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ANTI INFLAMMATORY, ANTIPYRETIC ACTIVITIES AND CHEMICAL COMPOSITION OF THE METHANOL LEAF EXTRACT OF FLAME THORN (*Acacia ataxacantha* D.C.) IN RATS

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Abstract: *Laboratory experiment was carried to evaluate the toxicity profile, anti-inflammatory and anti-pyretic activities of methanol leaf extract of Acacia ataxacantha in rats. 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. The induced inflammation produced a dose dependent significant ($p \leq 0.05$) reduction of inflammation at 200 and 400 mg/kg 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 differences between the treatment means. 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 in human and animals.*

Keywords: *Acacia ataxacantha, Chemical composition, Toxicity, Inflammation.*

Introduction

The plant Flame thorn, *Acacia ataxacantha* is a shrub that is widespread in tropical Africa (Habbal *et al.,* 2011). These medicinal plants have been used globally from ancient times as the major source of traditional medicine in the treatment of various diseases (Kareji 2013). It is reported that about 50% of orthodox drugs are derived from vegetable sources (Kareru *et al.,* 2007). Presently, there is increasing popularity in the use of medicinal plant as they are believed to be natural, beneficial, safe, available, accessible and free from adverse effects (Gilani *et al.,* 1999). Traditionally, the plant was reported to be used in the management of painful inflammation of the respiratory tract, tooth, ulcers and skin sores (Erasto *et al.,*2004). The analgesic and anti-inflammatory activities of the plant had also been evaluated (Eloff *et al.,* 2008). These assumptions are based on long term use of the plants, with little or no scientific data to support information on the efficacy and safety profiles of these medicinal plants (Eloff, 2004). However, researches have proven that not all medicinal plants are safe, as they produce toxic effect on evaluation, which can result

from inherent toxic effect of the active principle, overdosing, chronic use, interactions, allergies, contaminations (Chanda *et al.,* 2010).

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 (Anjim and Mir, 2010). An inflammatory response may be induced in a great variety of ways and these include; truma, 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 (Kareji, 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 (Arabski *et al.,* 2012). Pyrexia can also result from secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states (Cheikyoussef *et al.,* 2011). 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 (Bruneton, 2009). 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 (Arora *et al*., 2012). 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 (Kareru *et al*., 2007). Research have shown that herbal medicine contain phytochemicals that can help alleviate injuries and inflammatory conditions (Boubakar *et al*., 2012). The objectives of the study was to evaluate the anti-inflammatory and anti-pyretic effects of methanol leaf extract of *Acacia ataxacantha* in rats.

Materials and methods

Plant collection and identification

The fresh plant of *Acacia ataxacantha* D.C was collected from Lake Chad Research Institute of Maiduguri, Borno State, Nigeria, in August 2023. It was identified and authenticated by the Department of Forestry, Faculty of Agriculture, University Maiduguri. Where herbarium number (Voucher Number 564) was compared with existing specimen and deposited.

Preparation of Extract

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 analysis

Phytochemical screening of the plant was carried out according to the methods described by Wagner and Blat (2001) and Bruneton (2009) for the detection of plant secondary metabolites. Tannins, alkaloids, flavonoids, steroids, coumarins, saponins, naphthoquinones, triterpenes, lignans, pigments, anthracene derivatives have been investigated using tube test. Each extract (10 mg/ml) were deposited on TLC plate to confirm the results.

Experimental animals

Rats (190–240 g) were obtained from Animal Research Farm, Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, University, Maiduguri. The animals were maintained in a well-ventilated room under standard temperature and pressure, feeds and water administered ad libitum. Animals were handled in compliance with ARRIVE guidelines 2010 (Boubakar *et al.,* 2012) while the experiments were conducted according to standard protocols of National Institute of Health (NIH, 2015), guidelines for use and care of laboratory animals.

Equipment and instruments

Pestle and mortar, weighing balance "mg" (AE240 Switzerland), weighing balance "g" (Golden-Mettler USA), desiccator (MonaxScotland), water bath, evaporating dish, plain bottles, EDTA bottles, capillary tubes, haematocrit centrifuge (Denley, BS400, UK), centrifuge (Techmel and Techmel, TT-645P, UK), haematology analyser (PIOWAY HY-3400, Japan). Other materials include distilled water, methanol (BDH Chemical Ltd, Poole, England), chloroform (Sigma Chemicals Co. USA), 10% formalin.

Albumin- induced paw oedema in rats

This test was carried out using a modification of Wadood *et al*., (1989), as described by Arias *et al.* (2004). 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.

Statistical analysis

The data were 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 and Discussion

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

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 (Firras and Hassan, 2008).

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

Means followed by same letter are were represented using the following superscripts ^{a} = P < 0.05, ^{b} = P < 0.01 and ^{c} = P < 0.001

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 (Arora *et al.,* 2012). The ability of the extract to reduce the oedema induced by albumin suggests its anti-inflammatory activity of the plant extract.

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

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.

Table 4: Effect of extract on some renal biochemical parameters after 90 days of oral administration.

Biochemical Parameters	D/W 1ml/kg	MEAA 50 mg/kg	MEAA 200 mg/kg	MEAA 400 mg/kg
Urea (mg/dL)	3.18 ± 0.45	5.53 ± 0.69*	5.80 ± 0.31*	5.67 ± 0.42*
Creatinine (mg/dL)	35.82 ± 0.66	47.00 ± 3.61*	45.50 ± 1.50*	49.67 ± 5.46*
Na^+ (mmol/L)	141.20 ± 2.71	148.67 ± 2.40	156.50 ± 7.50*	156.67 ± 0.88*
K^+ (mmol/L)	5.00 ± 0.07	5.50 ± 0.17	5.40 ± 0.50	5.40 ± 0.16
Cl^- (mmol/L)	101.92 ± 1.12	107.23 ± 2.92	101.25 ± 0.85	104.80 ± 2.77
HCO_3^- (mmol/L)	25.94 ± 0.91	25.40 ± 0.47	27.35 ± 0.95	25.83 ± 1.34

The oral median lethal dose (LD_{50}) of the extract in rats was found to be greater than 5000 mg/kg body weight. There was significant increase ($p \leq 0.05$) in body weight of the rats that received extract at doses of 200 and 400 mg/kg when compared with the negative control group. There was no significant difference in the relative organ weights of all the tested doses. There was significant ($p \leq 0.05$) increase in the level of urea and creatinine, while the level of Na^+ ion significantly ($p \leq 0.05$) and dose dependently increase when compared with the negative control group in the liver function test (Table 4).

Table 5: Effect of extract on hematological parameters after 90 days of oral administration.

Haematological indices	D/W 1 ml/kg	MEAA 50 mg/kg	MEAA 200 mg/kg	MEAA 400 mg/kg
WBC (10^9/L)	7.10 ± 1.05	8.63 ± 1.29	5.55 ± 1.25	5.07 ± 0.04
RBC (10^{12}/L)	7.58 ± 1.46	6.83 ± 0.67	7.70 ± 0.01	7.40 ± 1.73
HGB (g/dL)	13.86 ± 0.16	13.33 ± 0.22	14.50 ± 0.20	14.00 ± 0.12
PLT (10^9/L)	724.00 ± 2.90	699.00 ± 5.80	706.00 ± 9.00	773.00 ± 9.60
PCV (%)	50.80 ± 0.20	48.00 ± 1.70	50.50 ± 0.50	51.00 ± 0.60
MCV(fL)	65.90 ± 1.50	70.20 ± 3.60	65.60 ± 0.30	68.40 ± 2.00
MCH (Pg)	18.10 ± 0.30	19.50 ± 0.50	18.50 ± 0.10	18.80 ± 0.50
MCHC(g/dL)	27.20 ± 0.30	27.90 ± 0.70	28.20 ± 0.20	27.90 ± 0.40

Data were presented as Mean ± SEM, n= 6. Statistical tool ANOVA (followed by Dunnett post-hoc test) $p \leq 0.05$ level of significance. D/W =Distilled water. MEAA =Methanol Extract of *Acacia ataxacantha*.

The analysis of haematological parameters is important in accessing toxic effect of substance because, it has a higher predictive value of toxicity in humans when tests involve rodents (Kereru *et al.,* 2007). There was no significant difference in the haematological parameters when the extract treated groups were compared with the negative control group. The extract on evaluation did not produce any significant difference in the level of hematological parameter (Table 5).

Discussion

Recent report showed that about 85% of people worldwide, particular in developing countries rely on traditional medicine and its practice for their primary health care need (Wagner and Bladt, 2001). The oral median lethal dose (LD_{50}) of the extract in rats was found to be greater than 5000 mg/kg body weight. This shows that on acute administration (orally), the extract is practically non-toxic (Wadood *et al.,* 1989). Reduction in body weight and relative organ weight is usually regarded as toxic effect of extract on animal, resulting to reduced food and water intake (Tandon *et al*., 2005). The extract caused an increase in body weight when compared with negative control group, hence showed relative safety of the extract on the rats.

The kidney is an important organ in the body that maintains homeostasis through its osmoregulatory function (regulation of electrolytes and blood pressure, maintenance of acid-base balance) (Shah *et al., 1*997). Urea is a by-product of protein metabolism that is excreted solely in the kidney (Sasidharan *et al.,* 2011) while, creatinine is a by-product of muscle metabolism (Saleem *et al.,* 2010) which is also excreted exclusively by glomerular filtration. Hence, the levels of creatinine, urea, sodium, potassium or chloride, are parameters used as a measure of kidney function (Saad *et al.,* 2011). The significant increase in the serum level of urea and creatinine in the renal biochemical parameters tested is an indication of kidney dysfunction (may signify decrease in kidney function due to toxic effect of extract). Sodium is an extracellular fluid ion that is filtered and reabsorbed in the kidney (Riffel *et al* 2002). The significant elevation in serum level of sodium ion in the serum electrolytes evaluated might be due to kidney dysfunction resulting from loss of excessive fluid (dehydration) and reduced water intake by the rats (Riaz *et al.,* 2011).

Liver is the major organ in the living system for metabolism of drugs and other xenobiotics. Alanine transaminase, aspartate transaminase and alkaline phosphatase are biomarkers used to evaluate liver function (Popova *et al.,* 2009). The significant increases in the level of AST might be an indication of liver damage, which is suggestive of hepatotoxic effect. Although, ALT and AST are largely used in the assessment of liver damage (as they cause hepatocytes inflammation, cellular leakage and damage to cell membrane) by drugs or any hepatotoxic substance (Ngoci *et al.,* 2014), but elevated level of ALT is more specific for liver related injuries or diseases (Nayak *et al.,* 2007). However, high level of AST is also indicative of liver damage, cardiac infarction and muscle injury (MacDonald *et al.,* 2010). ALT is present in the liver only in small quantities, the enzyme is secreted in the bile and substantial elevation of serum ALP is seen with mild intrahepatic or extra-hepatic biliary obstruction (Keymanesh *et al.,*2009).

The analysis of haematological parameters is important in accessing toxic effect of substance because, it has a higher predictive value of toxicity in humans when tests involve rodents (Mahesh and Satish, 2008). There was no significant difference in the haematological parameters when the extract treated groups were compared with the negative control group.

Conclusion

The methanol extract of *Acacia ataxacantha* is safe on acute administration, however prolong use may produce toxic effects on some organs (liver, kidney and stomach),

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