



## **Anti-inflammatory Activities of the Chloroform Extract of the Fruit of *Tetracarpidium conophorum* (Mull. Arg.) (Nigerian Walnuts)**

**Famobuwa E. Olaniyi<sup>1\*</sup>, Osho I. Bamidele<sup>2</sup>, Akinlami O. Omokehinde<sup>1</sup>  
and Agbowuro A. Ayodeji<sup>3</sup>**

<sup>1</sup>Department of Chemistry, Adeyemi College of Education, Ondo, Nigeria.

<sup>2</sup>Department of Animal Production and Health, Federal University of Technology, Akure, Nigeria.

<sup>3</sup>Department of Pharmaceutical Chemistry, University of Jos, Nigeria.

### **Authors' contributions**

*This work was carried out in collaboration among all the authors. Author FEO designed the study, wrote the protocol and the first draft of the manuscript. Author OIB managed the animals and wrote part of the manuscript, author AOO did the literature search. Author AAA collected all the data and performed the statistical analysis. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Objective:** To investigate the anti-inflammatory activity of the chloroform extract of the fruit of *Tetracarpidium conophorum* (CEFTC).

**Methods:** CEFTC was evaluated for anti-inflammatory activity using standard formalin-induced rat paw edema. The activities of the extract at different doses were compared to diclofenac, a standard anti-inflammatory drug.

**Results:** In all the experiments, the chloroform extract of *T. conophorum* at 400 mg/Kg showed anti-inflammatory effects which were significant ( $p=0.05$ ) and comparable to those of diclofenac, whereas CEFTC at 200 mg/Kg was pro-inflammatory. This suggests that the extract could be harmful at low doses.

\*Corresponding author: E-mail: [edyniyi@yahoo.com](mailto:edyniyi@yahoo.com);

**Conclusion:** The results showed that CEFTC at 400 mg/Kg possesses significant anti-inflammatory activity comparable to that of diclofenac, one of the non-steroidal anti-inflammatory drugs (NSAIDs). This supports its use as a potent anti-inflammatory drug in herbal medicine.

*Keywords: Tetracarpidium conophorum; fruit extract; inflammation; diclofenac; formalin.*

## 1. INTRODUCTION

Inflammation is the body's response to disturbed homeostasis caused by infection, injury or trauma resulting in systemic and local effects. An inflammatory reaction prevents the spread of infections and promotes the healing of any destroyed tissue [1]. Inflammation hastens the healing of wounds and infections, and unchecked destruction of the tissues will lead to extinction of the organism. However, inflammation which runs unhindered can lead to numerous diseases, such as hay fever, atherosclerosis, and rheumatoid arthritis. An inflammatory reaction may be propelled by infection trauma, thermal injury, chemical injury, and immunologically mediated injury [2]. Some of its symptoms are excessive heat, swelling, pain, and redness. It is a common factor in arthritic diseases or osteoarthritis. The rapid response to an injurious agent that serves to deliver mediators of host defence leukocytes and plasma proteins to the site of injury is known as acute inflammation. It has three major components: vasodilation, vascular leakage, oedema and leukocyte emigration (mostly polymorphonuclear cells). When a host encounters an injurious agent, such as an infectious microbe or dead cells, phagocytes that reside in all tissues try to eliminate these agents. At the same time, phagocytes and other host cells respond to the presence of the foreign or abnormal substance by liberating cytokines, lipid messengers, and the various other mediators of inflammation. Some of these mediators act on endothelial cells in the vicinity and promote the efflux of plasma and the recruitment of circulating leukocytes to the site where the offending agent is located. The recruited leukocytes are activated by the injurious agent and by locally produced mediators, and the activated leukocytes try to remove the offending agent by phagocytosis [2]. As the injurious agent is eliminated and anti-inflammatory mechanisms become active, the process subsides and the host returns to a normal state of health. If the injurious agent cannot be quickly eliminated, the result may be chronic inflammation. Chronic inflammation is a pathological condition characterized by recurrent active inflammation, tissue destruction, and attempts at repair. It is not characterized by the

classic signs of acute inflammation listed above [2].

The most commonly used drug for management of inflammatory conditions are non steroidal anti-inflammatory drugs (NSAIDs) [3], which have several adverse effects especially gastric irritation leading to formation of gastric ulcer [4]. Thus, there is a need to search for new anti-inflammatory agents with little or no side effect. Natural products of plant, animal or microorganism origin have been good sources of new bioactive compounds [5].

Walnut comprises such families as *Juglandaceae* (English walnut), *Euphorbiaceae* (African walnut), and *Olacaceae* (African walnut). Each family has its own peculiar characteristics, but they have some things in common such as the nuts. *Juglandaceae* is mostly found in the Southeast Europe, to Japan and more widely in the new world.

*Tetracarpidium conophorum*, a woody perennial climber that belongs to the family *Euphorbiaceae* is found in Nigeria and other African countries. Nigerian walnut is botanically known as *T. conophorum* (Mull. Arg.) Hutch. and Dalziel. Its common name is African walnut and in Nigeria it is called Nigerian Walnuts. It is known as "Asala" in Yoruba Land in Western Nigeria, "Okhue" or "Okwe" in Edo State, "Ekporo" by the Efiks and Ibibios of Cross River and Akwa Ibom states in Nigeria, commonly called "ukpa" and "awusa" in the Eastern, and Northern Nigeria respectively and it is known in the littoral and the western Cameroon as "Kaso" or "Ngak" [6]. Walnut, common name for small flowering plants are important for the nuts and timber most of them produce and for its representative genus. *T. conophorum* (Mull. Arg.) Hutch. and Dalziel. are a synonym of *Plukenetia conophora* Müll. Arg [7]. It is a dry nut, enclosed between two semispherical hard shells joined, and the fruit has a characteristic shape that resembles brains. *T. conophorum* plant is cultivated principally for the nuts which are cooked and consumed as snacks [8]. A bitter taste is usually observed on drinking water immediately after eating the nuts. This could be attributed to the presence of

chemical substances such as alkaloids. Their white, slightly bitter flesh consists mainly of a blend of vegetable fats (60%), followed by a very respectable amount of protein (24%) and a lower amount of carbohydrates (10%). It is a climbing shrub 10-20 Feet long and is found in the forest regions of Africa and India [8,9].

*T. conophorum* has pro-fertility activity [10]. Edem et al. [11] reported on the proximate composition, ascorbic acid and heavy metal contents of the nut. Oyenuga reported on the amino acid and fatty acid components of the nut and on the use of its leaf juice for the treatment of prolonged and constant hiccups [12]. Nwokolo also reported that the local method of processing the nuts has an effect on the nutrient and flavour of the nut [13]. Okafor reported on the use of *T. conophorum* seeds and processing waste in livestock feed formulation [14].

Medical studies have shown walnuts to improve several physical illnesses, promote weight loss (even though the caloric content is fairly high) and enhance overall health. These beneficial effects are probably linked to their high content in seldom-eaten omega-3 polyunsaturated fats, which are slowly, but steadily disappearing from our diets but are absolutely essential for the good functioning of our bodies. Walnuts are rich in linoleic and linolenic acids and in other health-related compounds such as high-biological-value proteins (e.g. arginine) fiber, vitamins, tannins, folates, and polyphenols which may provide additional antiatherogenic properties [15]. Walnuts contain polyunsaturated fatty acids, which may protect against cardiovascular disease and may enhance tocopherol absorption [16]. In this study, the blood glucose lowering effects of the hexane crude extract of *T. conophorum* on oral glucose load has been investigated on normal rats.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Plant Materials

Fresh walnuts were bought from Ondo market in Ondo Town, Nigeria during the month of July and identified by the Head, Department of Biology, Adeyemi College of Education, Ondo, Nigeria.

### 2.2 Extraction

1 kg of the fresh walnuts was pulverized and soaked with chloroform at room temperature (25-30°C). After 72 h, the chloroform extract was

filtered. This extract was dissolved in Tween 80 followed by normal saline to get the required concentrations of 200 and 400 mg/kg and were used for screening anti-inflammatory activity.

$$\% \text{ Yield} = \frac{\text{Weight of the extract}}{\text{Weight of crude}} \times 100$$

### 2.3 Phytochemical Screening

Phytochemical screening tests for alkaloids, glycosides, saponins, flavonoids, tannins, terpenoid, reducing sugar and soluble carbohydrates were performed according to the standard methods [17].

### 2.4 Animals

Wistar rats were obtained and housed in polythene cages at a population density of six rats per cage. Food and water were available *ad libitum* through 1-qt gravity-fed feeders and waterers. The room temperature was maintained at 29°C, and overhead incandescent illumination was maintained on 12-hour light-dark cycle. Daily maintenance was conducted during the first quarter of the light cycle. Rats were allowed to acclimatize for 7 days before use. Group sample size of 6 was used throughout the study. The animals were handled in accordance with international principles guiding the use and handling of experimental animals and were approved by the College Ethics Committee.

### 2.5 Chemicals and Drugs

All the chemicals and drugs used were of analytical grade.

### 2.6 Determination of Acute Toxicity

The lethal dose (LD<sub>50</sub>) of the plant extract was determined by method of Lorke [18] using 13 rats. In the first phase, rats were divided into 3 groups of 3 rats each and were treated with the ethanol extract of the plant at doses of 10, 100 and 1000 mg/kg body weight intra-peritoneally. They were observed for 24 h for signs of toxicity. In the second phase 4 rats were divided into 4 groups of 1 rat each and were also treated with the chloroform extract of the plant at doses of 1000, 1600, 2900 and 5000 mg/kg bodyweight (i.p). The median lethal dose (LD<sub>50</sub>) was calculated using the second phase.

### 2.7 Formalin-induced Oedema in Rats

Pedal inflammation was produced in rats according to the method described by Winter et

al. [19]. Four groups (comprising of six animals each) of rats were treated orally with 200 and 400 mg/Kg of the extract while the control and reference groups received saline (orally) and diclofenac (10 mg/Kg, orally) respectively. One hour after the administration of extract and diclofenac, 0.1 ml of 3% formalin was injected into the left hind paw of each animal under the sub plantar aponeurosis. Measurement of paw size was carried out by wrapping a piece of cotton thread round the paw and measuring the circumference with a metre rule. Paw sizes were measured immediately before and 1–4 hrs after formalin injection. Oedema inhibitory activity was calculated according to the following formula [20].

$$\text{Percentage inhibition} = \frac{(Ct - Co)_{\text{Control}} - (Ct - Co)_{\text{Treated}}}{(Ct - Co)_{\text{Control}}} \times 100$$

Where Ct = paw circumference at time t, Co = paw circumference before formalin injection and Ct – Co = Oedema.

Data were expressed as Mean ± Standard error of means of six experiments. Statistical comparisons were performed by one-way ANOVA followed by Dunnett's multiple comparison test, and the values were considered statistically significant when  $P = .05$ .

### 3. RESULTS AND DISCUSSION

#### 3.1 Extraction and Acute Toxicity

The yield of the extract was 1.20% w/w dry matter and the acute toxicity test of the extract produced no death or signs of toxicity after 48 h.

#### 3.2 Phytochemical Screening

As presented in Table 1, all the phytochemicals investigated were present except terpenoid. The anti-inflammatory activity of this plant extract may not be unconnected with the presence of these phytochemicals.

#### 3.3 Effect of CEFTC on Formalin-induced Paw Oedema in Rats

The chloroform extract at 400 mg/Kg showed remarkable activity against acute inflammation by suppressing the paw oedema. In the animals

treated with 400 mg/kg of the extract and in those treated with diclofenac (10 mg/kg), the reduction in oedema was the same 3 hours after formalin administration. Whereas, with 200 mg/Kg of the extract, the increase in paw size is almost at par with the control. 200 mg/kg of the extract did not show any oedema inhibition but increased throughout the duration of the experiment (Fig. 1). The maximum inhibition (50%) was achieved with 400 mg/kg of the extract within 4 hours of induction of inflammation (Fig. 2). In the control group, there was a progressive increase in paw oedema after injection of formalin, which reached maximum intensity within 3 hours (Fig. 1). This strongly agrees with the work of Kelechi and Uzoma [21].

**Table 1. Phytochemical screening**

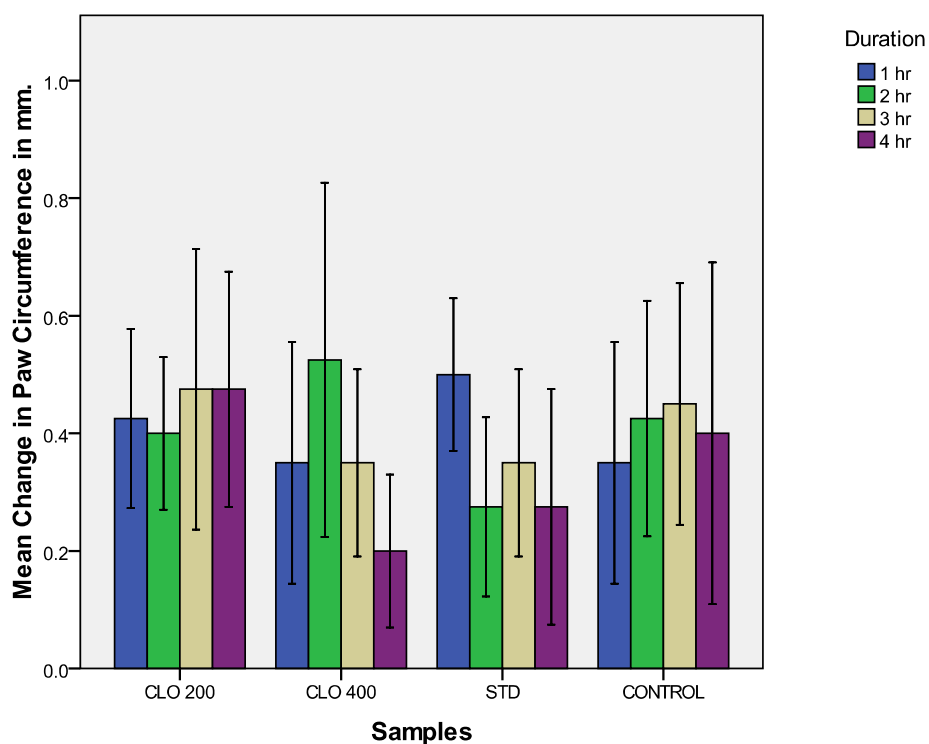
Phytochemicals	Results
Alkaloids	+
Glycosides	+
Saponins	+
Flavonoids	+
Tannins	+
Terpenoids	-
Reducing sugar	+
Soluble carbohydrates	+

+ = present; - = absent

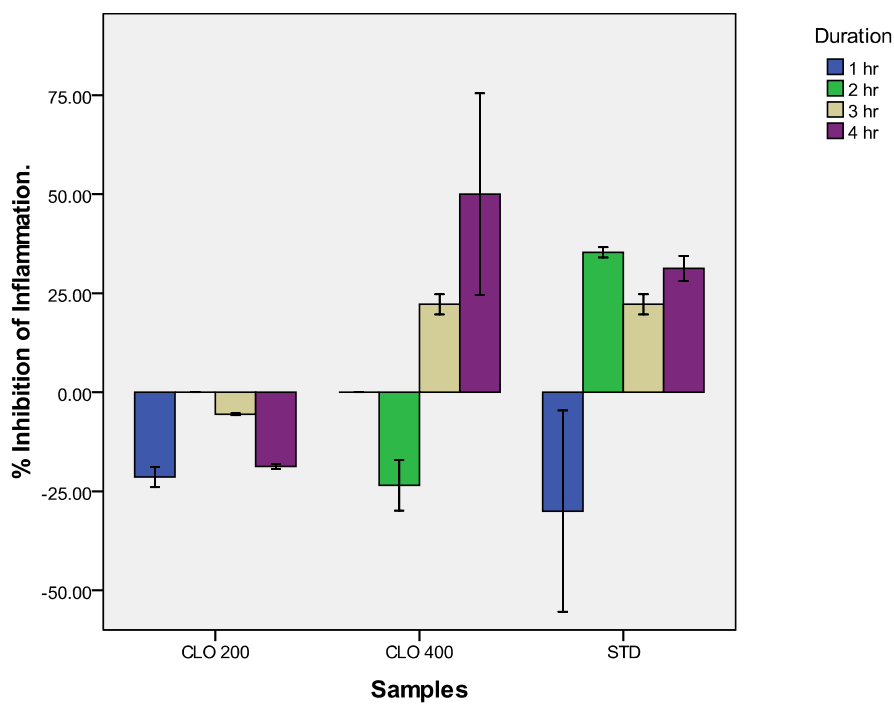
The model used in this study provide broad spectrum for evaluation of anti-inflammatory activity. The formalin-induced rat paw oedema, which is widely used as a working model of inflammation in the search of new anti-inflammatory agents [22] tested for activity against acute inflammation, the extract being administered orally. The results obtained with this model indicates that the fruit of *T. conophorum* possesses significant activity against acute inflammation at high doses.

In this experiment, CEFTC exhibited effects which were comparable, though at high dose, to that of diclofenac, an anti-inflammatory drug.

The mechanisms of the action of the active constituents of the plant may be attributed to the presence of the phytochemicals present and also the inhibition of the cyclooxygenase pathway of arachidonic acid metabolism, which is the general mechanism of action of Non Steroidal Anti-inflammatory Drugs [23].



**Fig. 1. Mean change in paw circumference in mm**  
 CLO 200 = 200 mg/Kg CEFTC; CLO 400 = 400 mg/Kg CEFTC; STD = 10 mg/Kg diclofenac



**Fig. 2. Percentage inhibition of inflammation**  
 CLO 200 = 200 mg/Kg CEFTC; CLO 400 = 400 mg/Kg CEFTC; STD = 10 mg/Kg diclofenac

#### 4. CONCLUSION

The results showed that CEFTC at 400 mg/Kg possesses significant anti-inflammatory activity comparable to that of diclofenac. This supports its use as a potent anti-inflammatory drug in herbal medicine.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

The authors declare that this work was not against public interest. Animal experiments were conducted in accordance with NIH guidelines for care and use of Laboratory animals (Pub. No. 85–23, Revised 1985).

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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