

# ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC LEAF EXTRACT OF ANTHONOTHA MACROPHYLLA (CAESALPINOIDEAE).

Ibitoye S. F.<sup>1</sup>, Ogbeche K. A.<sup>2</sup>, Ogunleye, D. S.<sup>1</sup>, and Ekor M.<sup>3</sup>

1. Department of Pharmaceutical Medicinal Chemistry Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu.

2. Department of Pharmaceutical Chemistry Faculty of Pharmacy, College of Medicine, University of Lagos, Idi-Araba

3. Department of Pharmacology, Obafemi Awolowo College of Medicine, Olabisi Onabanjo University, Shagamu

## Abstract:

The fresh leaves of *Anthonotha macrophylla* (family Caesalpinoideae) is used in the western part of Nigeria to treat swellings and general body aches. The present study evaluated the anti-inflammatory property of the ethanolic extract of *Anthonotha macrophylla* leaves against formalin induced oedema in rat hind paws using ibuprofen and aspirin as reference compounds.

Results indicate that the extract was very effective at 500mg kg<sup>-1</sup> dose ( $p < 0.001$ ). At 200mg kg<sup>-1</sup> dose, the anti-inflammatory activity was only significant after four hours, while the *Anthonotha Macrophylla* extract was not effective at 100mg kg<sup>-1</sup>. These suggest a dose dependent effect. The preliminary phytochemical studies on the plant revealed the presence of reducing sugars, saponins, cardiac glycosides, flavonoids, anthraquinones and tannins

**Key words:** Anti-inflammatory, Formalin Ibuprofen and Aspirin.

## Introduction

Inflammation is known to be a normal protective response to tissue injury caused by physical trauma, noxious chemicals or microbiologic agents. Inflammation is also the body's response to inactivate or destroy the invading organisms, remove irritants and set stage for tissue repair! Inflammation cannot be triggered by the release of chemical mediators from the injured tissues and migrating cells. The specific chemical mediators differ with the type of inflammatory process and include amines such as histamine, serotonin, lipids such as

prostaglandins and small peptides such as kinins.

*Anthonotha macrophylla* (A. Macrophylla) is a member of the plant Genera Leguminosae-Caesalpinoideae and Swartzieae and of the family caesalpinoideae. Widely distributed in the western part of Nigeria, where decoction of its leaves is commonly used to treat swellings, and general body aches<sup>2</sup>, mention has been made of the use of *A. macrophylla* roots, leaves and their ashes in West African medicine<sup>2</sup>. In the treatment of asthma and other cardiovascular diseases<sup>4</sup>. Drugs from other plants of caesalpinoideae family include sanna leaves and pods, cassia pods and tamarinds<sup>5</sup>. Report of any previous works on *A. macrophylla* has not been made. This paper reports the anti-inflammatory activity observed for the ethanolic extract of *A. macrophylla* leaves.

## Materials and Methods

### Plant collection and preparation:

Fresh leaves of *Anthonotha macrophylla* were collected at Ipara town (Ogun State, Nigeria) and authenticated by Mr. M.T.K. Odewo at forest Research Institute of Nigeria (Ibadan) and a voucher specimen was deposited (Voucher No. Fh1106133).

The leaves were dried in the oven at 45°C, minced into a fine powder, weighed (150 ± 0.1)g, transferred into a conical flask and 1 litre of 95% ethyl alcohol added. This was kept at room temperature for 48 hours, and later decocted thrice with 1 litre of 95% ethyl alcohol after heating for 2 1/2 hours at 45°C. The decoction was filtered, collected and concentrated by evaporation and freeze-dried to give a black crystalline residue (25.23g) 16.82% w/w of the dried starting material. The residue was refrigerated for

## Anti-inflammatory test

Thirty six male and female rats weighing between 80-100g obtained from the animal house of the University of Ibadan, were housed in group of six in six plastic cages. The rats were maintained on commercial rat tubes and water for a week to condition them to our laboratory environment prior to use. A preliminary screening test to ascertain the doses of the extract that could effectively prevent inflammation was done. The test for anti-inflammatory activity is based on the method of winter et al<sup>6</sup>, but the cotton and thread method of Bamgbose and Naomasi<sup>6</sup> was adopted for this investigation. A 0.15ml of 5% formalin in 0.9% saline was injected into the sub plantar tissue of the right hind paws of the animals in group one. The groups 2, 3 and 4 animals were pretreated orally with different doses of 100mg, 200mg and 500mg per kilogramme weight of animal. After an hour, a 0.15ml of 5% formalin in .9% normal saline was injected into the sub plantar tissue of the right hind paw of all the animals: 50mg/kg Ibuprofen (neutralized in 2% Na<sub>2</sub>CO<sub>3</sub> Solution) was administered orally to the animals in group five an hour before being treated as the group one animals, while the group six animals were orally pretreated with 10mg/kg aspirin an hour before the induction of inflammation. The paw size before and after induction of inflammation were determined by the method adopted<sup>6</sup>. Oedema rate or percentage increase in paw size as well as percentage inhibition of inflammation were calculated using an earlier reported expression<sup>7</sup>.

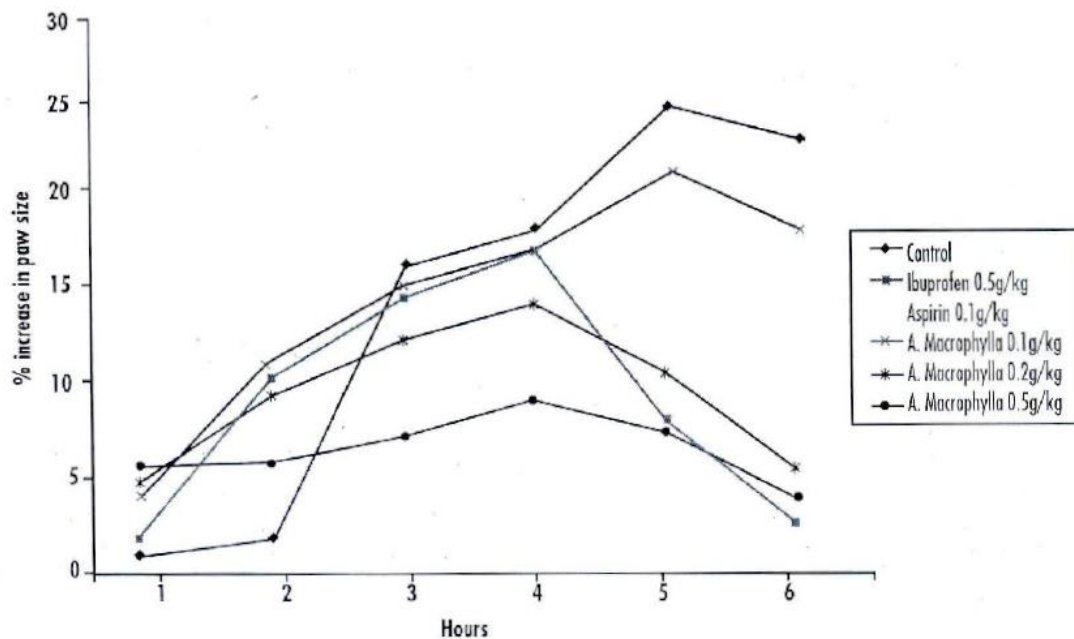
**Results**

The results of this experiment are shown in tables 1 and 2 and figures 1 and 2

Table 1: Effect of *A. macrophylla* extract, Ibuprofen and Aspirin on formalin induced inflammation in rats. (P<0.01)

Test Group	N	% INCREASE IN PAW SIZE (Mean ± SEM)				
		1 hour	2 hour	3 hour	4 hour	5 hour
Control	6	12.11±0.15	17.37±0.24	19.47±0.19	26.32±0.22	24.74±0.19
Ibuprofen 0.5g/kg	6	11.05±0.21	15.47±0.08	18.23±0.08	8.84±0.01	3.32±0.09
Aspirin 0.1g/kg	6	10.70±0.09	13.61±0.09	15.09±0.13	13.09±0.15	11.52±0.08
<i>Anthothona</i> <i>Macrophylla</i> 0.1g/kg	6	11.70±0.07	15.96±0.04	18.09±0.11	22.87±0.08	20.21±0.07
<i>Anthothona</i> <i>Macrophylla</i> 0.2g/kg	6	10.00±0.09	13.16±0.01	15.26±0.15	11.58±0.16	6.32±0.21
<i>Anthothona</i> <i>Macrophylla</i> 0.5g/kg	6	6.25±0.09	7.81±0.21	9.90±0.21	8.33±0.05	4.69±0.22

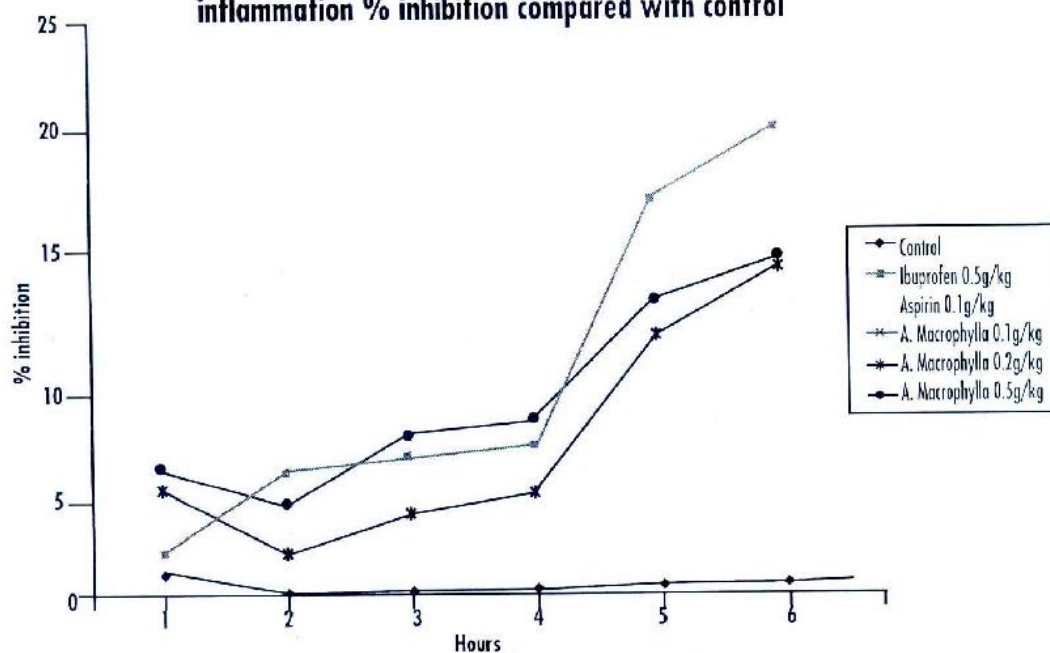
**Fig. 1** Effect of *A macrophylla* extract, ibuprofen and Aspirin on formalin induced inflammation in rats



**Table 2: Effect of Ibuprofen, Aspirin and extract of *A. Macrophylla* on formalin-induced inflammation percent inhibition Compared with control**

Test Group	Percent Inhibition with time (hour)				5 hour
	1 hour	2 hour	3 hour	4 hour	
Control	-	-	-	-	-
Ibuprofen 0.5g/kg	5.63	6.28	6.55	17.92	21.10
Aspirin 0.1g/kg	0.94	2.69	3.49	10.00	10.13
A Macrophylla 0.2g/kg	1.88	3.59	4.37	11.67	14.77
A. Macrophylla .5g/kg	4.23	7.18	7.86	13.33	15.19

**Fig 2: Effect of Ibuprofen, Aspirin and extract of *A macrophylla* on formalin induced inflammation % inhibition compared with control**



The results show that the ethanolic extract (0.2g/kg and 0.5g/kg) reduced the rat paw edema significantly ( $p < 0.05$ ) four and five hours after administration. Lower doses appeared less effective. Ibuprofen (0.5g/kg) effectively reduced the rat paw edema throughout the test period ( $p < 0.05$ ) and aspirin (0.1g/kg) measured after 4 and 5 hours. The preliminary phytochemical screening of plant indicated the presence of anthraquinones, flavonoids, saponins, tannins and sugars.

## Discussion and Conclusion

The rationale behind this work was to scientifically test investigate the traditional use of *A. macrophylla* in inflammation and asthma. The present results show that ethanolic extract of the leaves of *A. macrophylla* possess significant anti-inflammatory activity. The observed activity was dose dependent. This experimental model of inflammation is that for an acute phase, that may be modified to accommodate a prolonged phase<sup>8</sup>: such a model is considered simple and suitable for evaluating a potential anti-arthritic agents<sup>9</sup>. The percentage inhibition of the rat paw oedema was very high at 0.5g/kg dose of the extract; this was so throughout the duration of the test (5hours). The same result was obtained during the 4<sup>th</sup> and 5<sup>th</sup> hour of the test with the animals treated with

0.2g/kg of *A. macrophylla* extract may effectively control an acute inflammatory condition comparable to the reference drugs.

The results obtained for Ibuprofen and Aspirin confirmed the fact that the latter possess less-inflammatory property than the former<sup>7</sup>. The anti-inflammatory effect observed when 0.5g/kg of *A. macrophylla* extract was given seemed to be more pronounced than that of Ibuprofen and Aspirin as can be seen in figure 2. Ibuprofen and Aspirin are known to exert their anti-inflammatory effects by inhibiting the cyclo oxygenase enzyme responsible for prostaglandin synthesis<sup>10</sup>, since *A. macrophylla* extract elicited a comparable degree of anti-inflammatory action to Ibuprofen and Aspirin, the *A. macrophylla* extract might be acting through a similar mechanism. The experimental model adopted is that which involves the release of prostaglandin from tissue that consequently triggers the inflammatory process. The release of prostaglandin can be blocked by Non-steroidal anti-inflammatory (NSA) agents such as phenacetin and acetaminophen<sup>11</sup>. We have successfully demonstrated that ethanolic extract of *A. macrophylla* leaves possess a measurable anti-inflammatory property that is similar to that of the reference NSA agents (Aspirin and Ibuprofen used. The exact mechanism of the action of the *A. macrophylla* extract and the principle(s) responsible are being currently investigated in four research centers coordinated by us. The present report supports the folk medicinal use of this plant in inflammatory afflictions that are associated with pain.

## REFERENCES

1. Underwood JCE. General and Systematic pathology. 3rd edition. Churchill Livingstone; 2000. P. 201.
2. Cotran RS, Kumar V, Collins T. Robbins's pathological basis of disease. 6th edition Saunders company; 2001 P. 51.
3. L. Watson and M. J Dailwitz (1993). The Genera of Leguminosae-caesalpinoideae and Swartzioae. Descriptions, identification, and information retrieval version 29<sup>th</sup> Rev. 2000 P. 46-47.
4. Ake Assi ML (1984). Flore de la cote D'ivoire, etude description of biogeographical aequelques notes ethnobotanique: faculte De Science, University of Abidjan, published by Nouvelle Edition Afrika, Paris France P. 20-23
5. Evans W.C. and Trease (1998) Pharmacognosy (14<sup>th</sup> Edition), Baillere Tindall (publisher) P. 227-300
6. Bamgbose S.O.A. and Naomasi B.K. (1981) on Cryptolepine inhibition of Carrageenan induced oedema. *Planta medica* 42: 392-396
7. Kasim LS, S.F. Ibitoye, V.A. Oladimeji, A.O Fagbohun P. Aziba and M. Ekar: Anti-inflammatory property of ethanolic extract of *Gossypium Arboreum*. *J. Pharmaceut Sci. & Pharm Pract.* Vol. 6(2) April-June 2000. 46-48.
8. Mizushima Y., Tsukada W. and Akimoto T. (1972). A modification of rat adjuvant arthritis for testing anti rheumatic drugs. *Journal of Pharmacy and Pharmacology* 24, 781-785.
9. W.C. Bowman and M.J. Rand Experimental models of inflammation Text book of Pharmacology. 2<sup>nd</sup> edition Blackwell Scientific Publications. Chapter 13 page 16
10. Martin A. Shearn. Antipyretic Anti inflammatory Analgesics Review of Medical Pharmacology 7<sup>th</sup> edition F.H. Meyers and Goldfien Lange Medical Publications (Los. Altos, California 94022. P.280)
11. Martin A Shearn. Pharmacology effects and mechanism of Action of Salicylates; Review of medical Pharmacology (7<sup>th</sup> edition) by F.H. Meyers and Goldfien. Lange medical publications (Los. Altos, California 940022. P. 282.

