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Evaluation of Antipyretic Potential of the Ethanolic Leaf Extract of *Salacia lehmbachii* Loes

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Abstract: *Salacia lehmbachii* is an indigenous plant used in traditional medicine in Southern Nigeria for the treatment of fever and inflammatory diseases among other uses. The antipyretic effect of ethanolic leaf extract of *S. lehmbachii* leaf (100, 200 and 400 mg/kg) was evaluated in Wistar albino rats using brewer's yeast, amphetamine and 2, 4 dinitrophenol to induce fever, while the efficacy of the herbal drug was compared with 20 mg/kg paracetamol. Acute toxicity test (LD_{50}) of the herbal extract was conducted via oral route. The ethanolic leaf extract significantly ($p<0.05$) exhibited antipyretic activity in rats. The presence of diverse constituents including tannins, saponins, steroids, flavonoids and alkaloids could account for the potency of the ethanol leaf extract of this plant. The findings may be of clinical relevance and further substantiates the traditional use of the plant as antipyretic agent.

Keywords: Antipyretic, ethanol, herbal medicine, rats, *salacia lehmbachii*

INTRODUCTION

The use of medicinal plants plays an important role in the lives of rural people, particularly in remote parts of developing countries which are poorly served with health facilities. Before the availability of synthetic drugs man was completely dependent on natural medicinal plants for curing diseases (Singh *et al*., 2008). Different medicinal plant parts are used as raw drugs and they possess varied medicinal properties. The different parts used such as leaves,fruits, flowers, roots became more popular in recent years due to public awareness and increasing interest among consumers and scientific communities (Thiopeng *et al*., 2006). The beneficial effects of plant products typically results from the combinations of constituents such as flavonoids, alkaloids, steroids, tannins and saponins which are capable of producing physiological and pharmacological activities, including anti-oxidants, ant-inflammatory and antipyretic actions (Erdogul, 2002; Naresh *et al*., 2010). Natural products from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though little knowledge about their mode of action is available (Naresh *et al*., 2010). There is a growing interest in pharmacological evaluation of various plants used in Nigerian traditional systems of medicine. *S. lehmbachii* is a medicinal plant belonging to the family Celestraceae and is found in the tropical rain forest region of West, Central and East Africa. The leaves, fruits and roots are used medicinally. *S. lehmbachii* is one of such medicinal plants whose therapeutic application no doubt has a folkloric background. The lack of information on the scientific evaluation of this plant therefore necessitated the present study, which was designed to evaluate the antipyretic effect of the ethanolic leaf extract of the plant to ascertain the folkloric claim made by the indigenes.

MATERIALS AND METHODS

Plant collection: The fresh leaves of *S. lehmbachii* were collected in the month of November, 2009 from Ikot Arankere, Akwa Ibom State, Nigeria. The plant material was identified and authenticated by Mr. Frank. I Akpejoye, a taxonomist of the Department of Botany, University of Calabar, Nigeria, where a voucher specimen (No.688) was deposited at the herbarium for reference. The international plant name index is *Salacia lehmbachii* Loes. Bot. Jahrb. Syst. XLIV. (2-3) 173 (22 March, 1910). The leaves were cut into smaller pieces, dried at room temperature for 7 days and pulverized to dry powder using a mortar and pestle.

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Extraction of plant material: Three hundred and ninety-six (396) grams of the dry leaf powder was extracted in ethanol using soxhlet extractor (Friedrich Polzine, England) and dried in rotary evaporator (Techmel and Techmel, USA) at room temperature. The yield was 28 g (7% w/w). The dried extract was stored in a refrigerator at 4°C until used for the experiment. The dried extract was reconstituted in distilled water and administered to rats.

Phytochemical screening: The ethanolic leaf extract of *S. lehmbachii* was subjected to qualitative phytochemical analysis according to standard methods (Ajayi, 2008; Mukherjee, 2006).

Acute toxicity test: The LD_{50} of the leaf extract was tested to determine the safety of the agent according to the guidelines set by OECD (Organization for Economic Cooperation and Development) No. 423 (OECD, 2010). The study was carried out in two phases. In the first phase, nine mice were randomized into three groups of three mice per group and administered 10, 100 and 1000 mg/kg of the extract, orally. The animals were observed for the first 4 h and 24 h for signs of toxicity and mortality. The results of this phase informed the choice of doses for the second phase, in which 2000, 3000 and 5000 mg/kg were administered to another set of three mice per group. The mice were also observed for signs of toxicity such as paw licking, salivation, stretching of the entire body, weakness, respiratory distress, coma and death for 72 h.

Animals: Adult albino rats (160-200 g) of both sexes obtained from Animal house of the Department of Pharmacology, College of Medical Sciences, and university of Calabar, Nigeria. Animals were kept in cages and allowed free access to standard pellets and water. The experimental protocol was approved by the experimentation ethics committee on Animal use of the college of medical sciences, University of Calabar, Nigeria.

Antipyretic activity:

Yeast induced hyperthermia: Adult albinos rats (160-200 g) of both sexes fasted for 24 h but had water *ad libitum* were employed for the study. The rats were randomized into 5 groups of 6 in each cage. The basal temperature of each rat was taken and subcutaneously Injected with 20 ml.kg of 15% yeast suspended in methylcellulose to induce Pyrexia. Twenty four hours after yeast injection, the rectal temperature was again recorded and rats showing a minimum increase of less than 0.6°C were discarded (Akuodor *et al*., 2010a; Mukherjee *et al*., 2002). The extract understudy was administered at the dose of (100, 200 and 400 mg.kg) to rats in group 1, 2 and 3 respectively. The control group received distilled water, 20 mL.kg and the reference group received 20 ml.kg of paracetamol. All administered orally. The rectal temperature of each rat was again taken at 1 h interval for 5 h.

Amphetamine induced hyperthermia: Adult albino rats (160-200 g) of both sexes fasted for 24 h but had water *ad libitum* were employed for the study. The animals were randomized into 5 groups of 6 in each cage. The basal temperature of each rat was taken and 10 mg/kg of amphetamine was intraperitoneally injected into the rats. Hyperthermia developed 0.5 h following amphetamine administration. The extract was administered at the dose of (100, 200 and 400 mg.kg) to rats in group 1, 2 and 3, respectively. Paracetamol, 20 mg.kg and distilled water, 20 ml.kg were administered to control and reference groups. All administered orally. Rectal temperature of each rat was taken at 1 h interval for 5 (Mbagwu *et al*., 2007; Blackhouse *et al*., 1994).

2, 4-Dinitrophenol (DNP) induced pyrexia: Albino rats (160-200 g) of both sexes fasted for 24 h but had water *ad libitum* was employed for the study. The rats were randomized into 5 groups' of6 in each cage. The basal temperature of each rat was taken and 10 mg.kg of DNP was intraperitonealy injected to the rats. Hyperthermia developed 30 min after DNP administration. The extract (100, 200 and 400 mg.kg) to rats in group 1, 2 and 3, respectively. Paracetamol (20 mg/kg) and distilled water (20 mL/kg) were administered to control and reference groups. All administered by oral route. Rectal temperature of each rat was taken at 1 h interval for 5 h (Okokon and Nwafor, 2010).

Statistical analysis: Results obtained were expressed as mean$\pm$S.E.M. The data was analyzed using one-way ANOVA and differences between the means were considered significant at $p<0.05$.

RESULTS

Phytochemical analysis: Phytochemical screening of the extract revealed the presence of tannins, saponins, steroids, flavonoids, alkaloids and terpenes. These classes of compounds are reported to show important biological activities (Panda and Kar, 2007; Ghoghari and Rajan, 2006; Hollander-Hadacek, 2002).

Acute toxicity test: There was no mortality observed in mice after oral administration of the extract, even at doses as high as 5000 mg/kg signifying that the oral LD_{50} was $\geq$5000 mg/kg. Hence the experimental doses used (100, 200 and 400 mg/kg) were within safe margin.

Yeast-induced pyrexia: The result of the activity of the extract against yeast induced pyrexia is shown in Table 1. The extract exhibited significant ($p<0.05$) dose dependent antipyretic effect when compared to control. The leaf extract exhibited activity more than the standard drug, paracetamol.

Table 1: Effect of ethanolic leaf extract *S. lehmbachii* on yeast induced pyrexia in rat Rectal Temperature

Treatment	Dose (mg/kg)	0 h	24 h	1 h	2 h	3 h	4 h	5 h
Control	20 ml/kg	35.37±0.06	37.69±0.08	37.79±0.06	37.68±0.03	37.51±0.02	37.30±0.01	36.67±0.03
S.lehmbachii	100	35.50±0.13	37.46±0.13	36.73±0.09	36.32±0.08	35.99±0.09	35.64±0.07	35.35±0.05*
S.lehmbachii	200	35.40±0.05	37.52±0.06	36.72±0.06	36.25±0.06	35.88±0.08	35.60±0.07	35.24±0.02*
S.lehmbachii	400	35.57±0.08	37.61±0.07	36.78±0.03	36.30±0.03	35.81±0.02	35.53±0.03	35.15±0.02*
Paracetamol	20	35.34±0.06	37.36±0.05	36.80±0.08	36.36±0.04	36.12±0.04	35.50±0.05	35.35±0.04*

Data are expressed as mean ± SEM. *Significant at p<0.05 when compared to control. (n = 6)

Table 2: Effect of ethanolic leaf extract *S. lehmbachii* on amphetamine induced pyrexia in rat Rectal Temperature

Treatment	Dose (mg/kg)	0 h	0.5 h	1 h	2 h	3 h	4 h	5 h
Control	20 mL/kg	35.56±0.12	37.71±0.07	37.79±0.03	37.63±0.02	37.51±0.02	37.33±0.02	36.73±0.03
S.lehmbachii	100	35.37±0.04	37.54±0.06	36.75±0.04	36.43±0.02	35.79±0.02	35.50±0.04	35.27±0.04*
S.lehmbachii	200	35.34±0.07	37.55±0.06	36.56±0.03	36.22±0.02	35.75±0.02	35.41±0.02	35.16±0.02*
S.lehmbachii	400	35.33±0.04	37.55±0.04	36.70±0.07	36.30±0.03	35.63±0.05	35.28±0.05	35.11±0.02*
Paracetamol	20	35.34±0.02	37.30±0.03	36.63±0.06	36.43±0.05	36.19±0.02	35.45±0.02	35.23±0.03*

Data are expressed as mean ± SEM. *Significant at p<0.05 when compared to control. (n = 6)

Table 3: Effect of ethanolic leaf extract *S. lehmbachii* on DNP induced pyrexia in rat Rectal Temperature

Treatment	Dose (mg/kg)	0 h	0.5 h	1 h	2 h	3 h	4 h	5 h
Control	20 ml/kg	35.48±0.04	37.68±0.05	37.88±0.04	37.69±0.03	37.52±0.04	37.36±002	36.68±0.02
S.lehmbachii	100	35.46±0.09	37.57±0.06	36.43±0.05	36.19±0.02	35.63±0.06	35.36±0.06	35.21±0.04*
S.lehmbachii	200	35.45±0.12	37.62±0.04	36.36±0.05	36.17±0.03	35.57±0.05	35.31±0.04	35.13±0.03*
S.lehmbachii	400	35.34±0.03	37.48±0.03	36.59±0.02	36.29±0.02	35.56±0.02	35.32±0.02	35.10±0.00*
Paracetamol	20	35.36±0.02	37.32±0.03	36.57±0.04	36.35±0.03	36.17±0.03	35.42±0.04	35.24±0.04*

Data are expressed as mean ± SEM. *Significant at p<0.05 when compared to control. (n = 6)

Amphetamine-induced pyrexia: The antipyretic effect of the ethanol leaf extract on amphetamine induced pyrexia is shown on Table 2. The ethanol leaf extract caused significant (p<0.05) reduction in the temperatures of the treated rats when compared with the control. The antipyretic effect of the leaf extract was more than the standard drug, paracetamol.

2, 4-Dinitrophenol-induced pyrexia: Table 3 shows the effect of ethanol leaf extract of *S. lehmbachii* on dinitrophenol induced pyrexia. There was a progressive dose dependent reduction in the temperatures of rats treated with the extract. The ethanolic leaf extract significantly (p<0.05) caused reduction in hyperpyrexia when compared to control. The effect was more than the standard drug, paracetamol.

DISCUSSION

Fever could be induced by tissue damage, inflammation, infections, malignancy and other disease states. The infected or damaged tissue initiate the enhanced formation of pro-inflammatory mediators (cytokine like, interleukin 1β and TNF-α), which elevate the synthesis of prostaglandin E_2 near the hypothalamus, thereby causing the hypothalamus to elevate body temperature (Spacer and Brader, 1994; Akuodor *et al*., 2013). Most of the antipyretics inhibit cyclooxyginase-2 expression, hence inhibiting prostaglandin-biosynthesis (Evan *et al*., 2009) and consequently reduce the elevated temperature. For better characterization of the antipyretic activity of *S. lehmbachii*, three models of pyretics including brewer's yeast, amphetamine and dinitrophenol induced pyrexia were employed in this study. It is well known that pyretic activity involves stimulation of the region in the hypothalamus that controls body temperature; through prostaglandins synthesized within the central nervous system and that the blood-brain barrier prevents drug molecules or other chemicals from entering the central nervous system (Zakaria and Abdul, 2008).

Antipyretics such as paracetamol and other nonsteroidal anti-inflammatorydrugs (NSAIDs) reduce fever by depressing inflammatory messages at both peripheral sites of tissue inflammation and within central nervous system thermoregulatory sites. These agents suppress peripheral production of pyrogenic cytokines such as TNF-α and interleukins -1β, while lowering the thermoregulatory set point by inhibiting central cyclooxygenase production of Prostaglandin E_2 (PGE_2) (Sumanta *et al*., 2009; Rang *et al*., 1999; Afonoff and Neilson, 2001). Consequently, elevated plasma prostaglandin level, as observed in fever is suppressed. The ethanol leaf extract of *S. lehmbachii* at the three dose levels in the antipyretic evaluation showed significant (p<0.05) and dose dependent anal temperature decrease effective antipyretic activity evident in the reduction of temperature elevation in the three antipyretic models in rats. The observed antipyretic activity support the view that *S. lehmbachii* leaf extract has some inhibitory effect on prostaglandin biosynthesis because prostaglandin is believed to be involved in regulation of body temperature.

However, the active principle (s) responsible for the observed antipyretic activity of the leaf extract is yet to be identified. Further investigations are underway in our laboratory to isolate and characterize the specific active constituents of the leaf extract responsible for the observed action.

In conclusion, the results obtained in the present study clearly indicate that the ethanol leaf extract of *S. lehmbachii* possesses remarkable antipyretic activity.

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