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Evaluation of the Antipyretic and Analgesic Effects of the Hydroalcoholic Extract of *Leucas martinicensis*

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Abstract

Pain and fever are major public health issues in tropical areas. In sub-Saharan countries, particularly in Burkina Faso, *Leucas martinicensis* is commonly used as a medicinal plant because of its analgesic and antipyretic properties. This study aimed to provide scientific evidence supporting the use of the hydroalcoholic extract of *L. martinicensis* in the management of pain and fever. For each activity, five groups of rats were constituted. The control group, received distilled water; the reference group received diclofenac; and the other three groups received hydroalcoholic extract of *L. martinicensis* doses of 125, 250, and 500 mg/kg, respectively. To evaluate analgesic activity, 2.5% acetic acid was administered intraperitoneally to rats 30 minutes after treatment. The antipyretic activity was assessed in rats with pyrexia induced by injection of 20% brewer's yeast. the control group exhibited 45.6 ± 4.43 abdominal cramps after 20 minutes. In the presence of Diclofenac (50 mg/kg) and the extract, the number of cramps dropped to 18.6 ± 0,7; 29.5 ± 3.95; 25.8 ± 2.83; and 26.9 ± 5.38, respectively. These results showed evidence of a significant reduction in the number of abdominal cramps compared to those of the untreated group. The antipyretic test showed a significant decrease in the body temperature of rats during the first hours of treatment with the extract (250 and 500 mg/kg) and ASA (100 mg/kg) compared with the untreated group.

These results support the use of *L. martinicensis* in traditional medicine for the management of fever and pain.

Keywords

Antipyretic effect, analgesic effect, *Leucas martinicensis*, *medicinal plants*

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INTRODUCTION

Pain and fever, whether occurring separately or concomitantly, are among the main reasons for seeking medical care. In many countries, pain represents a major public health concern, with approximately 20–30 % of adults suffering from chronic pain (Rikard et al., 2023; Lucas and Sohi, 2024). In sub-Saharan Africa, a multi country surveillance study indicated that acute febrile illnesses are a major cause of morbidity and

mortality, with a substantial proportion of cases remaining undiagnosed (Belarbi et al., 2025).

Managing pain and fever requires the use of analgesic and antipyretic agents, respectively. These prescription drugs are often obtained through chemical synthesis and may be associated with adverse effects on human health and the environment. This concern has stimulated interest in natural products, including medicinal plant such as*Leucas martinicensis*, a plant traditionally used to manage of pain and fever.

Since the 2000s, the World Health Organization has promoted traditional medicine as a therapeutic alternative and has recommended that developing countries establish programmes focusing on the identification, cultivation and conservation of medicinal plants, as well as the use of innovative techniques to assess the quality and efficacy of traditional remedies. The present study was conducted within this framework and aimed to evaluate the antipyretic and analgesic effects of a hydroalcoholic extract of *L. martinicensis*.

MATERIALS AND METHODS

Plant material

Leaves of *L. martinicensis* were collected from the outskirts of Ouagadougou. Following botanical identification, voucher specimens were prepared and deposited at the Biodiversity Information Centre of Joseph KI-ZERBO University. The leaves were shade-dried, pulverised, and subsequently sieved. The resulting plant powder was macerated to obtain a hydroalcoholic extract.

Animal material

Male and female rats belonging to the Sprague–Dawley and Wistar strains were used for the analgesic and antipyretic assays, respectively, at the University of Lomé and the University Joseph KI-ZERBO. The animals were obtained from the respective university animal facilities. The rats were housed in rooms maintained at a stable temperature of 24 ± 2 °C and had free access to water and standard food. All experiments were performed following the guidelines of Animal Protection Ethics Committee

Methods

Evaluation of analgesic activity

Analgesic activity was evaluated using the acetic acid-induced writhing test, according to the method described by Koster et al. (1959) and subsequently used by Sagnan et al. (2017) and Etou Ossibi et al. (2023).

The test consisted of inducing nociceptive responses in rats by intraperitoneal administration of 2.5% acetic acid, 30 min after treatment with the extract or reference drugs. Intraperitoneal injection of 2.5% acetic acid induces a characteristic writhing response in rats, consisting of waves of abdominal muscle contractions and elongation followed by extension of the hind limbs.

The number of abdominal writhes induced by acetic acid was counted from 5 minutes after injection for a period of 30 min. The responses were recorded in rats treated with the hydroalcoholic extract, distilled water, or the reference analgesic drug.

Five groups of five rats each were constituted and treated as follows:

- Group 1: rats treated with distilled water 30 min before intraperitoneal injection of 2.5% acetic acid.
- Group 2: rats treated with diclofenac at a dose of 50 mg/kg body weight 30 min before intraperitoneal injection of acetic acid.
- Group 3: rats treated with the hydroalcoholic extract at a dose of 125 mg/kg body weight 30 min before intraperitoneal injection of acetic acid.
- Group 4: rats treated with the hydroalcoholic extract at a dose of 250 mg/kg body weight 30 min before intraperitoneal injection of acetic acid.
- Group 5: rats treated with the hydroalcoholic extract at a dose of 500 mg/kg body weight 30 min before intraperitoneal injection of acetic acid.

Evaluation of antipyretic activity

The antipyretic activity of the hydroalcoholic extract of *L. martinicensis* was evaluated in both male and female rats. The animals were weighed, and their baseline rectal temperatures were recorded.

Pyrexia was induced by subcutaneous injection of 20% w/v suspension of brewer's yeast (*Saccharomyces cerevisiae*) at a dose of 10 mL/kg into the dorsal region (Bhowmick et *al*., 2014 ; Ahmed et *al*., 2015 ; Safari et *al*., 2016). Following yeast administration, the animals were fasted for 18 hours.

Rectal temperature was then measured again in all rats. Pyrexia was confirmed when the rectal temperature increased by more than 0.5 °C. Animals showing a temperature increase of less than 0.5 °C were excluded from the experiment (Muhammad et al., 2012).

Rectal temperature was measured using a digital thermometer. The eligible animals were divided into five homogeneous groups of five rats each and received distilled water, the reference antipyretic acetylsalicylic acid (ASA), or the hydroalcoholic extract.

The treatments were administered orally as follows:

- Group I or control group: Rats received distilled water orally at a volume of 10 mL/kg body weight.
- Group II or reference group: Rats received acetylsalicylic acid, a therapeutically used antipyretic agent, orally at a dose of 100 mg/kg body weight.
- Group III: Rats received the hydroalcoholic extract of *L. martinicensis* orally at a dose of 125 mg/kg body weight.
- Group IV: Rats received the hydroalcoholic extract of *L. martinicensis* orally at a dose of 250 mg/kg body weight.
- Group V: Rats received the hydroalcoholic extract of *L. martinicensis* orally at a dose of 500 mg/kg body weight.

All treatments were administered immediately after pyrexia induction had been confirmed.

Statistical analysis

The results obtained for each parameter were analysed statistically using GraphPad Prism version 8.0 (GraphPad Software, Inc., California, USA). Data were expressed as the mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA), followed by Dunnett's multiple-comparison test, was used to compare the treated groups with the control groups. Differences were considered statistically significant when $p < 0.05$.

RESULTS

Analgesic Activity

Following pain induction via acetic acid injection, 45.6 ± 4.43 abdominal cramps were recorded after 20 minutes for Group 1 (the negative control group). With Diclofenac at 50 mg/kg, the number of cramps dropped to 18.6 ± 0.75 over the same time interval, while, the cramp counts for the groups treated with the extract were 29.5 ± 3.95, 25.8 ± 2.83, and 26.9 ± 5.38 for doses of 125, 250, and 500 mg/kg body weight, respectively. Highly significant, **p<0.01; ***p<0.001, reflecting the reduction in the number of abdominal cramps compared to those of the negative control group (Figure 1).

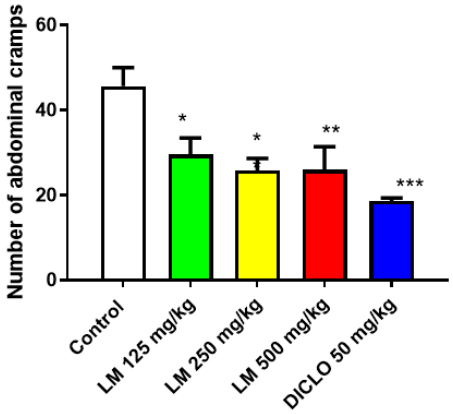


Figure 1: Effect of the hydroalcoholic extract of *Leucas martinicensis* (LM) leaves on the number of contortions induced by acetic acid (2.5%) injection (*p<0.05; **p<0.01; ***p<0.001).

Antipyretic activity

Induction of hyperthermia by administration of 20% brewer's yeast caused a increase of rats body temperature, 18 hours later (Figure 2). However, concerning animals treated with ASA and the extract, a decrease of the temperature was observed within the first hour. The doses of 125; 250; 500 mg/kg revealed a significant difference (p < 5%).

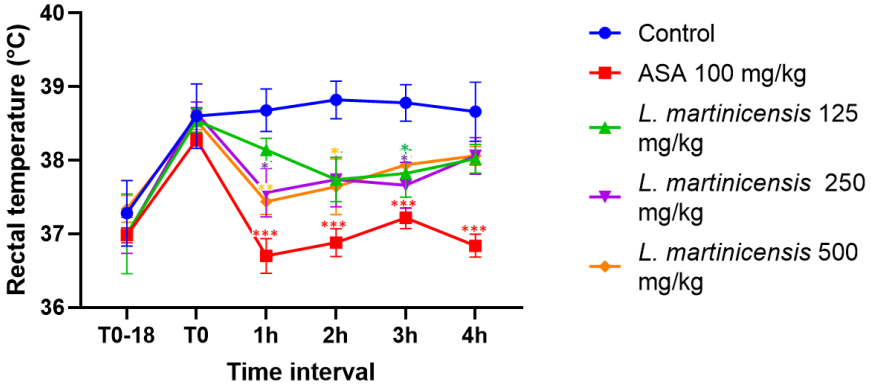


Figure 2. Hypothermic activity of hydroalcoholic extract of *L. martinicensis* (*p < 0.05; **p < 0.01; ***p < 0.001)

DISCUSSION

The results on analgesic activity showed that the hydroalcoholic extract of *L. martinicensis* leaves, administered at doses of 125, 250, and 500 mg/kg body weight, inhibited abdominal writhing of rats. This inhibition indicates a reduction in pain-related behavior, demonstrating that the extract possesses analgesic properties, and therefore relieves nociception. A previous studie investigated the analgesic activity of an alcoholic extract of *Leucas aspera*, a species belonging to the same genus as *L. martinicensis*, and also reported similar significant analgesic effects (Rahman et *al*., 2007). Acetic acid-induced pain results from the direct stimulation of acid-sensitive chemosensitive nociceptors and tissue damage at the visceral surface, leading to the release of algogenic mediators such as histamine, bradykinin, serotonin, and prostaglandins, as previously demonstrated by authors (Choi et *al*., 2006; Smith et *al*., 2010; Chen et *al*., 2011). Pretreatment with the hydroalcoholic extract of *L. martinicensis* leaves significantly reduced acetic acid-induced abdominal pain, as shown by the decrease in the number of rats' abdominal contractions in the present study. These findings suggest that the extract contains bioactive metabolites with analgesic activity. This analgesic effect may also interfere with the development of fever, which prompted us to investigate the extract's antipyretic properties.

Administration of the hydroalcoholic extract of *L. martinicensis* leaves significantly reduced rectal temperature in febrile rats. Similar findings have been reported for an extract of *Leucas lavandulaefolia*, a plant belonging to the same genus (Mukherjee et al., 2002). Fever is a response to pyrogenic stimulation and represents a natural defense mechanism of the organism. Endogenous cytokine pyrogens, including interleukin-1 (IL-1α and IL-1β), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), interleukin-8 (IL-8), tumor necrosis factor-beta (TNF-β), macrophage inflammatory proteins alpha and beta, interferon-alpha, interferon-beta, interferon-gamma, and prostaglandin E_2 (PGE_2), are involved in the development of pyrexia (Walter, 2003; Earn et *al*., 2014). Subcutaneous administration of yeast stimulates immune cells, particularly monocytes/macrophages and neutrophils. These cells subsequently produce and release cytokines that raise body temperature through activation of the thermoregulatory center in the hypothalamus (Dinarello, 1999).

CONCLUSIONS

This study demonstrated that the hydroalcoholic extract of *L. martinicensis* leaves exhibits both antipyretic and analgesic activities. These findings support the traditional use of *L. martinicensis* in African medicine for the management of fever and pain. From these results, further investigations into the mechanisms underlying the antipyretic and analgesic effects of the hydroalcoholic leaf extract of *L. martinicensis* to warrant the inocuity of traditional plants use.

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