



Investigation of the Anti-Pyretic Effect of the Aqueous and Ethanolic Leave Extracts of *Gnetum Africanum* on Brewers Yeast Induced Pyrexia on Wistar Rats

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ABSTRACT

The need for an alternative method of managing high body temperature has become necessary due to the toxicity of antipyretic drugs on various organs. *Gnetum africanum*, a wild liana found in Nigeria, Central Africa, Asia and South America, is believed to possess some phytochemical constituents that can help in the management of fever. 50 acclimatized male wistar rats weighing between 120g-200g were used for the study. Pyrexia was induced in some of the experimental animals by subcutaneous injection of the brewer's yeast suspension; their rectal temperatures were then measured and recorded after 24hrs. About 40 rats were selected for the study as they showed an increase in temperature up to 0.5°C. The animals were randomly assigned into 8 groups of 5 rats. Group A received normal rats chow and water ad libitum. Group B received 500mg/kg of the reference drug (Paracetamol) orally. Groups C, D and E received 125, 250 and 500mg/kg dosages of the aqueous extract orally while Groups F, G and H also received the same dosages of the ethanolic extract orally. After administration of the extracts, the temperatures of the animals were measured and recorded at 30minutes for 2 hours. The results of the study showed that the ethanolic extract reduce temperature significantly ($p \leq 0.05$) in the pyretic rats but at an earlier time interval especially seen with the 500mg/kg dosage of the ethanolic extract whereas the higher dosages of the aqueous extract were able to reduce temperature significantly ($p \leq 0.05$) in the rats but at a later time interval. The reference drug (Paracetamol) was able to reduce temperature significantly ($p \leq 0.05$) in the animals at an early time interval which could be compared with the 500mg/kg dosage of the ethanolic extract which reduced temperature significantly at a similar time interval. The findings therefore suggest that the ethanolic and aqueous leaves extract of *Gnetum Africanum* plant both possess an antipyretic property which was exhibited in a dose-dependent manner. However, the ethanolic extract seems to be more potent than the aqueous and the reference drug respectively.

Keyword: Temperature, fever, *Gnetum Africanum*, Acetaminophen, ethanol.

INTRODUCTION

The use of plants as an alternative form of therapy in primary health care of individuals and communities in most developing countries has increased in recent times as many plants and their derivatives have been discovered to be effective in the prevention and treatment of various diseases^[20]. Most body organs are efficient in their functioning at relatively constant temperature ranging from 36.1 to 38.5 °C and this is as a result of several variations which include circadian rhythm, vigorous exercise, variation in ambient temperature, results of food intake, Age factors, menstrual variations in females etc. Normal body temperature is regulated by temperature regulating centers found in the pre-optic area of the anterior hypothalamus which try to strike a balance between heat lost and heat gained^[9]. Pyrexia or fever is defined as sustained increase in body temperature above normal physiological range^[3]. Pyrexia or fever can be caused by infection and disease conditions. But in children, the cause could be as a result of recent immunization, teething, poor hygiene practices, malnutrition etc. While other conditions associated with fever include lethargy, depression, anorexia, sleepiness and inability to concentrate^[20]. *Gnetum Africanum* plant commonly called 'Ukazi' by the Igbos of the south-eastern Nigeria and 'afang' by the Ibibios/Efiks in the south-south region of Nigeria and several other trade names in the differently countries where it is found^[10] is a dioeciously wild liana which grows predominantly in humid forest zones in Nigeria, Cameroun, Central African republic and other subtropical Asia. *Gnetum Africanum* belongs to the family gnetacea and order of Gnetales^[13]. Its leaves are used in the preparation of soups in the southern Part of Nigeria and several other countries where the plant is found^[6]. The vegetable can also be consumed raw when it is prepared as salad mixed with dry fish or meat, other spices and palm oil^[6]. This study was aimed at investigating the antipyretic effects of aqueous and ethanolic leave extract of *Gnetum Africanum* on male wistar rats

and to find out if they can be used as an alternative form of therapy in reducing fever since there is paucity of information on the antipyretic effect of the plant on brewer's yeast induced pyrexia in wistar rats. It has been documented that non-steroidal anti-inflammatory drugs such as aspirin, paracetamol, Ibuprofen etc also serves as an antipyretic drugs. However, excessive consumption of these drugs has several adverse effects in the body. The toxicity of these drugs to various organs of the body when consumed for a long period of time has brought about the need for safer form of therapy involving the use of plants as antipyretics. Some several effects of the plants such as its anti-diarrheal effects have been documented^[5].

MATERIALS AND METHODS

PLANT COLLECTION, IDENTIFICATION AND AUTHENTICATION

Fresh leaves of *Gnetum Africanum* (GA) was purchased from Artisan Market in Enugu North Local Government of Enugu State Nigeria and a sample of the leaf was identified and authenticated at the Department of Botany of the University of Nigeria, Nsukka by a botanist (Mr. Chijioke Onyeukwu). A voucher specimen was deposited in the herbarium unit for further reference with no (UNH3a).

EXPERIMENTAL ANIMALS

Adult fifty male wistar rats of 12 weeks old and weights ranging from 120-200g were purchased from the Animals House Unit of the Department of Pharmacology & Therapeutics of the College of Medicine of the University of Nigeria, Enugu Campus. They were housed in a standard and well ventilated wire mesh cages under 12hr dark/light cycle at a room temperature of about 25°C. They were allowed for seven (7) days for acclimatization and were fed with super starter standard rat chow (Vital feeds Ltd) Jos, Plateau state Nigeria and clean drinking water *ad libitum*. All experiments and procedures were performed in accordance to the Guide for the Care and Use of

Laboratory Animals published by the National Research Council and was approved by the Ethics Committee of the College of Medicine of the University of Nigeria, Enugu Campus

PREPARATION OF THE AQUEOUS ETHANOLIC EXTRACT OF *GNETUM*

AFRICANUM LEAF

The aqueous and ethanolic extraction was done by the cold maceration method of ^[15].

PHYTOCHEMICAL SCREENING

The phytochemical screening of the extract of *Gnetum Africanum* was carried following the method of ^[18] at the department of Pharmacognosy of the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka and the Phytochemical constituents were indicated as follows; Alkaloids, flavonoids, glycosides, saponin, tannins, steroids, terpenoid, carbohydrates and proteins at different concentrations .

ACUTE TOXICITY TEST

The acute toxicity test (LD₅₀) was performed according to the method of ^[7].

INDUCTION OF PYREXIA

Pyrexia was induced in the animals according to the method of ^[17]. First the basal rectal temperature of the rats were measured and recorded by inserting the clinical thermometer (Dolphin Pharmaceutical Ltd Mumbai India) 3-4 cm into the rectum of the each rats and the values recorded after the thermometers beeps and displays the accurate reading. The pyrexia was induced into the animals through subcutaneous routes of drug administration of 10ml/kg per body weight of 20% w/v yeast ^[17] after their body weights were determined using a top loading digital weighing balance (Ohaus Scout Pro, Pine Brook, New Jersey USA). After the induction of pyrexia, animals that showed an increase in body temperatures at least by 0.5°C were selected for the study. After the induction of the pyrexia followed by treatments with the test plants, the rectal temperatures were measured after 30 minutes intervals for 2 hours respectively.

EXPERIMENTAL DESIGN

The forty (40) male wistar rats were divided randomly into eight groups of five rats each as stated

Group A: Animal allowed free access to normal diet and drinking water

Group B: Induced with pyrexia and received 500 mg/kg paracetamol

Group C: Induced with pyrexia and received 125mg/kg aqueous extract of *Gnetum Africanum*.

Group D: Induced with pyrexia and received 250mg/kg aqueous extract of *Gnetum Africanum*.

Group E: Induced with pyrexia and received 500 mg/kg aqueous extract of *Gnetum Africanum*

Group F: Induced with pyrexia and received 125mg/kg ethanolic extract of *Gnetum Africanum*

Group G: Induced with pyrexia and received 250mg/kg ethanolic extract of *Gnetum Africanum*

Group H: Induced with pyrexia and received 500mg/kg ethanolic extract of *Gnetum Africanum*

STATISTICAL ANALYSIS

All data were statistically analyzed and results expressed as mean±standard error of mean. One way analysis of variance (ANOVA) was done using the Graphpad Prism 7 Statistical software. Multiple comparisons were done using the Tukey (Post-hoc) test. P ≤0.05 were considered statistical significant and P≤0.01 were extremely significant ^[8]

RESULTS

The result of the phytochemical screening depicted on table 1, revealed the presence of high concentration of alkaloid, medium concentration of flavonoids, glycosides, saponin and a low concentration of tannins and steroids and terpenoid. Also, seen were high concentration of carbohydrates and a low concentration of protein was also seen to be present in the plant extracts while Table 2 shows the effect of *Gnetum Africanum* leaf extract on brewer's yeast induced pyrexia on rats .After the induction of pyrexia the rats in the control group A did not show any significant increase or decrease in the body

temperature owing to the fact that they were not induced to pyrexia and received normal rat chow daily *ad libitum* while the group B (500mg/kg) paracetamol showed a significant decrease in their body temperatures with respect to the time intervals. The difference in the body temperatures between the induction of pyrexia and the 120 minutes post treatment were as stated in fig 1 with group H (500mg/kg ethanolic extract) having the highest reduction in the rectal temperature by 1.18°C, followed by group B (500mg/kg paracetamol). Groups F, G, E, D and C with values of 1.08±0.10, 0.78±0.11, 0.74±0.02, 0.72±0.05, 0.60±0.04 and 0.10± 0.27 °C respectively. They were compared statistically for the level of significance and discovered that there were significant differences (p≤0.05) between group A compared with groups D and E on the 90th and 120th minutes of post induction/treatment periods and multiple comparison shows that group B was statistically significance compared to group C on the 90th and 120th minutes respectively. There were also extremely significant differences

(p≤0.01) between the control group A, group B and E on the 90th and 120th minutes respectively. There were also significant difference between group G compared with groups F and H on the 120th minutes treatment periods respectively.

Table 1. Result of the phytochemical screening of the *Gnetum Africanum* extracts Phytochemical concentration

Carbohydrate	+++
Reducing sugar	-
Alkaloids	+++
Glycosides	++
Saponin	++
Tannins	+
Flavonoid	++
Resin	-
Protein	+
Oil	-
Steroids	+
Terpenoid	+

+++ = High concentration
 ++ = moderate concentration
 + = low concentration
 = absent-

Table 2; Result of the Mean standard error of mean of the rectal temperatures (°C)

Groups	Initial temp (°C)	24hrs after Induction of pyrexia	30mins after treatment	60mins after treatment	90mins after treatment	120mins after treatment
A	36.94±0.12	36.92±0.13	36.93±0.13	36.91±0.13	36.89±0.12	36.92±0.13
B	36.74±0.16	37.52±0.16	37.24±0.14	36.90±0.15*	36.70±0.09**	36.44±0.2**
C	36.72±0.14	37.92±0.28	37.70±0.22	37.42±0.29	37.71±0.15 ^B	37.82±0.1 ^B
D	37.06±0.16	38.00±0.24	37.70±0.20	37.45±0.10	37.45±0.17*	37.40±0.2*
E	36.66±0.15	37.94±0.15	37.73±0.08	37.53±0.06	37.36±0.12*	37.22±0.1**

Values are expressed as significant difference *P≤0.05 compared with A; extremely significant difference **P≤0.01 compared with A. ^BP≤0.05 compared with

Table 3; Results of the Mean ±standard error of mean of the rectal temperatures (°C)

Groups	Initial temp (°C)	24hrs after Induction of pyrexia	30mins after treatment	60mins after treatment	90mins after treatment	120mins after treatment
A	36.94±0.12	36.92±0.13	36.93±0.13	36.91±0.13	36.89±0.12	36.92±0.13
B	36.74±0.16	37.52±0.16	37.24±0.14	36.90±0.15	36.70±0.09**	36.44±0.20**
F	37.02±0.12	37.80±0.23	37.56±0.16	37.28±0.09	37.13±0.12*	37.02±0.12* [®]
G	37.10±0.27	37.90±0.18	37.69±0.20	37.54±0.19	37.34±0.23*	37.16±0.20*
H	37.00±0.14	37.64±0.20	37.39±0.13	37.10±0.07*	36.81±0.08**	36.46±0.10* [®]

Values are expressed as significant difference *p≤0.05 compared with A; extremely significant difference **p≤0.01 compared with A. [®]p≤0.05 compared with G

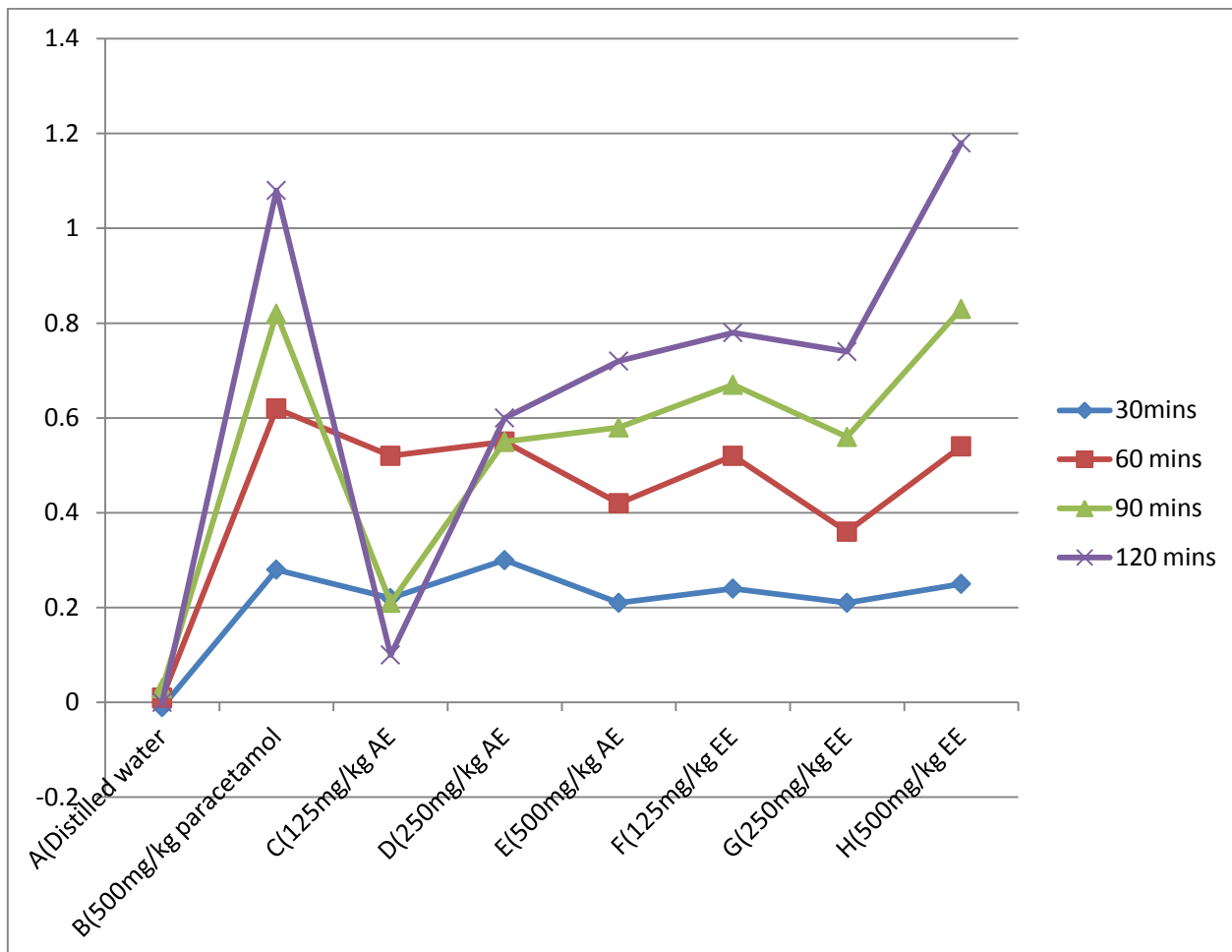


Fig i. Result of the mean differences between initial and post treatment temperatures in degree centigrade

DISCUSSION

The research investigated the antipyretic effect of the aqueous and ethanolic extracts of *Gnetum Africanum* in brewer’s yeast induced pyrexia on wistar rats. Pyrexia being a sustained increase in body temperature above the normal physiological range of 36.1-37.8°C (97-100°F) with an average of 37°C (98.6°F) caused by toxic substances that affect temperature regulating centers, also caused by pyrogens and other substances like protein, breakdown product of proteins and other substances especially lipopolysaccharide toxins released from bacterial cell wall which increases the set point of the hypothalamic thermostat^[9] and they are grouped as endogenous and exogenous pyrogens. Large amount of pyrogens are produced in the condition of fever with Interleukin-1 and TNF-alpha playing a central role by affecting release of neutrophils and enhancement of their functions. They cause vasodilatation and

increased of cell amongst other effects^[14]. Exogenous pyrogens that can cause fever include bacteria, viruses, protozoa, immune reactors, several hormones, medications and synthetic polynucleotide. These endogenous pyrogens interact with receptors on the endothelial cells of the brain^[14]. Possible mechanisms of actions of these pro-inflammatory mediators is to act on the pre-optic and anterior hypothalamus causing the production of prostaglandins E₂ from cyclogenase thereby leading to increase or elevation in body temperatures^[10]. According to^[2], this mechanism of fever induction is called the humoral hypothesis of inducing fever. Yeast induced pyrexia refers to an elevation in body temperature triggered by induction of yeast and its possible mechanism of action is by binding to the polysaccharide protein which is an immunological protein^[19]. Several endogenic cytokines factors are thus synthesized and released through this binding resulting in

prostaglandin E₂ synthesis and release^[11]. This leads to increase in temperature of set point of the thermoregulatory centre thereby inducing fever^[21]. Some activities seen when there is an increase in body temperatures include cutaneous vasoconstriction, piloerection, Epinephrine secretion, shivering, increase in muscle tone etc^[9] An increase in the point of temperatures regulating centre causes physiological mechanisms in the body to try to restore its temperature to normal through vasodilatation and sweating^[9], thus the set point is then reduced to a lower value. Non steroidal anti-inflammatory drugs (NSAID) are drugs which possess both anti-inflammatory and anti-pyretic properties. They include aspirin, ibuprofen, paracetamol etc and their mechanism of action is by inhibition of prostaglandin within the hypothalamus^[4] or by suppressing increase in Interleukin-1 production subsequent to Interferon production^[12]. The ability of the aqueous and ethanolic extracts of GA to significantly reduce temperatures in the rats induced with pyrexia might possibly be due to their pyretic properties which caused inhibition of prostaglandins in the brain^[12]. The presence of phytochemical constituents in the *Gnetum Africanum* leave extracts such as alkaloids, flavonoids, saponin, glycosides, tannins and terpenoid might also possibly enhance its antipyretic activity as indicated in the previous studies carried out by^[20] and^[1] that the presence of such bioactive agents in extracts of solvents could enhance their antipyretic activity. Furthermore, the possible mechanism by which extracts were able to reduce pyrexia might be through reduction in the rate of Interleukin-1 production^[20] which is similar to the mechanism of action of most antipyretic. As shown in this study, the lowest dose of the aqueous extract which is 125mg/kg body weight reduced temperatures significantly whereas a significant reduction in the temperature seen with the lowest dose of Ethanolic extract after 90th and 120th minutes treatment intervals. The result of this study therefore showed that both the aqueous leave extract and the ethanolic leave extract of

Gnetum Africanum reduced pyrexia in dose-dependent manner as higher doses of the extracts reduced temperature significantly at faster time interval than lower doses. The level of temperature reduction at the different post treatment time intervals in the animals administered with 500mg/kg of the ethanolic leave extract of *Gnetum Africanum* was also slightly similar to that seen in the group administered with the reference drug (paracetamol).

CONCLUSION

The data obtained in this study showed that both the aqueous and the ethanolic leaf extracts of *Gnetum Africanum* plant have antipyretic properties which were rather exhibited at different time intervals depending on the dosage of the extracts administered to the animals. This result suggests that the ethanolic leave extract of *Gnetum Africanum* might be more potent than the aqueous extract of *Gnetum Africanum* as the reduction in the temperature at the different treatment time intervals was more significantly observed with the ethanolic extract than the aqueous extract of the plants. Hence, the exact possible mechanism of action of the plant should be subjected to further studies including the effect of heat (cooking) on the pyretic activity owing to the fact that some of the plant is consumed as cooked recipes in soups while some is consumed raw as salad when added to our recipes. Based on these, the study has provided some basic information about the antipyretic activity of *Gnetum Africanum* which can partly contribute to its use as a form of herbal therapy.

ACKNOWLEDGEMENT

The authors sincerely acknowledged the technical assistance of the Laboratory Technologists (Mr. Ani Celestine Okafor and Mr. Ike Chibueze) who did most of the technical works. Moreover, Prof Anyaehie Bond Ugochukwu for his supervisory role in this research work.

CONFLICT OF INTEREST

The authors declared no conflict of interest during the course of this research

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