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Original Research Article

Antipyretic Activity of Orally Administered Extracts of *Newbouldia laevis* (Bignonaceae) in Mice

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Abstract

Purpose: Several plant species are used in the management and treatment of pyrexia. In this study, the antipyretic activity of extracts of *Newbouldia laevis* leaves was investigated on normal body temperature and baker's yeast induced pyrexia in mice.
Methods: The crude leaves were extracted with water, ethyl acetate and hexane. Pyrexia was induced with baker's yeast at a dose of 10 ml/kg of 30% w/v and the extracts were orally administered to groups of mice at 25, 50 and 100 mg/kg while 0.9% NaCl and paracetamol (20 mg/kg) were also administered and served as controls. The effect of the extracts on normal body temperature was assessed.
Results: Unlike the hexane extract, the aqueous and ethyl acetate extracts displayed significant antipyretic properties at all doses employed ($p<0.05$) but no significant effect on normal body temperature of the mice was observed similar to the effect of paracetamol.
Conclusion: Extract of the leaves of *N. laevis* exert antipyretic effects and these results support claims by traditional medicine healers for its use in fever reduction.

Keywords: *Newbouldia laevis*; Antipyrexia; Mice; Baker's yeast; Ethylacetate extract.

Indexing: Index Copernicus, African Index Medicus

Introduction

Nigeria is endowed with numerous plant species which are used for the treatment of various ailments because of their medicinal properties. One of such plants is *Newbouldia laevis* (Bignoniaceae) which is widely distributed and can also be found in the wild and nature reserves. *N. laevis* (Beauv.) Seeman ex Bureau popularly known as "fertlilty tree" or "boundary tree" is commonly employed medicinally in southeastern Nigeria, where the leaves are squeezed to treat eye troubles [1]. The roots, barks and leaves are also used during childbirth. Usually, an aqueous leaf extract of *N. laevis* is prepared and about 3 cups are consumed orally to induce or augment labor within a few hours [1]. An earlier study reported that the aqueous and ethanol extracts of the leaves of *N. laevis* increases the frequency of spontaneous uterine contractions and directly stimulates contraction of the uterus [2]. The leaves of *N. laevis* have also been reported to be used traditionally in the treatment of cough, fever, hypertension, infertility, wound dressing and circumcision in Nigeria [3]. Although the plant has been used in traditional settings to control fever, its pharmacological activities have not been documented. Previous phytochemical studies reported the presence of quercetin (a flavonoid) [6], tannins, flavonoids, terpenes, steroidal and cardiac glycosides [7] in the leaves.

This study is therefore aimed at the investigation of the antipyretic activities of the leaves of *N. laevis* by studying the effects of the aqueous extracts (ANL), ethylacetate extracts (ENL) and hexane extracts (HNL) of the plants on pyrexia induced by baker's yeast in mice.

Experimental

Drugs/Chemicals

Paracetamol (4-acetamidophenol, Sigma Chemical Company Taufkirchen, Germany) was dissolved in a

minimum amount of 5% Tween 80 and made up to 5 ml volume with sodium chloride solution; this was employed in this study as the positive control and administered orally. Dried baker's yeast (*Saccharomyces cerevisiae*, Vahine Monteux, France) was suspended in 0.9% sodium chloride solution and administered intraperitoneally (IP); 0.9% sodium chloride (Dana Pharmaceuticals, Nigeria) served as the negative control; Ethyl acetate, hexane (BDH Chemicals, England) and Tween 80 (Sigma Aldrich, UK) were employed as solvents in this study.

Plant Material

The fresh mature leaves of *Newbouldia laevis* were collected from Benin City in the month of September, 2009. They were identified by Dr JF Bamidele of Botany Department, University of Benin, Benin City, Nigeria and a herbarium specimen was deposited.

The leaves were air dried under a shade for two weeks after which they were powdered with a mortar and pestle. After weighing the dry powder (100 g each) they were placed into three glass tanks where maceration in 900 ml each of distilled water was done for 18 h for the aqueous extractions and 72 h for ethylacetate and hexane extractions respectively. Aqueous, ethylacetate and hexane extracts were filtered and concentrated at 40 $^{\circ}$C using a rotary evaporator. The concentrated extracts (12.03 g of aqueous, 9.68 g of ethylacetate and 9.57 g of hexane leaf extracts) were transferred into an oven set at 40 $^{\circ}$C and evaporated to dryness (giving a yield of 0.1203%, 0.0968%, and 0.0957% respectively) and stored in amber coloured bottles.

Animals

All experimental procedures on the animals in this study were as performed previously [8]. Briefly, in-bred Swiss albino mice of mixed sex (kept in separate cages according to sex) weighing between 20-30 g were obtained from the Animal House, Department of Pharmacology, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria. The animals were maintained according to standard nutritional and environmental conditions. Animal care and experimental procedures complied with standard guidelines for the use of laboratory animals (National Institute of Health USA: Public Health Service Policy on Humane Care and Use of Laboratory animals, 2002) [9]. This involved maintenance of animals under standard conditions with free access to standard diet (Bendel Feeds and Flour Mill, Ewu, Nigeria) and water. The animals were transferred into the laboratory 24 h before the experiment began and were housed in individual plastic cages lined with wood shavings. Each animal was used for one experiment only. The animals were exposed to natural lighting conditions and a room temperature of about 22-26 ºC ± 1 ºC.

Measurement of Temperature

Rectal temperature was measured by inserting a lubricated digital probe thermometer (model Panlab-0331) about 1 cm into the rectum of the animal as previously described [8].

Briefly, mice of either sex were divided into groups, comprising six in each group. The normal body temperature of each mouse was measured rectally before and every hour for the first 6 h and 24 h after administration of either 10 ml/kg 0.95% (w/v) NaCl IP (control), paracetamol (20 mg/kg) or extracts at doses of 25, 50 and 100 mg/kg orally. Thereafter, temperature measurements were performed daily for 14 days post treatment.

Study on Baker's Yeast-induced Fever

The animals had their basal rectal temperature measured and were injected IP with 10 ml/kg of 30% (w/v) suspension of baker's yeast in 0.9% NaCl (w/v) as previous study showed that administration of 10 ml/kg of 30% w/v baker's yeast to mice causes significant increases in temperature at the 6th and 7th hour [8]. Animals selected for the experiment were given 25, 50 or 100 mg/kg body weight of the extracts. Paracetamol and NaCl were also administered as described above. Seven hours after yeast injection, the rectal temperatures were measured every half hour for 3 h after dosing as previously described [8] using a lubricated digital probe thermometer (model Panlab-0331). The vehicle (5% Tween 80 solution) for the ethyl acetate and hexane extracts had been previously tested on yeast-elevated temperature in mice and observed to produce no significant change in temperature.

Data Analysis

Data were expressed as means ± SEM and analyzed using one-way analysis of variance (ANOVA). Post hoc analysis was carried out using Dunnett's Multiple Comparison Test with temperature at 7 h as control column. At 95% confidence interval, a p-value <0.05 was considered significant.

Results

The extracts at all doses employed, did not significantly affect normal body temperatures. When the results of the standard drug and extract treatment groups were compared with the control (NaCl) group, ANL and ENL significantly lowered body temperatures following administration of each extract (Figures 1 and 2 respectively). However, HNL did not significantly reduce the yeast-induced fever in these animals (Figure 3). It was also observed that ANL appeared more potent than ENL as seen in the temperature changes depicted in the figures (Figures 1-3), especially at 0.5 and 1 h. Antipyretic effects of

ANL started as early as 0.5 h and progressed throughout the duration of the experiment while the antipyretic effect of ENL 100 mg/kg started as early as 0.5 h while the 25 mg/kg dose started at 1 h. Paracetamol, the standard drug, at 20 mg/kg significantly reduced the yeast-induced elevation of mice body temperature.

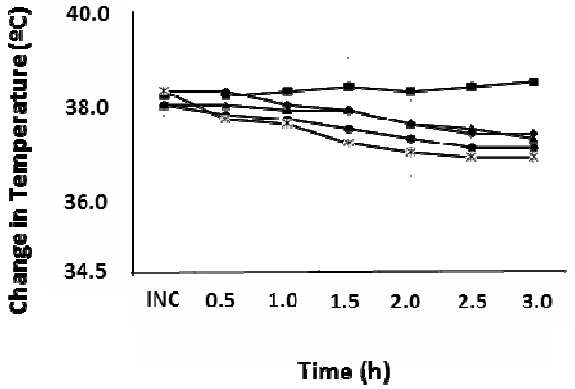

Figure 1: *Change in temperature with ANL in yeast-induced hyperthermia in mice. Inc: increase in temperature after yeast. ♦, ANL (25 mg/kg); ▲, ANL (50 mg/kg); •, ANL (100 mg/kg); ■, NaCl (10 ml/kg); X, PCM (20 mg/kg). n=6.*p<0.05; **p<0.01 n=6*

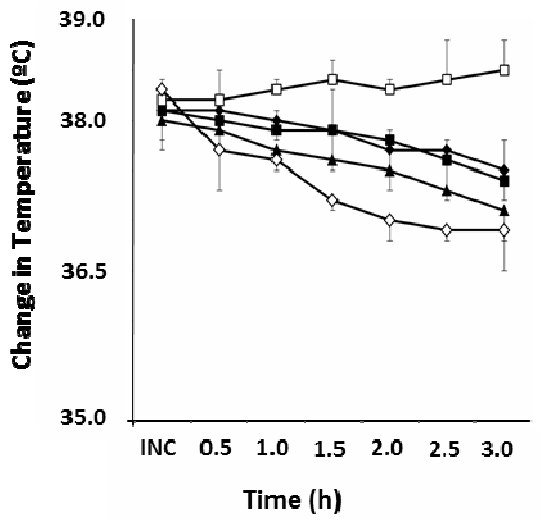

Figure 2: Change in temperature with ENL in yeast-induced hyperthermia in mice. Inc: percent increase in temperature after yeast. ♦, ENL (25 mg/kg); ■, ENL (50 mg/kg); ▲, ENL (100 mg/kg); □, NaCl (10 ml/kg); ◊, PCM (20 mg/kg).n=6. *p<0.05; **p<0.01; n=6

Discussion

The present results show that the aqueous and the ethylacetate extracts of *N. laevis* exhibit significant antipyretic activity against yeast-induced pyrexia in mice without significant alteration of normal body temperature an indication that the extract itself is not pyrexia-inducing.

Fever is a multifaceted response of the body to infections. Increase in the levels of prostaglandin E2 (PGE2) in specific regions of the brain leads to an elevation of the body's temperature. This temperature elevation alters the firing rate of neurons involved in the control of the hypothalamic thermoregulatory process [10]. The observation that the hexane extract failed to exhibit significant antipyretic action suggests that the active antipyretic phytochemical constituent(s) lies within the aqueous and ethylacetate extracts. The aqueous and ethylacetate extracts are therefore candidates for further research necessary for the isolation of the active phytochemical constituents. Hexane which is a non-polar solvent, extracts waxes, fats and fixed oils [11]. From the results it would seem that the antipyretic active constituents do not lie within waxes, fats or fixed/volatile oils. However, water which represents the aqueous phase is a highly polar solvent used in the extraction of polar phytochemical constituents such as sugars, amino acids and glycosides, while ethylacetate which is a solvent of medium polarity promotes the extraction of some semi-polar constituents such as alkaloids, aglycones and glycosides [11].

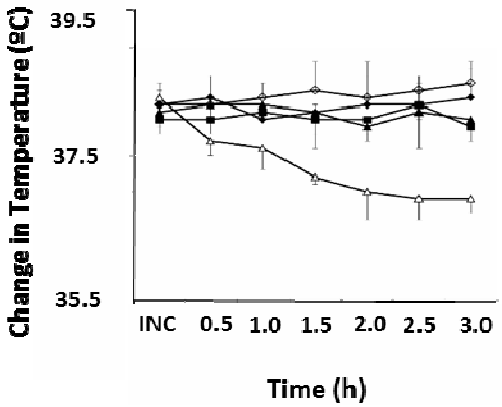

Figure 3: Change in temperature with HNL in yeast-induced hyperthermia in mice. Inc: percent increase in temperature after yeast. . ♦, HNL (25 mg/kg); ■, ANL (50 mg/kg); ▲, ANL (100 mg/kg); ◊, NaCl (10 ml/kg); Δ, PCM (20 mg/kg). n=6.

The reported presence of phytochemicals such as tannins, flavonoids, terpenes, steroidal and cardiac glycosides in the leaves may contribute to the antipyretic action observed [12]. Flavonoids have been reported to interfere with prostaglandins [13] and related flavonoid compounds have been reported to inhibit arachidonic acid peroxidation, which results in lower prostaglandin levels and thus reduction in fever [14] and the presence of flavonoids in the plant may also possibly contribute to the antipyrexia observed.

Prostaglandins play significant roles in the induction of fever. Yeast-induced pyrexia is primarily due to the production of prostaglandin E2 [15]. It is therefore likely that these extracts effectively reduces temperature via the inhibition of prostaglandins within the brain, particularly prostaglandin E2 possibly through inhibition of cyclooxygenase-3

(COX-3) or through stimulation of the system's own antipyretic substances as observed with vasopressin and arginine [16]. Antipyretics in use today such as the non-steroidal anti-inflammatory agents, (e.g. paracetamol used in this study) act through the inhibition of the enzyme cyclooxygenase which inhibits further synthesis of prostaglandins [17].

Conclusion

The result of this study has shown that the leaves of *N. laevis* have antipyretic activity. Though, the underlying mechanism is yet unknown, this finding supports the claims by traditional medicine healers in the use of the leaves in fever reduction.

Author's Contributions

BEE and OA conceived the study, sourced for the plant and performed all pharmacological experiments and data analysis, UOH and AO extracted the plant leaves while BEE and UOH prepared the manuscript which was approved by all authors.

Competing Interests

The authors declare no conflict of interest.

Acknowledgement

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