# EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF THE METHANOLIC LEAF EXTRACT OF *ADENODOLICHOS PANICULATUS* (HUA)

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## ABSTRACT

The plant *Adenodolichos paniculatus* is a shrub of up to 4m high of Savanna (bush and jungle) from Guinea to Northern Nigeria and across to Sudan. The plant has been reported to be used traditionally in tooth ache, blennorrhea and in treatment of burns. In this work, the analgesic and anti-inflammatory activities of the methanolic leaf extract of *Adenodolichos paniculatus* (MAP) were studied. MAP (75, 150 and 300 mg/kg i.p) was evaluated for analgesic and anti-inflammatory activities using acetic acid induced writhing and Carrageenan induced paw edema in mice and rats respectively. The methanolic leaf extract exhibited significant ($P < 0.05$) and dose dependent analgesic and anti-inflammatory effects comparable to that of the reference drug Ketoprofen (20 mg/kg body weight i.p) which is a standard analgesic and anti-inflammatory drug. The preliminary phytochemical screening of the MAP revealed the presence of flavonoids, tannins, glycosides, anthraquinones and phenols. The intraperitoneal median lethal dose ($LD_{50}$) of MAP in mice was found to be 1113.3mg/kg and the oral $LD_{50}$ in rats was found to be greater than 5,000 mg/kg. body weight. The findings suggest that the MAP possesses analgesic and anti-inflammatory activities, and supports the ethno medical claims of the use of the plant in the management of painful and inflammatory conditions.

**Keywords:** *Adenodolichos paniculatus*, analgesic, anti-inflammatory, phytochemistry and ketoprofen.

## INTRODUCTION

Medicinal herbs have been used as a form of therapy for the relief of pain throughout history[1]. The treatment of rheumatic disorder is an area in which the therapeutic efficacy. Taking into account the most important analgesic prototypes practitioners of traditional medicine enjoy patronage and success[2]. Natural products in general and medicinal plants in particular, are believed to be an important source of new chemical substances with potential (e.g. salicylic acid and morphine) were originally derived from the plant sources,

the study of plant species traditionally used as pain killers should still be seen as a fruitful research strategy in the search of new analgesic and anti-inflammatory drugs. Medicinal plants with anti-inflammatory activity are considerably employed in the traditional treatment of disorders of inflammation. The inflammatory responses involves a complex array of enzyme activation, mediator release fluid extravasations, cell migration, tissue breakdown and repair which are aimed at host defense and usually activated in most disease conditions[3]. These different reactions in the inflammatory response cascade are therapeutic targets which anti-inflammatory agents including medicinal plants interfere to suppress exacerbated inflammatory responses usually evoked in such disorders as rheumatoid arthritis, in infection or injury. Inhibition of the synthesis of pro-inflammatory prostaglandins is one of such therapeutic targets to which some of the potent anti-inflammatory agents of clinical relevance (e.g NSAIDs) owe their activity[4]. Several anti-inflammatory have also demonstrated the ability to inhibit the synthesis of synthesis of prostaglandins[5].

***Adenodolichos paniculatus*** (HUA) family Leguminosae:papilonoideae is a shrub of up to 4m high of savanna (bush and jungle) from Guinea to Northern Nigeria, and across to Sudan. The common name, "waken wuta" in Hausa, means fire beans. This is perhaps because the plant springs up freely after the bush [6]. The leaf is applied in Nigeria topically with butter oil as a dressing for burns and to wrap the hands or feet in staining with henna[6]. In Ubangi the leaf has been used in toothache[7]. In Central Africa Republic, the root decoction has been used for blenorrhoea and liver trouble [7]. The aim of the study is to evaluate the analgesic and anti-inflammatory properties of the methanol leaf extract of *Adenodolichos paniculatus* (MAP).

**Materials and methods**

The leaves of *Adenodolichos paniculatus* were collected from Kundun village, Birnin Gwari Road Kaduna State, in June, 2009. The leaves were identified and authenticated by Mallam Umar S. Gallah of the herbarium section, department of biological sciences, ABU Zaria. A voucher specimen (Number 3107) was deposited at the herbarium for future reference.

The Leaves were cleaned, air dried and crushed into powder with pestle and mortar. A portion of the powdered leaf was macerated with 70% methanol for 48 hours with occasional shaking. The solvent was

evaporated to obtain the concentrate of the crude extract and yield 8.44 %

**Experimental animals**

Swiss albino mice (18-30 g) and Wister rats (160-250 g) of both sexes were obtained from Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. The rats, maintained on rodent feed and water *ad libitum* were housed in propylene cages at room temperature throughout the study. All experimental protocols were approved by the University Animal Ethics Committee.

**Phytochemical analysis**

Adenodolichos paniculatus was subjected to phytochemical screening using standard protocol [8]

**Drugs / chemicals**

The following drugs and chemicals were used: Carrageenan (Sigma- Aldrich) Acetic acid (Ranbaxy Laboratories Ltd., Punjab), Ketoprofen (Lek Pharmaceuticals, Slovenia). The methanolic leaf extracts of Adenodolichos paniculatus (75, 150, 300 mg/kg)

**Acute Toxicity ($LD_{50}$) Studies in Mice (i p) and Rat (p.o.)**

The acute toxicity study ($LD_{50}$) of the methanolic extract was determined intraperitoneally in mice and rats orally (p.o) using the method described by[9]. The animals were fasted overnight and the $LD_{50}$ evaluation was carried out in two stages. In the first stage, three (3) groups of three (3) mice each case were treated with the extract at doses of 10, 100 and 1000 mg/kg body weight intraperitoneally (i.p). The mice were monitored for 24 hrs for signs and symptoms of toxicity, such as, paw licking, salivation sedation, convulsion and mortality. In the second phase, four (4) groups of one mouse each were further treated with the extract at doses of 200, 400, 800 and 1,600 mg/kg body weight respectively. The mice were also observed for 24 hrs for signs and symptoms of toxicity and mortality. The $LD_{50}$ value was then calculated as the geometric mean of the highest non-lethal dose (with no death) and the lowest lethal dose (where death occurred). For the oral $LD_{50}$ in rats the same procedures was employed but in the second phase three (3) groups of one rat were administered with the MAP at doses of 1600, 2900 and 5,000 mg/kg body weight respectively. .

**Analgesic Study**

The analgesic activity of the extract was evaluated using the following method.

**Acetic Acid-induced Writhes in Mice**

The experiment was carried out according to the method described by[10]. Thirty (30) mice were randomly divided into five (I-V) test groups of six (6) mice per group. Groups II, III and IV were pre-treated *i.p* with the extract at doses of 75, 150 and 300 mg/kg body weight respectively. Groups I and V were pre-treated (*i.p*.) with normal saline 10 ml/kg and ketoprofen 20 mg/kg body weight respectively. After 30 minutes, each mouse in the groups were then injected intraperitoneally (i.p.) with 1 ml/kg of aqueous (0.6% $^{w}/_{v}$) acetic acid solution and placed in a transparent cage. After a five (5) minute lag period, the number of writhes (abdominal constriction accompanied with full stretching of hind limbs) was counted for each mouse, using tally counters, for a period of 10 minutes. The numbers of acetic acid-induced writhes in groups II – IV were compared with that of group I (negative control) which received acetic acid and normal saline only.

**Anti-inflammatory Study**

**Carrageenan-induced Paw Oedema**

The test in rats was carried out according to the method described by[11]. Thirty (30) healthy rats were divided into five (5) groups of six (6) mice each. Groups I, II, and III were pre-treated with the extract at doses of 300, 150 and 75 mg/kg body weight (i.p.) respectively. Group IV and V were pre-treated with normal saline (10 ml/kg as control) and ketoprofen (20 mg/kg) respectively. After 30 minutes, 0.1 ml carrageenan suspension (1% $^{w}/v$ in normal saline) was injected into the sub-plantar region of the left hind paw of each rat. The paw diameter was measured with the aid of venier caliper, at 0, 1, 2, 3, 4 hours respectively after injection of carrangeenan.

**Statistical Analysis**

The data were expressed as mean ± SEM (standard error of mean) and analyzed statistically using student's t-test. P values less than 0.05 ($P < 0.05$) were considered to be statistically significant.

**RESULTS**

**Phytochemical screening**

The phytochemical constituents present in methanolic seed extract of *Adenodolichos paniculatus* include alkaloids, anthraquinones, carbohydrates, flavonoids, glycoside, tannins, saponins, and phenols. **(Table 1)**

**Acute toxicity ($LD_{50}$) study**

The behavioural signs of toxicity exhibited by mice that received extracts at dose of 100 mg/kg and above are itching, unsteady movement, excitation and hyperactivity. The intraperitoneal $LD_{50}$ of the extract in

mice was found to be 1131.3 mg/kg and oral $LD_{50}$ in rats was found to be greater than 5,000 mg/kg body weight.

**Acetic acid-induced writhing**

The extract significantly ($P < 0.05$) decreased the number of acetic acid-induced writhes in mice in a dose dependent manner. However, the effect of the methanolic leaf extract of the plant at 100 mg/kg body weight was greater than that of standard (ketoprofen) at a dose of 20 mg/kg body weight **(Table 2).**

**Carrageenan-induced paw oedema**

The extract significantly ($P < 0.05$) inhibited Carrageenan induced paw 0edema at third hour. However, this was considerably greater than that of Ketoprofen which afforded 48.1% protection **(Table3)**.

**DICUSSION**

The median lethal dose value of the extract found to be 1131.3mg/kg suggests that it is non-toxic at the graded doses (75, 150 and 300 mg/kg) used in the study. $LD_{50}$ values > 1000 mg/kg are considered as safe (Lorke, 1983). The oral $LD_{50}$ of the extract was estimated to be greater than 5000 mg/kg in both mice and rats. The Organization for Economic Cooperation and Development (OECD, Paris, France)[12] recommended chemical labeling and classification of acute systemic toxicity based on oral $LD_{50}$ values as: very toxic, $\leq$ 5 mg/kg; toxic, $> 5 \leq 50$ mg/kg; harmful, $> 50 \leq 500$ mg/kg; and not toxic or harmful, $> 500 \leq 2{,}000$ mg/kg. Based on this classification, the oral $LD_{50}$ up to 5,000 mg/kg established for both mice and rats indicated relative oral safety. Lack of overt toxicity signs in these experimental animals also pointed to that fact. Extracts of plants that contain flavonoids are known to modify the production of cyclo-oxygenase (COX-1 and 2) and lipoxygenase (LOX)[13] which are essential in prostaglandin biosynthesis. Flavonoids also possess anti-oxidant activity which is presumed to be responsible for inhibitory effect on several enzymes including those involved in Arachidonic acid metabolism [14].

The data presented here suggests that the MAP possesses anti-nociceptive and anti-inflammatory activities. The extract at the doses tested was shown to possess anti-nociceptive activity evident in the nociceptive model, signifying it possesses peripherally mediated activity. The abdominal constriction response induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics[15]. In general, acetic acid causes pain by liberating endogenous substances such as

serotonin, histamine, prostaglandins (PGs), bradykinins and substance P, which stimulate nerve endings. Local peritoneal receptors are postulated to be involved in the abdominal constrictions response. The method has also been associated with prostanoids in general, that is, increased levels of $PGE_2$ and $PGF_{2a}$ in peritoneal fluids[16], as well as lipoxygenase products. The significant reduction in acetic acid-induced writhes by the methanolic leaf extract of Adenodolichos paniculatus suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and relases of PGs[17] and other endogenous substances.

Carrageenan induced paw oedema is a commonly used primary test for the screening of new anti-inflammatory agents and is believed to be biphasic16. The first phase (1-2 hr) is due to the release of histamine or serotonin and the second phase of oedema is due to the release of prostaglandin[18]. The results of this study indicate that the methanolic extract of Adenodolichos paniculatus significantly reduced carrageenan induced paw oedema in rats. Therefore, the mechanism of action may be by inhibition of histamine, serotonin or prostaglandin synthesis. Usually most anti-inflammatory and analgesic drugs possess antipyretic activity. In general, non-steroidal anti-inflammatory drugs produce their antipyretic action through the inhibition of prostaglandin synthetase within the hypothalamus[19]. The anti-inflammatory and antipyretic activities of methanolic extract may be due to the presence of alkaloids, sterols and flavonoids.

## CONCLUSION

The results of the present study indicate the anti-inflammatory and analgesic activities of the leaves of Adenodolichos paniculatus. However, further investigation is required to isolate the active constituents responsible for these activities and to elucidate the exact mechanisms of action.

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**Table 1**: Phytochemical screening of MAP

| Phytochemical constituents | A. paniculatus |
|---|---|
| Flavonoids | + |
| Alkaloids | + |
| Glycosides | + |
| Tannins | + |
| Saponins | + |
| Cardiac glycosides | + |
| Phenols | + |

Key + = positive, - = negative

**Table 2: Effect of MAP on Acetic Acid-Induced Writhing in Mice**

| Treatment | Dose (mg/kg) | Mean Abdominal Writhes ± SEM | % Inhibition |
|---|---|---|---|
| Normal saline | 10 | 24.7 ± 1.2 | - |
| Extract | 75 | 17.2 ± 4.0[a] | 30.4 |
| Extract | 150 | 8.7 ± 1.9[d] | 64.8 |
| Extract | 300 | 7.2 ± 0.8[d] | 70.9 |
| Ketoprofen | 20 | 14.8 ± 2.8[b] | 40.1 |

Values presented as mean ± standard error of mean (SEM) and a,b,c and d represent $p<0.05$, $p<0.025$, $p<0.005$ and $p<0.0005$ level of significance respectively.

**Table 3**: **Effect of MAP on Carrageenan-Induced Paw Oedema in Rats**

| Treatment (Dose) | Mean Paw Oedema (cm) ± SEM (% Inhibition) | | | | |
|---|---|---|---|---|---|
| | **0 hr** | **1 hr** | **2 hr** | **3 hr** | **4 hr** |
| Normal saline (10 mg/kg) | 0.±0. | 0.162±0.009 | 0.170±0.024 | 0.160 ±0.013 | 0.130±0.018 |
| Extract (75 mg/kg) | 0.±0. | 0.102±0.021[b] (37.0) | 0.087±0.012[b] (48.8) | 0.100±0.009[b] (37.5) | 0.092±0.002[b] (29.2) |
| Extract (150 mg/kg) | 0.±0. | 0.072±0.016[d] (55.6) | 0.082±0.012[d] (51.8) | 0.085±0.013[d] (46.9) | 0.083±0.012[c] (36.2) |
| Extract (300 mg/kg) | 0.±0. | 0.052±0.010[d] (67.9) | 0.072±0.005[d] (57.6) | 0.063±0.006[d] (60.6) | 0.080±0.004 [d] (38.5) |
| Ketoprofen (20 mg/kg) | 0.±0. | 0.062±0.022 [c] (61.7) | 0.090±0.012 [b] (47.1) | 0.083±0.010 [c] (48.1) | 0.085±0.006 [c] (34.6) |

Values presented as mean ± standard error of mean (SEM) and

a,b,c and d represent $p<0.05$, $p<0.025$, $p<0.005$ and $p<0.0005$ level of significance respectively.