



## Anti-inflammatory effect of crude methanolic extract and fractions of African mistletoe *Tapinanthus bangwensis* (Engl. & K. Krause) on wistar albino rats

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### ABSTRACT

The anti-inflammatory activity of crude methanolic (MeOH) extract and its partitioned fractions [ethylacetate (EtOAc) and butanol (BuOH)] of leaves of *Tapinanthus bangwensis* on wistar albino rats was investigated. The methanolic extract and fractions (EtOAc and BuOH) at doses of 10 and 100mg/kg body weight exhibited varying degrees of anti-inflammatory activities with BuOH and EtOAc fractions (100mg/kg) showing high and significant ( $p \leq 0.05$ ) inhibition (67.32 and 38.56%) respectively at the 3<sup>rd</sup> h. The EtOAc and BuOH fractions (100mg/kg) reduced significantly ( $p \leq 0.05$ ) paw oedema volume ( $0.94 \pm 0.02$  and  $0.5 \pm 0.01$  ml) when compared to control (0.9% saline) ( $1.62 \pm 0.07$  ml) at the 3<sup>rd</sup> h. The administration of 10mg/kg dose of MeOH extract and its fractions ((EtOAc and BuOH) caused a mild inhibition at the 3<sup>rd</sup> h ( $4.56 \pm 0.26$ ,  $16.99 \pm 0.83$  and  $29.41 \pm 0.22\%$ ) respectively while mean percent inhibition was recorded as  $3.47 \pm 0.18$ ,  $13.35 \pm 0.33$ , and  $25.55 \pm 0.47\%$  respectively after four hours of carrageenan injection. However, the BuOH fraction (100mg/kg) gave the highest percent inhibition ( $67.32 \pm 0.17\%$ ) which was significant ( $p \leq 0.05$ ) at the 3<sup>rd</sup> h. The anti-inflammatory actions of the extract (MeOH) and partitioned fractions (EtOAc and BuOH) of *T. bangwensis* may be due to an inhibitory effect on mediators of inflammation. However, the results obtained indicate that the methanolic extract (MeOH) and partitioned fractions (EtOAc and BuOH) possess anti-inflammatory effect and this may justify the use of this plant in the management of inflammatory disease conditions.

**Key words:** anti-inflammatory, carrageenan, fractions, paw oedema, *Tapinanthus bangwensis*.

### INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects [1, 2]. The research into plants with alleged folkloric use as pain relievers and anti-inflammatory agents, should therefore be viewed as a fruitful and logical

research strategy in the search for new analgesic and anti-inflammatory drugs [3]. They form the basis for medical treatment in many developing countries [4, 5, 6, 7]. It is to this class that the African mistletoe (*T. bangwensis*); family-Loranthaceae belongs. Mistletoe is a semi-parasitic evergreen plant found growing on a host of evergreen and deciduous trees all year round, around the branches of the tree. It is an obligate parasite, obtaining part of its food from the host plant. It depends on its host for minerals and water only, but photosynthesizes its carbohydrate by means of its green leathery, oblong leaves [8]. In Nigeria and some other parts of Africa, mistletoe has been used traditionally as antihypertensive and antidiabetic plant [9, 10]. There have been reports on the phytochemical and antimicrobial properties of African mistletoe *Loranthus micranthus* [11].

Carrageenan-induced rat paw oedema is a widely used test to determine the anti-inflammatory activity, and it has been fully characterized in the past [12, 13, 14, 15]. More recently, it has been shown that cyclooxygenase-2 (COX-2) reaches maximal expression 1 h from carrageenan local injection [16]. Mouse paw oedema has been increasingly used to test new anti-inflammatory drugs as well as to study the mechanisms involved in inflammation. In literature, there are about 400 reports where mouse paw oedema has been used [17]. In 1987, Henriques and co-workers showed that carrageenan injection into the mouse paw induces a biphasic oedema [18]. The early phase (1 – 2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages [19, 20]. Considering that report on the anti-inflammatory activity of this plant is very scanty, the study was therefore aimed at investigating the anti-inflammatory activity of the methanolic extract (MeOH) and its partitioned factions (EtOAc and BuOH) with a view to justifying the use of the plant in the treatment of oedema, pain and inflammation.

## MATERIALS AND METHODS

### Plant Materials:

#### Collection and Authentication of plant materials

The African mistletoe (*T. bangwensis*) was collected from the orchard near the Vice Chancellor's lodge, Delta Park, University of Port Harcourt, Port Harcourt, Rivers State Nigeria. The plant was identified by Mr. N.L Edwin-Wosu of the Department of Plant Science and Biotechnology, University of Port Harcourt and Voucher specimens were deposited with the Herbarium in the Department.

#### Extraction of plant

The dried leaves of *T. bangwensis* were pulverized into a fluffy mass. Five hundred grams of the powdered leaves of *T. bangwensis* was extracted with 8 litres of 80 % MeOH using Soxhlet extractor for 24 h. The extract solution was evaporated to dryness under reduced pressure and below 40 °C to yield a crude methanolic extract.

#### Fractionation of the extract

The methanolic extract of *T. bangwensis* was suspended in H<sub>2</sub>O and partitioned successively with ethyl acetate (EtOAc) and n-butanol (BuOH). Each extract was evaporated to dryness under reduced pressure to yield ethyl acetate, n-butanol and aqueous fractions respectively.

**Experimental animals**

Wistar albino and rats weighing 120–180 gm of either sex from the Animal House of HEJ Research Institute of Chemistry, University of Karachi, Pakistan were used through out the study. They were kept under standard environmental conditions at 25 °C with 12:12 h light–dark cycle in ventilated plastic cages. Animals were fed with a standard rodent diet and water *ad libitum*. The experiment was performed in accordance with the guidelines established by the European Community for the Care and Use of Laboratory Animals and were approved by Institutional Animal Ethical Committee (IAEC) of University of Karachi.

**Acute toxicity test:**

Healthy Wister albino rats of both sexes weighing 170-200g maintained under standard laboratory conditions were used for acute toxicity test according to OECD guidelines 425 [21]. A total of five animals were used. Animals were administered a single oral-dose (2000mg/kg, body weight) of crude MeOH extract of leaves of *T. bangwensis*. Animals were kept overnight fasting prior to drug administration of crude extract by oral gavage. After administration of *T. bangwensis*, food was withheld for further 3-4 h. Animals were observed individually at least once during first 30 min after dosing, periodically during first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days.

**Anti-inflammatory activity**

The anti-inflammatory activity was determined in rats according to the method of Winter et al. [22] with slight modifications. The rats were randomly divided into four groups of 6 animals each. Before treatment, the average volume of the right hind paw of each animal was measured 3–4× by plethysmometer 7140, (Ugo Basile, Italy). The measurements which did not differ by more than 2% represent the initial paw volume ( $V_0$ ). Control (0.9% saline) or standard drug (Indomethacin at 10mg/kg body weight) or methanolic extract and partitioned fractions (ethylacetate and butanol) at 10 and 100 mg/kg body weight respectively derived from *T. bangwensis* were administered to the animals intraperitoneally. Thirty minutes after the administration of test compounds, each rat received in its right hind paw a subplantar injection of a 1%  $\lambda$ -carrageenan, (Type IV) suspension (0.05 ml per animal). The volume of the right hind paw was determined again at 1st, 2nd, 3rd and 4th hour after carrageenan treatment ( $V_f$ ). The percent inhibition in oedema volume was calculated as described [23].

$$\% \text{ inhibition of edema} = \frac{[(V_f - V_0)_{\text{control}} - (V_f - V_0)_{\text{treated}}] \times 100}{(V_f - V_0)_{\text{control}}}$$

Where,

$V_0$  = Paw volume before oedemogen administration (i.e. initial paw volume)

$V_f$  = Paw volume after oedemogen administration

$V_f - V_0$  = Paw volume

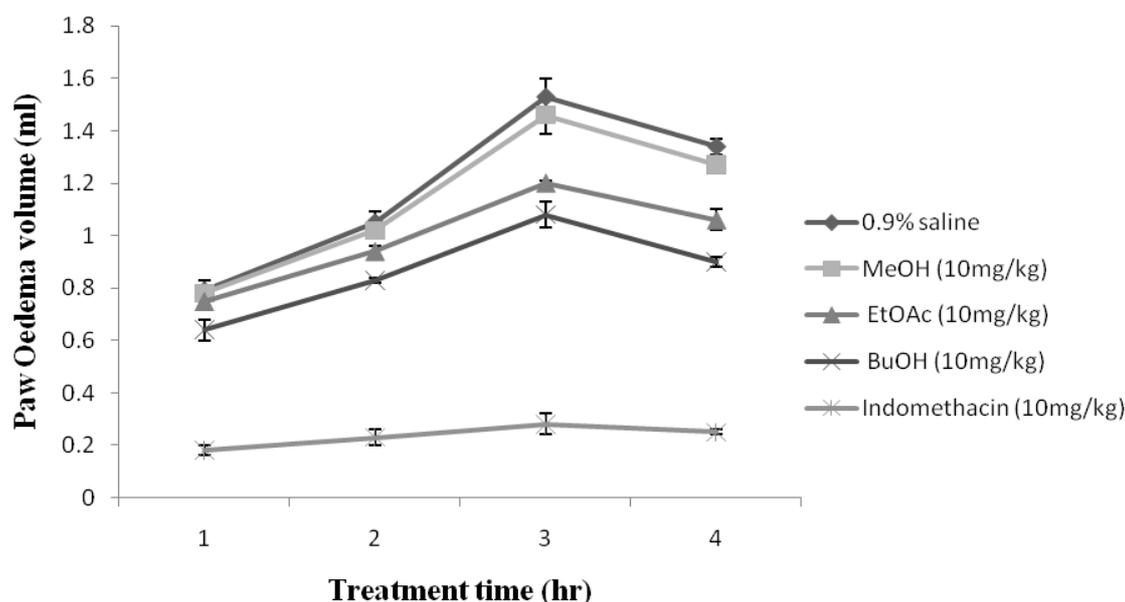
**Statistical analysis:**

The data were represented as mean  $\pm$  standard error of mean (SEM) or as percentages. The statistical significance was determined by one-way analysis of variance followed by Dunnet's test, with the level of significance set at  $P < 0.05$ . Since at 3rd hour of observation inflammation

is maximum and stable, the data at this particular time period is most reliable to analyse the drug - induced anti-inflammatory effects.

## RESULTS AND DISCUSSION

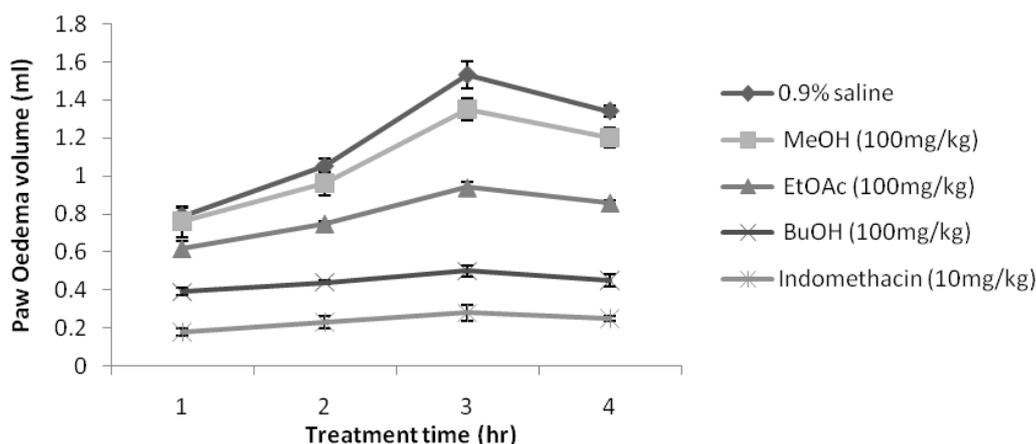
Result from our study on acute toxicity test showed no mortality or physical changes in skin and fur, eyes and mucus membrane (nasal), respiratory rate, circulatory signs (heart rate and blood pressure), autonomic effects (salivation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) among rats administered 2000mg/kg BW of crude MeOH extracts of *T. bangwensis*. Since none of the mentioned toxic signs and symptoms or mortality was observed in the animals at the above mentioned dose, 10 and 100mg/kg body weights of extracts were selected for evaluation of anti-inflammatory activity. The effect of methanolic extract and partitioned fractions (EtOAc and BuOH) of leaves of *T. bangwensis* in carrageenan-induced paw oedema in rats is shown in figures 1 and 2.



**Figure 1: Effect of the methanolic extract and partitioned fractions (EtOAc and BuOH) [10mg/kg body weight] and indomethacin (10mg/kg body weight) on carrageenan-induced hind paw oedema in rats.** Each point represents mean  $\pm$  S.E.M (n=6).  $p \leq 0.05$ . MeOH = methanol extract of *T. bangwensis*; EtOAc = ethylacetate fraction of *T. bangwensis*; BuOH = butanol fraction of *T. bangwensis*

Results show that the anti-oedematogenic response occurred in a dose - dependent manner. The EtOAc and BuOH fractions of *T. bangwensis* at a dose of 100mg/kg prevented the formation of oedema induced by carrageenan. The study of the acute anti-inflammatory test demonstrated that butanol fraction of *T. bangwensis* administered at a dose of 100mg/kg produced a maximum inhibition (67.32%) and mean percentage inhibition of 62.11% through out the four hours of observation. It was noticeable that EtOAc fraction at 100mg/kg showed a maximum percentage inhibition (43.28%) after four hours of observation. Among the treatment groups administered 10mg/kg, only BuOH fraction at the end of the 4<sup>th</sup> hr showed a percentage inhibition greater than 30% (Table 1) whereas maximum percentage inhibition for methanolic extract (11.76%) at a

dose of 100mg/kg was observed at the 3<sup>rd</sup> hr. However, indomethacin at 10mg/kg exhibited a maximum percentage inhibition (81.70%) at the 3<sup>rd</sup> hr. In general, the BuOH fraction at 100mg/kg produced the highest inhibition ( $67.32 \pm 0.17\%$ ) of paw oedema.



**Figure 2: Effect of the methanolic extract and partitioned fractions (EtOAc and BuOH) [100mg/kg] and indomethacin (10mg/kg body weight) on carrageenan-induced hind paw oedema in rats.**

Each point represents mean  $\pm$  S.E.M ( $n=6$ )  $p \leq 0.05$ . MeOH = methanol extract of *T. bangwensis*; EtOAc = ethylacetate fraction of *T. bangwensis*; BuOH = butanol fraction of *T. bangwensis*

**Table 1: Percentage inhibition of carrageenan-induced rat paw oedema by methanolic extract and partitioned fractions (EtOAc and BuOH) of leaves of *T. bangwensis***

Treatment	Percentage inhibition (%) at various times intervals (Mean $\pm$ SEM)				
	1h	2hr	3hr	4hr	Mean of % inhibition
<b>T.b MeOH</b>					
10mg/kg	1.27 $\pm$ 0.10	2.86 $\pm$ 0.16	4.56 $\pm$ 0.26	5.22 $\pm$ 0.20	3.47 $\pm$ 0.18
100mg/kg	3.73 $\pm$ 0.87	8.57 $\pm$ 0.62	11.76 $\pm$ 0.43	10.45 $\pm$ 0.80	9.38 $\pm$ 0.68
<b>T.b EtOAc</b>					
10mg/kg	5.06 $\pm$ 0.10	10.48 $\pm$ 0.14	16.99 $\pm$ 0.83	20.90 $\pm$ 0.25	13.35 $\pm$ 0.33
100mg/kg	21.52 $\pm$ 0.20	28.57 $\pm$ 0.14	38.56 $\pm$ 0.83	43.28 $\pm$ 0.32	32.98 $\pm$ 0.37
<b>T.b BuOH</b>					
10mg/kg	18.99 $\pm$ 0.52	20.95 $\pm$ 0.80	29.41 $\pm$ 0.22	32.84 $\pm$ 0.34	25.55 $\pm$ 0.47
100mg/kg	50.63 $\pm$ 0.23	58.10 $\pm$ 0.14	67.32 $\pm$ 0.17	66.42 $\pm$ 0.42	62.11 $\pm$ 0.24
<b>Indomethacin (10mg/kg)</b>	77.22 $\pm$ 0.62	78.10 $\pm$ 0.50	81.70 $\pm$ 0.52	81.34 $\pm$ 0.49	79.59 $\pm$ 0.53

Each value is mean  $\pm$ SEM ( $n=6$ ). *T.b MeOH* = methanolic extract of *T. bangwensis*; *T.b EtOAc* = ethylacetate fraction of *T. bangwensis*; *T.b BuOH* = Butanol fraction of *T. bangwensis*.

The present study shows that the phlogistic agent, carrageenan produced a time-dependent oedema in the control rats with a gradual increase reaching its maxima ( $1.53 \pm 0.10$ ml) at 3<sup>rd</sup> h followed by a decline at 4<sup>th</sup> h ( $1.34 \pm 0.07$ ml). The observation of maximum volume of hind paw

oedema at the 3<sup>rd</sup> h has been reported [24] and appears to be a reliable time for observing anti-inflammatory activity of compounds. The results from our study showed that the significant inhibitory activity ( $p \leq 0.05$ ) shown by the methanolic extract and partitioned fractions (EtOAc and BuOH) at a dose of 10 and 100mg/kg over a period of 4 h in carrageenan-induced inflammation was quite similar to that exhibited by the group treated with Indomethacin described in the control rats. The administration of methanolic extract and partitioned fractions (EtOAc and BuOH) of *T. bangwensis* at doses of 10 and 100mg/kg mildly inhibited the oedema even in the first hour with the lowest percentage inhibition of (1.27%) observed in the group administered 10mg/kg methanolic extract while the maximum percentage inhibition of (50.63%) among the treatment groups in the first hour was recorded in rats administered 100mg/kg butanol fraction. This finding corroborates earlier studies in the carrageenan-induced inflammation models that high anti-inflammatory activity observed at the first hour may be due to inhibition of mediators of the first phase of inflammation and prostaglandins and bradykinins which are released during the second phase of inflammation [25, 26, 27]. Based on this, it could be argued that the suppression of the first phase as exhibited by the administration of 10 and 100mg/kg of methanolic extract and fractions (EtOAc and BuOH) of leaves of *T. bangwensis* may be due to inhibition of the release of early mediators such as histamine and serotonin, and the action in the second phase may be explained by an inhibition of cyclooxygenase. The fact that the partitioned fractions (EtOAc and BuOH) of leaves of *T. bangwensis* at a dose of 100mg/kg inhibited the inflammatory oedema induced by irritant agent equipotently throughout the four hours of observation may be explained by the assumption that either the fractions has longer half life or is stable similar to indomethacin which not only inhibits initial inflammatory process where histamine, serotonin and kinin are the main mediators but also inhibited the oedema up till 4 h by inhibiting prostaglandin synthesis [28]. This could explain the fact that the EtOAc and BuOH fractions of leaves of *T. bangwensis* may contain compounds that maintain the level of activity for longer duration by non-selective interaction with the histamines, serotonins, kinins and prostaglandins.

Inflammation has been described as a response of living tissues to injury and it is known to involve a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair [29]. The most widely used primary test to screen new anti-inflammatory agents measure the ability of a compound to reduce local oedema induced in the right paw by injection of an irritant agent [21]. Carrageenan-induced paw oedema in rats is a classical model of acute inflammation [21] widely used in screening of drugs [30, 25]. Development of oedema in the paw of the rats after injection of carrageenan is a biphasic event. The early phase (1-2 h) of the carrageenan model is thought to be mainly mediated by histamine, serotonin and increased synthesis of prostaglandin in the damaged tissue surroundings. The late phase (3-4 h) has been shown to be sustained by prostaglandin release and mediated by bradykinins, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages [19, 20].

In a comparative study between the methanolic extract and the two fractions (EtOAc and BuOH), the results showed that the BuOH fraction had the highest anti-inflammatory action against carrageenan-induced inflammation. Our investigation showed that the highly polar methanolic extract of *T. bangwensis* at 10 and 100mg/kg was found not to have reduced paw oedema significantly ( $p \leq 0.05$ ) in the presence of the oedemogen. This suggests that methanolic

extract neither prevents mast cell degranulation nor interfere with histamine, serotonin and bradykinin action and hence may not have obvious role in preventing the initial phase of inflammation.

Our earlier study on the phytochemical constituents of the methanolic extract showed the presence of flavonoids, tannins, alkaloids, phlobatannins, anthraquinones and cardiac glycosides while three phenolic compounds of methyl gallic acid derivatives (Methyl syringate, 3, 4, 5- tri-methyl gallic acid and Methyl 4, 5 – dimethoxy-2 –hydroxybenzoate) were isolated from the EtOAc fraction of leaves of *T. bangwensis* (in press). The anti-inflammatory activity exhibited by the methanolic extract and fractions of *T. bangwensis* may be attributed to the presence of flavonoids [31, 32] and tannins [33] present in the plant.

Our finding from the acute toxicity test also suggests that the crude extract of leaves of *T. bangwensis* plant was safe in or non-toxic to rats at 2000mg/kg body weight and hence 10 and 100mg/kg body weight of crude Methanolic extract and fractions (EtOAc and BuOH) of *T. bangwensis* were selected for in-vivo study.

In conclusion, it is suggested that the EtOAc and BuOH fractions of leaves of *T. bangwensis* antagonize the initial and late phase of inflammation but with maximum inhibition during the late phase via inhibition of mast cell secretion and/ or possessing antihistaminic activity and interacting with arachidonate metabolism in different fashion. The highly polar methanolic extract was devoid of such effect. The results support the traditional use of this plant in inflammatory conditions and suggest the presence of biologically active components which may be worth further investigation and elucidation. However, further studies are needed to isolate and characterize the anti-inflammatory chemical constituents present in both the methanolic extract and BuOH fraction of the plant.

#### **Acknowledgement:**

The corresponding author wishes to acknowledge the Academy of Sciences for the Developing World (TWAS) for the award of the TWAS-ICCBS Postgraduate Fellowship and The International Center for Chemical and Biological Sciences (ICCBS) University of Karachi, Pakistan for providing the necessary facilities. Also acknowledged is Prof. Dr. Ahsana Dar of ICCBS.

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