

Acute Toxicity and Antipyretic Activities of a Methanolic Extract of *Alchornea cordifolia* Leaves

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ABSTRACT

Alchornea cordifolia (Euphorbiaceae) is a very prized plant among traditional healers in Africa. Its leaves are used for its antipyretic properties in traditional areas. The aim of our study is to determine the acute toxicity and the antipyretic activity of a methanolic extract of *Alchornea cordifolia* leaves. Acute toxicity was assessed by measuring mortality, changes in body weight, spontaneous movements, and normal rectal temperature in mice. Antipyretic activity was evaluated by brewer's yeast-induced hyperpyrexia in rats according to Teotino method (1963). The antipyretic effect of methanolic extract of *Alchornea cordifolia* leaves was compared with paracetamol (100 mg/kg bw) orally. Groups of mice treated with doses of 6500; 3250; 1625 and 812.5mg/kg of the extract did not show any mortality, nor significant alteration of body weight, nor alteration of spontaneous movements. However, incomplete reversed dose-dependent hypothermic activity was observed with doses of 50.78; 101.56; 203.12; 406.25; and 812.5 mg/kg p.o. of the extract, showing acute toxicity of this plant. In the antipyretic assay, the extract with doses of 50.78; 101.56; 203.12; 406.25; and 812.5 mg/kg p.o. exhibited a significant dose-dependent antipyretic activity similar to paracetamol (100 mg/kg bw) in rats. Thus *Alchornea cordifolia* may inhibit prostaglandins-biosynthesis from hypothalamus. Our results support claims on its traditional uses in management of fever. However *Alchornea cordifolia* may affect hypothalamus not only during fever but also when body temperature is normal.

Keywords: *Alchornea cordifolia*; Acute Toxicity; Brewer's Yeast; Hypothermia

1. Introduction

Alchornea cordifolia (Euphorbiaceae) is a plant that is widely used in traditional medicine in Africa, from Senegal to Cameroon, as a remedy for several diseases. Its leaves are renowned for being febrifuge [1]. Previous studies revealed no mortality in mice in acute toxicity tests, several pharmacological activities such as antimicrobial [2-4], antiameobic [5], antimalarial [6], anti-inflammatory [7,8], antispasmodic [9], antioxidant [10], antidiarrheic [11], and anti-stress properties [12]. However, there is no existing pharmacological data that indicated the antipyretic activity of *Alchornea cordifolia*. Thus, the aim of our study was to determine the acute toxicity and the antipyretic activity of the methanolic extract of *Alchornea cordifolia* leaves in rats.

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2. Materials and Methods

2.1. Plant Material

Fresh *Alchornea cordifolia* (Schum. and Thonn.) leaves were harvested in the Floristic National Center of Abidjan and the collected leaves were given to the botanic expert Prof Aké-Assi Laurent affiliated with the University of Cocody (Abidjan), who verified their uniformity with the specimens previously deposited and catalogued in the herbarium of the Floristic National Center of Abidjan. The leaves were collected and dried in darkness in a 25°C air-conditioned room

2.2. Experimental Animals

Mice (*Mus musculus*) (15 - 34 g) and rats (*Rattus norvegicus*) (weighing 150 - 286 g) were purchased from

Department of Nutrition and Pharmacology of the University of Cocody (Abidjan, Côte d'Ivoire). The animals were then bred at the Department of Pharmacology of the faculty of pharmacy of the University of Cocody. All animals were kept in the room maintained under environmentally controlled conditions of $24^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and 12 h light - 12 h dark cycle. The animals had free access to water and food and were acclimatized at least 1 week before starting the experiments. The animals were cared for and treated according to the principles of care for, and use of, laboratory animals, formally approved by the Board of Ethics of the Cocody University, in compliance with the Guide for Care and Use of Laboratory. And before the experiments they were fasted overnight with water ad libitum.

2.3. Chemicals

Methanol (Sigma), sodium carboxy-methyl-cellulose (CMC) Sigma (France). Paracetamol: (Doliprane 100 mgpoudre-pour solution buvable) (Sanofi Aventis), brewer's yeast Arkogélules (Arkopharma) were used in this study.

2.4. Methanolic Extract

The fine powder (200 g) of dried leaves were macerated for 24 h at room temperature in 700 ml of methanol. The methanolic extract obtained were dried in a rotavapor (Büchi R180) and conserved at 4°C , and aliquots of dried powder were used for pharmacological studies after suspending in 2% CMC.

For acute toxicity test, the stock solution was concentrated at 650 mg/ml. Three other ranges of concentration were prepared from this stock solution by successive dilutions in 1/2 (325 mg/ml, 162.5 mg/ml, and 81.25 mg/ml).

We used successively for the hypothermic activity and antipyretic activity, solutions concentrated at 81.25 mg/ml, 40.62 mg/ml, 20.31 mg/ml, 10.15 mg/ml and 5.07 mg/ml

2.5. Acute Toxicity Test

Mortality, body weight changes and spontaneous motor activity were assessed in mice and hypothermic activity in rats.

2.6. Spontaneous Motor Activity (SMA)

A multi-counter activity cage, Letica activity cages (LE 886) connected to AM1051 (Benwick electronics) data logger were used for this study. The AM1051 data logger is provided with two layers of infrared sensor placed horizontally to monitor the rearing, mobile and static activities, as well as the active and mobile times. Mice were singly placed in each cage and activity was automatically recorded by the instrument for 5 min. Each

mouse was used only once. The extracts (50.78; 101.56; 203.12; 406.25; and 812.5 mg/kg p.o.) were administered to five groups of mice ($n = 6$) and control group received CMC. SMA measurements started one hour after the administration of the extract and the fourth hour and subsequently every two days for 14 days after treatment.

2.7. Effect on Normal Body Temperature (Hypothermic Activity)

Rats weighing 150 - 286 g were divided into six groups of six animals. Initial rectal temperatures were recorded using a 12 channel electric thermometer (LETICA Scientific instruments), model 812 RS, PANLAB s. L, Barcelona. Then extract (50.78 mg/kg; 101.56 mg/kg; 203.12 mg/kg; 406.25 mg/kg; 812.5 mg/kg) was given orally. Temperature changes in body temperature values before and after drug administration were recorded.

2.8. Brewer's Yeast-Induced Hyperthermia in Rats

Rats weighing 180 - 220 g were divided into six groups of six animals. Initial rectal temperatures were recorded using a 12 channel electric thermometer (LETICA scientific instrument), model 812 RS, PANLAB s. L, Barcelona. Hyperthermia was induced in rats according to the method of Teotino *et al.* [8] by subcutaneous injection of 1 ml/100g body weight of 20% brewer's yeast. When the temperature was at a peak, 18 h after yeast injection, only rats which developed satisfactory pyrexia (0.6°C or more increase in rectal temperature) were used. Then extract (50.78 mg/kg; 101.56 mg/kg; 203.12 mg/kg; 406.25 mg/kg; 812.5 mg/kg) was given orally 24 h after yeast injection. Paracetamol (100 mg/kg, p.o.) served as the reference drug for comparing the antipyretic action of extract and the rectal temperatures of animals were recorded at 1h interval for 4 h following drug treatment.

The results are expressed as percentage [13,14]:

$$\text{Percent reduction} = \frac{(\text{yeast-induced pyrexia} - \text{post-treatment temperature}) \times 100}{\text{Yeast-induced pyrexia}}$$

2.9. Statistical Analysis

The results are expressed as mean value \pm S.E.M. Friedman's test was applied to the results. Mean values were considered significantly different when $P < 0.05$.

3. Results

3.1. Acute Toxicity

Mortality and body changes weight

No death was observed nor a significant changes in

weight (Table 1).

Spontaneous motor activity

After a two-week observation, the methanolic extract from *Alchornea cordifolia* engendered no significant modification of spontaneous motor activity.

Effect on normal body temperature

The doses of 203.125 mg/kg, 406.25 mg/kg and 812.5 mg/kg, presented hypothermic activity ($p < 5\%$). The methanolic extract of *Alchornea cordifolia* causes a hypothermia that is not totally reversed at the end of a four-hour time (Figure 1).

3.2. Antipyretic Activity

Figure 2 show the antipyretic activity. During four (04) hours, the methanolic extract of *Alchornea cordifolia*

administered at 812.5 mg/kg to the rat presented an antipyretic effect superposable to that of the paracetamol administered at 100 mg/kg. The doses of 203.125 mg/kg, 406.25 mg/kg presented also a statistically significant antipyretic activity ($p < 5\%$). The antipyretic effect is totally reversed after 4 hours.

Figure 3 shows the percentage of hyperthermia inhibition after two hours. We noted a reduction of the hyperthermia upper to 80% with 203.125 mg/kg, 406.25 mg/kg and 812.5 mg/kg. In doses lower than 203.125 mg/kg, we noted a percentage of reduction lower than 50%.

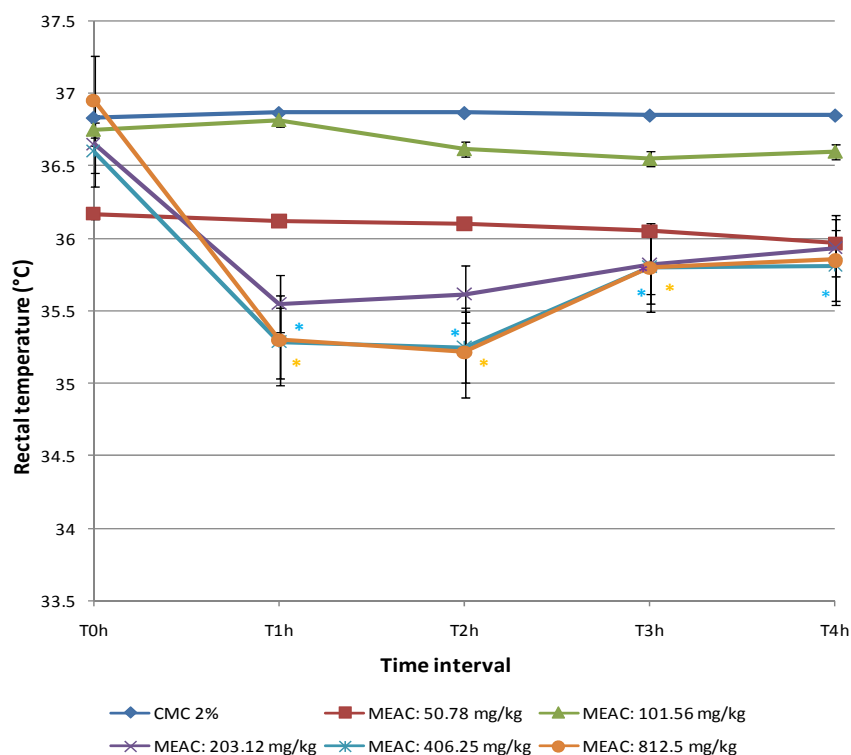
4. Discussion

Alchornea cordifolia is a plant which is very currently used in traditional medicine for the treatment of several

Table 1. Mortality and body changes weight.

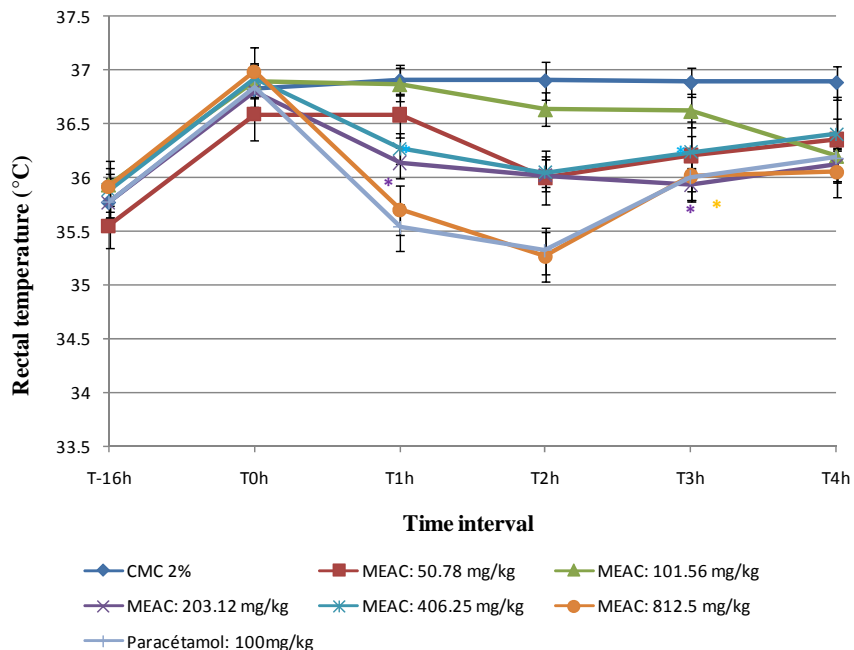
Substance	mice number at the beginning	mice number at the end	Average initial weight (g)	Average weight at the end (g)
CMC 2%	8	8	22.4	24.5
MEAC: 81.25 mg/kg	8	8	22.2	22.4
MEAC: 162.5 mg/kg	8	8	22.2	23.7
MEAC: 325 mg/kg	8	8	22.3	22.3
MEAC: 6500 mg/kg	8	8	22.2	23.6

Values represent the average evolution of the number of mice and the average of weight. Number of animals used ($n = 8$). * $p < 0.005$ compared to control by Friedman's test. †: $P < 0.05$: significantly different compared to control (CMC 2%).



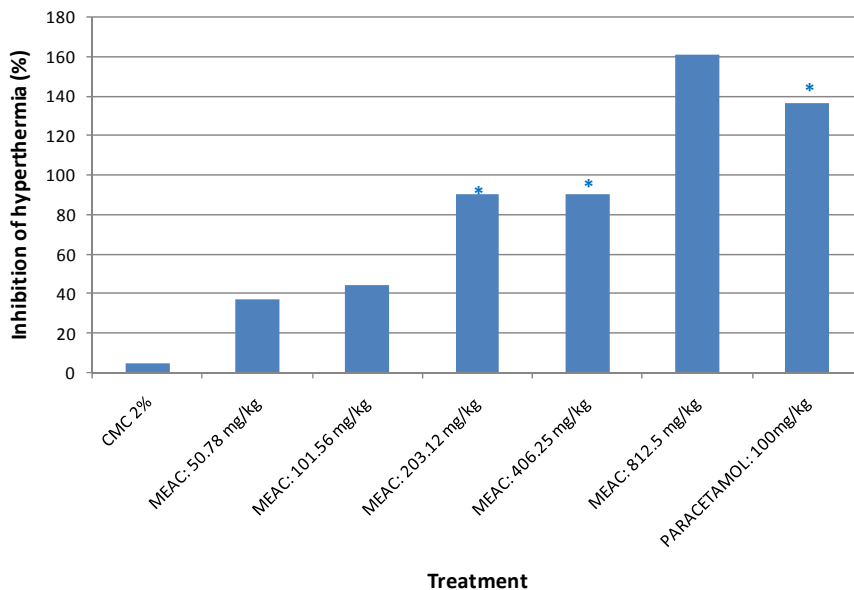
MEAC: Methanolic extract of *Alchornea cordifolia*; CMC: carboxymethylcellulose. Curves represent the average evolution of the rectal temperature in time. Number of animals used ($n = 6$). * $p < 0.005$ compared to control by Friedman's test. †: $P < 0.05$: significantly different compared to control (CMC 2%).

Figure 1. Hypothermic activity of methanolic extract of *Alchornea cordifolia*.



Curves represent the average evolution of the rectal temperature in time. Number of animals used (n = 6). * p < 0.005 compared to control by Friedman's test. *: P < 0.05: significantly different compared to control (CMC 2%).

Figure 2. Antipyretic activity of methanolic extract of *Alchornea cordifolia* and paracetamol on brewer's yeast induced pyrexia in rats.



The results are given as mean ± S.E.M. number of animals used (n = 6). * p < 0.005 compared to control by Friedman's test.

Figure 3. Inhibition of hyperthermia of methanolic extract of *Alchornea cordifolia* and paracetamol (100 mg/kg) at two hours.

diseases associated with fever. Our works aimed to search out a possible toxicity and an antipyretic activity. About acute toxicity, absence of death in the present study was also observed by Traoré [15] using 5000 mg/kg p.o. of leaves orally and by Umukoro [12] using 4000 mg/kg orally. The absence of modification in the spontaneous

movements shows that *Alchornea cordifolia* cannot modify the normal behavior in mice. However, the dose-dependent hypothermia observed in the present study after administration of *Alchornea cordifolia* extract showed acute toxicity of this plant. Hypothermia indicated alteration of preoptic anterior hypothalamus which is critical in

the neuronal network of thermoregulation. The hypothalamus regulated the set point at which body temperature is maintained. Hyperthermia inhibition observed in the present work varies from 89% to 160%. Evolution of hyperthermia inhibition due to *Alchornea cordifolia* at 812.5 mg/kg during four hour was similar to paracetamol (100 mg/kg p.o.) with a pick at two hours (**Figure 2**). The mechanism of the induced hyperthermia by brewer's yeast is mediated by prostaglandins biosynthesis [16,17]. Brewer's yeast administrated through subcutaneous route, behaves like an exogenous pyrogen. Thus, it produces an inflammatory process that according to Milton *et al.* [18] and Aronoff *et al.* [19], leads to the biosynthesis of chemical mediators as prostaglandins due to arachidonic acid. Among prostaglandins, PGE2 is strongly involved in the disturbance of the hypothalamic thermostat and so is responsible for fever [18,19]. Thus, the antipyretic activity of *Alchornea cordifolia* observed in this study would be related to the inhibition of the production of prostaglandins. Apart from their involvement in fever, prostaglandins are also known for their vasodilatation effect that generates red blotch and oedema as inflammation characteristics. Thus the hypothesis about the inhibition of the production of prostaglandins by *Alchornea cordifolia* could be uphold with the works by Osabede and Okoye [8] as well as those by Mavar-Manga *et al.* [7] highlighting the protecting effect of *Alchornea cordifolia* against oedema, the inflamemation model induced in rodent.

These antipyretic property lay in the saponins contain in *Alchornea cordifolia* leaves [5,9] that, according to Gepdiremen *et al.* [20] are strong prostaglandin inhibitors.

5. Conclusion

This antipyretic activity that is similar to paracetamol one could partly justify the traditional use of *Alchornea cordifolia* leaves against fever in the African societies. Considering the partially reversible hypothermic effect in a 4-hour observation time, we plan a more elaborate study on the neurotoxicity of that plant.

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