and Gambia to Western Ethiopia and Uganda where it is found in primary as well as secondary moist evergreen to dry semi-deciduous forest. In west and central Africa, its parts are generally used for the treatment of many ailments including malaria, fever, intestinal helminths, rheumatism, hypertension and other life-threatening diseases.[4] An infusion of the root and stem bark is drunk as a remedy for asthma; a liquid made from the stem bark and fruit is drunk once daily to treat impotence.[5] Other reported properties of *A. boonei* include: anti-viral, anti-microbial and antioxidant activities.[6] This study was aimed at investigating the effect of the ethanol extract of the stem bark of *A. boonei* on leucocyte migration in Wistar rats.

2. Materials and methods

2.1. Plant

Stem bark of *A. boonei* tree was collected from the Botanical Garden of the University of Nigeria, Nsukka, Enugu State, Nigeria. The botanical identification of the stem bark was done by Prof. (Mrs.) May Nwosu of the Department of Botany, University of Nigeria, Nsukka.

2.2. Preparation of the extract

Fresh stem bark of *A. boonei* tree was washed with distilled water and cut into smaller bits to increase their surface area for easier drying. The stem bark was shade-dried for a month and a half and homogenised into fine particles using an electric blender. A known weight (372 g) of the ground stem bark was macerated in 1500 ml of 80% ethanol for 24 h at room temperature. The mixture was filtered and the filtrate passed through a rotary evaporator to reduce the ethanol content. Thereafter, the filtrate was further concentrated using an oven at 50 °C and stored in a refrigerator until used.

2.3. Animals

Adult male Wistar rats of between 7 and 12 weeks old with average weight of 120 ± 20 g and albino mice weighing 30 ± 5 g were obtained from the Animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were acclimatised for one week under a standard environmental condition with a 12 h light and dark cycle and maintained on a regular feed and water *ad libitum*. There was adherence to the Principles of Laboratory Animal Care. The University Animal Research Ethical Committee approved the experimental protocol.

2.4. Acute toxicity study

The acute toxicity and lethality (LD_{50}) of the extract was determined using mice according to slightly modified method of.[7]

2.5. Chemicals and reagents

The chemicals used for this study were of analytical grade and procured from reputable scientific shops at Nsukka. They included: 80% ethanol (BDH Chemicals Ltd., Poole, England), indomethacin [standard anti-inflammatory drug (Sigma–Aldrich, Inc., St. Louis, USA)], 3% w/v agar suspension, 10% ethylenediaminetetraacetic acid (EDTA) (BDH Chemicals Ltd., Poole, England), phosphate buffer and distilled water.

2.6. Leucocyte migration test

The effect of the extract on *in vivo* leucocyte migration was determined in terms of the differential and total leucocyte counts by the method of.[8]

2.7. Statistical analysis

The data obtained from the laboratory were subjected to one-way Analysis of Variance (ANOVA). Significant differences were observed at $p \leq 0.05$. The results were expressed as means of five replicates ± standard errors of the means (SEM). This analysis was done using the computer software known as Statistical Package for Social Sciences (SPSS), version 18.

3. Results

3.1. The acute toxicity and lethality (LD_{50}) of the ethanol extract of the stem bark of Alstonia boonei

The result of this study shows that there was neither lethality nor any sign of toxicity in the four groups of three mice each that received 10, 100, 1000 mg/kg body weight of the ethanol extract of the stem bark of *A. boonei* and 5 ml/kg body weight of normal saline respectively at the end of the first phase of the

Table 1 – Effect of the ethanol extract of the stem bark of Alstonia boonei on agar-induced leucocyte migration in rats.

Groups	Treatments	Total leucocyte counts (cells mm^{-3})	Differential leucocyte counts (%)		
			Macrophages	Neutrophils	Lymphocytes
1	5 ml kg^{-1} of normal saline	3280 ± 77.96^{a}	25.35 ± 1.51^{c}	73.20 ± 3.65^{d}	18.65 ± 1.60^{a}
2	0.3 mg kg^{-1} of indomethacin	2163 ± 59.88^{b}	19.00 ± 1.14^{c}	67.69 ± 3.05^{d}	10.00 ± 0.95^{b}
3	400 mg kg^{-1} of the ethanol stem bark extract	2750 ± 68.75^{ab}	21.20 ± 1.30^{c}	68.60 ± 3.10^{d}	13.43 ± 1.12^{b}
4	800 mg kg^{-1} of the ethanol stem bark extract	2186 ± 61.17^{b}	20.40 ± 1.27^{c}	66.30 ± 2.97^{d}	10.20 ± 1.00^{b}
5	1200 mg kg^{-1} of the ethanol stem bark extract	1798 ± 47.33^{b}	18.85 ± 1.09^{c}	63.20 ± 3.00^{d}	8.40 ± 0.89^{b}

Values in the same column with different letter superscripts are significantly different ($p < 0.05$).

study. At the end of the second phase of the study, there was not death or obvious sign of toxicity in the groups of mice that received 1900, 2600 and 5000 mg/kg body weight of the ethanol extract of the stem bark of *A. boonei*.

3.2. *Effect of the ethanol extract of the stem bark of A. boonei on Leucocyte migration in terms of the total and differential leucocyte counts*

As shown in Table 1, there were statistically significant ($p < 0.05$) differences between the total leucocyte count of the Group 1 (control group) rats and those of the rats of groups 2, 4 and 5. The effect of the extract was comparable with that of the reference anti-inflammatory drug (indomethacin).

Table 1 also reveals that the extract at the tested doses exerted a marked inhibition in the migration of the differential leucocyte count (lymphocytes) into the peritoneal cavity. The effects of the extract with regard to the differential leucocyte counts were comparable with those of the standard anti-inflammatory drug (indomethacin).

4. Discussion

This study was carried out to examine the effect of the ethanol extract of the stem bark of *A. boonei* on agar-induced leucocyte migration in Wistar rats with a view to finding out if inhibition of leucocyte migration is a possible mechanism of anti-inflammatory action of the ethanol extract of the stem bark of *A. boonei*.

Acute toxicity test on the ethanol extract of the stem bark of *A. boonei* using mice showed an LD_{50} value of greater than 5000 mg/kg body weight which implies that the stem bark of *A. boonei* might be regarded as being safe with no risk of acute toxicity.

That the extract at the tested doses, evoked a marked dose-dependent inhibition of leucocyte migration into the peritoneum implies an anti-inflammatory effect of the extract. This effect might have been possible through the alteration of the activation of inflammatory cells. The neutrophils being higher in proportion than the lymphocytes probably may have led to the alteration in the migration of the inflammatory cells. The innate and adaptive mechanisms of the immune system could be modified by substances to either enhance or suppress their ability to resist invasion by pathogens.[9] Leucocytes are rapidly mobilised from the bone marrow into the blood during infections or inflammatory reactions. A blood neutrophilia is a characteristic feature of infections and inflammatory disorders, due to initially, the rapid mobilisation of neutrophils (being the body's first-line of defence) from the bone marrow reserve and their subsequent migration into the tissues.[10]

In conclusion, oral administration of the ethanol extract of the stem bark of *A. boonei* to Wistar rats caused a dose-related decrease in the migration of leucocytes in agar-induced inflammation indicating that this is a mechanism of anti-inflammatory effect of the extract.

Conflicts of interest

All authors have none to declare.

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