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Evaluation of Anti-Inflammatory and Antipyretic Activities of The Aqueous Stem Bark Extract of *Lophira Lanceolata* Van Tiegh. Ex Keay

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Abstract

The bark of *Lophira lanceolata* (Ochnaceae) has been used traditionally for the treatment of many disease conditions such as dysentery, headaches, diarrhoea, cough, abdominal pains, fungal infections, nociception and cardiovascular diseases. However, no specific work on its anti-inflammatory or antipyretic effect has been reported which forms the basis of this study. This study evaluates the in-vivo anti-inflammatory and antipyretic activities of the aqueous extract of the stem bark of *Lophira lanceolata* using laboratory animal model. The methods adopted for this study were formalin induced inflammation and yeast induced hyperpyrexia in rats. All the results were expressed as mean ± S.E. The data were statistically analysed by one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison and p-values 0.05 were considered as significant. The result of this study showed a significant anti-inflammatory effect different from the control (distilled water) at p 0.05 characterized by oedema formation, change in temperature, redness, and itching in the paw of the rats which is comparable with the standard indomethacin. The inhibition of oedema was statistically dose dependent as the group 4 rats which received 400 mg/kg body weight demonstrated more effect than the group 1 rats which received 100 mg/kg body weight of the aqueous extract of the stem bark of *Lophira lanceolata*. The aqueous *Lophira lanceolata* stem bark extract at the doses of (100, 200 and 400 mg/kg p.o) significantly ($p<0.05$) attenuates the pyrexia induced by yeast in rats in a manner statistically comparable with the standard drug Paracetamol, however, extract at the dose of 400 mg/kg body weight showed the highest antipyretic effect. Hence the antipyretic activity was also dose- dependent. The study demonstrated that the aqueous *Lophira lanceolata* stem bark extract possesses anti-inflammatory and antipyretic activities.

Keywords: Anti-Inflammatory, Antipyretic, Aqueous Stem Bark Extract, *Lophira Lanceolata* Van Tiegh. Ex Keay

Introduction

Lophira lanceolata (*L. Lanceolata*) commonly known as the dwarf red ironwood is a plant specie of the family Ochnaceae native to tropical West and Central Africa including Nigeria, Cameroon, Mali, Senegal, Ghana, Chad, Sudan and Uganda (Mapongmetsem, 2007). It is also found in Sokoto State North Western Nigeria. Various parts of the plant have been employed traditionally for the treatment of many ailments. It is popularly known and recognized by various tribes in Nigeria as Naminjin kade in Hausa (Abdullahi *et. al*., 2003). Ipondon in Yoruba (Georgia *et al*., 1968), Okopia in Igbo and Maganchi in Nupe (Sani *et al*., 2007). *L. Lanceolata* has been previously reported to possess anti-infertility (Etuk *et al*., 2009), anti-viral (Igboeli *et al*., 2015), anti-plasmodial and antioxidant (Onyeto *et al*., 2014), headache and blurred vision (Wang *et al*., 2016), antidiabetic, anti-helminthic and antimalarial respectively. *L. lanceolata* oil is used in the manufacturing of soap (Kouaro *et al*., 2010; Mapongmetsem, 2007). Chemical analysis of *L. lanceolata* oil revealed that it contains sodium, potassium, calcium, magnesium, zinc, iron, and phosphorus,

polyunsaturated fatty acids and essential amino acids (Lohlum *et al*., 2010; Nonviho *et al*., 2014). Inflammation is the immune system's response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation (Medzhitov *et al*., 2010), and acts by removing injurious stimuli and initiating the healing process (Ferrero-Miliani *et al*., 2007). Inflammation is therefore a defense mechanism that is vital to health (Nathan *et al*., 2010). Usually, during acute inflammatory responses, cellular and molecular events and interactions efficiently minimize impending injury or infection. This mitigation process contributes to restoration of tissue homeostasis and resolution of the acute inflammation. However, uncontrolled acute inflammation may become chronic, contributing to a variety of chronic inflammatory diseases (Zhou *et al*., 2016). At the tissue level, inflammation is characterized by redness, swelling, heat, pain, and loss of tissue function, which result from local immune, vascular and inflammatory cell responses to infection or injury (Tekeuchi *et al*., 2010). Important microcirculatory events that occur during the inflammatory process include vascular permeability changes, leukocyte recruitment and accumulation, and inflammatory mediator release (Ferrero-Miliani *et al*., 2007, Chertov *et al*., 2000). Various pathogenic factors, such as infection, tissue injury, or cardiac infarction, can induce inflammation by causing tissue damage. In response to tissue injury, the body initiates a chemical signaling cascade that stimulates responses aimed at healing affected tissues. These signals activate leukocyte chemotaxis from the general circulation to sites of damage. These activated leukocytes produce cytokines that induce inflammatory responses (Jabbour *et al*., 2009). Fever is one of the most common medical signs. It is part of about 30% of healthcare visits by children (*Sullivan et al., 2011)* and occurs in up to 75% of adults who are seriously sick (*Kiekkas et.al. 2013)*. While fever evolved as a defense mechanism, treating fever does not appear to worsen outcomes. Fever is often viewed with greater concern by parents and healthcare professionals than is usually deserved, a phenomenon known as fever phobia (*Crocetti et al., 2001*). Medicinal plants such as *L. lanceolata* and many others have anti-inflammatory activity and the need to explore their usefulness in inflammatory conditions is highly sorted. There are various medicines for controlling and suppressing inflammatory crisis; steroids, non-steroidal anti-inflammatory drugs, and immunosuppressant are the practical examples of these medications and nausea is associated adverse effect. In practice, our goal is to apply minimum effective dose by the highest efficacy and with the least adverse effects. Thus, we need to apply natural anti-inflammatory factors within medication therapy to achieve increased pharmacological response and the lowest degree of unwanted side effects. The search for natural compounds and phytoconstituents that are able to interfere with these mechanisms by preventing a prolonged inflammation could be useful for human health.

Materials and Methods

Materials: Beakers, flat bottom flask, round bottom flask, water bath, measuring cylinder, hand gloves, filter funnel, filter paper, syringe and needles, thermometer, vernier calliper, oven. Solvents/Regents/Drugs; distilled water, Paracetamol, indomethacin, formalin, Brewer's yeast suspension.

Animals: Healthy adult Wister strain of Albino rats of both sexes, two to three months old weighing between 120g – 170g were procured. The animals were allowed to acclimatize under laboratory conditions for a period of seven days prior to the experiment. The protocol was approved by the institutional ethical committee for use of animals in research.

Plant collection and identification: The plant stem barks were collected in March, 2021 from Zuru Local Government Kebbi State and was identified by Dr Halilu Mshelia of Pharmacognosy Department Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto, a voucher number (PCG/UDUS/OCHA/0001) was assigned and a specimen of the plant was kept in the herbarium of the Department of Pharmacognosy and Ethnopharmacy.

Preparation of aqueous plant extract: The barks

of Lophira lanceolata were dried under a shade until it could be powdered conveniently and the coarsely powdered plant was macerated and the constituents extracted where 2000gm of powder was mixed with 1200ml of water in a flat bottom flask, the mixture was kept for 48 hrs with intermittent shaking. Then it was filtered severally and the filtrate was evaporated to dryness in the oven at 60 C for 72 hours until a constant weight was obtained and the percentage yield was obtained afterwards.

Percentage yield calculation

Percentage was obtained using the equation:

$$\% \text{ yield} = \frac{\text{Mass of dried extract}}{\text{Initial mass}} \times 100$$

Dose preparation: Based on the acute toxicity studies conducted by Etuk *et al*. (2010) the dose of the extract was determined and the three treatment groups were given 100mg/kg, 200mg/kg and 400mg/kg respectively.

Anti-inflammatory activity: Animals were divided into five groups. Each group consists of five animals. Anti-inflammatory effect of the aqueous extract of the bark of *L. lanceolata* was assessed by employing formalin induced paw oedema in rats (Winter *et al*., 1962). The inflammation was produced by sub-plantar injection of 20uL of freshly prepared 2% formalin in the right hind paw. The extracts at doses of 100mg/kg, 200mg/kg, 400mg/kg, indomethacin 10mg/kg and distilled water (10m/kg, p.o) were given 1h prior to formalin injection, where group 1 received distilled water (10ml/kg) and group 2 received indomethacin (10mg/kg) while group 3,4 and 5 received 100mg, 200mg and 400mg/kg of the extract respectively. Increase in paw diameter was measured using vernier calliper. The measurement was done before and after 1-5 h following formalin injection.

Antipyretic activity using yeast induced hyperpyrexia: The antipyretic activity of the aqueous extract of *L. lanceolata* was assessed using yeast induced hyperpyrexia in rats (Vogel, 2002).

Five groups of five animals each were used. Before experimentation rectal temperature of the rats were recorded by inserting a well lubricated bulb of a thermometer in the rectum. Hyperpyrexia was induced in the rats by subcutaneous injection of 10ml/kg body weight of 15% aqueous suspension of brewer's yeast in the back below the nape of the rats. Pre drug control temperatures were taken at 24 h after the yeast injection to determine the pyretic response of the yeast. Aqueous *L. lanceolata* bark extract (100mg, 200mg and 400mg/kg body weight) and Paracetamol (150mg/kg) which served as the positive control were given orally 24 h after the yeast injection. The temperatures were recorded at 1-4 h after the drug treatment.

Statistical analysis

All the result was expressed as mean ± S.E. The data were statistically analyzed by one -way analysis of variance (ANOVA) followed by Dunnett's multiple comparison and p-values 0.05 were considered as significant.

Result

Percentage yield of the extract was found to be = 2.4 % w/w

Anti-inflammatory activity

The result of formalin induced paw oedema was analyzed using ANOVA and presented as mean ± SEM. The values obtained showed a significant difference from control at p 0.05 inhibition of inflammation (characterized by oedema formation, change in temperature, redness, and itching) in the paw of the rats but when compared with standard showed no significant difference. The values (diameter) obtained were lower than that of indomethacin (10mg/kg) which was used as positive control. The inhibition of oedema increased with increase in dosage from 100mg to 400mg/kg (table 1.0).

Table 1. Anti-inflammatory activity of *Lophira lanceolata* in rat (Paw diameter in mm)

Treatment (mg/kg)	0 hour	1st hour	2nd hour	3rd hour	4th hour	5th hour
Distilled water	1.69 ± 0.01	1.69 ± 0.01	1.68 ± 0.01	1.67 ± 0.01	1.67 ± 0.01	1.66 ± 0.01
LL 100	1.59 ± 0.09	1.57 ± 0.09	1.55 ± 0.08	1.51 ± 0.09	1.46 ± 0.09	1.43 ± 0.08
LL 200	1.55 ± 0.04	1.53 ± 0.05	1.49 ± 0.05	1.45 ± 0.05	1.37 ± 0.06*	1.32 ± 0.04*
LL 400	1.52 ± 0.01	1.51 ± 0.01	1.44 ± 0.02	1.40 ± 0.01	1.32 ± 0.01	1.31 ± 0.06*
Indomethacin	1.58 ± 0.11	1.56 ± 0.08	1.56 ± 0.10	1.50 ± 0.11	1.42 ± 0.09	1.33 ± 0.10*

Data presented as mean ± SEM, (n = 6) for all groups. * Significantly different at $p < 0.05$

Antipyretic activity

Following the subcutaneous injection of yeast suspension there was marked increase in the rectal temperature after 24 hours. However, treatment with the aqueous *L. lanceolata* bark extract at the doses of 100, 200 and 400 mg/kg significantly decreased the temperature of the rats in a dose dose-dependent manner. The antipyretic effect started as from the first hour of treatment and the effect was maintained for 4 hours. The result in the table 2 below showed treatment with extract and standard (Paracetamol) exhibited significant reduction in the elevated temperature when compared with control (distilled water) at p 0.05.

Table 2. Antipyretic activity of *Lophira lanceolata* using yeast induced hyperpyrexi in rats

		Rectal temperature after drug administration (^{o}C)				
Groups/Dose (mg/Kg) orally	Normal temperature	0 h	1 h	2 h	3 h	4 h
Distilled water 10ml	35.14 ± 0.19	39.90 ± 0.10	39.90 ± 0.10	39.84 ± 0.09	39.84 ± 0.05	39.48 ± 0.49
Paracetamol 150mg	35.22 ± 0.17	37.64± 0.11	37.06 ± 0.05	36.48 ± 0.08	36.02 ± 0.05*	35.50 ± 0.07
L. lanceolata (100mg)	35.22 ± 0.16	38.60 ± 0.43	38.14 ± 0.38	37.50 ± 0.01	37.04 ± 0.05	36.48 ± 0.08*
L. lanceolata (200mg)	35.28 ± 0.18	38.46 ± 0.31	37.54 ± 0.11	37.06 ± 0.11	36.64 ± 0.17	36.04 ± 0.09
L. lanceolata (400mg)	35.28 ± 0.18	37.82 ± 0.08	37.06 ± 0.09	37.06 ± 0.09	36.26 ± 0.13	35.78 ± 0.07

Data presented as mean ± SEM, n = 5 for all groups

Discussion

In the present study, formalin induced paw oedema and yeast induced hyperpyrexia methods in rats were adopted to evaluate the anti-inflammatory and antipyretic effects of crude aqueous extract of *L. lanceolata.* The formalin induced paw inflammation is accompanied by varieties of mediators which are liberated in two stages; the first being the release of serotonin and histamine while the second is mediated by prostaglandins. The cyclooxygenase products and the continuity between the stages are provided by kinins (Ageel *et al*., 2005; Adeolu *et al*., 2008). Meanwhile, the results of this study illustrates a statistically significant reduction ($P < 0.05$) in the diameter of the hind paw inflammation induced in the rats, the response which was linear and dose dependent with the highest activity observed at 400 mg/kg which is in agreement with the work of Abdulgafar *et al.* (2011) who reported the anti-inflammatory activity of *Schwenckia americana* using acetic acid induced writhing test and formalin induced pain in rat that showed significant percentage inhibition in a dose dependent manner. Also, the extract exhibited more reduction in the diameter of the oedema than standard indomethacin drug. It is well known that, non – steroidal anti-inflammatory drugs (NSAIDS) demonstrate their anti-inflammatory effect by blocking the prostaglandin synthesis (Gunaydin and Sirri Bilge, 2018). Yeast induced pyrexia is called pathogenic fever by increasing the synthesis of prostaglandin, and is considered as a useful model for the screening of plants materials as well as synthetic drugs for their antipyretic effect (Moltz *et al*., 1993; Jan *et al*., 2016). Several cytokines such as IL-8 and IL-1 are characterized as endogenous pyrogens and the inhibition of these pyrogens is responsible for antipyretic effect of drugs (Moltz *et al*., 1993). In this study, aqueous Lophira lanceolata bark extract significantly attenuates the pyrexia induced by yeast in rats, although the standard (paracetamol) exhibited more antipyretic effect than the extract. The reduction in rectal temperature was dose dependent as it reduced with increasing concentration of the extract from 100 to 400 mg/kg, with 400 mg/kg having the highest antipyretic activity. Therefore, *L. lanceolata* perhaps interfered with the release of prostaglandin and pyrogenic cytokines which revealed that, *Lophira lanceolata* bark extract possess anti-inflammatory and antipyretic effects. Previous studies have also shown that *Lophira lanceolata* contains flavonoids, anthraquinones, carbohydrate, glycoside, phenols, saponin steroid, tannin and a sugar as glucose (Sani *et al*., 2007). These phytochemicals are known for their antioxidant and other biological properties (Ahmad *et al*., 2011). Thus, the anti-inflammatory and antipyretic activities could also be associated with the presence of one or more of these phytochemicals identified in the plant extract.

Conclusion

This study demonstrated that the aqueous *Lophira lanceolata* stem bark extract possesses anti-inflammatory and antipyretic activities probably due to its ability to inhibit or interfere with the synthesis of prostaglandin and cytokines. The results obtained correlates with the reported traditional use of the plant as anti-inflammatory and antipyretic. Further studies could be carried out to elucidate the exact mechanism of action by which the extract exerts its anti-inflammatory and anti-pyretic activities.

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