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Imoh S Johnson
Department of Pharmacology & Toxicology, Faculty of Pharmacy, University of Uyo, Nigeria

Ette O Ettebong
Department of Clinical Pharmacology & Therapeutics, Faculty of Clinical Sciences, University of Uyo, Nigeria

Jude E Okokon
Department of Pharmacology & Toxicology, Faculty of Pharmacy, University of Uyo, Nigeria

Ekemini S Johnson
Department of Anatomy, College of Health Sciences, University of Uyo, Nigeria

Correspondence
Ette O Ettebong
Department of Clinical Pharmacology & Therapeutics, Faculty of Clinical Sciences, University of Uyo, Nigeria

Antipyretic potentials of ethanolic extract of *Hilleria latifolia* leaves in albino wistar rats

Imoh S Johnson, Ette O Ettebong, Jude E Okokon and Ekemini S Johnson

Abstract
Hilleria latifolia is a medicinal plant widely used to treat feverish conditions, including malaria, in the southern parts of Nigeria especially among the Ibibios. This work was aimed at assessing the antipyretic activities of this plant in adult albino wistar rats. Pyrexia was induced using Brewer's yeast, D-Amphetamine and 2, 4-Dinitrophenol. Animals with increased temperature of 1^0C were selected and randomized into 6 groups of six animals per group. Various doses of extract (250 – 750mg/kg) were thereafter administered intraperitoneally. The standard drug used was Acetyl salicylic acid (100mg/kg p.o) while distilled water (10 ml/kg p.o) served as negative control. Rectal temperatures were obtained at 0.5hr and thereafter hourly for 5 hrs. Data obtained were analyzed using One-way ANOVA and Tukey-Kramer multiple comparison post-test using Graph Pad (Version 3.10). The result showed that the extract dose- and time-dependently decreased rectal temperatures in the animals. This decrease was statistically significant ($p< 0.05 – 0.001$) compared to control. The result obtained with the highest dose (750 mg/kg) of the extract was comparable with that of the standard drug Acetyl salicylic acid. The antipyretic activity of the extract of *Hilleria latifolia* could be due to one or more active secondary metabolites present in it. These results depict that the extract of *Hilleria latifolia* possesses antipyretic properties which corroborates with the traditional use of the plant as a remedy in the treatment of febrile conditions.

Keywords: Antipyretic, extract, *Hilleria latifolia*, yeast, dose

1. Introduction
The temperature of the body is regulated almost entirely by nervous feedback mechanism and almost all these operate through temperature – regulating centers located in the hypothalamus. The temperature of the deep tissues of the body - the "core" of the body remains very constant within $\pm1^0$F ($\pm0.6^0$C) except when a person develops febrile illness [1]. Indeed, a nude person can be exposed to temperature as low as 55^0F or as high as 130^0F in dry air and still maintain an almost constant core temperature. Heat production is a principal by-product of metabolism. Also the signals generated by the temperature receptors of the hypothalamus are extremely powerful in controlling body temperature receptors in the other parts of the body play additional roles in temperature regulation. This is especially true of temperature receptors in the skin and in a few specific deep tissues of the body. Fever is caused by pyrogens – pyrogens can be either internal (endogenous) or external (exogenous) to the body. Exogenous pyrogens are proteins, brokendown products of proteins and certain other substances especially lipopolysaccharides toxins released from bacterial cell membranes. All endogenous pyrogens are cytokines – molecules that are a part of the immune system, produced by activated immune cells [2].

Hilleria latifolia is a perennial plant with more or less woody stems that can persist. It can grow up to two meters tall. It bears simple, alternate leaves up to 15 cm long, 6cm which have alternate margins. It is estipulate and petiolate. The leaves are ovately shaped or elliptical to broadly lanceolate. The leaf base is rounded to cuneate. It has a long acuminate apex. It bears bi-sexual flowers which are zygomatic. It possesses a pedicle usually 1-2mm long. The sepals are usually green to white in slender racemes up to 13cm long. The fruits are reticulate, glabrons, ellipsoid- globose about 2mm in diameter [3, 4]. The plant *Hilleria latifolia* occurs in tropical Africa, Guinea to Ethopia, Africa, Mozambique and North Africa. It also occurs in Madagascar and Srilanka. It can also be found in along the West African coast from Sierra Leone to Cameroun. It is native to South-America, naturalized in tropical Africa and Sri-lanka [5]. In Cote d'Ivoire, a decoction of the leaf is used to treat food poisoning, treating urethral discharge also. It may be taken alone or boiled in palm nut soup for the same purpose. In Nigeria, the leaf is boiled in soup to treat gonorrhea. In Congo, the leaves are used to treat gynaecological disorders in which purging is necessary and in combination with the stem sap of costus after the leaf decoction is taken orally to treat coughing of blood and a part of the flowers in orange juice is used to treat asthma [3, 4]. The sap is also used as ear drops to treat

ear infections. In Cote d'Ivoire, a decoction of the whole plant is taken orally for the treatment of urethral discharges. It may be taken or boiled in palm nut soup for the same purpose and guinea worms. A decoction of the leaf and twigs is used in Ghana for the treatment of jaundice. It's used as a steam bath for the same purpose. [6].

Also in Ghana, a poultice of fresh leaves or roots is applied to boils. A leaf decoction, in small doses is given for the treatment of leprosy. The leaves added to those of Piperguinea are applied to the body as a remedy for general oedema [5]. The leaves or the whole plant is utilized in the treatment of breast cancer [7]. A study by Abotsi *et al.* [8] showed that the ethanolic extract of the aerial part has anti- nociceptive, anti-inflammatory, anti-oxidant, anxiolytic and anti- depressant activities. A study conducted by Assob *et al*., [9] using the agar well dilusion and broth dilution methods observed that the ethyl acetate and methanol fractions were active against *Pseudomonas aeroginosa and Salmonella typhi* with MIC's of 0.62 $mgml^{-1}$ for both.A simple study by Antonia *et al.,* [10] investigating the pharmacognostic properties as well as the anti-microbial and antiplasmodial properties of *HLE* showed the presence of abundant calcium oxalate crystals in all plant parts. Tetragonal crystals were found beneath the epidermal cells and were not embedded in the palaside cells. This study was aimed at evaluating the anti-pyretic activities of the extract of *Hilleria latifolia*, which may provide necessary information in order to rationalize its use in therapeutics.

2. Materials and Methods

2.1 Collection of Plant Material

The plant material (*Hilleria latifolia)* was collected from a private garden in Ikono L.G.A in Akwa Ibom State, Nigeria. The plant was identified and authenticated by Professor Margaret Bassey, a certified plant Taxonomist in the Department of Botany and Ecological Studies, Faculty of Science, University of Uyo, Akwa Ibom State, Nigeria. A voucher specimen was deposited at the herbarium of the faculty and a herbarium specimen number UUH 3511 obtained.

Adult albino rats and mice were obtained from the Animal house of the University of Uyo, Akwa-Ibom State and maintained at the University of Uyo Animal House and fed with growers pellet feed with water given *ad libitum.*

2.2 Animal Stock

Adult albino rats were obtained from the Animal house of the University of Uyo, Akwa-Ibom State, Nigeria and maintained at the University of Uyo Animal House and fed with growers pellet feed with water given *ad libitum.*

2.3 Preparation of Extract

Fresh leaves of the plant were air-dried until it was very dry and pulverized into fine powder using a mixer grinder. 300g of the powder-dried leaf was obtained and subjected to cold percolation with 70% (v/v) ethanol at room temperature for 72 hours, then filtered. The filtrate was then concentrated under reduced pressure at 40^0C using a Rotary evaporator. The dried extract was stored at -4^0C in the refrigerator until needed.

2.4 Phytochemical screening

The extract obtained were subjected to qualitative phytochemical screening to identify presence or absence of secondary metabolites include such as flavonoids, phenolics, tannins, saponins, alkaloids, cardiac glycosides, sterols and terpenoids using methods of analysis as described by Harbone [11] and Kotake [12].

2.5 Evaluation of Antipyretic Activities of Extract

2.5.1 Effect of Extract on Yeast-induced Pyrexia in Rats

Adult albino rats of both sexes were used for this experiment. Their rectal temperatures were taken using digital clinical thermometer. The rats were then injected subcutaneously with 10 ml/kg of Brewer's yeast suspension in the back below the neck. Immediately after the yeast administration, feeds were withdrawn. 18 hrs post administration, rectal temperature were again recorded. Only animals with a body temperature increasing by 1^0C were randomized and divided into 5 groups of 6 animals per group. Group 1 animals received 10ml/kg of distilled water orally, and served as the control group. Group 2 – 4 animals were administered with between 250 – 750mg/kg of extract intraperitoneally respectively. Group 5 animals received acetylsalicylic acid (100mg/kg; p.o). Rectal temperatures were again recorded at 0.5 hr and then hourly for 5 hrs. the differences between the actual values and the starting values were recorded for each time interval [13].

2.5.2 D – Amphetamine-induced Pyrexia in Rats

Adult albino rats of both sexes were fasted for 24 hrs. but allowed access to water *ad libitum.* Basal rectal temperatures of these animals were obtained and 5mg/kg of amphetamine administered. Within 30 mins following the administration of amphetamine, the animals with increased temperature of 1^0C were selected and randomized into 6 groups of six animals per group. Group 1 received 10ml/kg of distilled water orally. Group 2 – 4 animals were administered with 250 – 750mg/kg of the extract intraperitoneally respectively. Group 5 animals were administered 100mg/kg of acetylsalicylic acid orally. Rectal temperatures were obtained at 0.5hr and thereafter hourly for 5 hrs [14, 15].

2.5.3 Effect of Extract on 2,4- Dinitrophenol (DNP)-induced Pyrexia in Rats

Adult albino rats of both sexes were fasted for 24hrs but allowed access to water *ad libitum.* Thereafter, their basal rectal temperatures were taken. DNP (10ml/kg) was thereafter administered to all the animals intraperitoneally. Pyrexia is expected to develop within 30mins following the administration of DNP. Animals with increase in temperature by 1^0C were selected and randomized into different groups of six rats per group. Group 1 served as control and received 10ml/kg of distilled water orally. Group 2-4 were administered with 250 – 750 mg/kg of the extract intraperitoneally respectively. Group 5 received 100mg/kg of acetyl salicylic acid orally. Rectal temperatures of all the groups were then recorded 0.5 hr, then at hourly interval for 5 hours [13, 15].

2.6 Statistical Analysis

Results were expressed as multiple comparison of Mean ± SEM. Significance was determined using One-way Analysis of Variance (ANOVA) followed by Turkey-Kramer multiple comparison post-test. Statistical software used was GraphPad Instat (Version 3.10). A probability level of less than 5% was considered significant.

3. Results and discussion

The plant *Hilleria latifolia* is used for the treatment of feverish conditions (of which malaria is one the most common causes) amongst the Ibibios and various other tribes in Nigeria and West Africa. *In vitro* studies have given

credence to folklore [10]. The phytochemical screening showed the presence of alkaloids, saponins, tannins, anthraquinones, cardiac glycosides, flavonoids and carbohydrates as shown in Table 1. The antipyretic activity of the extract of *Hilleria latifolia* could be due to one or more active secondary metabolites present in it. A number of studies have shown that these phytochemicals exhibit inhibitory actions on cyclooxygenase enzyme and as a result produce antipyretic activity by preventing the formation of prostaglandins or by increasing the concentrations of the body's own antipyretic components [13, 16]. Flavonoids like baicalin have been shown to exert antipyretic effect by suppressing TNF- α and its related compounds also exhibit inhibition of arachidonic acid peroxidation which results in reduction of prostaglandin levels therefore reducing fever [17]. Therefore, the presence of flavonoids in the *Hilleria latifolia* may be contributory to its antipyretic activity. Exogenous pyrogens like amphetamine, turpentine, lipopolysaccharides (LPS) and sulphur induce fever by their ability to act on macrophages, monocytes and other immune cells to release pro-inflammatory cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor a (TNF-a), which act as endogenous pyrogens [18]. Fever can be induced in experimental animals by intravenous or subcutaneous injection of pyrogens. To evaluate the antipyretic activity of test drugs, the most commonly employed method to induce fever involves injection of lipopolysaccharides (LPS) or Brewer's yeast in rabbits or rats [19]. In this study, subcutaneous injection of yeast suspension markedly increased the rectal temperatures 18 hrs post administration. The extract decreased rectal temperatures in a dose-dependent manner. This decrease was statistically significant ($p< 0.05 – 0.001$) as shown in Table 2. The most significant reduction was observed in the first two hours after the administration of extract. The highest dose produced effects similar to Acetyl salicylate acid suggesting possibly similar mechanism of action. This is in tandem with results obtained by Hassan [16] and Ettebong [13]. Yeast-induced pyrexia also called pathogenic fever and its aetiology may possibly be the production of prostaglandins. Therefore, inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action and the inhibition of prostaglandin can be achieved by blocking the cyclooxygenase enzyme activity [16]. Injection of Brewer's yeast subcutaneously induces pyrexia by increasing the synthesis of prostaglandins. It is a suitable model for the screening of natural or synthetic drugs or compounds for their antipyretic effect [16].

In the Amphetamine-induced pyrexia model, the extract showed significant ($p< 0.05 – 0.001$) and dose-dependent reduction in the elevated body temperature compared to control as shown in Table 3. The highest dose of the extract (750 mg/kg) compared well with Acetyl salicylic acid. Amphetamine is also an indirectly acting sympathomimetic agent. Its inhibition shows antagonistic effect on endogenous cathecolamines in central adrenergic neurons [13]. Behavioral changes in the rats such as increased alertness, aggressiveness and locomotor activity, stereotyped behavior (consisting of repeated actions like licking, gnawing and rearing) were and may be due to the fact that amphetamine is a psychomotor (CNS) stimulant [20]. One or more combination of the phytoconstituents may be responsible for the antipyretic activities observed with *H. latifolia* in this study, especially as synergy is an important concept in the pharmacology of phytochemicals of botanical medicines [21].

On the effect of 2, 4-Dinitrophenol- induced pyrexia in rats**,** the extract showed a dose and time related decrease in temperature compared to control. This decrease was statistically significant ($p< 0.05 – 0.001$) as shown in Table 4. DNP induces hyperthermia by uncoupling oxidative phosphorylation causing the release of calcium from mitochondrial stores and prevents calcium reuptake. This leads to free intracellular calcium, muscle contraction and hyperthermia [13]. Therefore the extract may have caused the stimulation of sarcoplasmic reticulum, muscle relaxation and hypothermia [13, 22]. The DNP model exhibited the highest antipyretic activity relative to the control followed by the Amphetamine- induced and yeast induced- models respectively.

Table 1: Phytochemical constituents of extract

Constituents	Relative Presence
Alkaloids	+++
Anthraquinones	++
Cardiac Glycosides	++
Carbohydrates	+++
Flavonoids	++
Saponins	+++
Tannins	++
Terpenes	_

Key Interpretation
Slightly positive (+), Moderately positive (++), Highly positive (+++), Negative (-).

Table 2: Antipyretic activity of extract on yeast-induced pyrexia in rats

Treatment/ Dose mg/kg	Time (Hours)							
	0	18	0.5	1	2	3	4	5
Control	34.90±0.03	36.10±0.02	36.10±0.05	36.20±0.02	36.30±0.11	36.30±0.02	36.40±0.02	36.50±0.03
Ext 250	34.80±0.05	36.00±0.07^b	35.90±0.03^b	35.80±0.18^b	35.50±0.04^b	35.30±0.06^b	35.10±0.05a	35.00±0.07
500	34.60±0.07	35.80±0.03^b	35.70±0.03^b	35.40±0.03	35.00±0.02^b	34.780±0.02^b	34.70±0.03	34.60±0.02^b
750	34.80±0.07	36.00± 0.03	35.90±0.05^b	35.50±0.05^b	35.10±0.05^b	35.00±0.03^b	34.90±0.06	34.80±0.05
ASA	34.70±0.03	36.10±0.03	35.90±0.05^b	35.40±0.03^b	35.00±0.04^b	34.90±0.04^b	34.80±0.02	34.80±0.02^b

Values represent Mean ± SEM. Significant $^a p<0.05$; $^b p< 0.001$ (N=6) Ext = Extract

Table 3: Antipyretic effect of extract on amphetamine- induced pyrexia in rats

Treatment/ Dose mg/kg	Time (Hours)							
	BT	0	0.5	1	2	3	4	5
Control	34.80±0.07	36.40±0.03	36.80±0.03	37.00±0.03	37.30±0.03	37.60±0.03	37.80±0.03	38.00±0.03
Ext 250	34.50±0.07	36.00±0.05^a	35.90±0.04^b	35.80±0.04^b	35.50±0.03^b	35.10±0.02^b	34.90±0.03^b	35.60±0.04^b
500	34.60±0.11	36.10±0.05^a	36.00±0.04^b	35.80±0.04^b	35.40±0.04^b	35.00±0.02^b	34.80±0.02^b	34.70±0.07^b
750	34.70±0.08	36.10±0.03^a	35.90±0.01^b	35.80±0.02^b	35.30±0.03^b	34.90±0.05^b	34.70±0.05	34.60±0.04^b
ASA	34.70±0.03	36.20±0.03^a	36.00±0.05^b	35.90±0.05^b	35.50±0.02^b	35.10±0.02^b	34.70±0.02	34.60±0.02^b

Values represent Mean ± SEM. Significant $^a p<0.05$; $^b p< 0.001$ (N=6) Ext = Extract

Table 4: Antipyretic activity of extract on 2,4-Dinitrophenol (DNP)- induced pyrexia in rats.

Treatment/ Dose mg/kg	Time (Hours)							
	BT	0	0.5	1	2	3	4	5
Control	34.70±0.02	35.90±0.08	36.10±0.06	36.90±0.07	37.36±0.02	37.63±0.02	37.±50.04	37.30±0.02
Ext 250	34.33±0.18	35.43±0.02[b]	35.80±0.02	35.83±0.02[b]	35.71±0.07[b]	35.35±0.02[b]	35.26±0.02[b]	35.30±0.03[b]
500	34.60±0.08	35.71±0.03[b]	35.90±0.02[a]	35.73±0.02[a]	35.33±0.02[b]	35.03±0.02[b]	34.81±0.02[b]	34.81±0.03[b]
750	34.80±0.13	35.95±0.01[a]	36.10±0.05[a]	35.60±0.02[b]	35.13±0.02[b]	34.70±0.02[b]	34.60±0.02[b]	34.60±0.02[b]
ASA	34.55±0.06	35.80±0.05[a]	35.90±0.03	35.30±0.03[b]	35.00±0.02[b]	34.80±0.03[b]	34.66±0.05[b]	34.66±0.05[b]

Values represent Mean ± SEM. Significant [a]$p<0.05$; [b]$p< 0.001$ (N=6) Ext = Extract

4. Conclusions

The extract of *Hilleria latifolia* at doses of 250-750 mg/kg has demonstrated significant antipyretic activities in yeast, Amphetamine and 2, 4 Dinitrophenol-induced pyrexia in rats. The antipyretic effect observed compared favourably with that of the standard drug Acetyl salicylic acid. This potential may be attributed to the phytochemical constituents present in the plant and lends support to the ethnomedicinal use of the plant in the treatment of diseases conditions associate d with fever.

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