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Evaluation of Antipyretic Effect of Methanol Root Extract of *Costus lucanusianus* in 2,4-Dinitrophenol and Yeast Induced Wistar Rats

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ABSTRACT

This study was conducted to evaluate the antipyretic effect of methanol root extract of *Costus lucanusianus* in 2,4-dinitrophenol and yeast-induced Wistar rats. The plant extract was screened for antipyretic activity in the rats. The methanol root extract at 100, 200, and 300 mg kg^{-1} body weight per day dose levels were used to treat the test groups. After 19 hours (yeast induced) or 1 hour (2, 4-dinitrophenol-induced), the rectal temperature of the different animals were measured and only rats that showed an increase of at least 0.6ºc or more in the rectal temperature were used for the study. The animals were divided into five groups. 100mg/kg (p.o) of Aspirin was administered as the reference drug. The control group was left untreated. The rectal temperature of the different groups was measured at 1,2,3,4 hours after drug administration and compared with the rectal temperature of rats in the control groups. The oral administration of methanol root extract of *Costus lucanusianus* significantly attenuated rectal temperature of yeast-induced pyrexia in wistar rats at all doses (100,200 and 300 mg/kg). The effect of 300mg/kg extract was comparable with that treated with Aspirin at the 3rd hour($p<0.05$) and the fourth hour($p<0.001$). These findings justify the traditional use of this plant in the treatment of pyrexia and validate its claim of being used in folklore medicine for the treatment of pyrexia.

INTRODUCTION

This research aims to evaluate the antipyretic effect of methanol root extract of *Costus lucanusianus* in 2,4-dinitrophenol and yeast-induced Wistar rats. Pyrexia, also known as fever and febrile response [1]is a condition defined as having a temperature above the normal range due to an increase in the body's temperature set-point. In healthy adult men and women, the ranges of normal healthy temperatures are as follows: oral, 33.2–38.2 °C (91.8–100.8 °F); rectal, 34.4–37.8 °C (93.9–100.0 °F); tympanic membrane (the eardrum), 35.4–37.8 °C (95.7–100.0 °F); and, axillary (the armpit), 35.5–37.0 °C (95.9–98.6 °F) [2]. The increase in set-point triggers increased muscle contraction and causes a feeling of cold[3]. This results in greater heat production and efforts to conserve heat. When the set-point temperature returns to normal, a person feels hot, becomes flushed, and may begin to sweat [4]. Pyrexia differs from hyperthermia in that hyperthermia is an increase in body temperature over the temperature set-point, due to either too much heat production or not enough heat loss [1]. Pyrexia is usually associated with pain and inflammations.

Fever is one of the most common medical signs. It is part of about 30% of healthcare visits by children [3] and occurs in up to 75% of adults who are seriously sick [5]. Fever occurs when the set-point of the anterior hypothalamic thermoregulatory center is elevated, caused by (Prostaglandin-E2) PGE2 synthesis which is stimulated when endogenous fever-producing agents (pyrogens) such as cytokines are released from white blood cells that are activated by infection, hypersensitivity, malignancy, or inflammation [6]. Fevers in humans do not typically go higher than 41–42 °C (105.8 to 107.6 °F) [7]. While fever is a useful defense mechanism, treating fever does not appear to worsen outcomes [8][9]. Fever is viewed with greater concern by parents and healthcare professionals than it usually deserves, a phenomenon known as fever phobia [3].

A fever can be caused by many medical conditions ranging from not serious to potentially serious. These include viral, bacterial, and parasitic infections such as common cold, urinary tract infections, meningitis, malaria, and appendicitis among others. Non-infectious causes of fever include vasculitis, deep vein thrombosis, side effects of medication, and cancer, among others [10]. Medications that lower fever conditions are known as antipyretics. They include analgesics such as paracetamol, aspirin; non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and naproxen. These drugs help to reduce elevated temperatures and

feverish conditions. However, they are associated with a side effect that causes serious dangers to the patients.

The use of herbs to treat disease is almost universal among non-industrialized societies and is often more affordable than purchasing modern pharmaceuticals [11]. *Costus lucanusianus* is commonly used as a medicinal plant in tropical Africa. This plant is locally used in situations of pains, inflammation, and dysmenorrhoea, and pyrexia [12]. Leaf sap is used as nose drops and leaf pulp is rubbed on the head to calm insanity. The juice from leaves has a wide reputation in folk medicine for the treatment of diarrhea, vomiting, and dysmenorrhea [13]. The plant is held to have febrifugal and analgesic properties [14]. This research was centered on investigating, analyzing, and justifying the antipyretic effect of methanol root extract of *Costus lucanusianus*.

MATERIALS AND METHODS

Study design

This experimental animal study was carried out in the Department of Biomedical Technology, University of Port Harcourt. The study was carried out on forty- two adult Wistar rats weighing about 200g -260 g. The experimental animals were made use of according to the Guide for the Care and Use of Laboratory Animals [15] and following the principles of Good Laboratory Procedure (GLP) [16].

All animals were acclimatized for at least 1 week in a room maintained under environmentally controlled conditions and a 12 hours light-dark cycle before starting the experiments. They were housed in polypropylene cages with paddy house bedding under standard laboratory conditions for an acclimatization period of 7 days before experiments. They had free access to water and a standard diet. The beddings were changed every 24 hours. Before the experiment, the animal has fasted overnight.

Collection and identification of plant materials

The roots of *Costus lucanusianus* were collected around the University of Port Harcourt Abuja campus. The plant materials were identified and authenticated at the herbarium, in the Department of Plant Science and Biotechnology, the University of Port Harcourt by a taxonomist, Dr . I. Agbagwa. The pictorial and voucher samples were deposited at the department with herbarium number UPH/V/1212.

Extraction method

After collection, the roots were shade-dried at room temperature (32 – 35ºC) to constant weight over seven (7) days. Fifty grams of *Costus lucanusianus* was weighed and ground to a fine powder. The cold maceration extraction method of Cowan (1999) was used. The pulverized dried roots were dissolved in 1000ml of seventy percent methanol inside a 2-liter conical flask. The flask was shaken vigorously at 30-minute intervals and left to stand for 72 hours at room temperature for effective extraction. The resultant mixture was then be filtered with Whatman's No. 1 filter paper and cotton wool to remove particles of the plant sample. The clear solution obtained was then concentrated with a rotary evaporator at 45ºC under low pressure and later transferred to evaporating dish over a steam bath. The solid dried powder that was obtained was stored in sterile pre-weighed screw-capped bottles and labeled accordingly. The extract was then stored at room temperature until when needed.

Preparation of the test solution

The digital chemical balance (Fuzhou Furico.,ltd, China) was used to weigh out the extract. The powdered extract of *Costus lucanusianus* was collected with a spatula. One gram (1g) of powdered extract of *C. lucanusianus* was weighed out and added to 10ml of DMSO, stirred thoroughly using a stirring rod to form a stock solution of 100mg/ml.

Experimental design and models

The body weights of the animals were measured using a weighing scale and recorded. They were randomly divided into 5 groups of 5 animals each in each experimental model.

Yeast induced pyrexia

The method described by Adams *et al* (1968) was used[17]. Yeast-induced pyrexia model was performed on female rats (weighing 200–260g). All the animals were fasted 18 h before the commencement of the experiment but were adequately supplied with water *ad libitum*. A digital thermometer (Wuxi Hong Guang Medical Equipment) was inserted 3 - 4 cm into the rectum to measure the initial basal temperature. Pyrexia was induced by subcutaneous administration of 20% w/v of brewer's yeast at a dose of 10 ml/kg body weight near the groin of the animals [18]. After 19 hours, the rectum temperature of the different animals was measured and only rats that showed an increase of at least 0.6ºc or more in the rectal temperature were used for the study. The animals were divided into five groups:

Group I: 100mg/kg (p.o) of Aspirin was administered. This group is known as the Standard group.

Group II: 100mg/kg (p.o) of the extract was administered to this group of animals.

Group III: 200mg/kg (p.o) of the extract was administered to this group of animals.

Group IV: 300mg/kg (p.o) of the extract was administered to this group of animals.

Group V: The animals in this group were orally treated with dimethylsulfoxide (10 ml/kg). This group is the Control group.

The rectal temperature of the different groups was measured at 1,2,3,4 hours after drug administration and compared with the rectal temperature of rats in the control groups. (Agbaje*et al*., 2008; Akindele *et al*., 2012).

2, 4-dinitrophenol-induced pyrexia

The animals in this group were deprived of food for 24 hours but water ad libitum will be provided. Their basal temperatures were taken before inducing pyrexia. Then 10mg/kg of DNP prepared in normal saline was intraperitonally injected into each rat. One hour after administering with DNP, their rectal temperatures were taken and confirmed for pyrexia. The animals were divided into five groups.

Group I: 100mg/kg (p.o) of Aspirin was administered. This group is known as the Standard group.

Group II: 100mg/kg (p.o) of the extract was administered to this group of animals.

Group III: 200mg/kg (p.o) of the extract was administered to this group of animals.

Group IV: 300mg/kg (p.o) of the extract was administered to this group of animals.

Group V: The animals in this group were orally treated with dimethylsulfoxide (10 ml/kg). This group is the Control group.

The rectal temperature of the different groups was measured at 1,2,3,4 hours after drug administration and compared with the rectal temperature of rats before treatment. [19][20]

Statistical analysis

The data were presented as Mean ± Standard Error of Mean (n=5). Results obtained were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons using Graphpad prism 5.0. The significant difference was considered at P<0.05, 0.01, 0.001.

RESULTS AND DISCUSSION

RESULTS:

The results obtained from the experimental study were shown below.

Table No. 1: Effects of Extract on Yeast Induced Pyrexia

	Dose	Intial(ºC) temperature	0hr(ºC)	Treatment			
				1hr(ºC)	2hr(ºC)	3hr(ºC)	4hr(ºC)
Aspirin	100mg/kg	37±0.43	38±0.19	38±0.15	37±0.20***	37±0.38*	37±0.29***
Costus lucanusianus	100mg/kg	38±0.09	39±0.18	38±0.25	38±0.15	38±0.06	38±0.13*
Costus lucanusianus	200mg/kg	37±0.08	39±0.17	38±0.19	38±0.18	38±0.20	37±0.33***
Costus lucanusianus	300mg/kg	36±0.15	38±0.24	38±0.32	38±0.07	37±0.20*	37±0.24***
Control- DMSO	10ml/kg	37±0.70	39±0.13	38±0.12	38±0.07	38±0.18	39±0.18

Values are presented in Mean ± SEM, n = 5. * Significant at P< 0.05 and * Significant at P < 0.001.**

Table No. 2: Effects of Extract On 2,4 Dinitrophenol Induced Pyrexia.

	Dose	Intial(ºC) temperature	0hr(ºC)	Treatment			
				1hr(ºC)	2hr(ºC)	3hr(ºC)	4hr(ºC)
Aspirin	100mg/kg	37±0.41	38±0.25	37±0.09**	36±0.41*	36±0.26***	36±0.23***
Costus lucanusianus	100mg/kg	37±0.47	38±0.31	37±0.12	36±0.35*	37±0.15	37±0.32
Costus lucanusianus	200mg/kg	37±0.12	39±0.40	38±0.44	37±0.40	37±0.18	38±0.17
Costus lucanusianus	300mg/kg	38±0.11	39±0.18	38±0.78	38±0.60	38±0.69	38±0.47
Control-DMSO	10ml/kg	38±0.17	39±0.10	38±0.59	38±0.36	38±0.15	38±0.12

Values are presented in Mean± SEM, n = 4. * Significant at P< 0.05, Significant at P< 0.01 and ***Significant at P < 0.001.**

DISCUSSION

Subcutaneous injection of Brewer's yeast induces pyrexia by increasing the synthesis of prostaglandin. It is considered a useful test for the screening of plant materials as well as synthetic drugs for their antipyretic effect [21][22]. Yeast-induced pyrexia is called pathogenic fever and its etiology could be the production of prostaglandins which set the thermoregulatory center at a higher temperature [23]. Brewer's yeast forms a linkage to an immunological protein called Lipopolysaccharide-Binding Protein (LBP). This link causes the production of endogenous pyrogens ultimately leading to the synthesis and release of prostaglandins [24]. The inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of Aspirin and the inhibition of prostaglandin can be achieved by blocking the cyclooxygenase enzyme activity[25]. They are known to also act by suppressing the production of pyrogenic cytokines such as TNF-α and IL-β [26]. There are several mediators for pyrexia and the inhibition of these mediators is responsible for the antipyretic effect [22]. The oral administration of methanol root extract of *Costus lucanusianus* significantly attenuated rectal temperature of yeast induced pyrexia in Wistar rats at all doses(100,200 and 300 mg/kg).the effect of 300mg/kg extract was comparable with that treated with Aspirin at the 3rd hour($p<0.05$) and the fourth hour($p<0.001$). Thus, it can be

postulated that methanol root extract of *Costus lucanusianus* contained pharmacologically active principle(s) that interfere with the release of prostaglandins. It is well known that pyretic activity involves stimulation of the region in the hypothalamus, which controls body temperature through prostaglandins synthesized within the central nervous system [27] and the blood-brain barrier prevents drug substances from having access to the central nervous system (CNS). The extract is likely to reduce pyrexia by reducing brain concentration of prostaglandin E2 especially in the hypothalamus through its action on COX-3 [28] or by enhancement of the production of the body's antipyretic substances like vasopressin and arginine [29]. The results reported in this study are in line with previous reports where extract/Fractions of plant decreased the body temperature induced with Brewers' yeast administration in experimental animals [18][30][31].

Intraperitoneal injection of 2,4 Dintrophenol(DNP) induces pyrexia by uncoupling of oxidative phosphorylation. DNP interferes with the final energy production pathway by preventing the uptake of inorganic phosphate molecules into the mitochondria. This results in the inhibition of all energy-requiring processes and the extra-mitochondrial accumulation of inorganic phosphate. DNP causes a shift in the proton electrochemical gradient which results in potential energy dissipating as heat, instead of being converted to ATP, with rapid consumption of calories. The heat production represents a failure in thermoregulatory homeostasis, leading to uncontrolled hyperthermia [32]. The oral administration of methanol root extract of *Costus lucanusianus* significantly attenuated rectal temperature of 2,4 dinitrophenol-induced pyrexia in Wistar rats. The effect of 100mg/kg extract was comparable with the group treated with Aspirin at the 2nd hour ($p<0.05$). This study correlates with the study carried out by Essien *et al*., 2016)[33] on the Pharmacological evaluation of the aqueous stem bark extract of *Bombax buonopozense* in the relief of pain and fever on 2, 4 dinitrophenols induced pyrexia in rats. The extract was found to have significant ($p<0.05$) and dose-dependent antipyretic activity when compared to control. However, the standard drug (aspirin) produced more activity than the extract. The antipyretic activity of many plants has been attributed to their saponins, terpenoids, flavonoids, and steroids contents [34]. Hence, it can be postulated that the methanol root extract of *C. lucanusianus* attenuated DNP-induced pyrexia by blocking the uncoupling of oxidative phosphorylation and converting potential energy into ATP instead of dissipating it as heat. Previous work on the phytochemical screening of aqueous leaf extract of *Costus lucanusianus* reveals the presence of saponins and tanins[35]. However, flavonoids, saponins, and tannins are known to inhibit

the synthesis of prostaglandins as reported by [36]. The presence of these phytochemicals can attribute to their ability to block the uncoupling of oxidative phosphorylation.

CONCLUSION

In this study, the extract was observed to inhibit greatly 2,4 Dintrophenol (DNP) and yeast-induced pyrexia. These findings justify the traditional use of this plant in the treatment of pyrexia and validate its claim of being used in folklore medicine for the treatment of pyrexia.

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