Original Research Article

ANTI-PYRETIC EFFECTS OF AQUEOUS EXTRACT OF *ALCHORNEA CORDIFOLIA* IN ALBINO WISTAR RATS

ABSTRACT

Anti-pyretic effect of aqueous leaf extract of *Alchornea cordifolia* on Wistar rats were investigated. Twenty Wistar rats of both genders weighing between 110-178g were used for this study. Baker's yeast was used for induction of pyrexia. The rats were grouped into five groups of 5 per group in each study. Groups 3, 4 and 5 received 400, 800mg/kg b.w of aqueous extract and reference drug respectively after induction. Groups 1 and 2 served as normal and negative controls. Rats were sacrificed and blood samples collected for hematological and biochemical analyses. Phytochemical screening of the plant revealed the presence of alkaloid, phenolic compounds, Tannins and Quinine. Result showed significant in ($p \leq 0.05$) in paw size, rectal temperature and number of writhing in group 2 and non-significant reduction ($p \geq 0.05$) in group 4 were seen when compared to group 1 and 2 respectively. Significant ($p \leq 0.05$) increase in C-reactive protein and nitric oxide concentrations were observed in groups 3 and 4 when contrasted to group 2 in the studies. Non-significant differences in all hematological parameters in all treated groups were observed when compared to group 2. Aqueous leaf extract of *Alchornae cordifolia* displayed pyretic effects at 800mg/ kg b.w after 4 hours of treatment.

1. Introduction

Pyrexia (also named fever) is the altering upward of the thermoregulatory set point, often secondary to the systemic inflammatory response to a stimulus such as infection. Fever has

been defined by The American College of Critical Care Medicine, the International Statistical Classification of Diseases and the Infectious Diseases Society of America as a core temperature of 38.3 °C or higher [1]. Pyrexia secondary to the systemic inflammatory response should be distinguished from hyperthermia resulting from excessive heat production, as observed in heatstroke and malignant syndromes, or from ineffective heat loss. Temperature levels encountered during hyperthermia are usually higher than during pyrexia because thermoregulation is abolished; indication of rapid temperature control is, therefore, indisputable to avoid irreversible tissue damage [2].

Fever is one of the most prevalent afflictions of the human race in the developing world [3]. There are several infectious diseases that involve fever as a symptom. Fever commonly develops in the course of microbial infection, where pyrogens released by the bacteria or virus act directly on the OVLT (organum vasculosum laminae terminalis) of the anterior hypothalamus, which responds by elevating core body temperature [4]. However, the endogenous release of pyrogens occurs in response to pathogenic protozoal life forms that use mosquitoes as their vectors.

The causes of Pyrexia of unknown origin can be considered in four categories: infective, inflammatory, neoplastic and miscellaneous. The relative prominence of each category has changed over time, with an increasing proportion of patients who remain undiagnosed, which may be up to 51% of cases [5]. Infectious causes account for 17–35% of cases, inflammatory causes 24–36%, neoplastic causes 10–20% and miscellaneous causes 3–15% [6]. An older multimorbid population, increased global travel, HIV infection, the increase in organ transplantation and immunomodulation for many diseases, evolving diagnostics and changing antimicrobial resistance patterns have all changed the management of patients with pyrexia

The recent global pandemic of COVID-19 has stimulated a renewed interest in discovering new therapies that act against fevers and fever-causing agents. Seeking curative drugs from plants is considered a rational approach because of recent developments in the context of synthetic derivatives of alkaloids from Cinchona, possibly contributing to patient recovery [7].

2. Materials and methods

Source of experimental animal

Wistar rats were purchased from the Department of Pharmacy, Faculty of Pharmaceutical Science, University of Port Harcourt, Rivers State, Nigeria. They were housed in different cages by groups with renewable bedding, and were fed with standard rat feed and clean water, allowing them to acclimatize for fourteen days under normal temperature, humidity and light-dark cycle for acclimatization.

Plant Collection and Identification

The plant was obtained from the botanical garden of the University of Port Harcourt, Rivers State, Nigeria. After collection, the plant was sent to the Department of Plant Science and Biotechnology (PSB), University of Port Harcourt, where it was properly identified.

Extract Preparation

The leaves were properly washed in running tap water and allowed to air dry for 2 weeks, and blended into fine powder form. Twenty five gram of the powder was macerated in 100ml of deionized water for 24hours under mechanical agitation at room temperature. The suspension was filtered using Whatman filter paper (25 mm) and dried in a water-bath at approximately 55 ^{o}C. Crude extract gotten was store at 4 ^{o}C before usage (EMEA, 2006)

Experimental design

Wistar rats of both sex weighing 110g to 178g were divided into five groups (n=4rats/group) of 20 rats for anti-inflammatory studies and anti-pyretic studies.

Experimental design table for anti-pyretic study

Groups	Description	Dose
Group 1	Normal control	No induction (Distilled water + Feed)
Group 2	Negative control	1ml of 0.4% yeast

Group 3	*Alchornea cordifolia* aqueous extract	1ml of 0.4% yeast + 400mg/kg
Group 4	*Alchornea cordifolia* aqueous extract	1ml of 0.4% yeast + 800mg/kg
Group 5	Reference drug	1ml of 0.4% yeast + 300mg/kg Aspirin

Phytochemical Screening

Alkaloid: The aqueous *Alchornea Cordiolia* leaves crude extract was dissolved in 2MHCl .The mixture was filtered and the filtrate was divided into 3 equal portions. One portion was treated with a few drops of Mayer's reagent; another portion with a few drops of Dragondroffs reagent and the other portion was treated with equal amount of Wagner's reagent. The creamy precipitate, orange precipitate and brown precipitate respectively indicated the presence of alkaloids [8].

Saponnin: The presence of saponnin was determined by frothing test. 0.5g of the extract was vigorously shaken with distilled water and was allow to stand for ten minutes and was classified for saponnin content as follows: Absence of froth indicate absence of saponnin and the presence of stable froth of more than 1.5cm indicated the presence of saponnins [9].

Coumarin: one ml of plant extract in a test tube was treated with 3-4drops of 1%potassium hydroxide solution (obtained by 1g of KOH in ethanol) and the appearance of a yellow color indicates the presence of Coumarin

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Tannins:Ferric Chloride test:0.5g of plant extract was stirred with distilled water and filtered. Two drops of 5%Ferric Chloride solution was added to the filtrate. Formation of a blue black, green blue green precipitate was taken for evidence of tannins [10].

Phenolic compound:0.5g of plant extract was stirred with distilled water and filtered .2-3,drops of ,1% neural Ferric Chloride solution was added ,(obtained by adding dilute ammonia until precipitate just begin to form and filtered).The appearance of violet color with ferric ion indicates the presence of phenolic compounds.(shindo's test)

Quinine: To the test sample in a test tube dilute sodium hydroxide was added .formation of blue green or red color indicates the presence of quinine. (shiondo'test)

Steroids: Liebermann-Burchard reaction was performed to assess the presence of steroids. Drops of chloroform ,3-4drops of acetic anhydride and drop s concentrated sulphuric acid were added down the side,appearance of purple colour that changes to blue or green color shows the presence of steroids.

Cardiac glycosides; Keller: kiiani test was performed to access the presence of cardiac glycosides .the crude dry powder of the plant was treated with I ml of Ferric chloride reagent (mixture of 1 volume of5% FeCl3 solution and 99 volume of glacial acetic acid).To the solution a few drops of concentrated sulfuric acid was added. Appearance of greenish blue color within a few minutes indicates the presence of cardiac glycosides.

Protein:million reagent; To the test sample in a test tube ,few drops of reagent was added and heated for two minutes ,formation of red precipitate indicates the presence of protein.

2.1 Biochemical assay

Nitric Oxide

Method: Modified Griess reaction described by Bredt and Snyder (1994).

Principle: The Griess reaction is a diazotation reaction of sulphanilamide and further condensation of the produced diazonium salt with naphthyl-ethylene diamine hydrochloride.

Procedure:

Total Nitric Oxide content is measured after the sample is incubated with Nitrate Reductase and NADH. The reductase join with NADH reduces Nitrate to Nitrite. After 20 minute incubation at 25^{o}C, color reagents A & B are added and incubated at 25^{o}C for 5 minutes. The amount of nitrate in the sample is calculated by taking the measured nitrite concentration and subtracted from overall nitric oxide concentration for the sample.

C-Reactive protein

Method: Enzyme immunoassay

Principle: This method is anchored on solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the on the C-reactive protein (CRP) molecule. This mouse monoclonal anti-CRP antibody for solid phase immobilization (on the microtiter wells). A goat anti-CRP antibody present in antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is reacted simultaneously with both antibodies, resulting in the CRP molecules being sandwiched between enzyme-linked antibodies and solid phase. After a 30-45-minutes incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A tetramethylbenzidine (TMB) reagent is introduced and incubated for 20 minutes, then formation of blue colour. The colour formation is stopped by introduction of 1N HCl transforming the colour to yellow. The amount of CRP equals the test sample colour.

Procedure: Serum was diluted 100 fold befor use. The coated wells needed in the holder was secured. 10 microliter of CRP standards was dispensed, before diluting specimens and control into the appropriate wells. 100 microliters of CRP enzyme conjugate reagent was then

dispensed. Thoroughly mix for 30 seconds. Incubate at room temperature (18-25^{O}C) for 30-45 minutes. The incubation mixture taken by flicking plate contents into a waste contains. Rinsing and flicking the microtiter wells repeatedly in penta-fold. 100µl of TMB were dispense into solution of each well and were gently mixed for five seconds. Incubation was done at 25^{o}C for 20mins, then halting with 100ul of stop-solution. It was then gently mixed for 20 seconds before reading at 450nm within 15 minutes

Interleukin-6

Method: Enzyme immunoassay

Principle: Enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The small titer plate provided in the test kit has monoclonal antibody just for IL-6.

Procedure: All samples and reagents were stored at 25^{O}C before starting. All reagents, standards, and samples were prepared as directed by the manufacturer's manual. Excess microplate strips were extracted from plate frame, and then re-introduced pouch foil containing the desiccant pack, and reseal. 100ml std. was poured into samples and control per well. The plate sealer provided was covered and incubated at 25^{o}C for 120 minutes. The cover was removed and liquid discarded into a waste receptacle. The plate was inverted on the bench top onto a paper towel and then taped gently blot any remaining liquid. 100ul of the prepared 1x biotinylated anti-human IL-6 anti-body introduced in each well, which was then cover with wash buffer. The process was then repeated for each well before adding 90µl of color developing reagent to all well. In all the well, the sealer provided was covered and stored in the dark for 30mins at 25^{0}C for change in colour measurement at 450nm.

Method of Data Analysis

All statistical analysis was done using the standard package for social science (SPSS version 23.0). The results were analyses using the one-way analysis of variance (ANOVA) with a significant difference at $p<0.05$. LSD & turkeys' multiple comparison was used to test for significant difference between the groups.

3. RESULTS

Table 1. Phytochemical Analysis of *Alcharnia cordifolia*

Alkaloid	**positive**
Phenolic_compound	**positive**
Tannins	**positive**
Flavonoid	**negative**
Saponnin	**positive**
Quinine	**Positive**
Coumarin	**negative**
Protein	**negative**
Cardiac glycoside	**negative**
Steroid	**negative**

Table 2: Effect of administration of aqueous leave extract of *Alchornea cordifolia* on yeast induced pyrexia in wistar rats

Group	Treatment	Initial rectal temperature (^{o}C)	Rectal temperature (^{o}C) after drug administration			
			1 hour	2 hours	3 hours	4 hours
1	Control (0.5ml distilled water)	36.50±0.20	36.65±0.22^{a}	36.15±0.06^{a}	36.15±0.02^{a}	36.50±0.04
2	Control (-ve) 0.4% yeast	36.85±1.80	38.75±0.53^{a}	39.13±0.50^{a}	37.55±0.10^{a}	37.43±0.19^{a}
3	0.4% yeast + 400mg/kg *Alchornea cordifolia*	38.35±0.51	39.03±0.34^{b,c}	38.73±0.18^{b}	37.20±0.31^{b}	37.50±0.25^{b}
4	0.4% yeast + 800mg/kg *Alchornea cordifolia*	37.43±0.38	37.75±0.39^{b}	38.00±0.40^{a}	36.35±0.26^{a,b,c}	36.70±0.20
5	0.4% yeast + 500mg/kg Paracetamol	37.08±0.53	37.10±0.26^{a,b,c}	37.28±0.25^{a,b}	37.08±0.21^{c}	36.40±0.64^{a,b}

Values are reported as mean ± standard error of mean (M±SEM) (n =4). Values with similar superscript letters indicate statistical significant differences ($p \leq 0.05$) down the column while those without superscripts show non-significant differences ($p \geq 0.05$) down the column when compared with the control and between groups.

Table 2 showed that rectal temperature in group 5 decrease significantly ($p \leq 0.05$) after one hour when compared with group 2, a significant increase ($p \leq 0.05$) in rectal temperature in group 3 after one hour when compared with groups 4 and 5, a significant increase ($p \leq 0.05$) in rectal temperature after one hour in group 3 when compared with group 5.

Table 2 revealed a significant decrease ($p \leq 0.05$) in rectal temperature in group 4 and 5 after two hours of observation when compared with group 2, a significant increase ($p \leq 0.05$) in rectal temperature in group 3 after two hours when compared with group 5.

Table 2 also revealed a significant decrease ($p \leq 0.05$) in rectal temperature in group 4 after three hours when compared with group 2, a significant decrease ($p \leq 0.05$) in rectal temperature in group 4 after three hours when compared with group 3, a significant decrease ($p \leq 0.05$) in rectal temperature of group 4 after three hours when compared with group 5.

Table 2 further showed a significant increase ($p \leq 0.05$) in rectal temperature in group 5 after four hours, non-significant decrease ($p \geq 0.05$) in rectal temperature after four hours in groups 3 and 4 when compared with group 2. The Table also showed a significant increase ($p \leq 0.05$) in rectal temperature in group 3, and a non-significant increase ($p \geq 0.05$) in rectal temperature in group 4 after four hours when compared with group 5.

Table 3: Effect of oral administration of aqueous leave extract of *Alchornea cordifolia* on interleukin-6, C-reactive protein and nitric oxide concentration of yeast induced-pyrexia in wistar rats

Group	Treatment	Interleukin-6 (pg/ml)	C-reactive protein (mg/l)	Nitric Oxide (mg/ml)
1	Control (0.5ml distilled water)	249.75±59.79[a]	0.06±0.00[a]	0.09±0.00[a]
2	Control (-ve) 0.4% yeast	280.10±44.14[a]	59.80±4.47[a]	39.23±10.56[a]
3	0.4% yeast + 400mg/kg *Alchornea cordifolia*	90.73±10.66[a,b,c]	0.071±0.01[a]	1.05±0.05[a]
4	0.4% yeast + 800mg/kg *Alchornea cordifolia*	13.68±41.48[a,b,c]	0.54±0.35[a]	0.72±0.27[a]
5	0.4% yeast + 500mg/kg Paracetamol	101.33±28.61[a,c]	0.28±0.14[a]	0.98±0.55[a]

Values are reported as mean ± standard error of mean (M±SEM) (n =4). Values with similar superscript letters indicate statistical significant differences ($p \leq 0.05$) down the column while those without superscripts show non-significant differences ($p \geq 0.05$) down the column when compared with the control and between groups.

Table 3 revealed a significant decrease ($p \leq 0.05$) in interleukin-6, C-reactive protein and nitric oxide levels in groups 3, 5 and 5 when compared with group 2, a significant decrease ($p \leq 0.05$) in interleukin-6 concentration in group 3 and 4 when compared with group 5. The C-reactive protein and nitric oxide levels in groups 3 and 4 revealed a non-significant increase ($p \geq 0.05$) when compared with group 5. Nitric oxide levels further showed a non-significant decrease in group 4, a non-significant increase ($p \geq 0.05$) in group 3 when compared with group 5.

4. Discussion of findings

On the anti-pyretic activities of *Alchornea cordifolia* as shown in Table 3, the study findings showed that the aqueous leave extract of *Alchornea cordifolia* at 400 and 800mg/kg b.w effect in ameliorating yeast induced pyrexia was not observed after one hour. The anti-pyretic activities of *Alchornea cordifolia* started manifesting between two to four hours of oral administration which indicate a time dependent effect. Anti-pyretic activities of *Alchornea cordifolia* at 800mg/kg was pronounced compared with the 400mg/kg b.w implying dose-dependent effect. A time dependent effect was observed in the 500mg/kg b.w paracetamol treated group after four hours showing a decline in the anti-pyretic effect as time elapse but extract exhibit the direct opposite in conformity with Nsonde *et al.* [11] who found that *Alchornea cordifolia* at 400 and 800mg/kg b.w showed pronounced antipyretic property comparable to paracetamol at 100 mg/kg per os.

In furtherance to the biochemical examination of antipyretic activities of leaf extract of *Alchornea cordifolia*, it was obvious that the extract at 400 and 800mg/kg b.w administered orally caused pronounced reduction ($p \leq 0.05$) in interleukin-6, C-reactive protein and nitric oxide levels which agrees with Effo *et al.* [12] finding that *Alchornea cordifolia* extract exhibit dose-dependent hypothermic activity. These biochemical parameters are usually elevated during swelling, hyperthermia and pain. Thus, the reduction in yeast-induced pyrexia seen could be an indication of the ability of the extract to cause alteration of pre-optic anterior hypothalamus which is critical in the mechanism of inducing hyperthermia by yeast mediated by prostaglandins biosynthesis [13]. Studies have established that brewer's yeast given sub-cutaneously accts like an external pyrogen [14] Thus, yeast induces elevation of body temperature that according to Aronoff *et al.* [15], leads to the bio-production of prostaglandins. Prostaglandins E2 is implicated in the upset of the hypothalamic thermostat thereby responsible for fever. [16] Therefore,

hypothermia seen may also be due to terminal interference with prostaglandins synthesis. These antipyretic property could attributed to the presence saponins in *Alchornea cordifolia* leaves [16]. Several recent studies have confirmed the use of medicinal plants I the management of pyrexia, inflammation and related health conditions [18-22]

Conclusion

This research findings indicates that *Alchornea cordifolia* possess a dose-dependent antipyretic effect properties. The anti-pyretic activities of *Alchornea cordifolia* was found to start manifesting between two to four hours after oral administration.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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