PHARMACOLOGICAL AND TOXICOLOGICAL STUDIES OF THE METHANOL EXTRACT OF *ACACIA ATAXACANTHA* LEAF D.C. (LEGUMINOSAE) IN MICE AND RATS

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DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS,
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MARCH, 2015

DECLARATION

I declare that the work in this thesis entitled "Pharmacological and toxicological studies of the methanol extract of *Acacia ataxacantha*leaf D.C. (leguminosae) in mice and rats" has been performed by me in the Department of Pharmacology and Therapeutics under the supervision of Prof. J. A. Anuka and Dr. J. I. Ejiofor. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has been previously presented for another degree at this or any other Institution.

MEDINAT YAKUBU ABBAS		MARCH, 2015
Name	**Signature**	**Date**

CERTIFICATION

This thesis entitled "PHARMACOLOGICAL AND TOXICOLOGICAL STUDIES OF THE METHANOL EXTRACT OF *ACACIA ATAXACANTHA* LEAF D.C. (LEGUMINOSAE)IN MICE AND RATS" by Medinat Yakubu ABBAS meets the regulations governing the award of the degree of Masters of Science of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my late father Justice A. A. Abbas who died on the 1st of November 2012, may Almighty Allah (SWT) grant him Aljanatul firdaus (Amin).

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LIST OF ABBREVIATIONS

5HT	Serotonin
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ANOVA	One way Analysis of Variance
ANS	Autonomic Nervous System
ASA	Acetylsalicylic acid
AST	Aspartate transaminase
CB	Conjugated bilirubin
CNS	Central Nervous System
COX	Cyclooxygenase
COX-2	cyclooxygenase-2
Hb	Haemoglobin
IASP	International Association for the Study of Pain
LD_{50}	Median lethal dose
MCHC	Mean Corposcular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
MEAA	Methanolic Extract of *Acacia ataxacantha*
NSAIDS	Non-Steroidal Anti-inflammatory Drugs
OECD	Organisation for Economic Cooperation and Development.
PCM	Paracetamol
PCV	Packed Cell Volume
PGE2	Prostaglandins E_2
PGF2	Prostaglandins F_2
PLT	Platelet

RBC	Red Blood Cell
RT	Rectal Temperature
TB	Total Bilirubin
TENS	Transcutenous Electrical Nerve Stimulation
WBC	White Blood Cell
W.H.O	World Health Organisation

ABSTRACT

Acacia ataxacantha is used in traditional medicine for management of pain and inflammation. Literature survey revealed that the safety and efficacy of such uses have not been scientifically validated. The present study was designed to study the pharmacological and toxicological activities of methanol extract of *Acacia ataxacantha* leaf in mice and rats. The acute and chronic toxicity studies were carried out using Lorke method (1983) and OECD guideline (1995) respectively. The extract was evaluated for analgesic activity using acetic acid, formalin and hot plate induced pain assays. Similarly, anti-inflammatory activity was evaluated using carragenaan and albumin induced inflammation, and antipyretic activity using yeast induced pyrexia. Phytochemical screening revealed the presence of flavonoids, alkaloids, glycosides, phenols, tannins and carbohydrate. The oral median lethal dose (LD_{50}) of the extract in both mice and rats was estimated to be > 5000 mg/kg, while the intraperitoneal LD_{50} in mice and rats were estimated to be 565.69 and 1,264.91 mg/kg respectively. There were significant increase in the level of liver AST, ALT and ALP of the animals after 90 days daily administration of the extract, suggesting that the extract could be hepatotoxic. Similarly, there was significant increase in the levels of urea and creatinine which may indicate toxic effect of the extract on the kidney. The histopathological evaluation revealed adverse effects on the morphology of some organs; liver (mild hepatocellular necrosis and kupfer cell hyperplasia), kidney (glomeruli necrosis and lymphocytes hyperplasia) and stomach (mild necrosis of the stomach mucosa). The extract at doses of 50, 100, 200 and 400 mg/kg significantly ($p< 0.05$) reduced the number of writhing induced by acetic acid when compared with the negative control. In the thermal pain induction at the 1st hour post extract administration, there was significant ($p < 0.05$) increase in pain threshold at 400 mg/kg only while, at the 2nd and 3rd hour there were significant differences ($p< 0.05$) at doses of 50, 100, 200 and 400 mg/kg when compared

with the negative control. There was significant ($p < 0.05$) reduction in pain perception at doses of 50, 100, 200 and 400 mg/kg (first phase) and at 200 and 400 mg/kg (second phase) of formalin pain induction. The carragenaan induced inflammation produced significant ($p < 0.05$) reduction of inflammation at 200 and 400 mg/kg (2^{nd} hour) while the significant reduction in oedema was observed, in the 3^{rd} and 4^{th} hour, at doses of 100, 200 and 400mg/kg when compared with the negative control. Similarly there was significant inhibition of inflammation at the 20^{th}, 40^{th} 60^{th} and 120^{th} minutes post extract administration in albumin induced inflammation. These findings suggest that the extract may contain bioactive compounds that possess analgesic and anti-inflammatory activities, thus supporting the ethno-medical use of the plant for the management of pain and inflammation. However, prolong use of the extract may produce some adverse effects on the liver, kidney and stomach mucosa.

CHAPTER ONE

1.0 INTRODUCTION

Traditional medicine is described as "therapeutic practices that have been in existence, often for hundreds of years before the development and spread of modern sciences and are still in use" (WHO, 2002). About 80% of Africa population use traditional medicine (TM) to help meet their primary health care needs, (WHO, 2002; Truter, 2007). This is due to the fact that traditional medicine is the most affordable and accessible health care system available (WHO, 2002; Stanley, 2004). Traditional medicine can therefore be defined as "the sum total of knowledge and practices, whether explicable or not, used in diagnosing, preventing or eliminating a physical, mental or social disease and which may rely exclusively on past experiences and observations handed down from generations to generations, verbally, or written". The practice utilizes vegetable, animal, minerals and other methods (Truter, 2007; WHO, 2013).

The World Health Organization encourages the inclusion of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries (WHO, 2002; Elujoba *et al*., 2005). The degree of sensitization and mobilization by the World Health Organization (WHO) has encouraged some African countries to commence serious development on Traditional African Medicine (Elujoba *et al*., 2005). Results obtained from such research into substances used in traditional medicine practices led to the discovery of relevant plants with useful chemical constituents that can be used in the treatment of various diseases e.g. *Artemisia annua, Ocimum gratissimum, Citrus aurantifolia, Xylopia aethiopica, Cajanus cajan, Crossopteryx febrifuga, Securinega virosa,* e.t.c(WHO, 2002;Rapheal, 2011*).*

A medicinal plant as defined by World Health Organization is “any plant in which one or more of its origin contains substance that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs”(WHO, 2008), examples of medicinal plant include;*Datura stramonium*produces atropine which is an anti-cholinergic agent used in asthma as bronchodilator, also *Papaver somniferum* produces morphine which is used in the management of pain, while *Digitalis lanata*producesdigoxin which is also used as a cardiotonic agent in the management of heart failure,(Elujoba *et al*., 2005).An example of precursor for synthesis of drug that are useful is *Agave sisalana* which produces saponin like nicogenins used in the production of steroidal drugs, (OAUC, 1985).

Pain (IASP)is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (Hasan *et al.*,2010). Pain is associated with manyhealth conditions e.g.headache, menstruation, cancer, sickle cell anaemia, arthritis and burns. Most inflammatory conditions are associated with pain and in most cases are responses to injurious stimulus, (Muhammed *et al*., 2012).

1.1 Statement of Research Problem

Pain of any type is the most common reason of physician’s consultation in various health care delivery systems Worldwide. Most pathological conditions are associated with pain, inflammation and/or pyrexia, (Muhammed *et al*., 2012). Studies have shown that pain poses a major challenge in public health, affecting about 7-30 % of the population (Wood, 2002). Chronic pain has a detrimental effect on physical, mental and psychological health as well as socio-economical wellbeing, affecting daily activity, labour and loss of revenue, (Wood, 2002).

Management of pain has not been effectively achieved due to inaccessibility, high cost and side effects associated with the currently used analgesics, (Mwale and Masika, 2010).Opioid analgesics (example morphine) are used usually to relieve moderate to severe pain particularly of visceral origin. Repeated administration of opioid analgesics can cause dependence and tolerance, some of the adverse effects associated with the use of opioid includes; addiction, respiratory depression, hypotension, constipation, drowsiness and hallucination (Almeida *et al*., 2001;Yerima *et al*., 2009).Opioid analgesics are often controlled drugs; hence, accessibility of these drugs most of the time may be difficult.

Non-opioid analgesics (example piroxicam) are particularly useful for treatment of pains of skeletal muscle conditions. However, there are serious adverse effects that limit the use of these drugs in the management of pains, for example, non–steroidal anti-inflammatory drugs (NSAIDS) like aspirin, ibuprofen, piroxicam cause gastro-intestinal irritation, with bleeding, increase in bleeding time, bronchospasm, dyspepsia, nephrotoxicity and skin reaction. Prolonged use of paracetamol can cause liver damage, (Hassan *et al.,* 2010; Usha, 2012). Thus the effective management of pains using these drugs can be seriously affected. More so, the high cost of these analgesics may prevent patient compliance and may also affect effective management of pain, (Mwale and Masika, 2010).

The widespread and growing use of traditional medicine (example *Acacia ataxacantha*) of unproven safety and efficacy has created public health challenges in terms of: policy, safety, efficacy, quality and rational use (WHO, 2002).Also the development of analgesics and anti-inflammatory agents that are more effective, safer and readily affordable is a challenging task for researcher, (Temdie *et al.,* 2012).

1.2 Justification for the Study

Pain is a significant health care problem and is estimated to cost US $ 560-635 billion annually (about US $ 2,000.00 for every person living in America), (San-Jose, 2011). Medicinal plants are used worldwide to treat many diseases and are valuable sources of lead compound for synthesis of modern drugs (Eto, 2012).Many rural dwellers depend largely on herbs for the treatment of painful conditions, inflammation and fever. However the unknown side effects of these herbs can pose health problems (Omogbai *et al*., 2010; Shekhawat and Vijayvergia, 2010). More so, medicinal plants have been found to be useful traditionally in the effective management of pain, examples include; S*tylosanthes fruticosa*, *Ficusglomerata*, *Bougainvilla spectabilis* and *Polyalthia longifolia*. Some of these plants have been verified scientifically to have analgesic and anti- inflammatory properties, examples include; *Spilanthes acmella*, *Vigna trilobata*, *Securinega virosa*, *Cissus guadrangularis*, *Lactuca scariola,* and *Crossopteryx febrifuga*, (Usha *et al.*, 2012), while some have not been verified scientifically examples include; *Acacia ataxacantha*, *Acacia hockii*, *Detarium senegalense*, *Afzelia Africana*, (Burkill, 1997).

Herbal medicines are generally regarded as safe, but studies have shown that not all natural products are safe (Ansari and Inamdar, 2010).*Acacia ataxacantha* (leaf) have been reportedly used traditionally in the management of pain. There are no documented data, at present, on the activity and safety profile of *Acacia ataxacantha*. The present study was designed to evaluate the analgesic, anti-inflammatory, antipyretics and some toxicity studies of the plant in rodents so as to scientifically describe the activities of *Acacia ataxacantha*which is already in use in traditional medicine.

1.3 Theoretical Frame Work

1.3.1 Median lethal dose (LD_{50}) determination

The method used to determine the median lethal dose was done as described by Lorke (1983). Using this method, it's possible to obtain adequate information on the acute toxicity and the LD_{50} of a compound with fewer experiment animals. It can also be used for every route of drug administration and applied to; drugs, agricultural and industrial chemicals. The test is done in two phase, such that the outcome of the first phase determine the doses for the second phase.

1.3.2 Chronic toxicity study

The chronic toxicity study provides information on the possible health hazards likely to arise from repeated exposure over a long time. The study will provide information on the toxic effects of the substance; indicate target organs damage and the possibility of accumulation of substance in organs which can be established using kidney function tests, liver function test, haematological analysis and histopathological evaluations. It can also provide an estimate of the no–observed -adverse effect level which can be used for establishing safety criteria for human exposure. Chronic toxicity studies are usually carried outin rodents. The three main routes of administration used in chronic toxicity studies are oral, dermal and inhalation, and the choice of the route of administration depends on the physical and chemical characteristics of the test substance and the predominant route of exposure in humans. Exposure via oral route is the most commonly used in chronic toxicity studies (OECD 452 Guidelines, 2008). The duration for chronic toxicity studies in rodents is usually ninety (90) days.

1.3.3 Analgesic studies

1.3.3.1 *Acetic acid-induced writhes in mice*

The method used was that described by Koster *et al.,* (1959). It was based on the fact that aqueous acetic acid (0.6% $^{w}/v$)causes writhes (a syndrome characterized by a wave of contraction of the abdominal musculature followed by extension of hind limbs) when administered intraperitoneally, which is a symptom of pain. The abdominal constriction response induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics. It is a chemical means of pain induction in laboratory animals, (mice and rats). Acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, prostaglandins (PGE_2 and $PGF_{2\alpha}$), bradykinins and substance P, which stimulate sensory nerve endings (Bentley *et al.,* 1983).

1.3.3.2 *Thermal method of pain induction*

The hot plate method is used to measure the response latencies to thermally induced pain (Eddy and Leimbach, 1953). Thermal nociception model such as hot plate is used to evaluate central analgesic activity. The paws of mice and rats are very sensitive to temperature at 55 ± 0.5°C, which are not damaging to the skin. The pain response is taken in form of jumping off the hot plate, licking or shaking of limbs. The response latency is usually measured in seconds. In order to prevent tissue damage a cut-off time of 30 seconds is imposed on all animals, (Muhammed *et al.,* 2012).

1.3.3.3 *Formalin induced paw licking in mice*

The method used was that described by Tjolsen *et al.,* (1992). This method was originally developed by Dubbuisson and Dennis, (1977). It was based on the fact that aqueous formalin (1%$^{v}/_{v}$) causes paw licking and biting when administered subcutaneously to the

right limb in mice, which is a symptom of pain. It is also a chemical means of pain induction in laboratory animals, e.g. mice. It involves two phases, namely early phase (which is usually measured in the first 5 minutes) is thought to be produced by direct activation of nociception neurons by formalin, whereas, the later phase (measured in the last 45 minutes, with a lag period of 10 minutes between both phases) reflects pain generated in acutely injured tissue, (Yerima *et al.*, 2009; Temdie *et al.*, 2013).

1.3.4 Anti-inflammatory studies

1.3.4.1 *Carragenaan induced hind paw oedema*

The test was conducted according to the method described by Winter *et al.*, (1962). Carragenaan (1% $^{w}/_{v}$) was used as inflammagen. Acute inflammation was produced by the sub-plantar administration of 0.1m1 of 1% $^{w}/_{v}$ of carragenaan (in distilled water) into the right hind paw of the rats. The extent of inflammation or inhibition was measured using Vernier caliper and compared with the control group for 0, 1, 2 and 3 hours respectively. The carragenaan induced hind paw oedema model in rats is known to be a non-specific, acute inflammatory model sensitive to cyclooxygenase (COX) inhibitors, which prevents prostaglandins synthesis. It involves three phases; first phase, which occurs between 0 to 2 hours post-injection of carragenaan, has been attributed to release of histamine or serotonin. The second phase (3^{rd} hour) which is due to liberation of kinins and the third phase which begins just after the kinins phase and it involves the release of prostaglandins and cyclooxygenase products, (Biswas *et al.*, 2011; Temdie *et al.*, 2013).

1.3.4.2 *Albumin induced paw oedema*

The test is carried out using a modification of the method described by Winter *et al*, (1962), as described by Akah and Nwambie (1994). In this method, 0.5 ml/kg of raw egg

albumin, was injected in the sub-plantar surface of the left hind-limb, pedal oedema (inflammation) was observed within 5 - 8 minutes after injection. A Venier caliper was used to measure the volume of paw oedema for a period of 120 minutes, with readings taken at 20 minutes intervals and recorded (cm). Egg albumin-induced inflammation model is a significant predictive test for acute anti-inflammatory activity, (Salawu *et al.*, 2008).

1.3.5. Yeast induced pyrexia

The procedure described by Al-Ghamdi (2001) was adopted for this study. 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. The body temperature of each albino wistar rat was recorded by measuring rectal temperature (RT) at predetermined intervals. Pyrexia is a result of secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states. The infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediators (cytokines like interleukin 1β, α, β and tissue necrotizing factor-α) which causes the synthesis of PGE_2 near pre-optic hypothalamus that cause the elevation the body temperature, (Salawu *et al.*, 2008).

1.5 Aim and Objectives of the Study

1.4.1 Aim of the study

The aim of the study is to evaluate the analgesic, anti-inflammatory, anti-pyretic and some toxicity studies of methanol extract of *Acacia ataxacantha*leaf in experimental animals (mice and rats).

1.4.2 Objectives of the study

The objectives of the study are to;

i conduct phytochemical screening on the methanol extract of *Acacia ataxacantha*leaf,

ii carry out acute and chronic toxicity studieson the methanol extract of *Acacia ataxacantha*leaf,

iii evaluate the analgesic, anti-inflammatory and anti-pyretic activitiesof methanol extract of *Acacia ataxacantha*leaf.

1.5 Research Hypothesis

Methanol extract of *Acacia ataxacantha* leaf contains phytochemical constituents that are safe and relevant in the management of pain, inflammation and pyrexia.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Pain

2.1.1 Definition of Pain

Pain is defined by the International Association for the study of pain (IASP) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" (Hasan *et al*., 2010). Pain can also be defined as a localized sensation of discomfort, distress or sensitization of specialized nerve fibres. It acts as a protective mechanism as it causes the subject to withdraw from the source of pain, (Hudspith *et al.*, 2005).

Pain is highly subjective to the individual experiencing it and there are no specific tests that can quantitatively or qualitatively measure pain intensity or severity. However, the perception of pain sensation depends upon the size of the area stimulated, the frequency of stimulus and application, the duration and location of the stimulus. Although pain is a definite and singular experience based upon activity in specific receptors, any single nociceptor's activity is influenced by simultaneous activity at nearby nociceptors. Thus the pain experience is a composite of concurrent inputs at multiple receptors. It is part of the body's defence system, triggering a reflex reaction to retract from a painful stimulus and helps the individual to adjust behaviour so as to avoid that particular harmful situation in future. Pain can also be expressed as a sign and symptom of an underlying disease, example of these diseases are angina, gout and others, (Rang *et al.*, 2003).

It is the most important symptom that brings the patient to the physician. Excessive pain may be unbearable and cause other effects such as; sinking sensation, apprehension,

sweating, nausea, palpitation and raise or fall in blood pressure, and tachypnea.

2.1.2 Types of Pain

Based on the primary mechanism of pain production, pain can be classified into two major types, namely; nociceptive and neuropathic pain.Nociceptive pain results from tissue damage and usually resolves when the initial tissue damage heals and is generally responsive to non-steroidal anti-inflammatory drugs (NSAIDs). Nociceptive pain can be subdivided into somatic and visceral pains.Somatic Pain is caused by injury to the skin or superficial tissues. Visceral Pain originates from the body's viscera or organs and it is noticed at the onset or early stage of disease. Visceral receptors are located within the body organs and internal activities. The amount of nociceptors located in these areas is low and so produces pain that is usually diffuse, dull, aching, cramping that is poorly localized and of a longer duration than the somatic pain. An example is gall bladder pain which can radiate to the scapula, (WHO, 2012)

Neurological disease affecting sensory pathway can produce severe chronic pain termed, "neuropathic pain" which is unrelated to any peripheral tissue damage. Also pathological stimulation that evokes sympathetic responses can produce severe pain (neuropathic pain). Neuropathic pain may occur whenever there is either damage or dysfunction of nerves in the peripheral or central nervous system (CNS). An example is diabetic neuropathy or pain due to herpes zoster infection (shingles). The qualities of neuropathic pain can be described as burning or electrical. This occurs with CNS disorders such as stroke and multiple sclerosis or with conditions associated with peripheral nerve. Neuropathic pain is relatively resistance to NSAIDs and opioids, although they may be helpful in certain situations, (Craig and Stitzel, 2002).

Traditionally, based on duration of occurrence, pain can be classified as acute or chronic.Acute Painis a type of pain that has sudden onset (felt immediately following injury), and severe in intensity, but is usually short-lasting. It arises as a result of tissue injury stimulating nociceptors and generally disappears when the injury heals (self-limiting in duration). Examples include; post operative pain, pain due to injury, pain resulting from childbirth and others. Acute pain may be accompanied by signs of automatic nervous system (ANS) activity such as tachycardia, hypertension, disphoresis, mydriasis and pallor that mimic those of anxiety, (WHO, 2012).

Chronic Pain is described as continuous or recurrent pain lasting for more than 3 months. It is much more subjective and not easily described as in acute pain. Chronic pain may begin as acute pain and persist for long periods or may reoccur due to persistence of noxious stimuli or repeated exacerbation of an injury. It may also arise and persist in the absence of identifiable pathophysiology or medical illness. This kind of pain can affects individual's personality, ability to function (work) and quality of life. It is rarely accompanied by autonomic symptoms. Examples include back pain, arthritis and fibromyalgia, (Rang *et al*., 2007; WHO,2012).

2.1.3 Pathophysiology of pain

The four basic steps involved in nociception include:

i. Transduction which is the transformation of noxious stimuli causing tissue injury to electrical signals in the peripheral neurons.
ii. Transmission involves the propagation of electrical signals along nociceptive nerve fibers.

iii. Modulation is the alteration (amplification or attenuation) of nociceptive signals in the spinal cord and at supraspinal sites.

iv. Perception is the process of the integration of nociceptive signals arriving to the brain with cognitive and emotional factors, yielding the subjective experience known as pain (Castañeda-Hernández and Bach-y-ita, 2003).

Pain mediators are substances that evoke pain when liberated in the tissue and they include; kinnins, biogenicamines, histamines, 5HT (serotonin) and metabolites of arachidonic acid (such as prostaglandins and leukoterienes).Among these substances released when tissue is damaged are histamine (from mast cells) and potassium (from damaged cells), both of which excite nociceptors thereby producing pain.Adenosine triphosphate may also exhibit this pain-producing effect by acting alone or in combination to sensitize nociception. One substance known to produce pain is bradykinin (a polypeptide produced by cleavage of plasma protein after tissue injury). At low concentration, bradykinin produces hyperalgesic (which may occur by stimulation of specific PGE_2) while at high concentration it causes direct stimulation of nociception.Also, the by-products of arachidonic acid metabolism (prostaglandins and leukotrienes) are synthesized at site of tissue damage. These compounds are present at high concentration in inflammatory fluids and are potent mediators of inflammation.

Prostaglandins are formed from arachidonic acid by the enzyme cyclo-oxygenase, of these, prostaglandins E_2 (PGE_2) is the most potent. PGE_2 is thought to produce hyperalgesic by direct action on the nociceptors. Prostaglandins may also sensitize nociceptors via coupling to a cyclic adenosine monophosphate system. Non-steroidal anti-inflammatory drugs (NSAIDs) owe their analgesic activity due to inhibition of cyclo-

oxygenase.Leukotrienes produced from arachidonic acid by enzyme lipoxygenase also produce hyperalgesic. However, pain producing actions of leukotrienes are not block by cyclo-oxygenase inhibitors, but by depletion of polymorphonuclear leucocytes.In contrast to substrate released in region of injury, nociceptors themselves discharge pain-enhancing substances such as substance *P* (a peptide), which is liberated from some *C* fibres and leaves (exits) pain transmission pathway in the dorsal horn, (Lamont *et al.*, 2000).

However, in the 1960's Melzack and Wall proposed the gate control theory of pain in which it was thought that painful stimulus acted upon sensitive receptors and caused electrochemical impulse to travel to the brain, which then initiated the physical and psychological response to brain. Although some of the key details of this theory have since been revised, it is still widely accepted to explain the way signals are collected, transmitted and interpreted within the central nervous system (CNS), as it allows for the existence of specific pain receptors as well as for role of the nervous system in pain mediation.Essentially, the gate control mechanism occurs as follows; afferent *C* fibres and A-α transmit pain signals to an area known as the substantial gelatinosa, located in the dorsal horn of the spinal cord. Cells within the dorsal horn collected and interpret these signals and send them to transmission cells with terminals projecting to distance sites outside the dorsal horn. Some of the *C* and A-α fibres terminates in the dorsal root horn, whereas others form a complex known as the lateral spinothalamic tract. Pain impulses travel up along this tract to the thalamus and from there to the cerebral cortex of the brain, competing nerve impulses (example, stimulus from different nerve branch) can block pain signals at the nervous system "gates", diminishing the intensity of the pain-relaying messages. Other controls that descend from the brain to inhibit firing of responsive neurons in the dorsal horn exist and therefore stop or halt pain signals.

2.1.4 Opioid Receptors

The main pharmacological effects of opiates have been found to be mediated by three (3) classes of opioid receptors, which have been identified in various nerve sites and other tissues. These are mu (μ), delta (δ), Kappa (k). A fourth subtype, sigma (σ), which was also postulated in other to account for the dysphoric effect (anxiety, hallucination bad dreams) produced by some opiates(Rang *et al*., 2003).

The mu (μ) receptors are thought to be responsible for most of the analgesic effects of opioids, and most of the analgesic opioids are μ-receptor agonists. Mu (μ) receptors are located primarily in pain-modulating areas of the CNS and induce central analgesic and some of the unwanted effects of opiates such as respiratory depression, euphoria, sedation and dependence.Kappa (k) receptors are responsible for analgesia at the level of the spinal cord and brain and are found in high concentration in the cerebral cortex and substantial gelatinosa of the dorsal horn. Because they produce analgesia without inducing opioid habituation (dependence), there is a great interest in the development of k-specific receptor agonists.Delta (δ) receptors are probably more important in the periphery, but may also contribute to analgesic. The receptors are located in the limbic areas of the brain and in the spinal cord and may play a role in euphoria that selected opioids produce. Delta (δ) receptors have also been implicated for its analgesic effect at the spinal cord. The delta (δ) receptors are not selective opioid receptors because they also serve as site of action of psychotomimetic drugs such as phencyclidine. They are believed to be associated with glutamate-activated in channels, and may account for the dysphoric produced by some opioids.All opioids receptors are linked through G-protein to inhibition of adenylate cyclase (Rang *et al*., 2003).

2.1.5 Endogenous Peptides

Endogenous opioids (peptides) namely, endorphins, enkephalins and dynorphins are found in varying concentration in the CNS. Dynorphins and enkephalins appear to be responsible for intrinsic regulation of pain perception within the medulla, while endorphins and enkephalins probably serve as regulator of pain perception within the substantial gelatinosa. Each of the endogenous opioids has greater preference for the opioid receptors type, example, β-endorphin and enkephalins are more active at mu (μ) and delta (δ) receptors, while the target site for dymorphins is the Kappa (k) receptor (Herfindal and Gourley, 2000 ; Rang *et al*., 2003)

2.2 MANAGEMENT OF PAIN

2.2.1 Pharmacological Treatment

Medically, the management of pain has given rise to a distinction between acute and chronic pain. The pharmaceutical substances used usually to relieve pain are called 'analgesics' and the processes of pain relieving is called 'analgesia'.Analgesia is an alteration of the sense of pain without loss of consciousness. The body possesses an endogenous analgesic system, which may be supplemented with pain-relieving drugs (analgesics) so as to regulate nociception and pain sensation. Analgesia may occur at the level of central nervous system (CNS) or in peripheral nerves and other nociceptive areas(Goodman and Gilman, 2006).

Analgesics can be broadly classified into two namely; opioid analgesics (narcotic analgesics) and non-opioid analgesics (Usha*et al*., 2012).Opioids are natural or synthetic compounds that produce morphine-like effects. They act by binding to specific opioid receptors in the central nervous system (CNS) to produce effects that mimic the action of

endogenous peptide neurotransmitters, the epiopeptins (endorphins, enkephalins and dynorphins). Examples are; morphine, fentanyl, pentazocine, methadone, etc. The non-steroidal anti-inflammatory drugs (NSAIDs) and paracetamol are examples of non-opioid analgesics, (Katzung, 2004).

2.2.2 Non-Pharmacological and Complementary Treatment of Pain

These include the following:

- Radiotherapy – This type of treatment is used in the management of bone metastasis.
- Physiotherapy – The main aim of this therapy is to alleviate suffering and restore normal body function, so physiotherapy and active mobilization must be considered early. They include spinal manipulation, massage, application of heat or cold and exercise.
- Psychological techniques – such intervention can enhance coping skills and reduce disability and social isolation associated with long-term painful conditions,these include counseling, self-relaxation, self-hypnosis, bio-feedback, stress management as well as cognitive behavior technique.
- Stimulation therapies - acupuncture has been used successfully in Chinese medicine for centuries. It causes the release of endogenous analgesics (endorphins) within the spinal cord. Also, transcutaneous electrical nerve stimulation (TENS) may have a similar mechanism of action as acupuncture and can be used in both acute and chronic pain.
- Herbal medicine and homeopathy – this are widely used for pain, but often with little evidence for efficacy as well as safety regulations when compared to conventional drugs (Goodman and Gilman, 2006; WHO, 2013).

2.3 INFLAMMATION

Inflammation occurs when immunological component cells are activated in response to foreign organisms or antigenic proteins. The outcome of this inflammatory response for the host may either be beneficial (when it causes invading organisms to be phagocytosed or neutralized), or deleterious (as in the case of arthritis, when it leads to the destruction of bone, cartilage which ultimately result in the limitation of joint function). Inflammation is also described as a protective response to localized origin elicited by injury, destruction of body tissues which serves to destroy, dilute or sequester both the injurious agents and injured tissue. It is characterized in the acute form by classical signs of pain, redness (flushing), swelling (flaring) and loss of function. There is also infiltration of the interstitial spaces (oedema) in some cases depending on the cause of inflammation. Histologically, there is increased dilation of the capillaries, with increased permeability and blood flow. This results in the observed signs termed "flushing and flaring" of the tissue.

2.3.1 Inflammatory Responses

An inflammatory response may be induced in a great variety of ways. These include trauma, injury, antigens (viral, bacteria, protozoa and fungi), some chemicals such as 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. The cellular damage associated with inflammation acts on cell membrane,which activates leucocytes to release lysosomal enzymes, such as arachidonic acid (liberated from precursor compounds) and various eicosanoids are synthesized in the inflammatory responses. Leukotrienes have a powerful chemotoxic effect on eosinophils, neutrophils

and macrophages and promote bronchial constriction and alteration in vascular permeability. Kinins, neupeptides and histamine are also released at the site of injury. Stimulation of the neutrophil membranes produces oxygen-derived free radicals. Superoxide is formed by the reduction of molecular oxygen, which may stimulate the production of other reactive molecules such as hydrogen peroxide and hydroxyl radicals. The interaction of these substances with arachidonic results in the generation of chemotatic substances, thus perpetuating the inflammatory process.A local inflammatory response is usually accompanied by systemic changes such as fever, malaise and an increase in circulating leucocytes, such signs and symptoms are often helpful in diagnosis of various inflammatory responses.

Inflammation is basically a protective mechanism in the body. The leakage of water and proteineous substances into the injured area bring about humoral factors including antibodies into the area of injury. These may serve to dilute soluble toxic substances and normalizes the system. The migration of leucocytes to the local site brings about destruction of these injurious agents.In certain situation where the cause of inflammation is due to infections, such as rheumatoid arthritis and rheumatoid fever, this may be uncontrolled by the body immune system and may require the use of anti-inflammatory and other adjuvants for treatment(Taylor, 2002).

Inflammation can be acute or chronic in nature. Acute inflammation progresses mostly with a sudden onset and it is of short duration, with characteristic classical signs of predominantly vascular and exudative processes. Chronic inflammation progresses slowly and usually persists for a longer duration of time. It is directly marked by new connective tissue formation. It could result due to untreated acute inflammation. Other types of

inflammation are inflammation due to traumatic ulcer and exudating fibrinous inflammation (Taylor, 2002).

Inflammation is divided into three (3) phases; acute, immune response and chronic inflammation.The initial response to tissue injury is mediated by autacoids (histamine, serotonin) and usually preceded by the development of immune response.This results when immunologically competent cells are activated in response to foreign organizations or chronic inflammatory response. The immune response is protective in nature as it leads to phagocytosis of invading organisms. Though immune response to inflammatory may be defensive, it could be deleterious if the underlying cause is not well treated and may progress to chronic inflammation. Chronic inflammation involves the release of mediators such as interleukin I, II and III, interferon and platelet derived growth factor. One of the most important conditions involving these mediators is rheumatoid arthritis in which chronic inflammation results in pain and destruction of bone and cartilage that can lead to severe disability. The cell damage, which is a characteristic of inflammation act on cell membrane to cause leukocytes to release lysosomal enzymes. This involves also liberation of prostaglandins and leucotrienes, which are mediators of inflammation, (Rang *et al.*, 2003).

The signs observed following inflammation includes vascular and cellular changes.In vascular changes; following a mild injury, there is fleeting constriction of the smallest arterioles in theviable tissue close to the site of injury lasting for a period of 5 – 10 minutes. This isfollowed by dilation of the same arterioles leading to engorgement anddilation ofthe capillaries, which extends over to an area farther to the contributingcomponents.These causes capillary engorgement and constriction at the site of

injury.

There is alsoconstriction of the smallest veins (venules), which is closely localized inthe central areaaround the site of injury. Along with the changes in blood vessels, thereis net movement ofwater into the extracellular fluid, causing an excess of fluid accumulation in theextracellularcompartment which is referred to as oedema. The vessel walls also become morepermeable to largeproteinous molecules that circulate in blood plasma, thesemolecules then leak out intothe tissues. In contrast to the vascular response, the cellular response in inflammation is varied and serves to characterize the different types of inflammation. Participating cells are derived from two sources, the circulating blood and the local connective tissue. The circulating leucocytes are divided distinguishable cell types, namely, granulocytes (eosinophil, neutrophil and basophil), small and large lymphocytes and monocytes. Fibroblasts and most cells are solely connective tissue cells. Macrophages and giant cells arise in the inflammatory locus probably from circulating monocytes (Usha *et al*, 2012).

2.3.2 Management of Inflammation

The treatment of patient with inflammation involves two primary goals. Firstly, the relief of pain, which often is the presenting symptoms and the major continuing compliant of the patient and secondly, the slowing or arrest of tissue-damaging process.Non-steroidal anti-inflammatory drugs (NSAIDs) are often used in the reduction inflammation which often results in the relief of pain for significant period. Furthermore most of the NSAIDs are appropriate for the treatment of both acute and chronic inflammatory condition. NSAIDs possess analgesic, anti-inflammatory and anti-pyretic properties. The term “Non-steroidal” is used to distinguish these drugs from steroids, which also have among other effects a similar eicosanoid-depressing, anti-inflammatory action. These steroids are also use in

alteration of modulation of inflammatory conditions in some cases. Examples include dexamethasone, triancinolone, prednisolone, prednisone and budesonide(Rang *et al.*, 2003).

2.4 PYREXIA

Pyrogens are substances, typically produced by a bacterium, which produces fever when introduced into the blood. 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. Pyrexia can also be said to be a result of secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states. The infected or damaged tissue initiates the enhanced 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. Elevated body temperature that is not caused by a resetting of the temperature set point in the hypothalamus is commonly called hyperthermia. The common symptoms of pyrexia are as follows; fever, chills, shivering, malaise and pains (back or leg), (Kumar *et al.*, 2009; Felton, 2013).

2.4.1 Pathophysiology of Fever

The thermoregulatory center is located in the anterior portion of the hypothalamus. When the vascular bed surrounding the hypothalamus is exposed to certain exogenous pyrogen (bacteria) or endogenous pyrogens (interleukin-1, interleukin-6, tumour necrosis factor), arachidonic acid metabolites are released from the endothelial cells of this vascular network. Among these metabolites, prostaglandins E_2 cross the blood brain-barrier and

diffuse into thermoregulatory area of the hypothalamus, triggering series of events that ultimately increases the set point(Felton, 2013).

2.4.2 Treatment of Pyrexia using Analgesics

Most of the anti-pyretic drugs inhibit COX-2 expression thus inhibiting PGE_2 biosynthesis to reduce elevated body temperature. Examples include; aspirin, ibuprofen, paracetamol, glucocorticoids, (Dinarello and Gelfand, 1998; Salawu et al, 2008).

2.5 Medicinal Plants with Analgesic, Anti-inflammatory and AntipyretcProperties

Medicinal plants are known to provide a rich source of raw materials, with potential therapeutic effects, for use in traditional medicine practice in Africa and other parts of the developing world, (Shekhawat and Vijayvergia, 2010).Research and development from traditional medicinal preparations have led to discovery of many potent drugs which are used in modern clinical practices. Example is the development of NICOSAN™ from medicinal plants (*Piper guineenses* seed, *Pterocapsus osum* stem, *Eugenia caryophyllum* fruit, and *Sorghum bicolor* leaves) in Nigeria, which is used in modern clinical practice to manage of sickle cell anaemia, (Yusuf, 2011).

A number of medicinal plants are naturally endowed with analgesic and anti-inflammatory properties. Examples include; *Papaver somniferum,* from which morphine was isolated in 1806 and is regarded as a prototype opiate analgesic drug.The plant extract of *Lactuca scariola* and *Artemisia absinthium* were evaluated and shown to have potent and significant analgesic and anti-inflammatory activities (Fayyad *et al*., 1992).Kolaviron, a defatted seed extract of *Garcinia kola* (bitter kola) was found to have weak analgesic but very strong anti-inflammatory activities (Olaleye *et al*., 2000).The root bark extract of *Khaya senegalensis* has prominent use in the treatment of fever in Senegal. Studied carried

out on its methanol root bark extract showed that it possess analgesic property (Ogunlela, 2002).*Ammania baccifera* Linn, whole plant was investigated and found to have potent analgesic activity which confirms its folkloric use in India for treatment of rheumatic pain (Dhanapal *et al*., 2004).The aqueous extract of *Spilanthes acmella* was investigated and found to possess significant analgesic and anti-inflammatory potentials (Chakraborty *et al*., 2004).

The crude methanol extract of *Vigna trilobata* (Fabaceae) was shown to possess potent analgesic potentials, which confirms its folkloric use in Ayuveda medicine in the relief of pain, (Rafeeq *et al*., 2007).Similarly, *Glycine tomentella* Hayata (Fabaceae) was evaluated and found to possess potent analgesic and anti-inflammatory potentials which confirm its ethnomedical use in the Knimen area of Taiwan as anti-inflammatory agent for the treatment of rheumatic illness, gout, arthritis and body aches (Tsung-Chun *et al*., 2007). The methanol extract of *Schwenkia Americana* was also found to contain bioactive constituents which possess analgesic and anti-inflammatory activities (Hassan *et al*., 2008).Similarly, *Cissus guadrangularis* was found to have analgesic activity which confirms its use as analgesic agent in eye and ear diseases (Mate *et al*., 2008).

Crossopteryx febrifuga preparations have been used traditionally in Nigeria for treatment of pain and malaria for many years. Its efficacy is widely acclaimed among the Hausa tribe in Nigeria (Audu, 1989). However, the methanolic stem bark extract of *Crossopteryx febrifuga* was found to contain biologically active substances that possess analgesic and anti-inflammatory activities, (Salawu *et al*., 2008). Also the root extract of *Securinega virosa* is used in West African sub-region for its analgesic property. Studies showed that methanol root bark extract of *S virosa*possess analgesic as well as anti-inflammatory

activities, (Magaji *et al*., 2009).*Commelina benghalensis,* was reported to possess pain-relieving, anti-inflammatory, febrifugal activities in India and China. Studies carried out on the analgesic activity of the different fractions of the aerial parts showed that, *C. benghalensis* possesses significant analgesic action, (Hasan *et al*, 2010).The methanol leaf extract of *Madhuca indica* GMEL was also evaluated for its anti-inflammatory, analgesic and antipyretic properties. The plant showed analgesic, anti-inflammatory as well as antipyretic effects similar to those observed for the standard drugs (phenylbutazone and paracetamol) used for the experiment, (Shekhawat and Vijayvergia, 2010).Similarly, the plant, *Stereospermum kunthianum* was reported to be used in the treatment of inflammatory conditions and pain in Mali. Studies have also shown that the methanol extract,as well as the fractions of this plant possess significant analgesic activity, (Omogbai *et al*., 2010).

Aqueous extract of *Aloe ferox* leave was reported to possess some analgesicand anti-inflammatory activity, giving scientific based evidence in support for its acclaimed worldwide acceptance as an anti-inflammatory properties, (Mwale and Masika, 2010).Similarly, the ethanol extract of the root of *Aconitum heterophyllum* was evaluated and found to possess anti-inflammatory effect. This supports the traditional claims and provides a scientific basis for its use in inflammatory diseases, (Verma *et al*, 2010).Aqueous extract of *Ipomoea pes-tigridis* whole plant was evaluated for its analgesic effect and was found to be capable of inhibiting non-inflammatory reaction as well as inflammatory pain. These provide scientific based evidence for its use in herbal remedy in the treatment of painful conditions like headaches, swellings, (Ramesh, 2010).

Different parts of the tree *Dalium guineense* have been reportedly used traditionally in

Enugu-Nigeria, in the treatment of headache, pains (bark), fever, prenatal pain and oedema (leaves). Studies on the analgesic activity of the methanol stem-bark of the plant showed significant analgesic activity, (Ezeja *et al*., 2011).The ethanol extract of *Cymbidium aloifolium*leaf was demonstrated to possess anti-inflammatory and analgesic activities, which scientifically provides evidence for its traditional use, in Pakistan, to treat boils, fever, burns, and sore, (Howlader *et al*, 2011).

The methanol extract of *Markhamia tomentosa*leaf have been investigated for its analgesic and anti-inflammatory effects, and was found to possesss anti- inflammatory, as well as both central and peripheral antinociceptive activities, (Temdie *et al.,* 2012).Whole plant of *Viola betonicifolia* was reported to possess anti-pyretic as well as diaphoretic activity from folkloric investigation, in Pakistan. However, studies showed that the methanol extract of the whole plant have marked antipyretic, analgesic and anti-inflammatory activities, (Muhammed *et al*., 2012).Also, the methanol extract of *Luffa echinata* seed was evaluated and found to have antioxidant, anti-inflammatory as well as analgesic effects, this provides scientific based evidence for its acclaimed free radical scavenging, antioedema and antipyretic activities, (Sharma *et al*., 2012).

Ethanol extract of the mushroom *Ganoderma applanatum* was evaluated and shown to possess analgesic as well as anti-inflammatory activity, which give scientific evidence to support the acclaimed wide range of pharmacological activities, (Ede *et al.,* 2012).Other plants reported to possess potential analgesic and anti-inflammatory activities are; *Melenthera scandens, Abutilon indicum, Cussonia paniculata, Oscillatoria willei, Mondia whytei, Pfaffia glomerata, Swertia chirata, Kigelia africana, Microtrichia perotitii*(*D.C*), *Anisopus mannii, Zizyphus rugosa, Solanum trilobatum*(Linn), *Anogeissus acuminate,*

(Usha *et al.,* 2012). *Schewenkia americana,* (Hassan *et al.,* 2008), *Leonotis leonurus*(Maphosa *et al.,* 2012). There were reports on the anti-inflammatory and analgesics

activities of*Ananas comosus, Boswellia serrata, Calotropis gigantea, Calotropis procera, Camellia sinensis, Cannabis sativa, Curcuma longa, Mangifera indica, Ricinus communis,* (Anilkumar, 2010).

2.6 Scientific studies done on *Acacia ataxacantha*

The ulcer protective effect of the methanol extract of *Acacia ataxacantha*leaf was carried out using indomethacin and stress induced ulcer in rats. The extract was found to possess ulcer protective effectwhich validates its use locally in Senegal in the treatment of ulcer, (Akapa*et al.,* 2014).

Plate I:*Acacia ataxacantha* D.C in its natural habitat, showing the stems and leaves

2.7 *Acacia ataxacantha*

Acacia ataxacantha is a shrubby scrambler, with stem measuring up to 10cm long forming thickets 4-5cm deep. It is very thorny, and sometimes grow on rocky hills in the forest region, and in dry savanna, from Senegal to Nigeria, and widespread in tropical Africa. The plant has hooked prickles that are scattered along the stems and the leaf rachis. The leaves have about 8-15 pairs of pinnae; petiole with a stalked gland at the base. The flower has axillary spikes with creamy-white colour. The pods are straight, slender, dehiscent, papery reddish-brown. (Hyde *et al*, 2013; Akapa *et al.,* 2014)

2.7.1 Taxonomy

The current name is *Acacia ataxacantha* D.C, family; Leguminosae-Mimosoideae

The synonyms of *Acacia ataxacantha* include;

Acacia eriadenia Benth.

Acacia lugardiae N. E. Br. (Hyde *et al.*, 2013)

The common names of the plant in English are, flame thorn, Benin rope acacia andbenirope. In Nigeria, the Hausa tribe call it “dufuwa”, “duhuwa”, “kwandariya”, “sarkakiyaa” and “sarkakkiya”, while the kanuri tribe called it “dusu”.The Edo tribe call it “ukhuenkhuen”, the Adamawa call it “kwaraje”, the Igbo call it “uke” and the Yoruba’s named the plant “ewon adele” (Burkill, 1997).

2.7.2 Chemical constituents

Unnamed alkaloids are thought to be present in the leaves and stems of *Acacia ataxacantha*, (Burkill, 1997). Alkaloids, polyphenols, flavonoids, saponins, tannins and terpenoids were found to be present in the methanol extract of*Acacia ataxacantha* leaf (Akapa*et al.,* 2014).

2.7.3Ethnomedical uses

Preparations of different parts of *Acacia ataxacantha* are used in traditional medicine as pain relieverand as anti-inflammatory agent. The stem-back infusion is prepared in Soudano-guinea region 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).Decoction of *Acacia ataxacantha*is prepared in Ubangi for tooth ache; the sap is rubbed on the head for headaches and the pulped leaf is made into poultice for burns and sore on the head. The dried powdered leaf is also applied to chancre on the penis as an anti-infective agent.The Wolof and Serer of Senegal take the powdered leaf internally for treatment of syphilis.An aqueous maceration of the root in association with extracts of*Capparis tomentosa* (Capparacae) and *Securidaca longipedunculata* (Polygalaceae) are taken as draught by Fula in Senegal for hernia, helminthiasis, sores and wounds, (Burkill, 1997). In Namibia it is use in combination with other plants for treating HIV/AIDS,(Hedimbi and Chinsembu, 2012). The leaves and roots have also been reportedly used for vitamins, minerals, digestive system disorders, chest ailments, infestations, pain reliever and respiratory system disorders (Akapa *et al.,* 2014). Also in Abeokuta, the pods and seeds have been documented to be used as herbal remedy for treatment of stomach ailments and dysentery (Akapa *et al.,* 2014).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Animals

- Swiss albino mice
- Wistar rats

3.1.2 Chemicals

- Acetyl salicylic acid (Aspirin 300mg, Mayer & Baker Ltd, Dagenham England)
- Acetic acid (0.6% $^{w}/_{v}$), Mayer & Baker Ltd, Dagenham England
- Brewer's yeast
- Carrageenan suspension (1% $^{w}/_{v}$ in normal saline), Sigma-Aldrich Germany
- Chloroform (Sigma Chemicals Co, USA)
- Concentrated HCl (BDH Chemicals Ltd Poole England)
- Ferric chloride (BDH Ltd Poole, England)
- Formaldehyde (Sigma-Aldrich Laborchemikalein GmBH Germany)
- Formalin (1% w/v) (Sigma-Aldrich Laborchemikalein GmBH Germany)
- Distilled water
- Egg-albumin
- Methanol (70% v/v), BDH Chemicals Ltd Poole England
- Morphine sulphate B.P 25 g (Martindale Pharmaceuticals Ramford Essex, U.K)
- Normal saline (0.9% NaCl Isotonic Solution).
- Piroxicam (Felxicam 20mg, HOVID Bhd, Malaysia)

3.1.3 Equipment

- Analytical Balance (Mettler Instrument Corporation, U.S.A.)
- Animal Cages
- Beakers
- Conical Flasks
- Digital thermometer (Omron® Healthcare, China)
- Evaporating Dishes
- Filter (Whatman filter paper No. 1)
- Hot Plate (Gallenkamp, Thermostat, England)
- Measuring Cylinder
- Mortar and Pestle
- Needle and Syringe
- Reagent Bottle
- Stop Clock
- Venier Caliper
- Water Bath (MacDonald, England)

3.2 METHODOLOGY

3.2.1 Collection of plant materials

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 Mr. 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.

3.2.2Preparation of extract

The leaves of the plant were separated from the tree branch, cleaned, air-dried under the shade for fourteen days (2 weeks) and crushed into coarse powder using pestle and mortar. Five hundred grams (500 g) of the coarse powder 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 was concentrated to dryness using evaporating dish over a water-bath, maintained at a temperature 50-60 ^{o}C to give 31.94% $^{w}/_{w}$ of the extract.

3.3 Phytochemical Screening of the plant extract

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

3.4 Toxicity Studies

Acute toxicity was carried out in mice and rats according to the method of Lorke (1983), while chronic toxicity was carried out in rats according to OECD guidelines, (1995).

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

The oral and intraperitoneal 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. In the first stage, nine mice were randomly divided placed into threegroups of three mice each. Groups one, two and three were treated with the extract at doses of 10 100 and 1000 mg/kg body weight i.p. respectively. The mice were monitored for 24 hrs for signs of toxicity, and mortality. In the second phase, four mice were put in four groups of one mouse each. Groups one, two, three and four were administered the extract at doses of 200, 400, 800 and 1600 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).This experimental procedure was also done using rats.

3.4.2 Chronic toxicity study

Twenty four adult rats (Wistar strain), were randomly divided into four groups of six rats each. Group I served as the control and received feed and water only while, the animals in group II, III and IV received the extract at doses 50, 200 and 400mg/kg body weight respectively. The treatment was done once daily for 90 days. The extract was administered orally throughout the period. All the animals were observed daily for signs of toxicity and mortality. The body weight of each rat was measured at 9 am once weekly using a sensitivebalance before the commencement of dosing.At the end of the treatment period, the animals were humanely sacrificed in chloroform chamber and blood samples were collected for sera preparation by cardiac puncture. The sera preparations were used

subsequently for biochemical and haematological studies.

Biochemical studies

After the animals were anaesthetized in a chloroform chamber, they were opened up surgically, blood samples were collected by cardiac puncturing into plain samples bottles. The blood was then allowed to clot and centrifuged at 3500 rpm for 10 minutes. The serum was separated and stored at -4°C until used. The serum was analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphate (ALP), serum bilirubin, serum urea, creatinine, chloride, sodium, potassium, bicarbonate using kits obtained from Reckon Diagnostic Limited, India.The biochemical analysis was carried out at the Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital (ABUTH), Zaria.

Heamatological studies

Blood samples were collected into heparinised bottles for determination of packed cell volume (PCV), haemoglobin concentration (Hb), platelet count, white blood cell count (WBC) and differential lymphocyte count, mean corpuscular haemoglobin concentration (MCHC), using an automated haematological machine (Cell- Dyn TM Abbot, USD). The haematological analysis was carried out at the Department of Haematology, Ahmadu Bello University Teaching Hospital (ABUTH), Zaria.

Histopathological Studies

Vital organs like the brain, liver, heart, kidney and lungs were removed, weighed and preserved, in 10% formalin until used. Tissues were processed histologically by the

method described byDisbrey and Rack, (1970).Histological examination was done in the Department of Anatomy, A.B.U, Zaria.

3.5 Analgesic Studies

The analgesic activity of the extract was evaluated using the following methods;

3.5.1 Acetic acid-induced writhes in mice

The experiment was carried out according to the method described by Koster*et al*., (1959).Thirty six mice were randomly divided into six groups of six mice per group. Mice in group I (control) were pre-treated with normal saline (10ml/kg). Animals in groups II, III, IV and V were pre-treated with the extract at doses of 50, 100, 200 and 400mg /kg body weight respectively, while group VI was pre-treated with piroxicam 10mg/kg (standard drug). All treatments were administered orally. One hour post-treatment,each mouse in the groups was then injected (intraperitoneally) with 10 ml/kg of aqueous solution of acetic acid (0.6% $^{w}/_{v}$) and placed in a transparent cage. After five minutes lag period, the number of writhes (abdominal constriction accompanied with backward stretching of hind limbs) were counted for each mouse, using tally counters, for a period of 10 minutes. The number of acetic acid-induced writhes in groups II – VI were then compared with that of group I (control), acetic acid treated mice only.

The percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \frac{\text{Mean No. of writhing (control)} - \text{Mean No. of writhing (test)}}{\text{Mean number of writhing (control)}} \times 100$$

3.5.2 Hot plate assay

The thermal method was used to measure the response latencies (Eddy and Leimbach, 1953) of mice when exposed to hot plate. Effect of the extract was evaluated using hot plate method in mice, in which the temperature of the hot plate was maintained at 55 ± 0.5 °C; and the animals which responded when placed on the hot plate within a period of 30 seconds were selected for the experiment. Responses include, shaking or licking of paw or jumping off the hot plate. One hour post-treatment, the mice were then divided into six groups of six mice each. Group I was pre-treated with normal saline (10ml/kg) and serves as the control group.Groups II, III, IV and V were pre-treated with extract at doses of 50, 100, 200 and 400 mg/kg body weight (orally) respectively. Group VI was pre-treated with morphine (4 mg/kg body weight, (orally), which served as the positive control. After one hour of post-treatment, each mouse was placed on the hot plate and the responses were recorded as index of response latency. Readings were taken at 0, 1, 2, 3 and 4hours respectively.

3.5.3 Formalin induced paw licking

The method used was that described byTjolsen *et al*. (1992).This method was originally developed by Dubuisson and Dennis, (1977). Thirty rats were randomly divided into six groups of six rats per group. Groups I was pre-treated with normal saline(10ml/kg) and served as the negative control. Groups II, III, IV and V were pre-treated with extract at doses of 50, 100, 200 and 400 mg/kg body weight respectively, while group VI was pre-treated with morphine(4mg/kg) and serve as the positive control. All drugs were administered orally. One hour post-treatment, each mouse in all the groups was then injected (subcutaneously), under the plantar surface of the left hind paw, with 0.2ml of freshly prepared formalin (1.0% $^{w}/_{v}$) and placed in a transparent cage and observed for an

hour. The severity of the pain response was recorded for each rat based on the following scale:

Scale 0 the rat walked or stood firmly on the injected paw;

Scale 1 the injected paw was raised or partially elevated;

Scale 2the injected paw was clearly lifted off the floor;

Scale 3 the rat licked, chewed or shook the injected paw.

Anti-nociceptive effect was determined in two phases; the early phase (phase1) was recorded during the first fiveminutes, while the late phase was recorded during the last forty fiveminutes with a ten minutes lag period between the two phases.

3.6 Anti-inflammatory Studies

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

3.6.1 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 (10ml/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) 10mg/kg body weight, which serve as the positive control. One hour post-treatment, 0.1 ml of carrageenan 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 was measured with the aid of Venier caliper, at 0, 1, 2, 3, 4 hours respectively after injection of carragenaan.

3.6.2 Hind 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 sexes were divided into five groups of five rats and pre-treated as follows: Group I received normal saline (10 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.

3.7 Antipyretic Study

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

3.7.1 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 (10ml/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(150mg/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 120minutes.

3.8 STATISTICAL ANALYSIS

The data wereexpressed as mean ± SEM and analyzed usingoneway analysis of variance (ANOVA) followed by Dunnett-t post-hoc test.Values of $p<0.05$ were considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 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.

4.2 Phytochemical Constituents

Preliminary phytochemical screening of methanol extract of *Acacia ataxacantha* leaf revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols, saponins, tannins (Table 4.1).

4.3 Toxicity Studies

Median lethal dose (LD_{50}) and chronic administration of extract for 90 days was used to access the safety profile of the extract of *Acacia ataxacantha* in mice and rats.

4.3.1 Median lethal dose (LD_{50}) in mice and rats

The median lethal dose (LD_{50})of the methanol extract of *A*cacia *ataxacantha* leaf in mice and rats via oral and intraperitoneal routes were estimated as shown in (Table 4.2).

4.3.2 The effect of the extract on kidney function parameters in rats

There was statistically significant increase($p < 0.05$) in the serum levels of creatinine, urea and sodium ion when compared with control groupin rats following 90 days oral administration of methanol extract of *Acacia ataxacantha* leaf(Table 4.3).

4.3.3. The effect of the extract on serum liver enzymes in rats

There was statistically significant increase in the levels of aspartate transaminase (AST) ($p< 0.001$), alanine transaminase (ALT) ($p < 0.05$ at 50 and 200 mg/kg, $p < 0.01$ at 400 mg/kg respectively), and alkaline phosphatase (ALP)($p < 0.05$ and $p < 0.001$ at 200 and 400 mg/kg respectively) when compared with the control group in rats following 90 days oral administration of methanol extract of *Acacia ataxacantha*leaf (Table 4.4)

4.3.4 The effect of the extract on haematological parameters in rats

There is no statisticallysignificant difference in the haematological parameters evaluatedin rats treated with theextract when compared with the control animals(Table 4.5).

4.3.5The effect of the extract on histology of different organs in rats

4.3.5.1 The Heart

There is no histological change inthe heart (normal cardiac cells and cardiac muscles) tissues of rats treated with the extractwhen compared with the control group,(plate II).

4.3.5.2 The kidney.

The control animals had normal glomerulus and renal tubular structures while animals treated with the extract at doses of 50, 200 and 500mg/kg had changes in kidney morphology (moderate glomerular necrosis and lymphocytes hyperplasia) (plate III).

4.3.5.3 The liver.

Animals treated with 50, 200 mg/kg of the extract had normal liver architecture. However, rats treated with the extract at 400 mg/kg had hepatocellular necrosis with kupfer cells hyperplasia, when compared with the control animals, (plate IV).

4.3.5.4 The spleen.

All the rats treated with different doses (50, 200, and 400 mg/kg) of the extract had normal spleen structures (normal red and white pulp distribution)(plate V).

4.3.5.5 The stomach.

Rats treated with 50 and 200 mg/kg of the extract had normal stomach histology. However animals given extract at dose of 400 mg/kg showed mild mucosal necrosis, (plate VI).

4.4 Analgesic Studies

4.4.1 Acetic acid-induced writhes in mice

The extract at doses of 50, 100, 200 and 400 mg/kg statisticallysignificantly ($p < 0.05$) reduced the number of writhes (19.40, 17.40, 14.60 and 12.60 respectively) induced by acetic acid when compared with the control animals (35.40)(Figure4.1).

4.4.2 Hot plate assay(thermally induced pain) in mice

The extract statistically significantly ($p < 0.05$)increased the reaction time to the thermally induced stimulus in mice in all the tested doses. The highest statistical significant increase in reaction time to thermal stimulus was observed in the second and third hour post-treatment for all doses tested(Table 4.6).

4.4.3 Formalin–induced pain in rats

The extract at doses of 50, 100, 200 and 400 mg/kgsignificantly ($p < 0.05$) reduced the paininduced by formalin in the early phase while, in the late phase there was significant ($P < 0.05$) reduction in pain threshold at doses of 200 and 400 mg/kg body weight only. However, the mean pain scores(early phase) of the extract at 100 and 200mg/kg body

weight were the same(2.4) and hence the effect produced was statistically significant but, dose-independent (Figure 4.2).

4.5Anti-inflammatory Studies

4.5.1 Carragenaan-induced paw oedema

The extract showed statistical significantinhibition($p < 0.05$)of inflammation at the 3rd and 4th hour after carragenaan administration.Also the percentage inhibition was highest at the 3rd (20 % at doses of 200 and 400 mg/kg) and 4th hours (26 %, 18 % and 30 % at doses of 100, 200 and 400 mg/kg) respectively(Figure 4.3).

4.5.2Albumin-induced paw oedema

At a dose of 200 mg/kg, there was statistical significant decrease($p < 0.05$) at 40th and 120th minutes only, while at 400 mg/kg body weight there was significant decrease ($p < 0.05$) in paw oedemaat 20th, 40th, 60th and 120th minutes respectively. Also for the standard drug, there was significant decrease($p < 0.05$) at all the tested times. The inflammatory responses obtained using albumin was not consistent when compared with carragenaan induced inflammation in group I (normal saline 10 ml/kg) (Table 4.7).

4.6Antipyretic Study

4.6.1 Yeast induced pyrexia

The extract produced no significant reduction in yeast-induced pyrexia in rats, when administered to the animals (Table 4.8).

Table 4.1: Phytochemical Constituents ofExtract of *Acaciaataxacantha*Leaf

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

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

Table 4.2: LD_{50} values of methanolextract of *Acacia ataxacantha*leaf

Species	Route of administration	LD_{50} (mg/kg body weight)
Mice	Intraperitoneal	565.69
	Oral	> 5000
Rats	Intraperitoneal	1264.91
	Oral	> 5000

Table 4.3: Effect of the methanol extract of *Acacia ataxacantha*leaf on someRenal biochemical parameters after 90 days of administration

Biochemical parameters	D/W	MEAA 50mg/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
CI^- (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

Data were presented as Mean ± SEM
n= 6, *=P< 0.05 level of significance
Statistical tool: ANOVA (one way analysis of variance)
D/W= Distilled water
MEAA=Methanolextract of *Acacia ataxacantha*

Table 4.4: Effect of the methanol extract of *Acacia ataxacantha*leaf onLiver function parameters after 90 days of administration

Treatment (mg/kg)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TB (mg/dL)	CB (mg/dL)
D/W	23.80±1.20	33.80±2.08	56±2.03	26.20±0.73	22.80± 3.76
MEAA 50	38.33±0.67***	48.30±3.53*	68±6.00	25.33±1.67	18.67±1.33
MEAA 200	34.00±2.00***	47.50±1.50*	78±6.00*	20.50±2.50	14.00±1.20
MEAA 400	42.00±2.65***	50.00±4.04**	83±6.24***	22.00±1.70	14.33±1.77

Data were presented as Mean ± SEM
n= 6, * = P< 0.05, **= P < 0.01, ***= P< 0.001 level of significance
Statistical tool: ANOVA (one way analysis of variance)
D/W = Distilled water
MEAA=Methanolextract of *Acacia ataxacantha*
AST= Aspartatetransaminase
ALT=Alaninetransaminase
ALP=Alkaline phosphatase,
TB= Total bilirubin
CB=Conjugated bilirubin

Table 4.5: Effect of the methanol extract of *Acacia ataxacantha*leaf on Haematological parameters after 90 days of administration

Haematological indices	D/S	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 (one way analysis of variance)
MEAA= Methanolextract of *Acacia ataxacanta*
WBC= White blood cell
RBC= Red blood cell
HGB= Haemoglobin
PLT= Platelet count
PCV= Packed cell volume
MCV= Mean corpuscular volume
MCH= Mean corpuscular haemoglobin
MCHC= Mean corpuscular haemoglobin concentration.

Table 4.6: Effect of methanol extract of *Acacia ataxacantha*leaf on thermally induced pain (hot plate) assay in mice

Treatment (mg/kg)	Mean Response Latency (seconds)				
	0hr	**1hr**	**2hr**	**3hr**	**4hr**
N/S	1.22 ± 0.06	3.60 ± 0.24	3.60 ± 0.68	4.20 ± 0.48	3.20 ± 0.67
MEAA 50	1.30 ± 0.07	3.38 ± 0.58	8.40 ± 0.67*	11.00±1.18**	6.20 ± 0.86
MEAA 100	1.24 ± 0.07	3.60 ± 0.25	9.80 ± 1.49**	12.2 ± 1.01**	5.20 ± 0.37
MEAA 200	1.24 ± 0.05	4.40 ± 0.81	9.60 ± 0.87**	12.4 ± 0.81**	3.20 ± 0.57
MEAA 400	1.22 ± 0.05	7.00 ± 1.04*	13.00±0.89**	11.40±0.79**	3.60 ± 0.81
Morphine 4	1.24 ± 0.07	14.60±1.26***	18.80±0.37**	8.60 ± 1.07**	3.60 ± 0.74

n = 6, *, **, ***, represents $p < 0.05$, $p< 0.01$, $p< 0.001$ level of significance respectively
Statistical tool: ANOVA (one way analysis of variance)
N/S =Normal saline
MEAA=Methanol extract of *Acacia ataxacantha*

Table 4.7: Effect of methanol extract of *Acacia ataxacantha*leaf on albumin induced hind paw oedema in rats

Treatment (mg/kg)	Volume of Oedema (cm)/(% Inhibition)					
	20min	40min	60min	80min	100min	120min
N/S	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±0.21[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±0.01[b] (15.50)	0.37±0.04[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±0.01[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±0.02[b] (18.10)	0.34± 0.01[b] (30.00)

n = 5 [a]= P < 0.05, [b] = P < 0.01, [c]= P < 0.001
Statistical tool: ANOVA (one way analysis of variance)
N/S= Normal saline
MEAA=Methanol Extract of *Acacia ataxacantha*
ASA=Acetyl salicylic acid

Table 4.8: Anti-pyretic effects of the methanol extract *Acacia ataxacantha*leafin rats

Treatment (mg/kg)	0min	30min	60min	90min	120min
N/S	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]

n = 5, [a] = P<0.05 level of significance
N/S= Normal saline
Statistical tool: ANOVA (one way analysis of variance)
MEAA=Methanol Extract of *Acacia ataxacantha*
PCM= Paracetamol

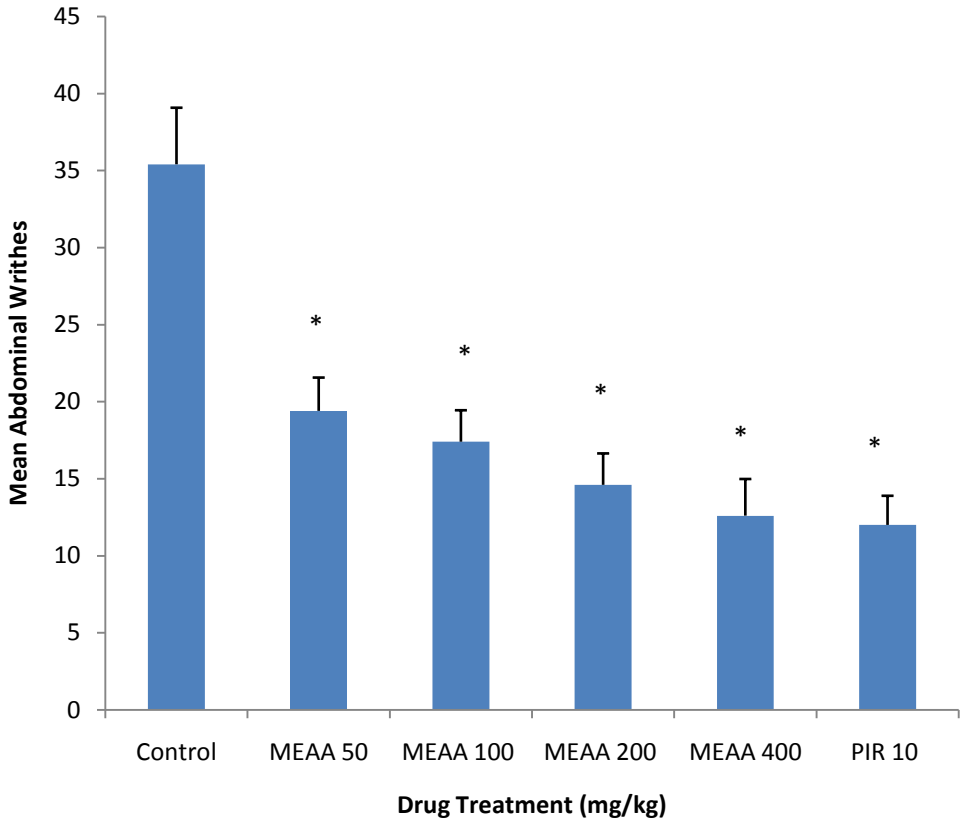

Figure 4.1: Effect of methanol extract of *Acacia ataxacantha* leaf on acetic acid induced abdominal writhes in mice

n= 6, * = P < 0.05 level of significance
Statistical Analysis: ANOVA (one way analysis of variance)
MEAA= Methanol extract of *Acacia ataxacantha*.

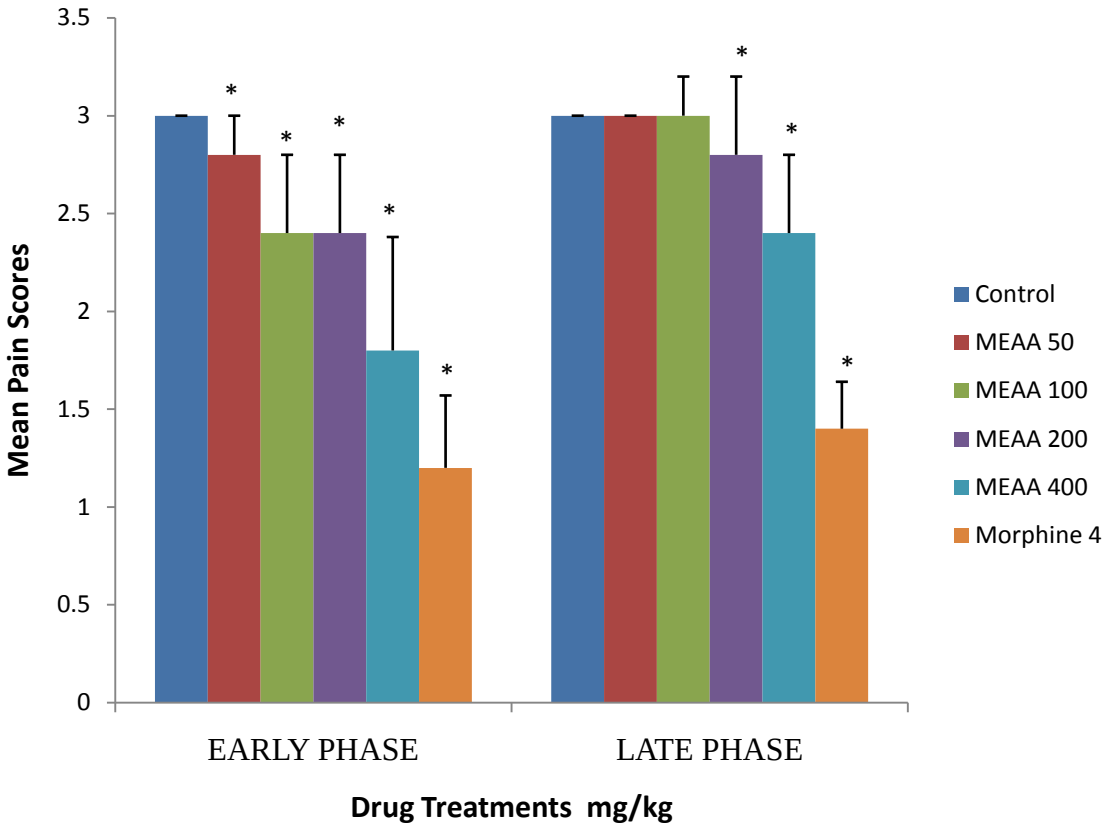

Figure 4.2: Effect of methanol extract of *Acacia ataxacantha*leaf on formalininduced pain in mice.

n= 6, * = P < 0.05 level of significance
Statistical Analysis: ANOVA (one way analysis of variance)
MEAA= Methanol extract of *Acacia ataxacantha*

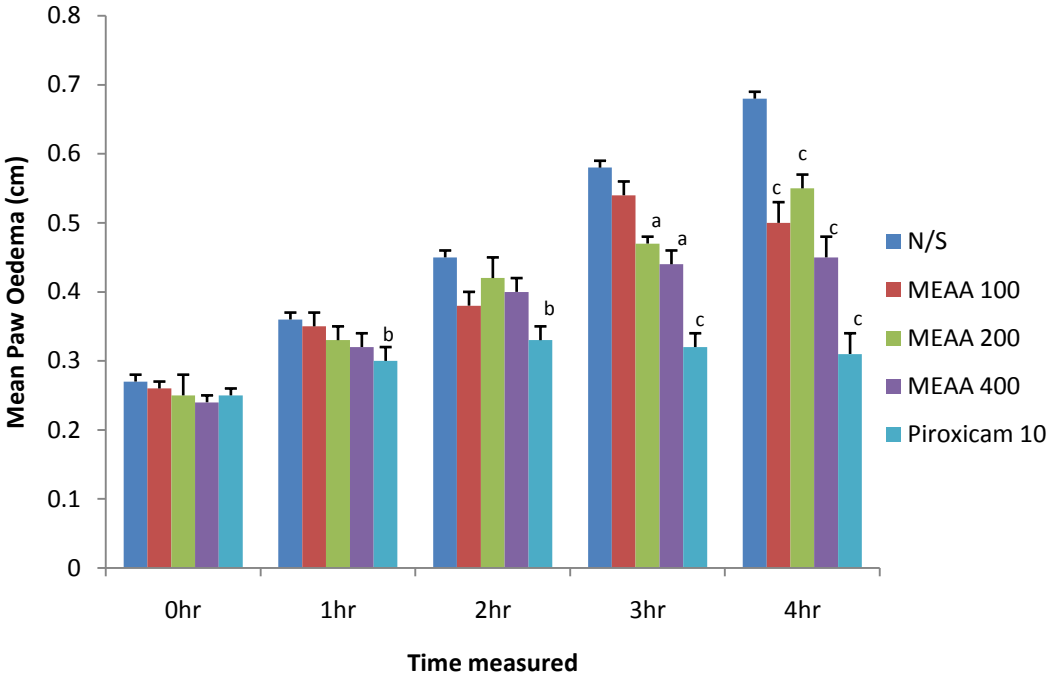

Figure 4.3: Effect of methanol leaf extract of *Acacia ataxacantha*leaf on carragenaan induced paw oedema in rats.

n = 5, a= P<0.05, [b]= P<0.01, [c]= P<0.001 levels of significance
Statistical Analysis: ANOVA (one way analysis of variance)
N/S= normal saline
MEAA=Methanol Extract of *Acacia ataxacantha*.

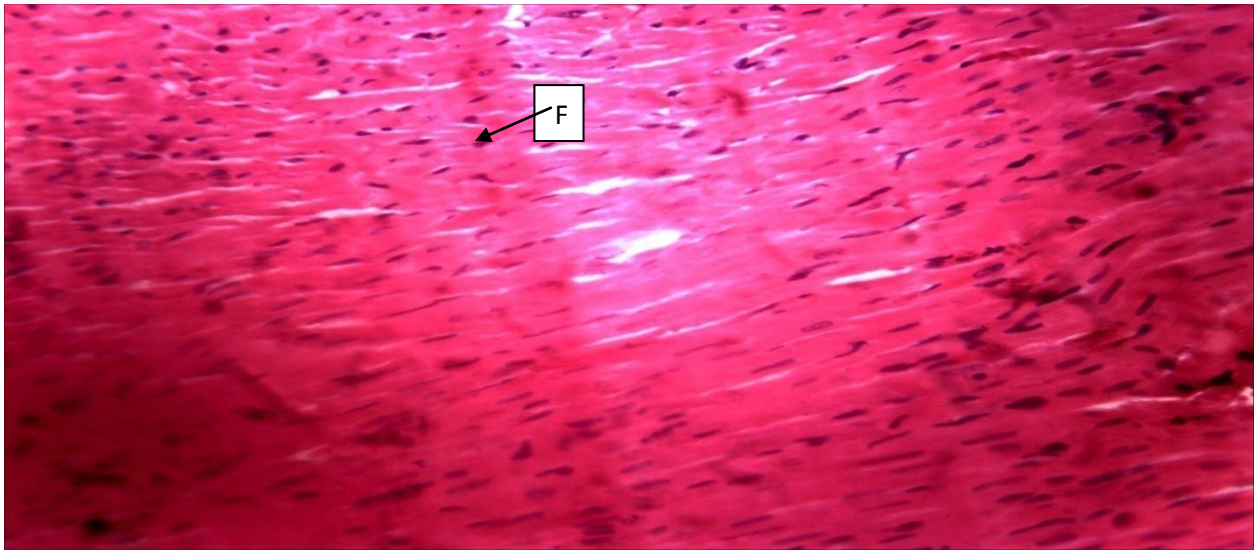

Plate IIi: Photomicrograph of a section of heart of a rat administered normal saline (10 ml/kg) for 90 days. (H & E x 250) showing normal cardiac muscle cells (F)

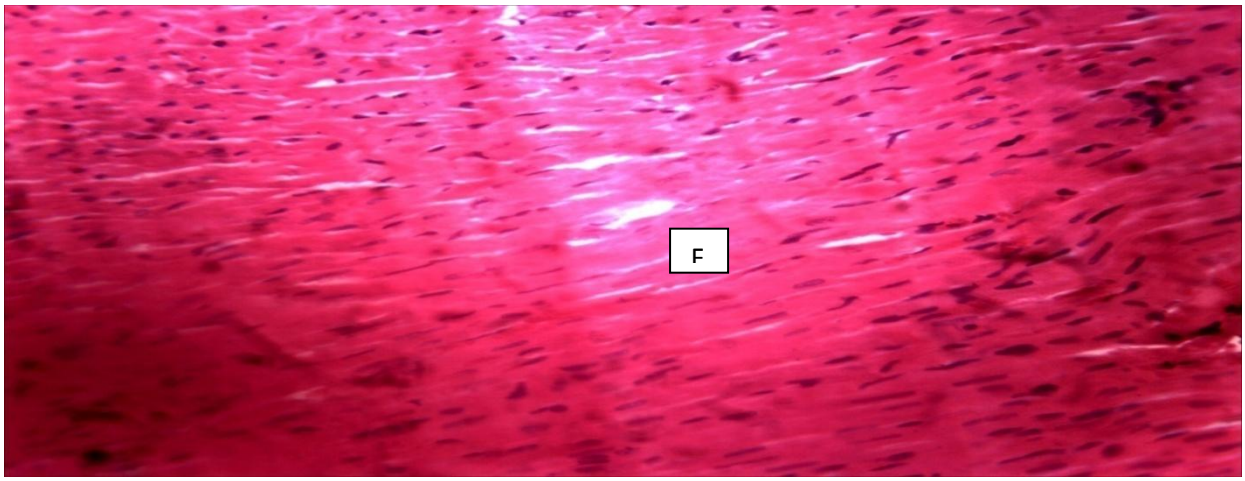

Plate IIii: Photomicrograph of a section of heart of a rat administered with methanol extract of *Acacia ataxacantha*50 mg/kg for 90 days. (H & E x 250) showing normal cardiac muscle cells (F)

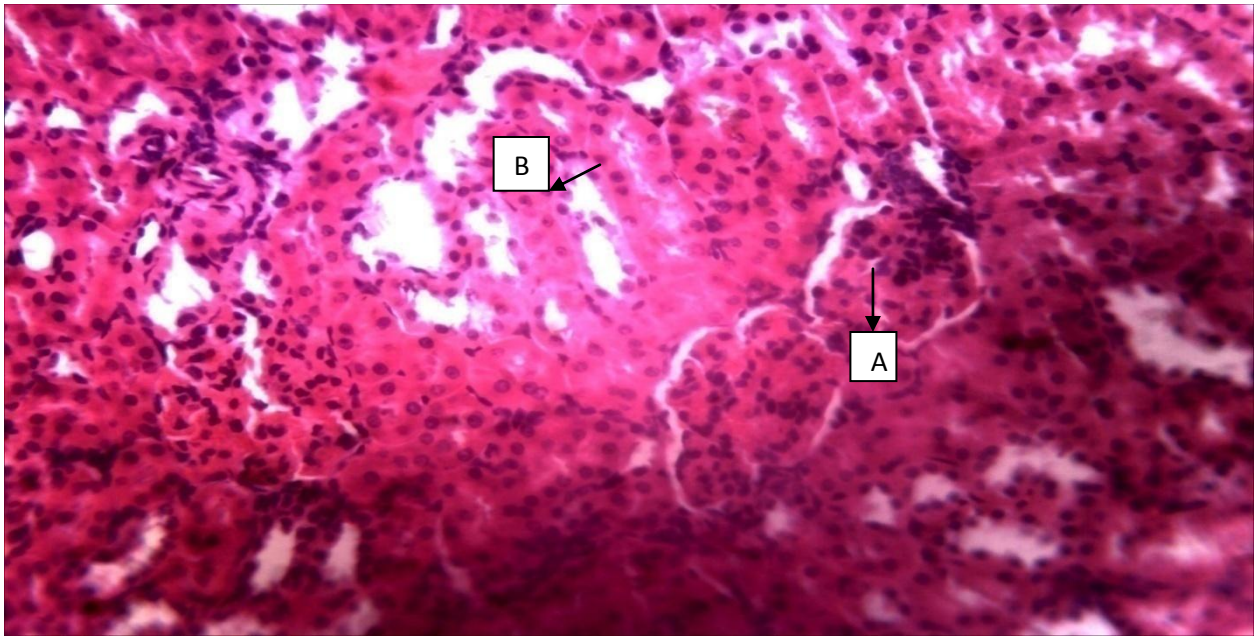

Plate IIIi: Photomicrograph of a section of kidney of a rat treated with normal saline (10 ml/kg)for 90 days. (H & E x 250) showingnormal glomerulus (A) and renal tubules (B)

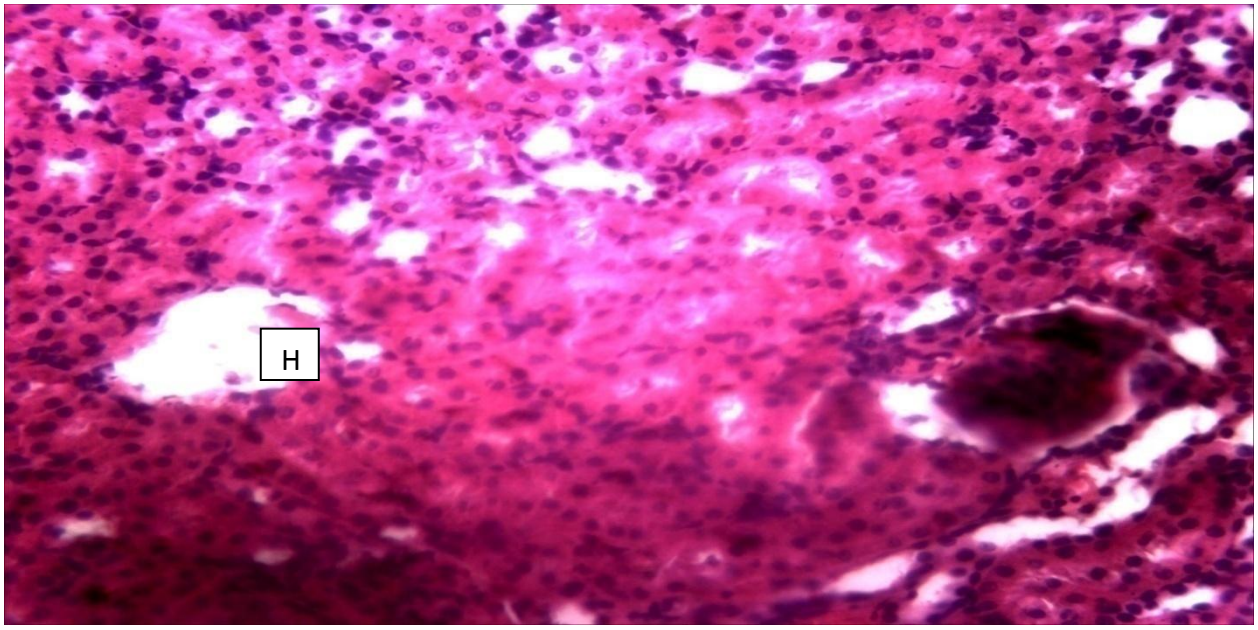

Plate IIIii: Photomicrograph of a section of kidney of a rat treated withmethanol extract of *Acacia ataxacantha*(50 mg/kg)for 90 days. (H & E x 250) showing complete glomeruli necrosis (H).

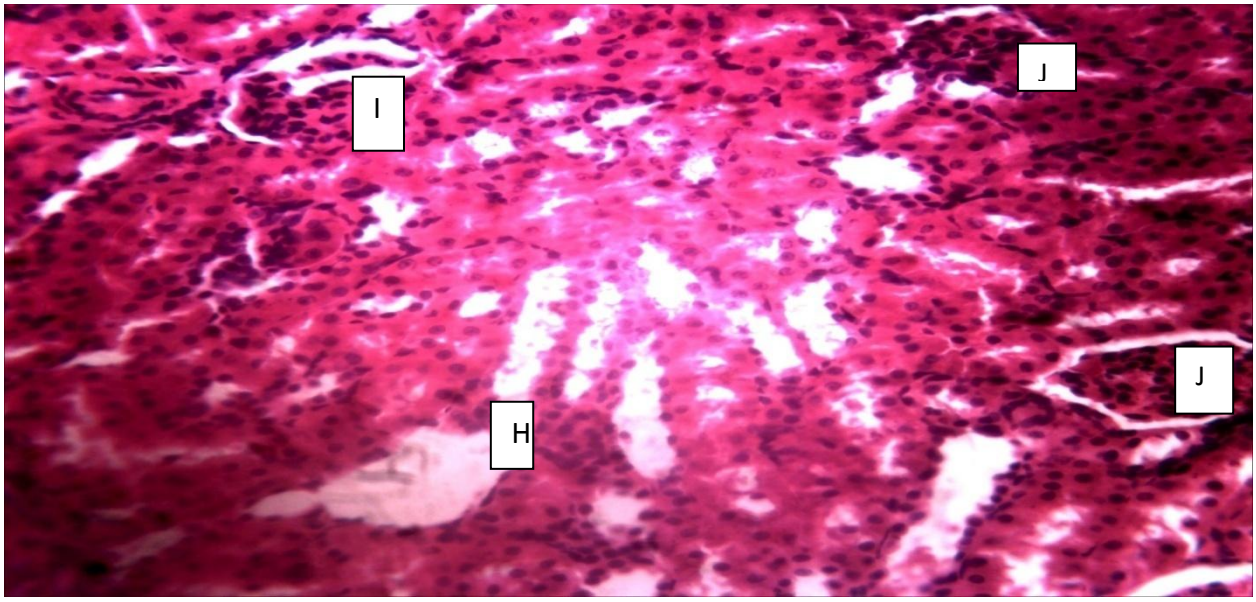

Plate IIIiii: Photomicrograph of a section of Kidney of a rat treated with methanol extract of *Acacia ataxacantha*(200mg/kg) for 90 days. (H & E x 250) showing complete glomeruli necrosis, moderate glomeruli necrosis (I) and Lymphocyte hyperplasia (J)

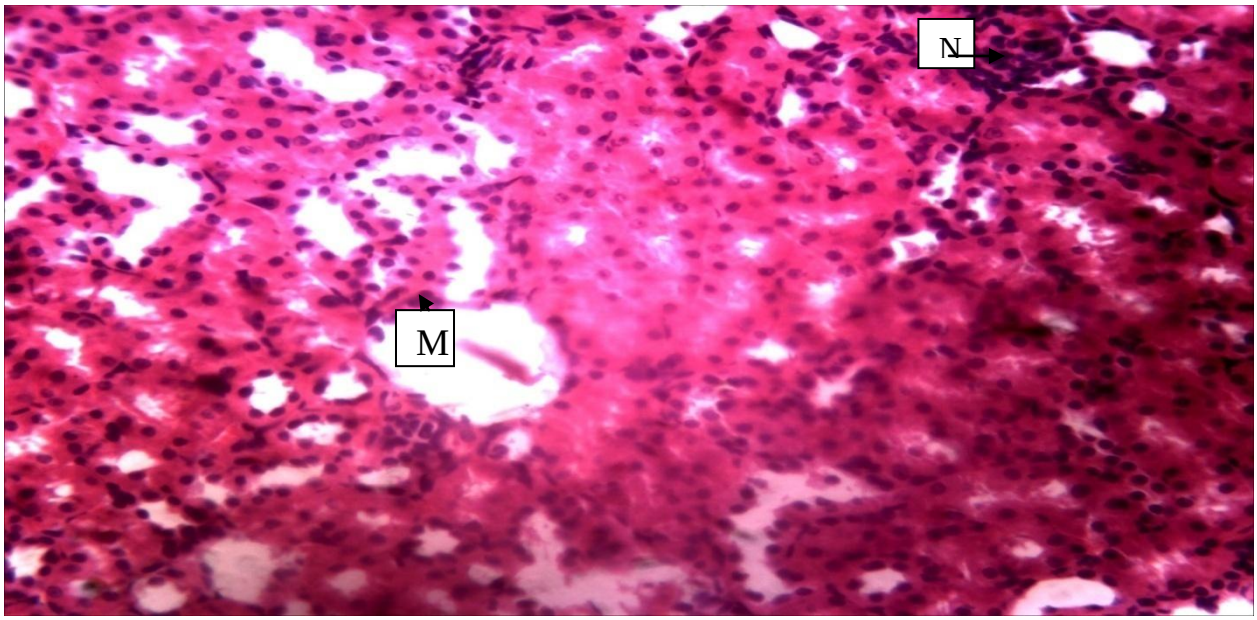

Plate IIIiv: Photomicrograph of a section of Kidney of a rat treated with methanol extract of *Acacia ataxacantha*(400mg/kg) for 90 days. (H & E x 250) showing complete glomerular necrosis, moderate glomerular necrosis (M) and Lymphocyte hyperplasia (N)

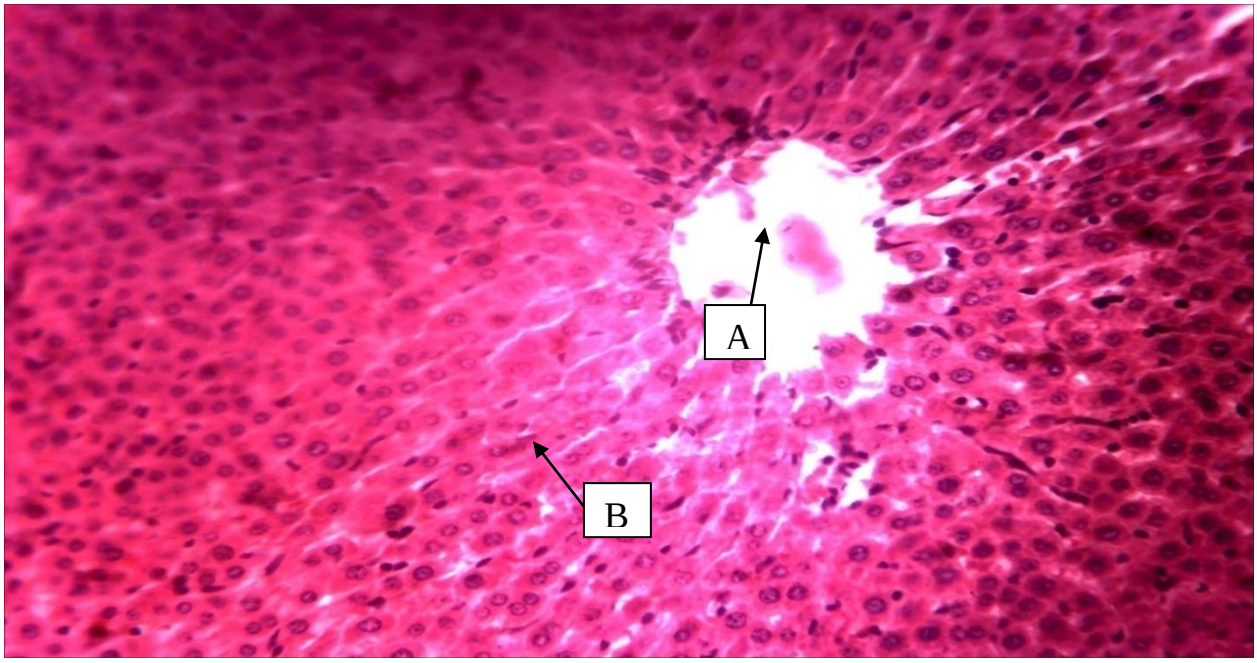

Plate IVi: Photomicrograph of a section of Liver of a rat treated with normal saline(10 mg/kg) for 90 days. (H & E x 250) showing central vein (A) and normal hepatocytes (B)

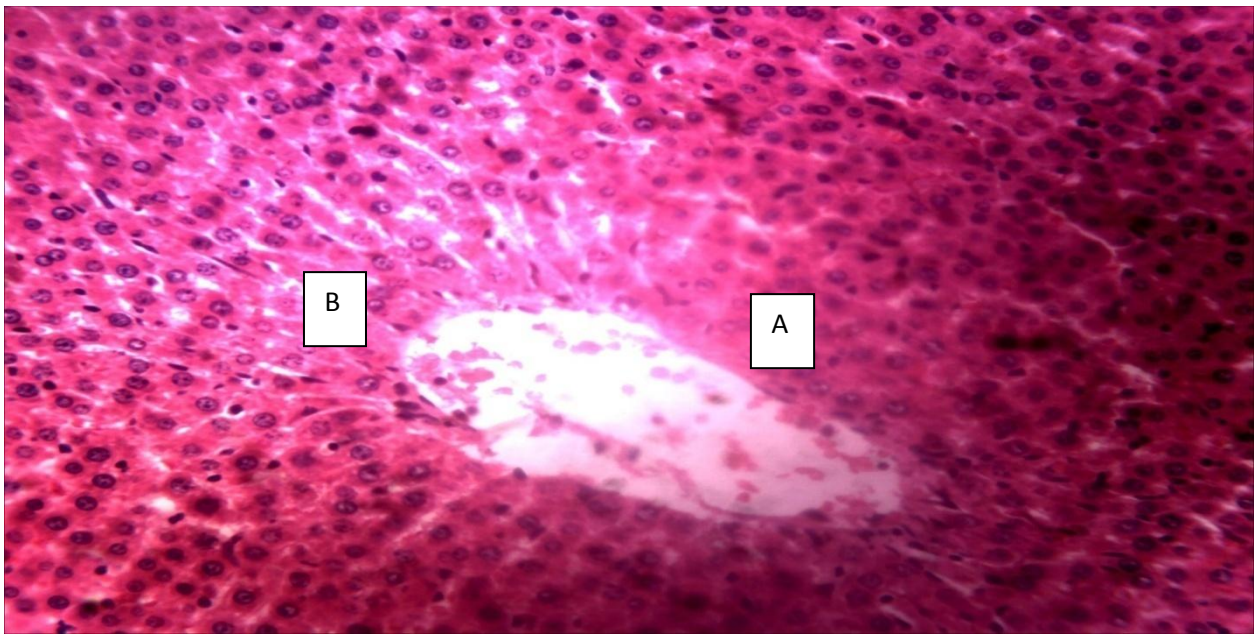

Plate IVii: Photomicrograph of a section of Liver of a rat treated with methanol extract of *Acacia ataxacantha* (50mg/kg) for 90 days. (H & E x 250) showing central vein (A) and normal hepatocytes (B)

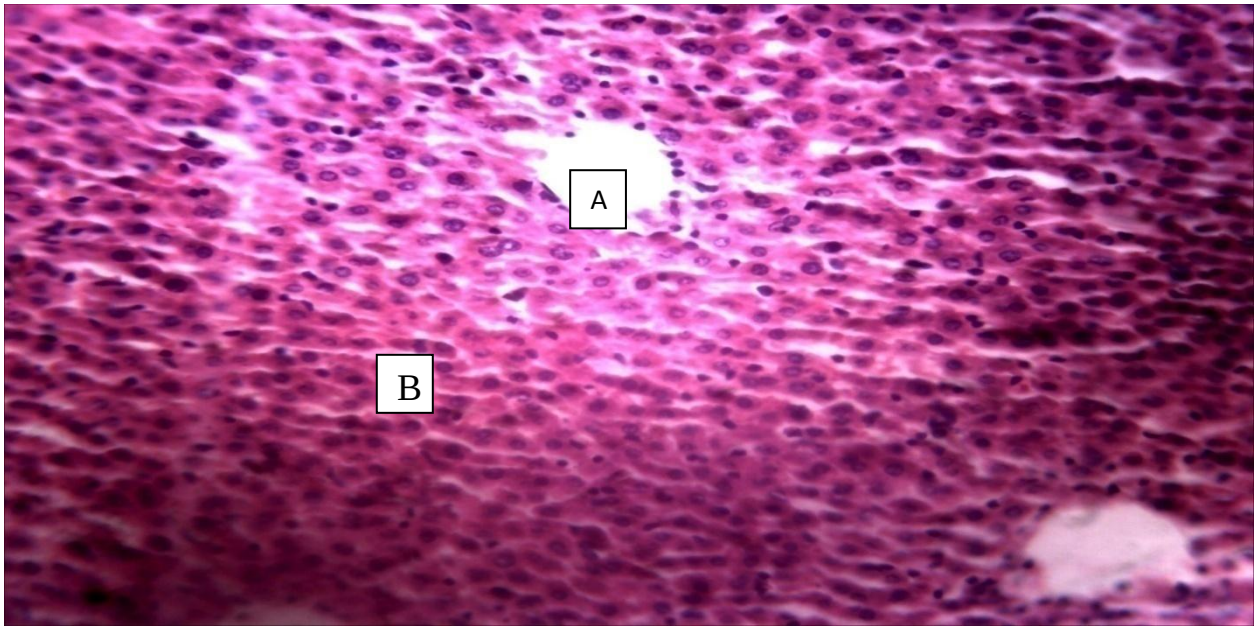

Plate IViii: Photomicrograph of a section of Liver of a rat treated with methanol extract of *Acacia ataxacantha* (200mg/kg) for 90 days. (H & E x 250) showing central vein (A) and normal hepatocytes (B)

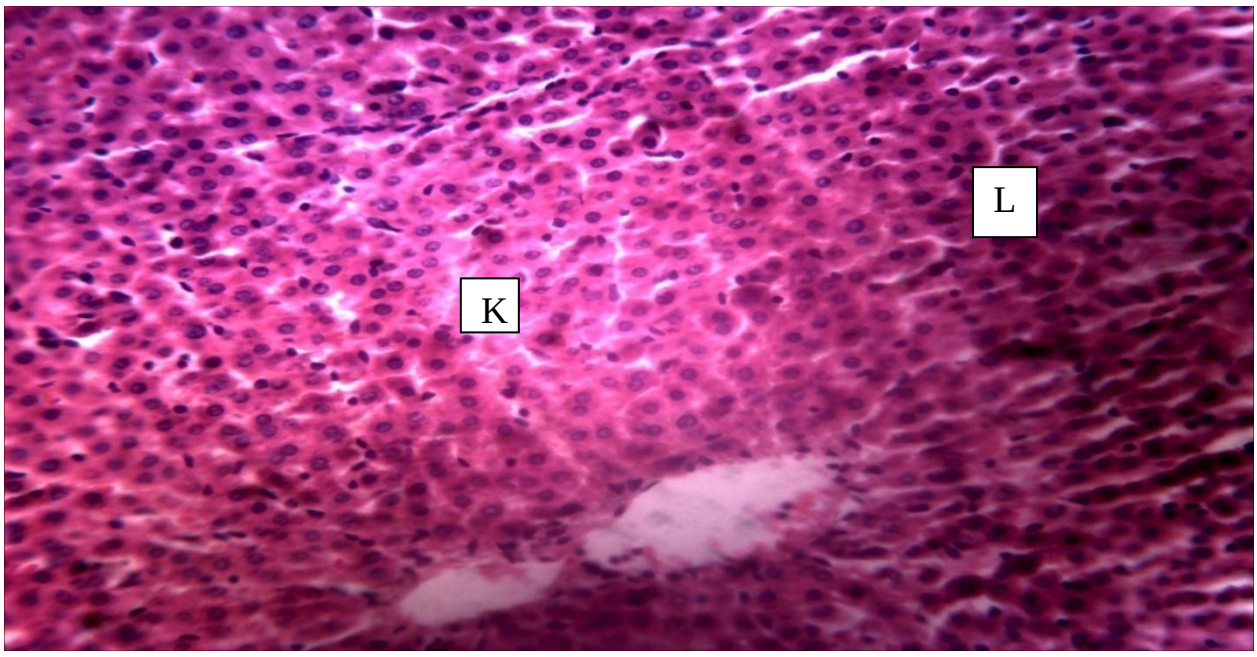

Plate IViv: Photomicrograph of a section of Liver of a rat treated with methanol extract of *Acacia ataxacantha* (400mg/kg) for 90 days. (H & E x 250) showing mild hepatocellular necrosis (K) and kupfer hyperplasia (L)

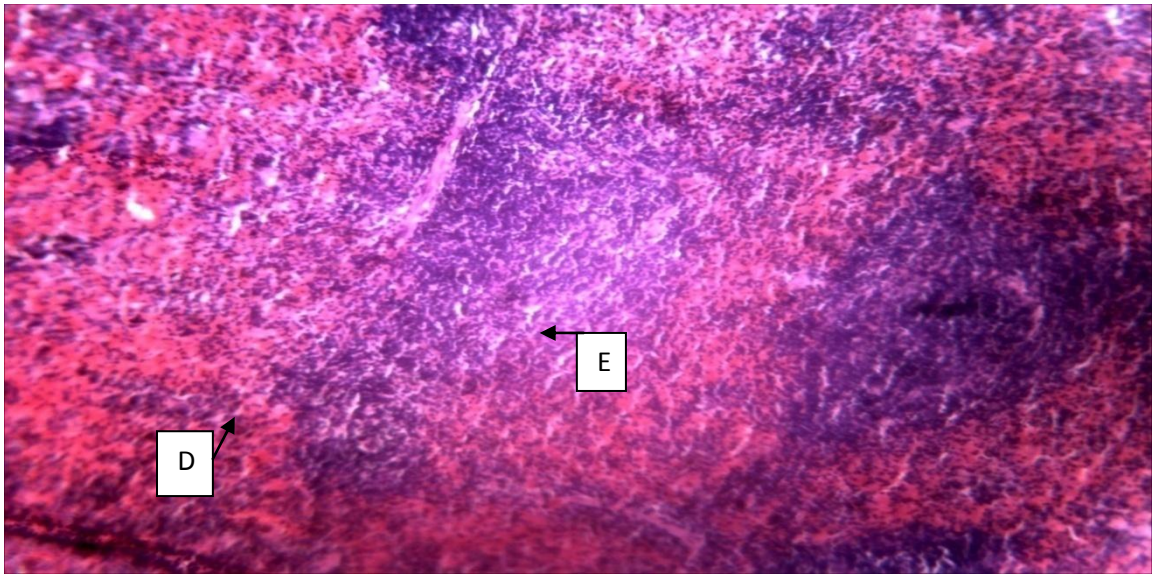

Plate Vi: Photomicrograph of a section of Spleen of a rat treated with normal saline(10 mg/kg) for 90 days. (H & E x 250) showing normal red pulp (D) and normal white pulp (E)

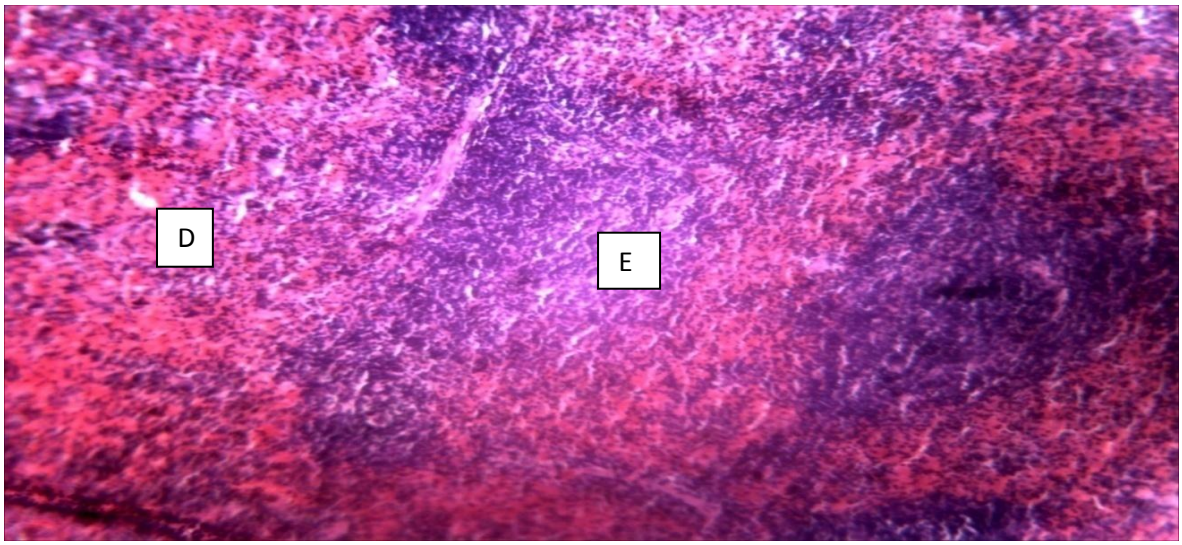

Plate Vii: Photomicrograph of a section of Spleen of a rat treated with methanol extract of *Acacia ataxacantha*(50 mg/kg) for 90 days. (H & E x 250) showing normal red pulp (D) and normal white pulp (E)

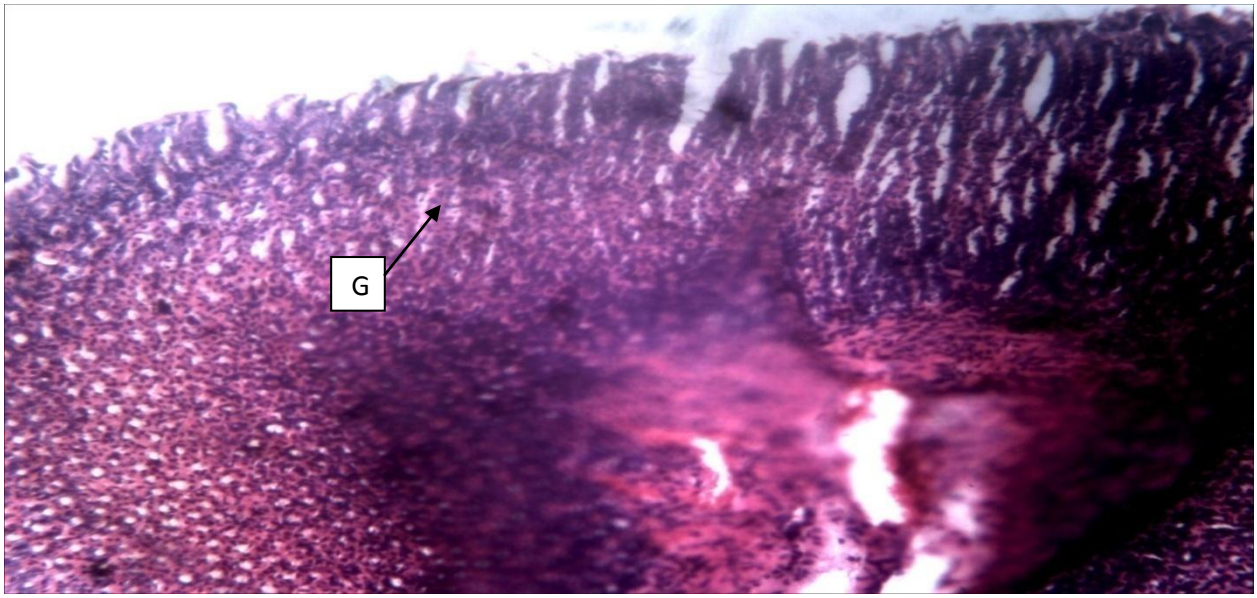

Plate VIi: Photomicrograph of a section of Stomach of a rat treated with normal saline(10 mg/kg) for 90 days. (H & E x 250) showing normal mucosa of the stomach (G)

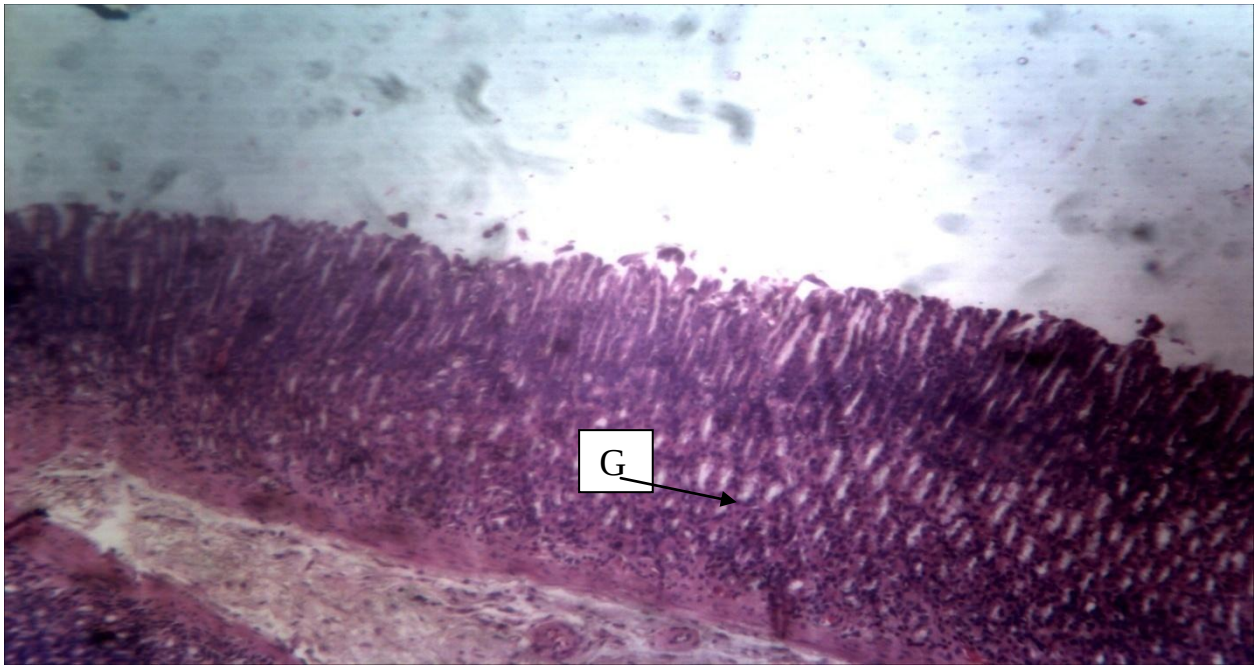

Plate VIii: Photomicrograph of a section of Stomach of a rat treated with methanol extract of *Acacia ataxacantha*(50mg/kg) for 90 days. (H & E x 250) showing normal mucosa of the stomach (G)

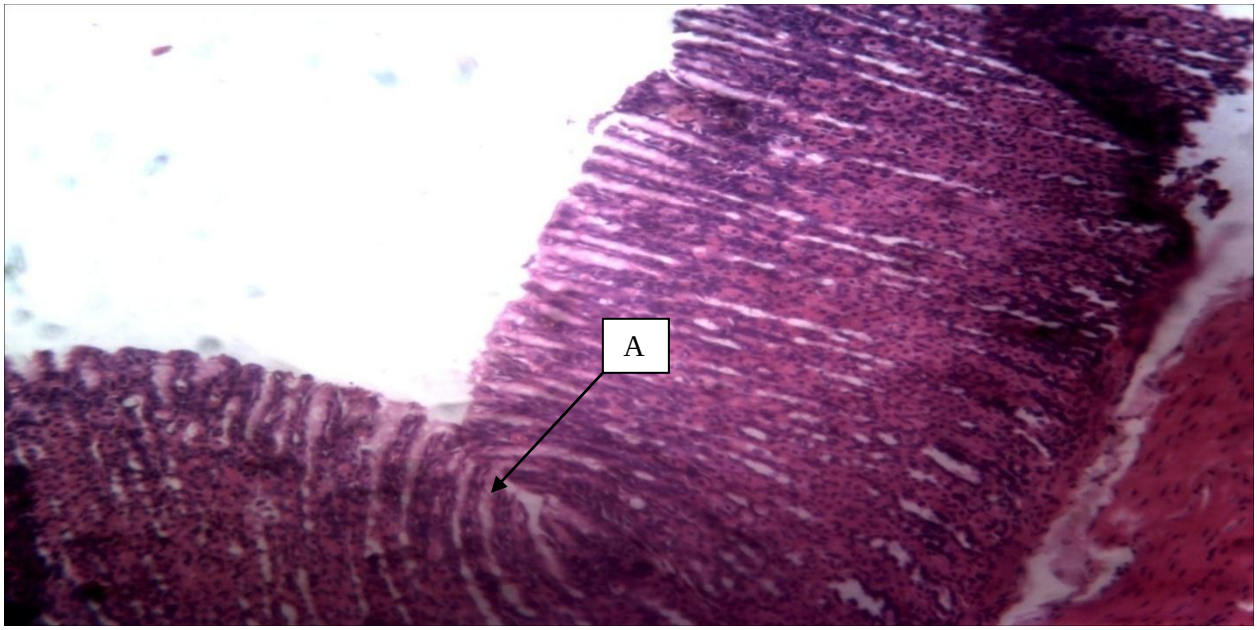

Plate VIiii: Photomicrograph of a section of Stomach of a rat treated with methanol extract of *Acacia ataxacantha*(200mg/kg) for 90 days. (H & E x 250) showing normal mucosa of the stomach (G)

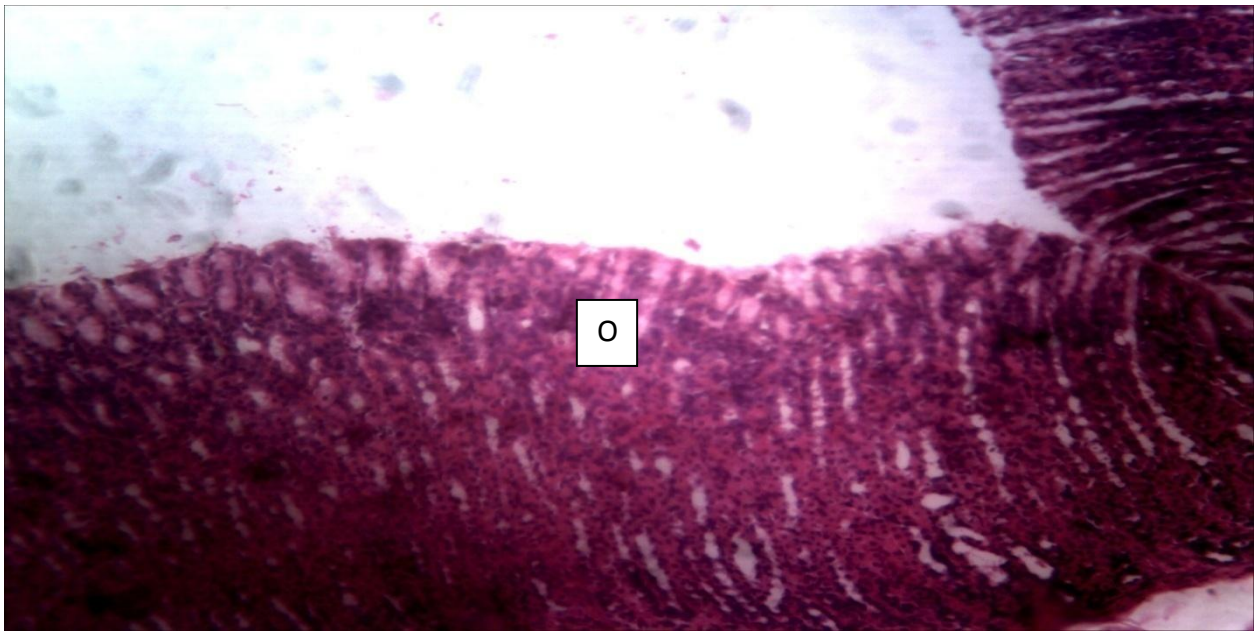

Plate VIiv: Photomicrograph of a section of Stomach of a rat treated with methanol extract of *Acacia ataxacantha*(400mg/kg) for 90 days. (H & E x 250) showing mild necrosis of stomach mucosa (O)

CHAPTER FIVE

5.0DISCUSSION

Flavonoidsandtannins found in the plant extract have been shown to possess analgesics and anti-inflammatory properties (Ahmadiani *et al.*, 2000). Alkaloids, saponins, phenol containing substances have also been found to possess analgesics as well as anti-inflammatory activities (Chen *et al*., 2005; Choi *et al.,* 2005; Uche and Aprioku, 2008). Phytochemical screening of the extract of *Acacia ataxacantha* leaf revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenol, saponins, reducing sugars, steroids and tannins. The therapeutic benefits of traditional remedies are often attributed to the combination of different bioactive constituents (Chindo*et al*., 2003; Maphosa, *et al.,* 2012) and might probably contribute in part to the analgesic and anti-inflammatory activities of the methanol extract of *Acacia ataxacantha*leaf.

The oral median lethal dose(LD_{50}) of the extractin both mice and rats were found to be greater than 5000 mg/kg body weight, while the intraperitoneal LD_{50} was calculated to be 565.69 mg/kg and 1264.91 mg/kg in mice and rats respectively. These shows that the extract when administered orally is practically non-toxic and it is slightly toxic when administered intraperitoneally, (Loomis and Hayes, 1996; Lorke 1983).

Urea is a by-product of protein metabolism that is excreted solely in the kidney, (Schwart and Garrison, 2008) while, creatinine on the other hand is a by-product ofmuscle metabolism, (Stephens *et al.,* 2006) which is also excreted exclusively by glomerular filtration in the kidney. Hence urea and creatininelevels areparameters used as a measure of kidney function.The significant increase in the serum level of urea and creatinine in the

renal biochemical parameters tested is an indication of kidney dysfunction. The elevation of serum level of creatinine and urea at all doses of extract testedmay signified decrease inkidney function due to toxic effect of extract on the kidneys.

Sodium is an extracellular fluid ion that is filtered and reabsorbed in the kidney (Craig and Stitzel, 2002). The significant elevation in serum level of sodium ion in the serum electrolyte evaluated might be due tokidney dysfunction resulting fromloss of excessive fluid (dehydration) and reduced water intake by the rats, (Tietz*et al.*,1994).

Changes in the level of liver enzymes AST, ALT and ALP, have been used in evaluating function of the liver. The statistical significant increases in the level of aminotransferases (AST, ALT and ALP) might be an indication of liver damage caused by oral administration of plant extract, which is suggestive of hepatotoxic effect.ALT and AST are largely used in the assessment of liver damage by drugs or any hepatotoxic substance (Dobbs *et al*., 2003). Elevated level of ALT is more specific for liver related injuries or diseases, (Schwartz and Garrison, 2008). High level of AST is also indicative of liver damage due to necrosis or viral hepatitis, cardiac infarction and muscle injury thus, it is not a specific parameter for assessing liver injury (Xu *et al*., 2002).Though 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 extrahepatic biliary obstruction, (Schwartz and Garrison, 2008).

The analysis of haematological parameters is relevant to risk evaluation of substances because the haematological system has a higher predictive value of toxicity in humans when tests involve rodents, (Olslon *et al.,* 2000). There was no significant difference in the

haematological parameters when the extract treated groups were compared with the control group, hence the extract may not have any toxic effects on the haematological indices.

Histopathological evaluation of the 90 days oral administration of the methanolic leaf extract of *Acacia ataxacantha*in rats showed that the morphology of kidney, liver and stomach mucosa were adversely affected, suggesting that the extract could be toxic to the hepatocytes, kidney and stomach mucosa.

The anti-nociceptive activity of methanol extract of *Acacia ataxacantha*leafwas evaluated using acetic acid induced writhing, thermally induced pain and formalin induced pain models in laboratory animals. The models selected enable the investigation of both peripheral and central mediated analgesia, (Tjolsen *et al.*, 1992).

The abdominal constriction response induced by acetic acid is a sensitive procedure for evaluating peripherally acting analgesics, (Kumar*et al*., 2009). The methanol extract of *Acacia ataxacantha* significantly ($p<0.05$) and dose dependently reduces the number of acetic acid induced writhes in mice when the extract treated groups were compared with the control group. This suggests that the analgesic effect maybe mediated via the inhibition of the synthesis and release of prostaglandins in peritoneal fluids, leukotrienes and other endogenous substances that play a key role in pain mediation(Kumar *et al.*, 2009). The extract ata dose of 400mg/kg showed almost similar analgesic activity(64%) when compared with the standard drug, [(piroxicam 10mg/kg), 65%], this suggests a promising anti-nociceptive activity of the extract when pain is induced using acetic acid.In general intraperitoneal administration of acetic acid causes pain by liberating endogenous

substances such as serotonin, histamine, prostaglandins, bradykinins and substance P, which stimulates nerve endings. The abdominal constriction response is also thought to be mediatedby local peritoneal mast cells, acid sensing ion channels and prostaglandins, (Kawshik*et al*., 2005; Speroni*et al*., 2005).

Hot plate method is a central model of evaluating analgesic activity of compound that has selectivity for opioid-derived analgesics (Temdie *et al*., 2012). It is a thermal mechanism of inducing pain in laboratory animals that is supraspinally mediated, (Hasan *et al.,* 2010). The significant prolongation of mean reaction time in this studyshowed that the analgesic activity of the extract might be mediated via the central mechanism of mediating analgesia (Hasan *et al.,* 2010; Sourabie *et al.,* 2012).

The extract of *Acacia ataxacantha* at all doses tested significantly inhibits the early phase (5-10mins) of formalin induced pain which, suggest its possible involvement in central mechanism of analgesia (Yerima *et al*, 2009). The inflammatory phase (15-60mins) of formalin-induced pain was also inhibited by the extract at doses of 200mg/kg and 400mg/kg which, suggest that the extract exhibits peripheral mechanism of analgesia (Chen *et al*., 1995).Formalin method of inducing pain is a well established, valid model for the study of central sensitization events at the spinal level after peripheral inflammatory state (Diaz and Dickenson, 1997). It is a specific chemical model of inducing pain in animal model which, is characterized by two distinct phases of nociception. The early phase is thought to be produced by direct activation of nociceptive neurons by formalin, and reflects centrally mediated pain while, the later phase reflects pain generated in acutely injured tissue or due to inflammatory mediators like histamine, serotonin, prostaglandins and bradykinins, (Tjolsen, *et al.,* 1992; Dharmasiri *et al.,* 2003).It has been

reported that centrally acting analgesics, such as morphine, inhibits both phases of formalin-induced pain, while peripherally acting analgesics, such as acetylsalicylic acid, reduces nociception only at the late phase of analgesia (Tjolsen*et al.*, 1992). The ability of the extract to inhibit both phases of formalin induced pain indicates that it has both peripheral and central mechanism of analgesic effects.

The anti-inflammatory effect of the extract was evaluated using Carragenaan and Albumin induced paw oedema in rats. The anti-inflammatory effect of methanol extract of *Acacia ataxacantha* was significant ($p<0.05$) at the third and fourth hour respectively when compared with the negative control group. The result obtained from carragenaan assay correlated well with that of the second phase formalin induced pain, thus confirming the anti-inflammatory effect of *Acacia ataxacantha* earlier reported in formalin- induced pain model.Carragenaan induces non-specific inflammation which results from sequential action of several mediators of inflammation. This model is highly sensitive to non-steroidal anti-inflammatory drugs and has been accepted as a phlogistic tool for investigating new anti-inflammatory drugs (Temdie*et al*., 2012).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 (3^{rd} hour) is due to liberation of prostaglandins, bradykinins, lysosomes, cyclooxygenase products (Perianayagan*et al*., 2006; Fotio*et al*., 2009).

Albumin-induced hind paw oedema test is useful in detecting activity in acute inflammation (Akah and Nwambie, 1994).Egg-albumin is a cheap phlogistic agent which when injected into the sub-plantar surface of the hind-paw produces oedema after 7minutes. The method is non-specific because, the oedema produced (displaced volume) is

not sustained throughout the experimental period. There was significant difference when the extract treated groups were compared with negative control group, which suggests that the extract has anti- inflammatory activity against albumin induced oedema. The standard drug (acetylsalicylic acid 150mg/kg) exhibit statistical significance at all time when readings were taken.

There was no significant ($p<0.05$) difference in the mean basal temperature between the various doses of extract test when compared with the negative control group. This implies that the extract has no significant anti-pyretic activity. Pyrexia is a result of secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other disease states. The infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediators (cytokines e.g. interleukins Iβ, α, β and TNF-α) which increases the synthesis of PGE2 near the pre-optic hypothalamus area, thereby triggering the hypothalamus to elevate the body temperature (Spacer and Breder, 1994).

CHAPTER SIX

6.0 CONCLUSION

The methanolic extract of *Acacia ataxacantha* possesses significant pain-relieving action, as well as anti-inflammatory activities, with no significant antipyretic activity.Hence, it can be concluded that the methanolic extract of *Acacia ataxacantha* (leaf) contain bioactive constituents with relevant analgesic and anti-inflammatory activities as claimed from the folkloric investigation earlier reported.However prolong use of the drug may produce toxic effects on some organs; liver, kidney, stomach, as evident from the liver function test, kidney function test, as well as histopathological analysis.

6.1 RECOMMENDATIONS

1. Further works should be carried out in order to separate the fraction of the extract that produced the observed pharmacological effects
2. Also works should be carried in order to isolate the bioactive constituents responsible for the observed pharmacologic effects
3. Pharmacodynamic studies should be undertaken to establish the analgesic and anti-inflammatory mechanisms of action of the plant extract.

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APPENDICES

Appendix I: Effect of Methanolic Leaf Extract of *Acacia ataxacantha* on Acetic Acid induce Writhes in Mice

Treatment	Dose mg/kg	Mean Abdominal Writhes	% Inhibition
Control	-	35.40±3.67	-
MEAA	50	19.40±2.16*	44.57
MEAA	100	17.40±2.04*	50.29
MEAA	200	14.60±2.04*	58.29
MEAA	400	12.60±2.38*	64.00
Piroxicam	10	12.00±1.89*	65.71

Data were presented as Mean ± SEM. * = $P < 0.05$, n= 6.
Statistical Analysis: ANOVA (one way analysis of variance)
MEAA= Methanol extract of *Acacia ataxacantha*

Appendix II: Effect of Methanolic Leaf Extract of *Acacia ataxacantha* on Formalin Induced Pain in Rat

Treatment	Dose (mg/kg)	Mean Pain Score	
		Early Phase(5-10min)	Late Phase(15- 60min)
Control	-	3.00 ± 0.00	3.00 ± 0.00
MEAA	50	2.80 ± 0.20*	3.00 ± 0.00
MEAA	100	2.40 ± 0.40*	3.00 ± 0.00
MEAA	200	2.40 ± 0.40*	2.80 ± 0.20*
MEAA	400	1.80 ± 0.58*	2.40 ± 0.40*
Morphine	4	1.20 ± 0.37*	1.40 ± 0.24*

Data were presented as Mean ± SEM,* = $P < 0.05$, n = 6,
Statistical tool: ANOVA (one way)
MEAA = Methanolic Extract of *Acacia ataxacantha*.

Appendix III: Effect of Methanol Extract of *Acacia ataxacantha*Leaf on Carragenaan Induced Paw Oedema in Rats

Treatment (mg/kg)	**Mean Paw Oedema (cm^3) ± SEM (% Inhibition)**				
	Ohr	1hr	2hr	3hr	4hr
Control	0.27±0.01	0.35 ± 0.01	0.45 ± 0.02	0.58 ± 0.02	0.674±0.019
MLEAA 100	0.27±0.01	0.35 ± 0.02 (0.50)	0.38 ± 0.04 (16.00)	0.55 ± 0.12 (5.50)	0.498±0.026*** (26.00)
MLEAA 200	0.25±0.01	0.33 ± 0.02 (7.90)	0.42 ± 0.01 (5.80)	0.46 ± 0.02* (20.00)	0.552±0.016*** (18.10)
MLEAA 400	0.24±0.01	0.32 ± 0.02 (9.60)	0.40 ±0.02 (12.00)	0.46 ± 0.01* (20.90)	0.47±0.012*** (30.30)
Piroxicam10	0.25±0.01	0.30 ±0.03** (16.38)	0.33±0.02** (26.70)	0.33 ± 0.04*** (43.50)	0.306±0.022*** (54.60)

Data were presented as Mean ± SEM,n = 5, *= P<0.05, **= P<0.01, *** = P<0.001, Statistical tool: ANOVA (one way)
MEAA=Methanolic Extract of *Acacia ataxacantha*.