EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 7
39-42
Year: 2016

EFFECTS OF CHLOROFORM AND METHANOL EXTRACTS OF THE ROOT BARK OF *FICUS EXASPERATA* ON FORMALIN-INDUCED RAT PAW OEDEMA

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Article Received on 02/05/2016 **Article Revised on 23/05/2016** **Article Accepted on 14/06/2016**

ABSTRACT
The results showed that methanol extract (MET) at 375 mg/Kg possesses better anti-inflammatory activity than the extract of chloroform (CLO) and diclofenac at 1-3 hours after formalin administration and compared favourably with both at 4 hr. This supports the use of this plant extract as a potent anti-inflammatory drug in herbal medicine.

KEYWORDS: Inflammation, *Ficus exasperata*, Oedema, Methanol, Chloroform, Diclofenac, Formalin.

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 . 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. 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].

The genus Ficus consist of woody trees, shrubs, vines, epiphytes, and hemiepiphytes[6]. They are collectively known as fig trees or figs. They are native throughout the tropics with few species extending into the semi-warm temperate zones.The use of medicinal plants to improve health is as old as humanity. Among these plants, none may be older than the fig[7]. A number of Ficus species are used for medicinal purposes in Ayurvedic and Traditional Chinese Medicine especially amongst people where these species grow. These uses, however, originated and are most widely found in the Middle East. In Iran, a decoction of the fruits of Ficus carica is taken orally for bronchitis, cystitis and nephritis[8]. Pharyngitis and stomatitis are treated with an oral decoction of the dried shoots[9]. In West Africa and Papua New Guinea,

the dried leaf buds of F. septica are taken orally for headache and gastroenteritis.

A number of Ficus species have shown diverse biological and pharmacological activities. They have been investigated as potential repository of natural products for the treatment of various diseases including tumors, inflammatory diseases, wound healing and as antioxidants.

MATERIALS AND METHODS

Collection of Plant Materials

The roots of *Ficus exasperata* were collected in a plantation in Ondo, Ondo State, Nigeria. The samples were identified at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria, where a voucher specimen has been deposited in the herbarium.

Extraction

500 g of the root bark of *F. exaspetrata* was pulverized and soaked separately with chloroform and methanol at room temperature (25-30°C). After 72 h, the extracts were filtered and later dissolved in Tween 80 followed by normal saline to get the required concentration375 mg/Kg body weight and were used for screening anti-inflammatory activity.

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

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.

Chemicals and Drugs

All the chemicals and drugs used were of analytical grade.

Determination of Acute Toxicity

The lethal dose (LD50) of the plant extract was determined by method of Lorke[10] 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 (LD50) was calculated using the second phase.

Formalin-induced Oedema in Rats

Pedal inflammation was produced in rats according to the method described by Winter et al.[11] Four groups (comprising of six animals each) of rats were treated orally with 375 mg/Kg of MET and CLO extracts 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[12].

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

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.

RESULTS AND DISCUSSION

Extraction and Acute Toxicity

The yield of the CLO extract was 0.5% W/W while that of MET extract was 0.71% W/W dry matter and the acute toxicity test of the extracts produced no death or signs of toxicity after 48 h.

Effect of CLO and MET extracts on Formalin-induced Paw Oedema in Rats

Both extracts at 375 mg/Kg body weight showed remarkable activity against acute inflammation by suppressing the paw oedema. An hour after formalin administration, the reduction in oedema was the same in the animals treated with both extracts and higher than those treated with 10 mg/Kg of diclofenac. The percentage reduction of oedema at 2 and 3 hours, after formalin administration in animals treated with MET extract was higher than those of CLO and the standard, diclofenac. Whereas, at 4 hr the inhibition of oedema by both extracts is almost at par with the standard (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[13].

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[14] tested for activity against acute inflammation, the extracts being administered orally. The results obtained with this model indicates that the root bark of *F. exasperata* possesses significant activity against acute inflammation at the later phase of the experiment.

In this experiment, the MET extract exhibited effects which were better than those of CLO and diclofenac, an anti-inflammatory drug. The mechanisms of the action of the active constituents of the plant may be attributed to the inhibition of the cyclooxygenase pathway of arachidonic acid metabolism, which is the general mechanism of action of Non Steroidal Anti-inflammatory Drug[15].

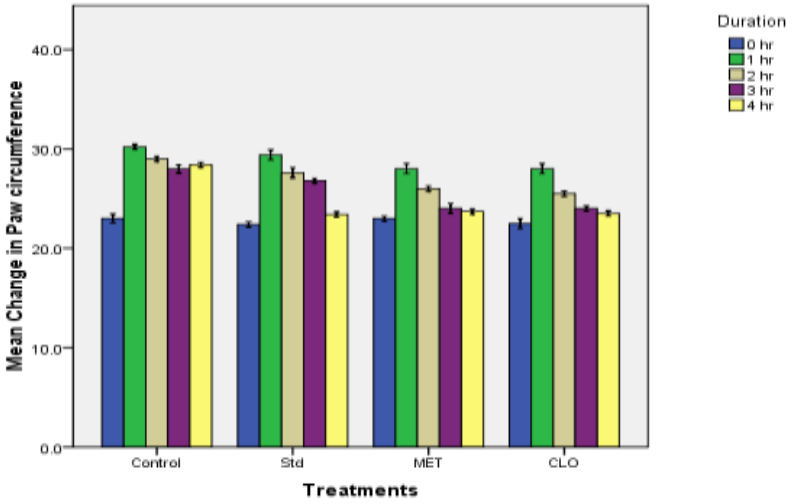

Figure 1: Mean change in paw circumference in mm.
Std = Diclofenac; MET = Methanol extract; CLO = Chloroform extract.

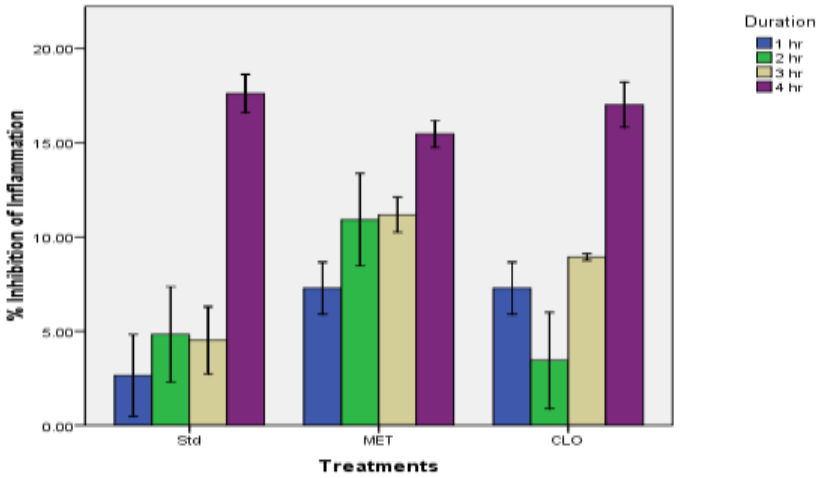

Figure 2: % Inhibition of Inflammation.
Std = Diclofenac; MET = Methanol extract; CLO = Chloroform extract.

CONCLUSION

The results showed that MET at 375 mg/Kg possesses significant anti-inflammatory activity than those of CLO and diclofenac at 1-3 hours after formalin administration and comparable to both CLO and diclofenac at 4 hr.. This supports the use of this plant extract 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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