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ANTI INFLAMMATORY AND ANTIPYRETIC ACTIVITIES OF THE METHANOL LEAF EXTRACT OF *Acacia ataxacantha* D.C. (LEGUMINOSAE) IN MICE AND RATS

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ABSTRACT
Acacia ataxacantha (Leguminosae) has been reported to be used in traditional medicine for management of pain and inflammation. The present study was designed to evaluate the anti inflammatory and antipyretic activities of methanol leaf extract of Acacia ataxacantha in rats. The acute toxicity study was carried out using Lorke method (1983). The doses (100, 200 and 400 mg/kg body weight of the extract) selected for the study were based on the calculated LD_{50}. Anti-inflammatory activities were investigated using the carragenaan and albumin induced paw edema, while the antipyretic activity was evaluated using yeast induced pyrexia method. With respect to the negative control (group 1), the carragenaan induced inflammation produced a dose dependent significant ($p \leq 0.05$) reduction of inflammation at 200 and 400 mg/kg (3^{rd} h) while a significant ($p \leq 0.05$) reduction in oedema was observed at doses of 100, 200 and 400mg/kg (4^{th} h). Similarly there were significant inhibitions ($p \leq 0.05$) of inflammation at the 20^{th}, 40^{th}, 60^{th} and 120^{th} minutes post extract administration in albumin induced hind-paw inflammation. The data obtained from the antipyretic study showed no significant effect. These findings suggest that the extract may contain bioactive compounds that possess anti-inflammatory activities, thus supporting the ethno-medical use of the plant in the management of painful inflammation.
Key words; Acacia ataxacantha, Inflammation, Pyrexia, Carragenaan, Albumin, Yeast

INTRODUCTION

Inflammation occurs when immunological competent cells are activated in response to injury or irritants or foreign organisms (antigenic proteins). The outcome of this inflammatory response for the host may either be beneficial (invading organisms are phagocytosed or neutralized), or deleterious (arthritis). It is characterized in the acute form by classical signs of pain, redness (flushing), swelling (oedema), heat (warmth) and loss of function (Perianayagam, 2006; Stankov, 2012). An inflammatory response may be induced in a great variety of ways and these include; trauma, injury, antigens (viral, bacteria, protozoa and fungi), some chemicals (turpentine, croton oil) and other foreign substances which evoke immune responses. The character of the injury, its severity and the site of injury, each modified the progression of inflammatory responses as does the therapeutic intervention being administered (Stankov, 2012).

A local inflammatory response is usually accompanied by systemic changes such as fever (pyrexia), malaise and an increase in circulating leucocytes (Stankov, 2012; Kumar *et al.,* 2013).

Pyrexia (fever) is elevated body temperature (> 37.8 °C orally or > 38.2 °C rectally) or an elevation above a person's known normal body temperature (Felton, 2013). Pyrexia can also results from secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states. The infected or damaged tissue enhances the formation of pro-inflammatory mediators (cytokines like interleukin 1β, α, β and TNF-α) which increase the synthesis of PGE_2 near pre-optic hypothalamus area, thereby triggering the hypothalamus to elevate the body temperature (Spacer and Breder, 1994). Non-steroidal anti-inflammatory drugs (NSAIDS) and paracetamol are employed in the management of inflammatory pain. Due to cost of drug and adverse effects associated with the use of these drugs, majority of people in developing countries rely on medicinal plants in management of inflammatory injuries and pain (Stankov, 2012; Kumar *et al.,* 2013). Hence, the World Health Organization (WHO) encourages the inclusion of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries, despite the huge advancement made in orthodox medicine (WHO, 2002; Elujoba *et al*., 2005; Kareru *et al.,* 2007). Research have shown that herbal medicine contain phytochemicals that can help alleviate injuries and inflammatory conditions (Verma *et al.,* 2011).

Preparations of different parts of *Acacia ataxacantha* are used in traditional medicine as pain reliever, and as anti-inflammatory agent. The stem-back infusion is prepared Guinea as mouth-wash for dental caries and tooth ache. The leaves are prepared and inhaled into the respiratory tract, especially when accompanied with pain (Burkill, 1997; Akapa *et al.,* 2014). There are no scientific data on the acclaimed anti-inflammatory and anti-pyretic activities of the plant. Hence, the present study was designed to evaluate the anti-inflammatory and anti-pyretic effects of methanol leaf extract of *Acacia ataxacantha* in mice and rats.

MATERIALS AND METHODS

Preparation of Extract

The fresh leaves of *Acacia ataxacantha* D.C was collected from Bassawa area of Zaria, Kaduna State, Nigeria, in April 2013. It was identified and authenticated by Mallam Umar. S. Gallah of the Department of Biological Sciences, Faculty of Sciences, Ahmadu Bello University, Zaria. Where herbarium specimen (Voucher Number 1924) was made and deposited. The leaves of the plant were separated from the tree branch, cleaned, air-dried under the shade, and crushed into coarse powder using mortar and pestle. Five hundred grams (500 g) of the powdered plant was cold macerated with 2.5 litres of 70% $^{v}/_{v}$ methanol (in water) for 72 hours. The resultant mixture was filtered using Whatman filter paper (No.1) and concentrated to dryness using evaporating dish over a water-bath. The temperature of the water-bath was maintained at 40-50 ^{o}C to give a constant weight of the dried extract.

Phytochemical Screening of the Plant Extract

Phytochemical screening tests for detecting presence of various chemical constituents were employed (Trease and Evans, 2002).

Acute toxicity (LD_{50}) study in mice and rats

The oral median lethal doses (LD_{50}) of the methanol extract *Acacia ataxacantha* leaf was determined in mice using the method described by Lorke (1983). Briefly, the animals were fasted overnight and the LD_{50} evaluation was carried out in two stages. The first stage, nine mice were randomly divided and placed into three groups of three mice each. Groups I, II and III were treated orally with the extract at doses of 10, 100 and 1000 mg/kg body weight, respectively. The mice were monitored for 24 hrs for signs of toxicity and mortality. In the second phase, four mice were administered with the extract at doses of 1000, 1600, 2900 and 5000 mg/kg body weight, respectively. The second phase design was determined by the result obtained from the first phase (phase I). The mice were also observed for 24 hrs for signs 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).

Anti-inflammatory Studies

The anti-inflammatory studies were carried out using carragenaan-induced paw oedema and albumin-induced paw oedema in rats.

Carragenaan-induced paw oedema in rats

The test was carried out according to the method described by Winter *et al*., (1962). Thirty rats were divided into five groups of five rats each per group. Groups I was pre-treated with normal saline (1 ml/kg) which serve as the negative control. Rats in groups II, III, IV were pre-treated with the extract at doses of 100, 200 and 400 mg/kg body weight (orally) respectively, while group V was treated with piroxicam (orally) 10 mg/kg body weight, which serve as the positive control. One hour post-treatment, 0.1 ml of Carragenaan suspension (1.0 % $^{w}/_{v}$ in normal saline) was injected into the sub-plantar region of the left hind paw of each rat. The paw diameter of the rats was then measured with the aid of Venier caliper, at 0, 1, 2, 3, 4 hours respectively after injection of carragenaan.

Albumin- induced paw oedema in rats

This test was carried out using a modification of Winter *et al*., (1962), as described by Akah and Nwambie (1994). Twenty five rats of either sex were divided into five groups of five rats and pre-treated as follows: Group I received normal saline (1 ml/kg) which serve as the negative control. Groups II, III and IV received 100, 200 and 400 mg/kg of the extract respectively, while rats in group V rats received acetyl salicylic acid (150 mg/kg). All drugs were administered orally. One hour post-treatment, rats in each group were injected with 0.5 ml/kg raw egg albumin (phlogistic agent) in the sub-plantar surface of the left hind-paw. Paw oedema was measured with Venier caliper every 20 minutes for a period of 120 minutes at 20, 40, 60, 80, 100 and 120 minutes after albumin administration. Pedal oedema (inflammation) was evident within 5 - 8 minutes, following fresh egg albumin (0.5 ml/kg) injection into the sub-plantar region of the left hind paw in rats.

Antipyretic Study

The antipyretic activity of the extract of *Acacia ataxacantha* was evaluated using Brewer's yeast-induced pyrexia in rats.

Effect on yeast-induced pyrexia

The procedure described by Al-Ghamdi (2001) was used for this study. The body temperature of each albino Wistar rat was recorded by measuring rectal temperature (RT) at 20 minutes intervals for 60 minutes. Fever was induced in the rats by injecting 15 % ($^{w}/_{v}$) suspension of brewer's yeast (*Saccharomyces cerevisiae*) at a dosage of 1 ml/kg body weight subcutaneously per rat. The rectal temperature of each rat was again recorded after 24 hour of yeast administration. Rats that did not show a minimum increase of 0.5 ^{o}C in temperature 24 hours after yeast injection were discarded. Twenty-five selected rats were grouped into five and immediately treated as follows: group I received normal saline (1 ml/kg) and served as negative control, group II, III and IV received extract at doses of 100, 200 and 400 mg/kg body weight respectively, while group V received Paracetamol (150 mg/kg) body weight, which served as the positive control. All drugs were given orally. Rectal temperature of all the rats was then recorded by inserting digital thermometer into the rectum of each rat at thirty minutes interval for 120 minutes.

Statistical Analysis

The data will be expressed as Mean ± SEM and analyzed using one way analysis of variance (ANOVA), followed by Dunnett-t post-hoc test. Values of $p \leq 0.05$ were considered statistically significant.

RESULTS

Yield of Extracts

Five hundred grams of dried powder of *Acacia ataxacantha* leaf yielded 159.69 g (31.94% $^{w}/_{w}$) of the dried extract on extraction Preliminary phytochemical screening of methanol extract of *Acacia ataxacantha* leaf revealed the presence of the following secondary metabolites (Table .1).

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Table 1: Phytochemical Constituents of Methanol Extract of *Acacia ataxacantha* Leaf

Chemical Constituent	Inference
Alkaloids	+
Anthraquinones	-
Carbohydrates	+
Flavonoids	+
Glycosides	+
Phenols	+
Reducing sugar	+
Saponins	+
Steroidal glycosides	+
Tannins	+
Triterpenes	-

Key + = (Positive) present and - = (Negative) absent

Table 2: Effect of methanol extract of *Acacia ataxacantha* leaf on albumin induced hind paw oedema in rats

Treatment (mg/kg)	Volume of Oedema (mm)/(% Inhibition)					
	20min	40min	60min	80min	100min	120min
N/S(1mg/ml)	0.45±0.11	0.52±0.02	0.64±0.33	0.58±0.02	0.48±0.02	0.49±0.11
MEAA (100)	0.48±0.01 (-6.20)	0.41±0.02 (19.10)	0.64±0.03 (0.93)	0.49±0.03 (13.70)	0.50±0.09 (-5.00)	0.48 ±0.01 (2.00)
MEAA (200)	0.35±0.2[c] (21.00)	0.34±0.04[c] (33.20)	0.52±0.03[a] (14.00)	0.45±0.03 (22.40)	0.49±0.01 (-4.60)	0.36 ±0.21[a] (26.50)
MEAA (400)	0.34±0.0[b] (15.50)	0.37±0.0[b] (27.70)	0.53±0.03[a] (17.70)	0.48±0.02 (16.80)	0.43±0.01 (10.10)	0.38± 0.01[a] (22.40)
ASA (150)	0.35±0.0[b] (22.20)	0.26±0.01[c] (49.20)	0.52±0.01[a] (19.90)	0.42±0.03[a] (26.90)	0.39±0.0[b] (18.10)	0.34± 0.01[b] (30.00)

Data were represented as Mean ± SEM. N/S= Normal saline, MEAA=Methanol Extract of *Acacia ataxacantha*, ASA=Acetyl salicylic acid. n = 5

Statistical analysis: ANOVA (one way analysis of variance) and Statistical significance levels were represented using the following superscripts [a] = P < 0.05, [b] = P < 0.01 and [c] = P < 0.001

Table 3: Anti-pyretic effects of the methanol extract *Acacia ataxacantha* leaf in rats

Treatment (mg/kg)	Mean Rectal Temperature (◦ C)				
	0 min	30 min	60 min	90 min	120 min
N/S (1mg/ml)	38.94±1.02	38.01±0.92	38.01±0.01	39.02±0.11	38.74±0.01
MEAA (100)	38.91±1.41	37.92±0.14	37.04±0.24	39.91±0.43	39.30±0.21
MEAA (200)	38.27±0.10	38.29±0.11	38.80±0.37	38.91±0.01	38.71±0.01
MEAA (400)	38.41±0.12	37.88±1.14	39.24±0.91	39.02±0.01	38.51±0.22
PCM (150)	38.11±0.19	38.95±0.52	38.58±0.11	38.4±0.11[a]	38.0±0.01[a]

Data were represented as Mean ± SEM. N/S= Normal saline, MEAA=Methanol Extract of *Acacia ataxacantha*, PCM= Paracetamol. n = 5

Statistical analysis: ANOVA (one way analysis of variance)

Statistical significance difference from the control is at p ≤ 0.05. ([a] = p ≤ 0.05)

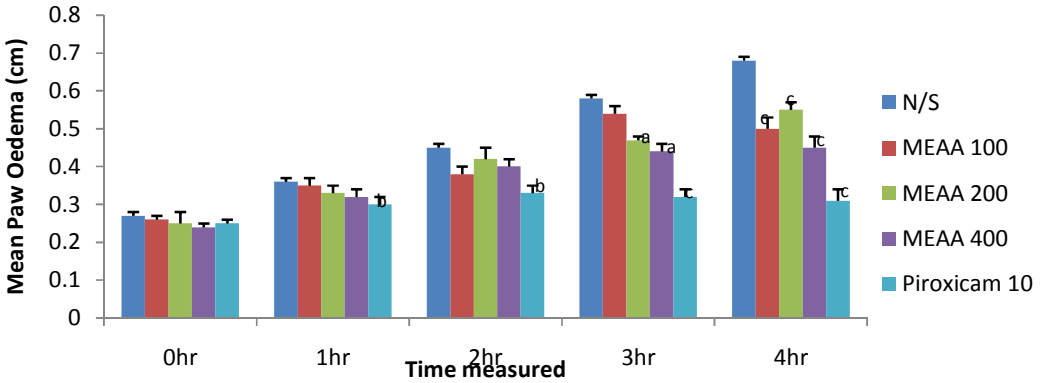

Figure 1: Effect of methanol leaf extract of *Acacia ataxacantha* leaf on carragenaan induced paw oedema in rats.

Data were represented as Mean ± SEM. N/S= Normal saline, MEAA=Methanol Extract of *Acacia ataxacantha*. n = 5

Statistical analysis: ANOVA (one way analysis of variance) and Statistical significance levels were represented using the superscripts [a] = P < 0.05, [b] = P<0.01 and [c] = P<0.001

DISCUSSION

The oral median lethal dose (LD_{50}) of the extract was found to be greater than 5000 mg/kg body weight. This suggests that the extract is practically non-toxic when administered orally (Loomis and Hayes, 1996; Lorke, 1983).

The data represented above (Fig.1) showed that the methanol extract of *Acacia ataxacantha* possess significant anti-inflammatory activity. Swelling of the hind-paw oedema occurred progressively from time 0-2 h after carragenaan injection in all the groups (I-V), but at the 3rd h oedema reduction occurred in a dose dependent manner and was significant ($p \leq 0.05$) only in rats group pre-treated with extract at doses 200 and 400 mg/kg body weight. However, at the 4th h there was significant ($p \leq 0.05$) difference in the swelling of oedema at 100, 200 and 400 mg/kg when compared with the normal saline (negative control) group (Fig.1).The result obtained from carragenaan assay correlated well with that of the second phase of formalin induced pain reported earlier (Abbas *et al*., 2016), thus confirming the anti-inflammatory effect of *Acacia ataxacantha*. This model is highly sensitive to non-steroidal anti-inflammatory drugs and has been accepted as an experimental procedure for investigating new drugs possessing anti-inflammatory properties (Temdie *et al*., 2012). Carragenaan induces non-specific inflammation which results from sequential action of several mediators of inflammation such as; prostaglandins E series, histamine, bradykinins, leucotrienes and serotonin, all of which causes pain and fever (Asongalem *et al.,* 2004). Carragenaan-induced inflammatory process is believed to be a biphasic process. The initial phase which (0-2hours) is mediated by histamine and serotonin release while, the second phase (3rd hour) is due to liberation of prostaglandins, bradykinins, lysosomes, cyclooxygenase products (Perianayagan *et al*., 2006; Fotio *et al*., 2009). The ability of the extract to significantly reduce the inflammatory response induced by carrageenan, suggests its anti-inflammatory activity.

The oedema (displaced volume) induced by egg albumin in the hind paw of rats was not sustained throughout the experimental period, as peak oedema was observed for all the groups at 60 minutes and a decreased oedema was observed till 120 min. There was significant ($p \leq 0.05$) difference in oedema at doses of 200 and 400 mg/kg body weight when compared with the normal saline (negative control) group at 20, 40 and 60 minutes respectively (Table 2). The standard drug (acetylsalicylic acid 150mg/kg) exhibit statistical significance ($p \leq 0.05$) at all the time when hind paw oedema readings was taken. Albumin-induced hind paw oedema test is used in investigating activity in acute inflammation (Akah and Nwambie 1994). The ability of the extract to reduce the oedema induced by albumin suggests its anti-inflammatory activity of the plant extract.

There was no significant ($p<0.05$) difference in the mean rectal temperature between the various doses of extract test when compared with the negative control group (Table 3). This implies that the extract has no significant anti-pyretic activity.

Phytochemical screening of the extract of *Acacia ataxacantha* leaf revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenol, saponins, reducing sugars, steroids and tannins (Table 1). Alkaloids, flavonoids and tannins have been found to alleviate inflammatory processes through the inhibition of prostaglandins pathway (Zhaohong, *et al.,* 2006; Salawu *et al.,* 2008).

CONCLUSION

The methanol extract of *Acacia ataxacantha* leaf was found to contain bioactive constituents with relevant anti-inflammatory effect, which supports the traditional uses in the management of inflammatory pains.

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