Research Article

Evaluation of antipyretic activity of *Leucas Ciliata* leaves extract in rats

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Abstract

Background: Fever, also known as pyrexia, is a common medical sign characterized by high body temperature. Causes include infections, immune reactions, and tissue destruction. Antipyretic drugs inhibit COX-2 expression to reduce temperature but are toxic to hepatic cells and heart muscles. Natural COX-2 inhibitors have lower selectivity and less toxic. The demand for herbal medicines is increasing due to their fewer side effects. Hairy Tufted *Leucas*, a shrub in India, has high levels of flavonoids with antioxidant and hepatoprotective properties. **Objective:** This investigation aims to determine the antipyretic potential of *Leucas ciliata* leaves. **Material and methods**: The study involved drying and powdering dried leaves, grinding them into a coarse powder, and macerating them with ethanol for 7 days. The ethanol extract was then evaporated in a water bath. The extract was tested on albino rats, which were subjected to Brewer's yeast-induced pyrexia and Lipo-polysaccharides induced pyrexia treated with the herbal extract orally. The study involved rats administered herbal extracts orally at doses of 200 mg/kg and 400 mg/kg, distilled water, paracetamol and indomethacin as a drug control. The rats' rectal temperature was recorded before and after the dosing. Antipyretic activity was assessed by comparing the initial rectal temperature before and after the administration. Phytochemical screening included detecting alkaloids, carbohydrates, saponin, tannins, and flavonoids. **Results and conclusion**: The herbal ointment was found to be physically stable at different temperatures for four weeks. The study was confirmed the antipyretic activity of the extracts. The study examines the effect of herbal extract on yeast-induced pyrexia in rat rectal temperature, comparing it to control and paracetamol. Phytochemical such as alkaloids, carbohydrates, saponin, tannins, flavonoids are present in *Lucas ciliata*. The Antipyretic activity observed can be attributed to the presence of flavonoids.

Keywords- Pyrexia, *Leucas ciliata*, Lipo-polysaccharides, Brewer's yeast, paracetamol, antipyretic

Introduction

Fever (also known as pyrexia) is when a human's body temperature goes above the normal range of 36.5 -37.5^{0}C (98-100^{0}F); it is a common medical sign. Fever is usually accompanied by different general symptoms, such as sweating, chills, sensation of cold and other subjective sensations. Missing of these symptoms during high temperature may be a sign of a serious illness (Mackowiak, 1998). Causes of fever include infections caused by parasites, viruses, bacteria, rickettsia, Chlamydia, immune reactions including the defects in collagen, immunological abnormalities and acquired immune deficiency. Other causes of fever are destruction of tissues, such as trauma, local necrosis (infarction), and inflammatory reaction in tissues and vessels (phlebitis, arthritis), pulmonary infarction, and rhabdomyolysis (Kluger, 1992). Most of the antipyretic drugs inhibits COX -2 expression to reduce the elevated body temperature by inhibiting prostaglandin E2 (PGE2) biosynthesis (Botting, 2004). Moreover, these synthetic agents irreversibly inhibit COX-2 with high selectivity but are toxic to the hepatic cells, glomeruli, cortex of brain and heart muscles, whereas the natural COX-2 inhibitors have lower selectivity with some side effects. A natural antipyretic agent with reduced or no toxicity is therefore essential. The demand for herbal medicines is increasing rapidly due to their fewer side effects (Gupta et al., 2013).

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Leucas ciliata (Lamiaceae) commonly known as Burumbi is shrub having ovate/lanceolate leaves with serrate margins. It is frequently distributed along the ghats and in places along forest edges in Ahmednagar, Dhule, Kolhapur, Mumbai, Satara, and Thane regions of Maharashtra in India. Traditionally *L. ciliata* is used for wound healing and as antidote for snakebite. In Chinese medicine it is used for its antibacterial and antifungal activity. Preliminary phytochemical analysis of *L. ciliata* leaves indicated presence of relatively high levels of flavonoids. Several flavonoids have been reported to possess antipyretic and hepatoprotective properties. Hence the present investigation was undertaken to determine the antipyretic potential of *L. ciliata* leaves (Qureshi et al., 2010).

Leucas ciliata is commonly known as Hairy tufted *Leucas*, botanical name is *Leucas ciliata* var. oblongifolia Family-Lamiaceae (Mint Family). Hairy Tufted Leucas is an annual or perennial herb, 30-75 cm high. Stem is greenish woolly, lower portion of stem and branches prostrate, rooting frequently. Branches are pale green, bluntly quadrangular, grooved, densely hairy with white shining hairs, hairs 2.5-3 mm long, spreading. Leaves are opposite, leaf-stalk 8-15 mm long, elliptic to broadly ovate, rounded to wedge-shaped at base, broadly blunt to rounded at tip, margin rounded toothed with 9-14 teeth, upper surface pale green, densely hairy with antrorse hairs. Flowers are borne in 10-15-flowered whorled clusters at branch ends, each subtended by a pair of bracteate leaves with a pair of tender leaves projecting above the whorls, 1.5-2.5 cm across. Flowering calyx is 7-8 mm long, (fruiting calyx 10–11 mm long), straight, bell-shaped, broad above, narrow towards base, yellowish brown with dense spreading hairs outside.

Materials and methods

Collection and authentication of plant material

The leaves of *Leucas Ciliata* leaves belonging to family-Lamiaceae was collected during October 2023 from Radhanagari, Kolhapur district, Maharashtra state, India. The samples were authenticated by Botany Department of Bhogawati Mahavidyalay.

Preparation of plant extract

Leaves were dried in a shade and then powdered to get a course powder. This powder was stored in air tight container and used for extraction. The dried leaves were ground into coarse powder with the motor grinder. Thereafter, 500 g of the leaf powder was macerated with 4 L of ethanol for 7 days and agitated intermittently. Evaporation of the resultant solution in water bath afforded 32 g (6.4 % yield) of the ethanol extract.

Phytochemical Screening

1. Detection of alkaloids

Extract was dissolved individually in dilute Hydrochloric acid and filterd.

a) Mayer's Test

Filtrate was treated with Mayer's reagent (Potassium Mercuric lodide) Formation of yellow coloured precipitate indicates the presence of alkaloids.

b) Wagner's Test

Filtrate was treated with Wagner's reagent (Solution of iodine in potassium iodide) Formation of yellow or brown ppt. indicates the presence of alkaloids.

c) Hager's Test

Filtrate was treated with Hager's reagent (Picric acid) Formation of yellow coloured precipitate indicates the presence of alkaloids.

d) Dragendroff's Test

Filtrate was treated with Dragendroff's reagent (Solution of Potassium Bismuth lodide Formation of red precipitate indicates the presence of alkaloids.

2. Detection of Carbohydrates

Extract was dissolved individually in 5 ml distilled water and filtered. The filtrate was used to test for the presence of carbohydrates.

a) Molish Test

2-3ml of test solution, add few drops of alcoholic alpha-naphthol solution, shake and add conc. H2SO4 from sides of the test tube. Violet ring formation at the junction of two liquids indicates the presence of carbohydrates.

b) Fehling's test

In order to perform this test Fehling's A and B solution were mixed in equal proportion and test solution was added in it

followed by heating. Transformation of solution color to yellow and finally brick red precipitate indicates the presence of reducing sugars in the solution.

c) Benedict's Test

The solution was mixed with few drops of benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath,observed for the formation of redish brown precipitate to show a positive result for the presence of carbohydrate

3. Test for Saponin

a) Foam test

If the solution of extract with water shows foaming upon vigorous shaking and it is persistent in nature then saponin is present. Extract was diluted with distilled water to 20 ml and this was shaken in graduated

4. Test for Tannins

a) Gelatine test

To the extract, 1% gelatine solution containing sodium chloride was added Formation of white precipitate indicates the presence of Tannins.

b) Lead Acetate

A few drops of extract were treated with basic lead acetate. The of white precipitate indicates the presence of tannins

5. Test for Flavonoids

i) 5ml of 95% ethanol was added with few drops of conc. HCI, 0.5g of magnesium and testsolution turning of solution to pink colour gives presence of Flavonoids.

ii) 1 ml of filtrate with 2ml of dilute NaOH show development of golden yellow colour.

iii) 1ml of the filtrate add of FeCl3 reagent (mixture of 1 vol of 5% $FeCl_3$) and a few drops of conc. H2SO4.greenish blue colour appears within few minutes.[49,50]

Physical stability test of the herbal ointment was carried out for four weeks at various temperature conditions like 2°C, 25°C and 37°C. The herbal ointment was found to be physically stable at different temperature i.e. 2°C, 25°C, 37°C within four weeks.

Animals used

Albino rats (wistar strain) of either sex weighing about 180-200g were used in this study. The animals were kept in the standard metabolic cages in groups of four per cage, with free access to standard diet and water ad libitum. They are maintained at room temperature under suitable nutritional and environmental conditions throughout the experiment.

Experimental Design

Group I (Normal Group): Rats were subjected to the group received distilled water

Group II (Control Group): Rats were subjected after yeast administration subcutaneous injection of 10ml/kg of 15% w/v Brewer's Yeast suspended in 0.5% w/v methyl cellulose solution and did not undergo any treatment.

Group III (Treatment Group): Rats were subjected to yeast induced pyrexia and treated with 200 mg/kg of herbal extract orally administrate.

Group IV (Standard Group): Rats were subjected to yeast induced pyrexia and treated with paracetamol (45mg/kg) orally administrate.

Induction of Brewer's Yeast – induced pyrexia

Yeast induced pyrexia was used to evaluate the antipyretic activity of the extract. The rats were divided into four groups of six animals and the body temperature of each rat was recorded by measuring rectal temperature at predetermined time intervals.

Fever was induced by injecting 15% suspension of Brewer's yeast (Saccharomyces cerevisiae). In brief, the rats were allowed to remain quiet in the cage for sometimes.

A body temperature monitoring thermometer was inserted 3-4cm deep into the rectum, after fastened the tail, to record the basal rectal temperature.

The animals were then given a subcutaneous injection of 10ml/kg of 15% w/v Brewer's yeast suspended in 0.5% w/v methyl cellulose solution and the animals were returned to their housing cages.

Lipo-polysaccharides induced Pyrexia

Lipopolysaccharides from Gram-negative bacteria (E. coli), induce fever in rat after intravenous injection. Only lipopolysaccharide fractions are suitable, which cause after 60 min an increase of body temperature of 1 °C or more. Present study was conducted for 8 hrs.

Table 1: Preliminary phytochemical constituents present in ethanolic extract of *Leucas ciliata* leaves extract

Sr. No.	Chemical Test	Ethanolic extract of *Leucas ciliata*
1	Alkaloids	
	Mayer test	Positive
	Wagner's test	Negative
	Hager's test	Positive
	Dragondraff's test	Negative
2	Carbohydrates and glycosides test	
	Molisch's test	Positive
	Fehling's test	Positive
	Benedict's Test	Negative
3	Saponins	
	Foam test	Negative
4	Tannins	
	Gelatine test	Positive
	Lead Acetate	Positive
5	Flavonoids detection	Positive

Table 2: Effect of herbal extract on yeast induced Pyrexia in rat rectal Tempearature (^{0}F)

Sr. No	Treatment	Rectal temperature (^{0}F)(Mean SEM)After yeast administration						
		Basal	0h	19.5 H	20H	21H	23H	25H
1.	Normal	96.4±0.215	96.7±0.286	96.2±0.136	95.7±0.156	94.9±0.235	97.1±0.156	96.1±0.277
2.	Control	96.1±0.126	109.6±0.245	109.2±0.149	108.8±0.259	107.1±0.189	106.8±0.188	103.2±0.189
3.	F1=200mg/kg	96.8±0.154	108.6±0.285	106.3±0.258	104.9±0.158	104.1±0.193	102.2±0.136	99.3±0.256
4.	Paracetamol 45mg/kg	96.4±0.148	109.2±0.123	105.1±0.287	103.8±0.363	100.3±0.360	98.7±0.136	97.2±0.156

Drug Administration

Nineteen hour after yeast injection, the rats were again restrained in individual cages to record their rectal temperature. Immediately the extracts F1 were administered orally at doses of 200 mg/kg to the treatment groups of animals, the next group received distilled water and fourth group received 45 mg/kg of paracetamol as drug control.

Rectal temperature of all the rats was recorded at 19h immediately before, extract, vehicle or paracetamol administration and again at 1h interval up to 23hrs after yeast injection.

Evaluation of parameter

By comparing the initial rectal temperature (^{0}C) prior to the yeast injection with the rectal temperature (^{0}C) following the 18-hour yeast injection at various time intervals, antipyretic activity was assessed.

Results and discussion

Alkaloids was found by mayer test and hager's test. Carbohydrates and glycosides was present by performing molish's and fehlings test. Detection test of Tannins and flavonoids also shows positive test.

Yeast administration induced a pronounced febrile response in the Control group, as evidenced by a sharp rise in rectal temperature from a basal value of 96.1 ± 0.126°F to a peak of 109.6 ± 0.245°F at 0 hours. This elevated temperature persisted throughout the observation period, declining only marginally to 103.2 ± 0.189°F by 25 hours, confirming sustained fever in untreated subjects.

In contrast, both treatment groups (F1=200 mg/kg and Paracetamol=45 mg/kg) demonstrated significant antipyretic activity. The Paracetamol group exhibited rapid and robust fever reduction: after peaking at 109.2 ± 0.123°F (0h), temperatures declined steadily, reaching 100.3 ± 0.360°F by 21 hours and returning to near-baseline levels (97.2 ± 0.156°F) by 25 hours. This marked a 12.0°F

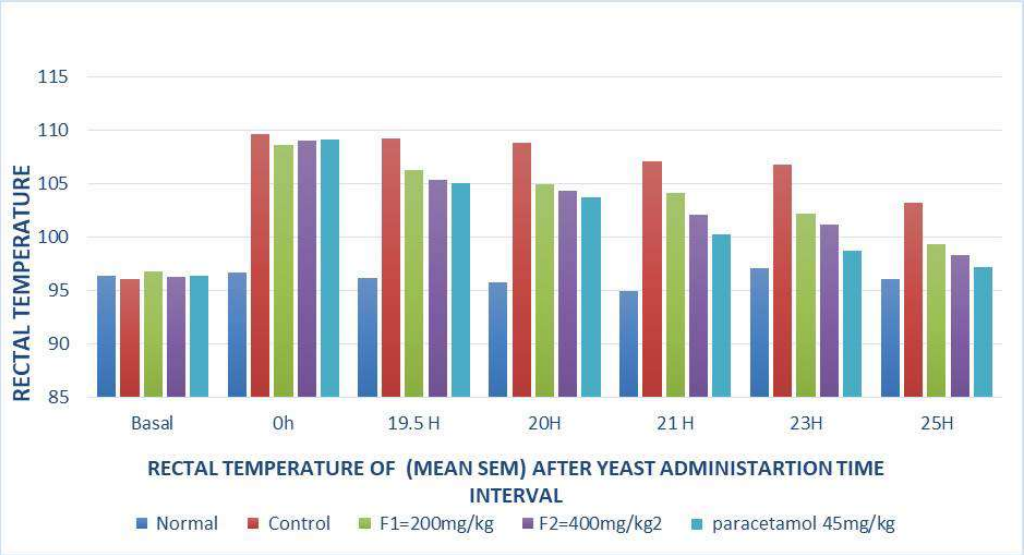

Figure 1: Antipyretic effect of herbal extract by yeast induced pyrexia

Table 3: Effect of herbal extract on lipo-polysaccharides induced Pyrexia in rat rectal temperature (^{0}F)

Sr. No	Treatment	Rectal temperature (^{0}F) (Mean Sem) After lipo-polysaccharides administration								
		Basal	1 hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr
1.	Normal	96.4±0.215	96.9±0.415	96.7±0.565	96.4±0.296	95.6±0.613	96.4±0.295	97.4±0.965	96.3±0.361	96.6±0.215
2.	Control	96.2±0.794	97.4±0.285	99±0.315	101.3±0.398	106.4±0.828	103.9±0.525	104.8±0.836	104.8±0.521	105.8±0.456
3.	F1=200mg/kg	96.2±0.293	97.8±0.356	99.2±0.244	101.8±0.411	104.9±0.932	103.5±0.566	101.9±0.415	98.5±0.812	96.9±0.123
4.	F2=400mg/kg	96.3±0.438	97.9±0.879	99.5±0.525	101.6±0.456	104.3±0.746	103.8±0.685	101.2±0.433	98.7±0.699	95.9±0.827
5.	Indomethacin 2.5mg/kg	96.1±0.416	98.1±0.213	99.6±0.287	101.7±0.363	103.5±0.360	102.6±0.136	100.5±0.156	98.4±0.538	95.3±0.841

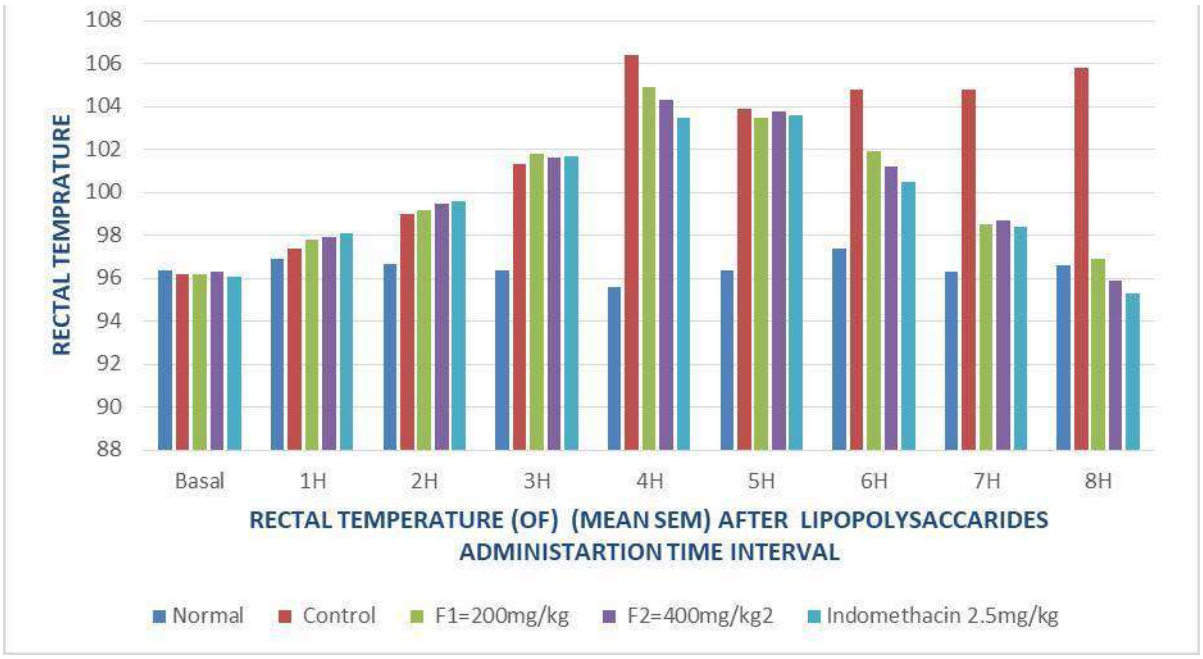

Figure 2: Antipyretic effect of herbal extract by lipo polysaccharide induced pyrexia

reduction over 25 hours, highlighting its potent and time-dependent efficacy.

The F1=200 mg/kg group showed a slower but statistically significant antipyretic effect. Starting at 108.6 ± 0.285°F

(0h), temperatures decreased gradually to 99.3 ± 0.256°F by 25 hours, reflecting a 9.3°F reduction. While effective, its action was delayed compared to Paracetamol, with notable reductions first observed at 20 hours (104.9 ± 0.158°F vs. Control's 108.8 ± 0.259°F).

The Effect of ethanolic extract of *Lucas Ciliata* Plant on brewer's yeast induced pyrexia has been shown in Fig No.1. Treatment with extract at dose of 200 mg per kg body weight and paracetamol at dose of 45 mg per kg were decreased body temperature of yeast induced rat. the result obtained from extract and standard treated groups were compared with the control group. A decrease in temperature was observed.

The study compares rectal temperature changes (mean ± SEM) in different treatment groups after lipopolysaccharide (LPS) administration. Normal (untreated) subjects showed stable temperatures (96.4–97.4°F). Control (LPS-only) exhibited a sharp fever response, peaking at 106.4°F at 4h, remaining elevated (105.8°F at 8h).F1 (200mg/kg) and F2 (400mg/kg) treatments reduced fever severity, with F2 showing better efficacy: temperatures peaked at 104.9°F (F1) and 104.3°F (F2) at 4h, then declined to near-normal levels by 8h (96.9°F for F1, 95.9°F for F2). Indomethacin (2.5mg/kg), a reference drug, showed the strongest antipyretic effect, with temperatures peaking at 103.5°F (4h) and returning to 95.3°F by 8h. Higher doses (F2) and Indomethacin demonstrated faster recovery, suggesting dose-dependent efficacy.

Discussion

400 mg per kg dose of a Lucas Ciliata was a good antipyretic agent because it was able to decrease body temperature in albino rat. phytochemical such as alkaloids, Carbohydrates, saponin, tannins, Flavonoids are present in Lucas Ciliata Plant. The Antipyretic activity observed can be attributed to the Present flavonoids.

Conclusion

The study showed that extract of *Lucas Ciliata* leaves possessed antipyretic effect in yeast induced and lipo-polysaccharides elevation of body temperature in experimental rat.

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