

TOXICOLOGICAL, ANTIPYRETIC, ANALGESIC AND ANTI-INFLAMMATORY
PROPERTIES OF METHANOL LEAF EXTRACT OF *DALBERGIA SAXATILIS*
HOOK. F (FABACEAE) IN RATS AND MICE

BY

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DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS,
FACULTY OF PHARMACEUTICAL SCIENCES,
AHMADU BELLO UNIVERSITY, ZARIA

AUGUST, 2014

DEDICATION

This work is dedicated to my late brother Abubakar Sadiq, may his soul rest in Jannatul Firdausi. ILAHEE

DECLARATION

I declare that the work in this thesis entitled '**Toxicological, Antipyretic, Analgesic and Anti-inflammatory Properties of Methanol Leaf Extract of *Dalbergia saxatilis* Hook . f (Fabaceae) in Rats and Mice**' was carried out by me in the Department of Pharmacology and Therapeutics, under the supervision of Dr. A.U. Zezi, Dr. A.H. Yaro, and Dr. U.H. Danmalam. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other University.

HASSAN, FATIMA ISMAIL

.....

Signature of student

.....

Date

CERTIFICATION

This thesis entitled “TOXICOLOGICAL, ANTIPYRETIC, ANALGESIC, AND ANTI-INFLAMMATORY PROPERTIES OF METHANOL LEAF EXTRACT OF *DALBERGIA SAXATILIS* HOOK.F (FABACEAE) IN RATS AND MICE” by Fatima Ismail HASSAN, meets the regulations governing the award of the degree of Master of Science in Pharmacology, of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

The decoction of the leaves of *Dalbergia saxatilis* is used in Traditional Medicine for various ailments such as cough, small pox, skin lesions, bronchial ailments and toothache. The study was aimed at establishing a safety profile, evaluating phytochemical constituents and some Pharmacological properties of methanol leaf extract of *Dalbergia saxatilis*. Acute toxicity study was carried out as described by Lorke (1983) in both mice and rats. Sub-chronic toxicity studies were carried out in rats in accordance with WHO (1992) and OECD 407 (1995) guidelines. Phytochemical constituents were screened as described by Brain and Turner (1975) and Evans (2002). The antipyretic effect was investigated using Brewer's yeast induced pyrexia in rats, hotplate method and acetic acid induced writhing test in mice were used to evaluate the analgesic effect, while Carrageenan induced paw oedema model in rats was used to investigate the anti-inflammatory effect of the extract. The extract was administered at doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg. The median lethal dose in both mice and rats was found to be above 5000 mg/kg. There was no significant increase in body weight at all doses tested, except in the 500 mg/kg group in the fourth week at $p < 0.05$. No statistically significant difference was observed in the relative organ weight ratio at all doses of the extract. Significant increase in alanine and aspartate transferases was observed at doses of 250 mg/kg and 500 mg/kg at $p < 0.01$ and $p < 0.05$, and 250 mg/kg at $p < 0.01$ respectively. A statistical significant reduction in number of white blood cells was observed at all the doses of the extract, at $p < 0.01$, $p < 0.001$ and $p < 0.01$. A statistical significant reduction in sodium and chloride concentrations at $p < 0.05$ was observed at doses of 250 mg/kg and 500 mg/kg, for bicarbonate in 250 mg/kg at $p < 0.05$ respectively. Histopathology revealed normal cardiac cells for heart, damaged mucosa with ulceration at 1000 mg/kg for stomach, moderate lymphocyte hyperplasia at 250

mg/kg, and intense lymphocyte hyperplasia at 1000 mg/kg for spleen. The liver showed vascular congestion with slight lymphocyte hyperplasia at 250 mg/kg, slight lymphocyte hyperplasia at 500 mg/kg, and slight peri-vascular necrosis at 1000 mg/kg. The kidneys showed massive necrosis of the glomerulus with tubular distortion, lymphocyte hyperplasia, intense glomerular and tubular necrosis at 250 mg/kg, 500 mg/kg, and 1000 mg/kg respectively. The extract was found to contain alkaloids, flavonoids, tannins, saponins, cardiac glycosides, and triterpenes. Statistical significant reduction in rectal temperatures were observed in standard group at 21st and 23rd hour, and in 500 mg/kg and 1000 mg/kg doses of the extract at 23rd hour at $p < 0.05$ compared with the 19th hour. The extract and standard significantly decreased the number of writhes caused by acetic acid at $p < 0.001$. There was statistical significant increase in reaction time in standard group, extract 250 mg/kg, and 1000 mg/kg from 60 minutes at $p < 0.05$ and $p < 0.01$, from 30 minutes in 500 mg/kg group at $p < 0.05$ and $p < 0.01$ compared with 0 minute. A statistical significant decrease in paw sizes at $p < 0.05$ and $p < 0.01$ compared to the peak increase in both standard and test groups were observed. The extract was found to possess constituents that may be associated with its antipyretic, analgesic and anti-inflammatory effects observed at doses tested. It has relative acute safety, but toxic on prolonged use.

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LIST OF ABBREVIATIONS

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
Anis	Anisaldehyde
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
AV	Atrioventricular
BNF	British National Formulary
CGRP	Calcitonin gene-related peptide
cm	centimetre
COX	Cyclooxygenase
CRPS	Complex regional pain syndrome
DNA	Deoxyribonucleic acid
DRG	Dorsal root ganglion
DS	<i>Dalbergia saxatilis</i>
DW	Distilled water
EDTA	Ethylene diamine tetra acetic acid
FeCl ₃	Ferric chloride
g	gram
Hb	Haemoglobin
HCl	Hydrochloric acid
IASP	International Association for the Study of Pain
IL	Interleukin
INF	Interferon
kg	kilogram

L.B	Liebermann Burchard
LD ₅₀	Median Lethal dose
LYMPH	Lymphocytes
MCHC	Mean corpuscular haemoglobin concentration
mg	Milligram
mg/dL	Milligram/decilitre
ml	Millilitre
mmol/l	Millimole/litre
mRNA	Messenger Ribonucleic acid
NEUT	Neutrophils
NICE	National Institute for Health and Clinical Excellence
NID	Non-infectious inflammatory disease
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
NSAIDS	Non-steroidal anti-inflammatory drugs
OECD	Organisation for Economic Cooperation and Development
PCV	Packed cell volume
PGE	Prostaglandin E
PLT	Platelet
PO	Per oral
ROW	Relative organ body weight
SEM	Standard error of mean
TLC	Thin layer chromatography
TNF	Tumour necrosis factor
U/L	Unit/litre

UV	Ultraviolet
W.H.O	World Health Organisation
WBC	White blood cell

CHAPTER ONE

1.0 INTRODUCTION

Toxicology is the science that deals with the study of the adverse effects caused by chemicals or physical agents in living organisms under specific conditions of exposure (Doull *et al.*, 2008). It is a science that attempts to qualitatively identify all the hazards; such as organ toxicities associated with a substance, as well as to quantitatively determine the exposure conditions under which those hazards are induced. It also experimentally determines the occurrence, nature, incidence, mechanism, and risk factors for the adverse effects of a toxic substance (James *et al.*, 2000).

Toxicity studies are conducted to provide greater understanding of the potential intrinsic hazard of the test item and to estimate safety margins (Robbinson *et al.*, 2009). These safety margins are used to determine an initial safe starting dose for clinical trials, a safe dose for continued use in humans through longer clinical trials, and ultimately to achieve successful review of registration dossiers to support marketing approval and use of new medicines within the wider population.

Pain can be defined as the effect produced in consciousness by the arrival of nerve impulses generated by noxious stimuli in the brain (Mishra *et al.*, 2011). It is sometimes interpreted as a suffering that results from the perception of painful stimuli. It is also 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 (Merskey and Bogduk, 1994).

Pain is always personal; no two people experience it in the same way, which makes it very difficult to define and treat (Macintyre *et al.*, 2010). It may affect ability to work, relate with family and friends. Activity levels and even sleep may be affected. All of this may become overwhelming and can lead to increasing pain and distress (Chair *et al.*, 2010).

Pain is a multidimensional sensory experience that is unpleasant and associated with hurting and soreness. It may vary in intensity, quality and duration from mild, moderate, or severe, sharp, burning or dull and transient, intermittent, or persistent. It can also be superficial or deep, localized or diffused (Woolf, 2004). Pain can be acute or chronic. Acute pain is usually of short duration and the cause often identifiable such as disease and trauma, while chronic pain persists after healing is expected to be completed, or is caused by a chronic disease which causes continuous pain and re-occurs at intervals for months or even years (Rajagopal, 2006).

Pain can also be classified into nociceptive and neuropathic based on its mechanism. Nociceptive pain is caused by activation of nociceptors, which are sensory neurons found throughout the body. A nociceptor is a type of receptor sensitive to a noxious stimulus. Neuropathic pain is initiated or caused by a primary lesion or dysfunction of the nervous system (Patel and Kopf, 2010). When tissue damage occurs, chemical substances that modulate the transmission of pain are released into the extracellular tissue. They activate the pain receptors by irritating nerve endings. These chemical mediators include histamine, substance P, bradykinin, acetylcholine, leukotrienes, and prostaglandins (Helms and Barone, 2008).

Inflammation is defined as the series of changes that occur in a living tissue when it is injured provided that the injury is not of such a degree that causes destruction. It is characterised by redness, swelling, heat and pain and atimes loss of function (Punchard *et al.*, 2004). Inflammation is a defensive mechanism of the body to remove injurious stimuli and initiate healing process for the tissue, but if it runs unchecked, it can lead to onset of certain diseases as vasomotor rhinnorrhoea, rheumatoid arthritis, and atherosclerosis (Sharma *et al.*, 2010).

The heat sensation in inflammation is due to increased movement of blood through dilated vessels into the cooled extremities, which also results in increased redness due to the additional number of erythrocytes passing through the area. The swelling is as a result of increased passage of fluid from dilated and permeable blood vessels into the surrounding tissues, infiltration of cells into the damaged area, and in prolonged inflammatory responses deposition of connective tissue. Pain is due to the direct effects of mediators and the stretching of sensory nerves due to ooedema (Punchard *et al.*, 2004).

Inflammation removes foreign matter from the body, disposes damaged cells, and initiates wound healing. It is controlled by mast cells that are in close proximity to autonomic nerves. Mast cells are constituents of connective tissues containing large granules that contain heparin, serotonin, bradykinin, and histamine (Kumar *et al.*, 2012). Fever is defined as the elevation of core body temperature above normal. In normal adults, average oral temperature is 37°C (98.6°F). Fever of an unknown origin is defined as a febrile illness lasting more than 3 weeks, with temperatures exceeding

38.3°C on several occasions, and lacking a definitive diagnosis after one week of evaluation in hospital (Dalal and Zhukorsky, 2006).

Fever is also known as pyrexia may be due to infection, inflammation, or any tissue damage and disease state. It arises as a secondary impact of infection, malignancy or other diseased states. Antipyretics are agents that reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat in the hypothalamus centrally which regulates the set point at which body temperature is maintained. In fever, this set point is elevated by the factors mentioned above. These drugs reduce elevated body temperature and are known to act centrally on the temperature regulation centre in the brain or peripherally through vasodilatation and heat dissipation. They reset the hypothalamic thermostat and rapidly reduce fever by promoting heat loss through sweating and they also act by inhibiting the synthesis of prostaglandin E₂ (Tirumalasetty *et al.*, 2012).

Fever is a medical sign of several disease conditions characterized by elevation of body temperature above 36.5–37.5°C. The most common of these diseases especially in Africa is malaria, pneumonia, typhoid, urinary tract infection, brucellosis, typhus or septicaemia and infective endocarditis (Ezeonwumelu *et al.*, 2012).

Dalbergia is a large genus of trees, shrubs, lianas, and woody climbers in the pea family also known as Fabaceae. It consists of over 200 species accepted and widely distributed in the tropical and subtropical regions of the world (Saha *et al.*, 2013). Various species have been reported to be used traditionally as aphrodisiac, abortifacient, expectorant, anti-helmentic, antipyretic, appetizer, antiemetic, burning sensation, cure of skin

diseases, anti-ulcers, and cure of diseases of the blood. It is also use for dyspepsia, dysentery, diseases of the eye and nose, syphilis, stomach troubles, leprosy, leucoderma, scabies, ringworm, and obesity (Vasudeva *et al.*, 2009).

Several phytoconstituents have been isolated from the various species of the genus such as flavonoids, isoflavonoids, glycosides, steroids, quinines which have potent biological activities (Vasudeva *et al.*, 2009). The family Fabaceae contain isoflavonoids such as genistein, daidzein and glycitein from soy and red clover and biochanin A and formononetin from red clover which are among the most important and distinctive classes of naturally occurring flavonoids that occur in plants belonging to the subfamily Papilionoideae of the family. Isoflavonoids possess biological activities which include antimicrobial, oestrogenic and insecticidal activities (Amin *et al.*, 2012).

1.1 Statement of Research Problem

Plants used in traditional medicine are relatively safe, but some may have undesirable adverse effects which may be due to over dosage or certain factors and these may lead to toxicity and death (Okigbo *et al.*, 2009).

Dalbergia saxatilis is a medicinal plant used for the treatment of pain, inflammation, and pyrexia, and has not been subjected to intensive toxicological evaluation. The use of analgesics also known as pain killers is the most common method in treatment of pain. These pain medications may cause serious side effects in some individuals. Orthodox drugs used in the treatment of pain include, non-steroidal anti-inflammatory drugs, opioid and non-opioid analgesics (Feinberg *et al.*, 2012).

Non-steroidal anti-inflammatory drugs such as aspirin, ibuprofen, diclofenac and naproxen in the treatment of mild to moderate pain, inflammation and pyrexia are limited by their significant side effects such as gastrointestinal tract irritation, blood disorders, liver damage, renal damage, tinnitus, hypersensitivity reactions etc (Mishra *et al.* , 2011).

Opioid analgesics, though very effective in chronic pain management, are associated with problems such as addiction and tolerance, and side effects such as constipation, weight gain and loss of libido. Opioids are also associated with dependence, and abrupt withdrawal can lead to withdrawal symptoms such as tiredness, diarrhoea, abdominal cramps and sweating (Stannard *et al.*, 2007).

1.2 Justification

Majority of the world population depend on traditional medicine such as herbs for treatment of various ailments. Present day medicine was derived from herbal traditions (Ezeonwumelu *et al.*, 2012). The use of traditional medicine is rapidly growing; most people are working in the field of ethnomedicine due to accessibility and affordability. Hence, there is need for the establishment of toxicological profiles of these medicines (Salawu *et al.*, 2009).

Several species of *Dalbergia* genus are widely used in traditional medicine systems and relatively few of these have been investigated from an evidence-based pharmacological approach. The species are used in traditional system of medicines all over the world in the treatment of various ailments like diarrhoea, pain, inflammation, pyrexia, leucoderma, dyspepsia, dysentery, syphilis, gonorrhoea, stomach ache, leprosy, eye

diseases, scabies, pain, and ringworm (Saha *et al.*, 2013). The anti-inflammatory, analgesic and antipyretic activities of *Dalbergia saxatilis* have not been established scientifically.

Problems associated with drugs used in fever, pain and inflammation are alarming, which necessitate the need for development of new drugs and variety of treatment option from bioactive constituents obtained from plants used in traditional medicine (Stark *et al.*, 2013).

Toxicity studies are conducted to provide greater understanding of the potential intrinsic hazard of a test item and to estimate safety margins. These safety margins are used to determine an initial safe starting dose for clinical trials, a safe dose for continued use in humans through longer clinical trials, and ultimately to achieve successful review of registration dossiers to support marketing approval and use of new medicines within the wider population (Robbinson *et al.*, 2009).

1.3 Theoretical Framework

Several plants used in Traditional Medicine have not been screened for efficacy and toxicity. Different procedures and models are in place for estimation of safety profile and evaluation of Pharmacological properties.

In this study, an acute toxicity study was carried out to determine the level of safety for the methanol leaf extract of *Dalbergia saxatilis* using Lorke's method 1983. The method can be used in different species, via several routes of administration. Result of the median lethal dose (LD₅₀) obtained which is an index of acute toxicity enabled in

selection of doses used in the experiments. A 28 days sub-chronic toxicity studies was carried out to determine the effect of the plant for long time use. Detailed studies were carried out using different models for pyrexia, pain, and inflammation.

1.3.1 Sub-chronic toxicity studies

This method has been used to evaluate long time toxicity effect of chemicals. The determination of oral toxicity using repeated doses may be carried out after initial information on toxicity has been obtained by acute testing in the assessment and evaluation of the toxic characteristics of a chemical (OECD, 1995). The method provides information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time.

1.3.2 Brewer's yeast induced pyrexia

This is a suitable model for screening natural or synthetic drugs and compounds for their antipyretic effect. Injection of Brewer's yeast subcutaneously induces pyrexia by increasing the synthesis of prostaglandins (Devi *et al.*, 2003).

1.3.3 Acetic acid induced writhing

Pain is induced in rats or mice by intraperitoneal injection of irritants such as phenyl quinine and acetic acid into the peritoneal cavity where the animal reacts with characteristic stretching and abdominal movement (Milind and Monu, 2013). Acetic acid causes writhes by increasing the release of prostaglandins in peritoneal fluids as well as lipoxygenase products, which enhances inflammatory pain by increasing capillary permeability (Khan *et al.*, 2010).

1.3.4 Hot plate method

The test consists of placing a rat or mouse on a heated plate at a constant temperature. A plate heated to a constant temperature produces two behavioural components that can be measured in terms of their reaction times, such as paw licking and jumping (Milind and Monu, 2013). The method as described by Eddy and Liembach 1953 is the most common thermal nociception model used for evaluating central analgesic efficacy of drugs or compounds. This method is considered to be selective for the centrally acting analgesics; therefore, effect of any drug or compound on this pain model indicates that it could have centrally acting anti-nociceptive activity (Khan *et al.*, 2010).

1.3.5 Carrageenan induced paw oedema model

Carrageenan induced rat paw oedema is a well established animal model for evaluating the anti-oedematous effect of drugs or compounds (Sharma *et al.*, 2010). Formation of oedema caused by carrageenan is said to be in two phases; the first hour after carrageenan injection (first or early phase), involves the release of serotonin, histamine and bradykinin while the second or late phase (2 –5 hours) with increased oedema formation that remains up to the fifth hour involves the release of prostaglandins (Khan *et al.*, 2009).

1.4 Hypothesis

The methanol leaf extract of *Dalbergia saxatilis* has toxicological property, and contain bioactive compounds that have antipyretic, analgesic and anti-inflammatory effects.

1.5 Aim and Objectives

The aim of this work is to establish the safety profile and efficacy of methanol leaf extract of *Dalbergia saxatilis* in treatment of pyrexia, pain and inflammatory conditions.

1.5 Specific Objectives

1. To conduct toxicological studies on the methanol leaf extract of *Dalbergia saxatilis*.
2. To determine the phytochemical constituents of the extract.
3. To study the antipyretic effect of the extract.
4. To study the analgesic effect of the extract.
5. To study the anti-inflammatory effect of the extract.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Toxicology

Toxicology is an applied science that incorporates biology, chemistry, physiology, pathology, physics, statistics, and sometimes immunology or ecology to help solve problems in forensic medicine, clinical treatments, pharmacy and pharmacology, public health, industrial hygiene, veterinary science, agriculture, as well as giving basic insight into how an organism functions (Casarez, 2001). Toxicology, like medicine, is both a science and an art. The science is defined as the observational and data-gathering phase, whereas the art consists of utilization of data to predict outcomes of exposure in human and animal populations (Doull *et al.*, 2008).

Toxicology can be defined as a branch of science that deals with poisons, where a poison is defined as any substance that causes a harmful effect when administered, which can be designed or accidental to a living organism (Doull *et al.*, 2008). Toxicology also includes the study of harmful effects caused by physical phenomena, such as radiation of various kinds and noise. Toxicity cannot be defined as a single molecular event but a cascade of events starting with exposure, proceeding through distribution and metabolism, and ending with interaction with cellular macromolecules (usually DNA or protein) and the expression of a toxic end point (Hodgson, 2004).

Toxicity can be acute or chronic depending on the duration of exposure. Acute toxicity is an adverse effect that is manifested within a relatively short period of time, ranging from almost immediately to within several days following exposure, while chronic

toxicity is a permanent or lasting adverse effect that is manifested after exposure to a toxicant over a long period of time, ranging from months to years (Casarez, 2001).

2.1.1 Importance of dose-response relationship in toxicology

For a substance to cause an adverse effect, it must come in contact with the organism, which is termed exposure. This exposure may be via air, water food or medication. Dose can be defined as the amount of hazardous substance administered to an organism at specific times or intervals, while response is any change in an organism's normal state, and degree of response depend on the amount of toxicant at the target site (James *et al.*, 2000). The relationship between the dose of a toxicant and the response produced follows a predictable pattern. As the dose of a toxicant increases, the response also increases, which may be either in terms of the proportion of the population responding or in terms of the severity of the graded responses. For most toxicants, at very low amounts, there will be no detectable effect of the toxicant, while in midrange of doses; the amount of the damage will increase as the dose increases. Larger amounts of toxicants will cause more severe biological responses until a maximum level of damage is reached. Further toxic effects may also appear along with increased doses, depicting both dose response and dose effect relationships (Ballantyne *et al.*, 1993; Casarez, 2001).

Appropriate dose selection is essential to establishing toxicity. The primary parameter used in dose selection is the tolerability of the test item in animals. Tolerability can be determined by observations such as clinical signs, reductions in body weight or a decrease in food consumption. Important parameters such as systemic exposure and histopathology may also be used to support dose selection. Depending on the nature of

the test item, other specific parameters may be used, these may include, changes in haematological parameters for certain anticancer drugs or blood pressure or electrocardiogram effects for compounds targeting the cardiovascular system (Robinson *et al.*, 2009).

2.1.2 Routes of exposure to toxic substances

Results obtained from toxicity studies can be different for the same dose, depending on the route of exposure to the toxicant. Hazardous substances can be inhaled, ingested, absorbed through the skin, or injected (Everhard *et al.*, 1976). The pathway by which a substance enters the body determines the amount, rate and extent of absorption, and organs that are initially exposed to the largest concentration of the substance. Water and lipid solubility characteristics of a toxicant affect its absorption across the lungs after inhalation, the skin after dermal application, or the gastrointestinal tract after oral ingestion and the effect differs for each organ. The rate and site of absorption may also determine the rate of metabolism and excretion of the toxicant (James *et al.*, 2000).

2.1.3 Some target organs in toxicology

Liver: it is the largest organ in the body, accounting for about 5 percent of the body mass. It is the principal organ for biotransformation, and type of injury depends on the type of toxicant and duration of exposure (James *et al.*, 2000). The metabolic function of the liver can be affected by diet, hormonal activity and alcohol consumption. The liver's increased vulnerability to toxic attack is due to its importance. It can affect, or be affected by, toxicants ingested orally or administered intraperitoneally, because it is the first organ perfused by blood containing the substance (Cullen and Ruebner, 1991). It consists of enzymes such as alanine transferase, aspartate transferase and alkaline

phosphatase, change in the levels of these enzymes are usually indicative of liver function abnormalities (Giboney, 2005).

Kidney: biotransformation also takes place in the kidney, it is susceptible to toxic agents as it constitutes 1 percent of the body's weight, but receive 20-25 percent of the blood flow during rest. As a result, large amounts of circulating toxicants reach the kidneys quickly. The kidneys have high oxygen and nutrient requirements because of their workload and they filter one-third of the plasma reaching them and reabsorb 98-99 percent of the salt and water. As they are reabsorbed, salt concentrates in the kidneys. Changes in kidney pH may increase passive diffusion and cellular concentrations of toxicants, while active secretion processes may concentrate toxicants (Goldstein and Schnellman, 1996). Kidney disease or damage may result in accumulation of Nitrogenous waste products such as urea and creatinine, and imbalance in sodium, potassium, chloride, and bicarbonate levels, as these electrolytes helps the body to maintain healthy water balance and stabilise its acid level (Briggs *et al.*, 1996).

Blood: This system can be damaged by agents that affect blood cell production such as bone marrow, blood components such as platelets, red blood cells, and white blood cells, or even the oxygen-carrying capacity of red blood cells (Ellenhorn, 1997). When bone marrow is injured or suppressed, the number of specific types of blood cells or all blood cells may decline or even disappear. The decline in the number of cells from a specific blood cell lineage has its own diagnostic term and is based on the expected normal range that exists in healthy subjects (James *et al.*, 2000). Example, a decrease in the normal number of circulating red blood cells leads to the clinical condition of

anemia, a decrease in platelets is known as thrombocytopenia, and a decrease in white blood cells is called leukopenia.

Spleen: this filters bacteria and particulate matter, especially deteriorated red blood cells from the blood. The spleen forms all types of blood cells in the embryo, while in the adult, it produces only certain kinds of leukocytes (James *et al.*, 2000). Disease or damage to the spleen may lead to accumulation of dead and abnormal cells, bacteria and other toxicants in the blood (Goerge-Gay and Parker, 2003).

2.2 Pain

Pain is basically a sensation, but has strong cognitive and emotional components, which can be described in terms of suffering. It is associated with avoidance motor reflexes and alterations in autonomic output. Pain can be classified into two categories: adaptive and maladaptive. Adaptive pain contributes to survival by protecting the organism from injury or promoting healing when injury has occurred, while non-adaptive pain is an expression of the pathologic operation of the nervous system (Woolf, 2004).

An influential conceptual model describes pain in terms of three hierarchical levels:

- i. A sensory-discriminative component. Example; location, intensity and quality;
- ii. A motivational–affective component. Example; depression and anxiety; and
- iii. A cognitive-evaluative component. Example; thoughts concerning the cause and significance of the pain.

Nociception can be defined as the process by which information about tissue damage is conveyed to the central nervous system. There can be pain without nociception such as phantom limb pain or nociception without pain. There are four classic descriptions of pain processes:

- a) Transduction: the conversion of the energy from a noxious thermal, mechanical, or chemical stimulus into electrical energy by sensory receptors called nociceptors.
- b) Transmission: this involves the transmission of these neural signals from the site of transduction to the spinal cord and brain.
- c) Perception: the appreciation of signals arriving in higher structures as pain.
- d) Modulation: descending inhibitory and facilitatory input from the brain that influences nociceptive transmission at the level of the spinal cord (Berry *et al.*, 2001).

Perception of pain can be influenced by several psychological factors such as the processes of attention, memory or learning, thought, beliefs, mood, behavioural responses, and ability to cope. Although pain may be an indicator of tissue damage, it may also be experienced in the absence of an identifiable cause. The degree of disability experienced varies in relation to the experience of pain and also in response to pain alleviation methods (Macintyre *et al.*, 2010).

2.2.1 Acute pain

Acute pain is one of the activators of the complex neurohumoral and immune response to injury and both peripheral and central injury responses have a major influence on acute pain mechanisms. Acute pain and injury of various types are interrelated and if prolonged, there will be severe adverse effects on outcome (Kehlet and Dahl, 2003; Chapman *et al.*, 2008). Acute pain is one of the important triggers of the injury response, as the magnitude and duration of the response is related to the magnitude and duration of the stimulus, effective pain relief can have a significant impact on the injury response (Liu and Wu, 2008).

There are psychological changes associated with acute pain which have been considered less important than those associated with chronic pain; however they are no less important. Sustained acute nociceptive input, which occurs after surgery, trauma or burns, can also have a major influence on psychological function, which may alter pain perception. Failure to relieve acute pain may result in increased anxiety, inability to sleep, demoralisation, a feeling of helplessness, loss of control, inability to think and interact with others in the most extreme situations (Cousins *et al.*, 2004).

Adverse Physiological Effects of Acute Pain

Clinically significant injury responses can be broadly classified as inflammation, hyperalgesia, hyperglycaemia, protein catabolism, increased free fatty acid levels and changes in water and electrolyte flux. There are also cardiovascular effects of increased sympathetic activity and diverse effects on respiration, coagulation and immune function (Liu and Wu, 2008).

Hyperglycaemia:

This is broadly proportional to the extent of the injury response. Injury response mediators stimulate insulin-independent membrane glucose transporters glut-1, 2 and 3, which are located diversely in brain, vascular endothelium, liver and some blood cells. Circulating glucose enters cells that do not require insulin for uptake, resulting in cellular glucose overload and diverse toxic effects. Excess intracellular glucose non-enzymatically glycosylates proteins such as immunoglobulins, rendering them dysfunctional. Alternatively, excess glucose enters glycolysis and oxidative phosphorylation pathways, leading to excess superoxide molecules that bind to nitric oxide (NO), with formation of peroxynitrate, ultimately resulting in mitochondrial

dysfunction and death of cells served by glut-1, 2 and 3. Myocardium and skeletal muscle are protected from this toxicity because these two tissues are served by glut-4, the expression of which is inhibited by injury response mediators (Carli and Schricker, 2009).

Lipotoxicity:

Free fatty acid levels are increased due to several factors associated with the injury response and its treatment and can have detrimental effects on cardiac function. High levels of free fatty acid can depress myocardial contractility, increase myocardial oxygen consumption (without increased work) and impair calcium homeostasis and increase free radical production leading to electrical instability and ventricular arrhythmias (Korvald *et al.*, 2000).

Protein catabolism:

The injury response is associated with an accelerated protein breakdown and amino acid oxidation, in the face of insufficient increase in protein synthesis. Following abdominal surgery, amino acid oxidation and release from muscle increased by 90% and 30% respectively, while whole body protein synthesis increased only 10% (Harrison *et al.*, 1989). Protein represents both structural and functional body components, thus loss of lean tissue may lead to delayed wound healing, reduced immune function and diminished muscle strength all of which may contribute to prolonged recovery and increased morbidity (Windsor and Hill, 1988; Watters *et al.*, 1993).

Types of Acute pain

Acute illness; it is a type of pain associated with an acute illness such as appendicitis, renal colic, and myocardial infarction.

Perioperative (includes postoperative); pain that is present in a surgical patient because of pre-existing disease, the surgical procedure such as associated drains, chest, or nasogastric tubes or combination of disease-related and procedure-related sources. Examples include head and neck surgery and abdominal surgery.

Burns; this is the type of pain that results from thermal or chemical burns such as fire and chemical exposure.

Procedural Pain; associated with a diagnostic or therapeutic medical procedure such as catheter placement, circumcision, chest tube placement, immunization, and suturing.

Obstetrical Pain; related to labour and delivery such as childbirth by vaginal delivery or caesarean section (Jacox *et al.*, 1992; Ready *et al.*, 1995).

2.2.2 Chronic pain

Chronic pain can be defined as pain that persists beyond the normal time of healing, and is associated with protracted illness or is a severe symptom of a recurring condition, and has a prolonged duration of 3 months or beyond. It can also be defined as pain persisting beyond the expected time frame for healing or that occurs in disease processes in which healing may never occur (Ospina and Harstall, 2002).

Chronic pain has a devastating effect on the lives of patients and families and creates a high amount of distress and disability. Patients with chronic pain report severe impairments on multiple quality-of-life measures that consider physical, social and psychological well-being domains. Many patients undergo progressive physical

deterioration caused by sleep and appetite disturbances, decrease in physical activity, and high risk of excessive medication (Marcus, 2000).

Chronic pain is a significant problem in the paediatric population, conservatively estimated to affect 15% to 20% of children. Children and their families experience significant emotional and social consequences as a result of pain and disability. The financial costs of childhood pain may also be significant in terms of healthcare utilization as well as other indirect costs, such as parents lost wages due to time off work to care for the child. In addition, the physical and psychological sequelae associated with chronic pain may have an impact on overall health and may predispose for the development of adult chronic pain (Feinberg *et al.*, 2012).

Chronic pain is a major public health issue. According to World Health Organisation/International Association for the Study of Pain (WHO/IASP), 1 in 5 people suffer from moderate to severe chronic pain worldwide. Several specific chronic pain syndromes have been recognized in people such as; low back pain, fibromyalgia, chronic pancreatitis, chronic active hepatitis, metastatic bone cancer pain, trigeminal neuralgia, postherpetic neuralgia, migraine, cluster headache, chronic pelvic pain, rheumatoid arthritis, osteoarthritis, phantom limb pain, reflex sympathetic dystrophy, diabetic neuropathy, giant cell etc. chronic pain can be classified into malignant and non-malignant, and treatment largely depends on type of chronic pain syndrome (Katz *et al.*, 2008).

The aetiology of chronic pain is often unclear and multi-factorial, which is one of the main challenges with the diagnosis and treatment of the disorder. There is a school of

thought that considers chronic pain to always have originated from an acute pain. However, other authorities recognise that chronic pain may occur for no identifiable reason and not following an episode of acute pain. It can also be attributed to a wide range of identifiable causes such as acute injury, arthritis, migraine, and fibromyalgia (Katz *et al.*, 2008).

Types of Chronic pain

Arthritis pain

Arthritis pain can result from more than 100 rheumatic diseases, which cause pain, stiffness, and swelling of joints as well as damage to supporting structures. Osteoarthritis and rheumatoid arthritis are the most common types of arthritis. Osteoarthritis is characterized by a progressive loss of articular (joint) cartilage, mostly affecting weight-bearing and frequently used joints such as hip and knee. It often manifests as deep aching pain, stiffness, and limited range of motion. Rheumatoid arthritis is a common inflammatory arthritis of unknown aetiology that affects multiple joints (Berry *et al.*, 2001).

Fibromyalgia

Fibromyalgia syndrome is a chronic condition which presents as chronic generalised musculoskeletal pain, fatigue and wide variety of other symptoms. The causes of the disease are unclear. Some authorities consider the disorder to be a form of non-articular rheumatism, while others consider the condition to arise as a result of nerve dysfunction due to augmented processing of pain and sensory information. Fibromyalgia can occur following infection or trauma, which is known as reactive fibromyalgia. Individuals with this form of fibromyalgia have more perceived disability, self-reported pain and

affective distress than those with idiopathic onset of the disorder (Berland *et al.*, 2009; White and Harth, 2001).

Low back pain

Low back pain is one of the most common causes of chronic pain and disability. There are a number of reasons why this type of pain can occur; obesity, manual labour, carrying heavy weights such as loaded backpacks, pregnancy, poor posture, abnormal curvature, degenerative disease, and inflammatory disease. Although disability from this condition is not widespread, the reasons an individual may become functionally incapacitated by the condition are complex. Recent thought is that one of the substantial contributors to why an individual may become disabled by the condition is the beliefs and values the individuals hold (Waddell, 2004).

Substances such as tobacco and alcohol can aggravate pain. Cigarette smoke triggers the release of pro-inflammatory cytokines, increasing inflammation and intensifying pain. Smoking makes the bones weak and increases the prevalence of osteoporosis, spinal degenerative disease, and impaired bone and wound healing (Feiberg *et al.*, 2012). Alcohol can enhance the effects of certain prescription drugs as well as markedly increase potential toxic side effects; such as liver damage when used in conjunction with acetaminophen. Chronic alcoholism frequently leads to permanent damage to the liver. Alcohol also affects the heart and blood vessels by decreasing normal function, leading to heart disease. Bleeding from the oesophagus and stomach frequently accompany liver disease caused by chronic alcoholism. Many medications cannot be given to patients with abnormal liver function, thus making it more difficult to treat chronic pain (Passik, 2005).

2.2.3 Pain physiology

The physiologic sources of pain can be divided into nociceptive pain and neuropathic pain:

Nociceptive Pain and Mechanism

Nociceptive pain is caused by the ongoing activation of A- δ and C-nociceptors in response to a noxious stimulus such as injury, disease, and inflammation. Pain arising from skin or subcutaneous tissue (superficial) or bone, muscles, blood vessels and connective tissues (deep somatic) is called somatic pain, whereas, that arising from visceral organs and linings of the body cavities is called visceral pain. Somatic pain is characterised by sharp, burning, dull, aching, cramping pain; while visceral pain is poorly localized, diffuse, deep cramping or splitting, sharp, and stabbing (Helms and Barone, 2008).

Generally, there is a close connection between pain perception and stimulus intensity, and the pain is indicative of real or potential tissue damage. Differences in how stimuli are processed across tissue types contribute to the pain's varying characteristics. Injury to tissue causes cells to break down and release various tissue by-products and mediators of inflammation such as prostaglandins, substance P, bradykinin, histamine, serotonin, and cytokines). Some of these substances activate nociceptors and most sensitized nociceptors increase their excitability and discharge frequency. Ongoing activation of nociceptors may cause nociceptive pain (Berry *et al.*, 2001).

The peripheral nerve ending (nociceptor) transmits pain impulse to the dorsal horn of the spinal cord, where it gets modified before onward transmission to the brain. Any pain caused primarily by stimulation of the nociceptor can be said to be nociceptive.

This does not mean that all nociceptive pains are similar in presentation or management because, for example, pain arising from smooth muscle spasm can be very different from the pain of skeletal muscle spasm (Rajagopal, 2006). Nociceptive pain system is a key early warning device, an alarm system that announces the presence of a potentially damaging stimulus. This type of pain must be controlled only under specific clinical situations, such as during surgery or medical procedures that damage tissue and after trauma. It is important that this system not be chronically disabled, because loss of its protective function inevitably leads to tissue damage, including self-induced mutilation of the tongue and lips, destruction of joints, loss of the tips of fingers, and pressure ulcers (Woolf, 2004).

Nociceptive pain is therefore a vital physiologic sensation. Lack of it in patients with congenital insensitivity to pain due to a mutation of the nerve growth factor tyrosine kinase A receptor, which results in a loss of high-threshold sensory neurons, reduces life expectancy (Miranda *et al.*, 2002). If tissue damage occurs despite the nociceptive defensive system, for example, through trauma, surgery, or inflammatory diseases, the body's imperative shifts from protecting against noxious, potentially damaging stimuli to promoting healing of the injured tissue (Woolf, 2004).

Nociceptors are unspecialised, free, unmyelinated nerve endings that convert a variety of stimuli into nerve impulses, which the brain interprets to produce the sensation of pain. The nerve cell bodies are located in the dorsal root ganglia, or for the trigeminal nerve in the trigeminal ganglia, and they send one nerve fibre branch to the periphery and another into the spinal cord or brainstem. There are two types of nerve fibres; small diameter, unmyelinated nerve that conduct the nerve impulse slowly, known as the C

fibres, and larger-diameter, lightly myelinated nerves that conduct nerve impulses faster, known as A δ fibres (Patel and Kopf, 2010).

Primary afferent nociceptors

Thermal, mechanical, or chemical stimuli that have the potential to cause damage produce cutaneous pain by acting on primary afferent nociceptors; these are generally the initial structures involved in nociceptive processes. Nociceptors are widespread in skin, muscle, connective tissues, blood vessels, and viscera. Nociceptive afferents lack specialized terminal structures and are morphologically free nerve endings. The peripheral axon terminal is not only transduces mechanical, thermal, or chemical energy into series of action potentials relayed to the spinal cord, it also releases peptides in response to injury such as substance P, calcitonin gene-related peptide (CGRP), and neurokinin A that contribute to peripheral inflammatory processes (Julius and Basbaum, 2001).

Visceral afferents

Information from nociceptors is transmitted by visceral afferents from visceral organs to the spinal cord and then in the spinothalamic tracts to the brain. The cell bodies are located in the dorsal root ganglia, and fibers travel with sympathetic and parasympathetic axons. Stimuli that are usually painful when applied to the skin, such as thermal and mechanical stimuli, are not usually painful when applied to the viscera. For example, the brain or bowel can be cut without any sensation of pain. Visceral pain arising from hollow organs commonly results from distension or prolonged contraction of smooth muscle wall of the structure. Visceral pain is frequently associated with accentuated visceral autonomic reflexes, which manifest as nausea and sweating. The

poor localization of visceral pain may be a consequence of the low number of afferent fibers compared with the size of the surface that is innervated; these fibers converge on dorsal horn neurons over a wide number of segments (Ringkamp and Meyer, 2008).

Silent nociceptors

Silent nociceptors are inactive under most circumstances but become active following inflammation and sensitization by nerve growth factor. They have been identified in joint capsules and the walls of viscera. Following sensitization they become responsive and discharge vigorously, even during ordinary movement or visceral distension within the physiological range; they also display changes in receptive fields. This class of nociceptor may contribute to the mechanical allodynia and hyperalgesia associated with peripheral inflammation in arthritis and visceral pain states, such as cystitis or inflammatory bowel disease (Giordano, 2005).

Neuropathic pain and Mechanism

Neuropathic pain is caused by impulse generation within the pathway proximal to the nociceptor which could be in the nerve, the spinal cord or the brain. This type of pain is chronic, often intractable due to peripheral nerve injury. Injury to the peripheral nerves can cause spontaneous paresthesias, numbness, pain with movement, tenderness of a partly denervated body part, and pain that are electric shock-like, burning, shooting, or tingling (Helms and Barone, 2008). It can be subdivided into three: neural injury pain, nerve compression pain and complex regional pain syndrome (CRPS). Neuropathic pain may result from lesions to the peripheral nervous system, as in patients with diabetic neuropathy, post-herpetic neuralgia, or lumbar radiculopathy, or to the central nervous

system, such as in patients with spinal cord injury, multiple sclerosis, or stroke (Woolf, 2004).

Pain along the distribution of one or multiple peripheral nerves, caused by damage to the affected nerve that have peripheral neuropathic pain as a symptom are painful diabetic neuropathy, post-herpetic neuralgia, trigeminal neuralgia, pain after surgery and neuropathic cancer pain. Pain due to a primary lesion or dysfunction of the central nervous system that have central neuropathic pain as symptom, are post-stroke pain, some cancer pain, and pain associated with multiple sclerosis (Berry *et al.*, 2001).

Neuropathic pain can be intermittent or constant, and spontaneous or provoked. Typical descriptions of the pain include terms such as shooting, stabbing, electric shock-like, burning, tingling, tight, numb, prickling, and itching. Symptoms can be described as allodynia (pain caused by a stimulus that does not normally provoke pain), hyperalgesia (an increased response to a stimulus that is normally painful), dysesthesia (an unpleasant abnormal sensation that can be spontaneous or evoked), abnormal skin sensitivity, and hyperpathia (NICE, 2010).

Peripheral processes in neuropathic pain

Peripheral nerve injury results in a number of biochemical, physiologic, and morphologic changes at the peripheral and spinal level that reflect altered afferent sensory input and which may themselves act as a generator of pain. Nerve damage results in an inflammatory response around the site of injury, with increased production of compounds, including nerve growth factor and other tropic factors that normally modulate neuronal growth. Normal sensory processing and primary afferent phenotype are critically dependent on a balance of both retrograde and anterograde axonal

transport of tropic factors typified by nerve growth factor. For example, following nerve injury there is a loss of the primary afferent peptide transmitters substance P and calcitonin gene related peptide (CGRP), and corresponding upregulation of neuropeptide Y and galanin within the dorsal root ganglion and dorsal horn of the spinal cord. This complex pattern of neuropeptide changes reflects altered tropic factor availability, and the exogenous application of nerve growth factor after experimental nerve injury can partially reverse such phenotypic changes. Although altered neuronal phenotype within the DRG and dorsal horn may contribute to the development of pathologic pain states, certain changes, such as the upregulation of neuropeptide Y, may also represent adaptive analgesic responses to injury (Vranken, 2009).

Central processes in neuropathic pain

Under normal circumstance, a painful stimulus result in the release of excitatory amino acids, neurotrophins and peptides from the central terminal of the nociceptive A δ and C-fibers in the dorsal horn. The neurotrophins activates tyrosine kinase receptors B, substance P and neurokinin A interact with the neurokinin 1 and 2 receptors, and contribute to the induction of dorsal horn sensitization. The CGRP is responsible for Ca²⁺ influx and increases the release of substance P and excitatory amino acids. The CGRP strengthen the process of sensitization (Vranken, 2009).

NMDA-mediated mechanisms underlying central sensitization may occur at both pre- and postsynaptic sites in the dorsal horn. Presynaptic sensitization, in conjunction with retrograde transmission by nitric oxide, may further enhance glutamate release from the primary afferent. At the postsynaptic site, NMDA receptor activation in second-order neurons results in phosphorylation of the NMDA receptor, such that the voltage gating

of the receptor for subsequent stimuli is removed. Functionally this is manifest as increased gain of transmission for a given afferent input, and the earliest stages in this process are demonstrated electro physiologically within the dorsal horn as ‘windup’. Wind-up is a progressive increase in the frequency of firing of second-order spinal neurons elicited by a peripheral stimulus sufficient to excite C fibers at a frequency above 0.5 Hz (Rajagopal, 2006; Vranken, 2009).

2.2.4 Pain management

Pain management or treatment can be broadly divided into two; pharmacologic and non-pharmacologic.

2.2.4.1 *Non-pharmacological treatment of pain*

Pharmacological treatment is the mainstay of pain treatment. However, optimal pain management also includes psychological, physical rehabilitative, and in some cases, surgical treatment strategies. A variety of comfort-producing measures have been implemented for pain management such as, endo-tracheal tube suctioning, repositioning in bed, massage, oral care, and reassurance. Other measures include application of heat or cold, massage, therapeutic touch, guided imagery, and relaxation techniques. This is common for critically ill patients (Helms and Barone, 2008).

Non-pharmacologic measures should supplement, not replace, the use of medications. In addition to supplementing the pain-relieving effects of analgesics, non-pharmacologic approaches offer other advantages. Example, they can improve mood, reduce anxiety, increase patient’s sense of control, strengthen coping abilities, assist with sleep, relax muscles, and improve quality of life (Wallace, 1992).

Psychological Approaches

Psychological interventions used in pain management include contingency management, cognitive behavioural therapy, biofeedback, relaxation, descriptions, and psychotherapy. Patients in whom psychological interventions may be most appropriate include those who express interest in such approaches, manifest anxiety or fear, have inadequate pain relief after appropriate pharmacologic interventions, or experience chronic or recurrent pain. Psychological preparation such as preparation for surgery or for an invasive procedure or psychological intervention such as relaxation may help to control the affective dimension of pain when it is acute. This, in turn, helps minimize the biological stress response as well as emotional distress and suffering that the patient experiences. Methods such as relaxation and imagery are simple and can be taught quickly, whereas others require more time. Patient education materials, example, printed instruction sheets, audiotapes can supplement, but not replace, clinician efforts to instruct patients in these methods (Berry *et al.*, 2001).

Physical Rehabilitative Approaches

These methods of pain management are appropriate for many types of pain and are essential in patients with chronic non-cancer pain. In addition to relieving pain, such methods can reduce fear and anxiety, improve physical function, and alter physiological responses to pain. Treatments used in physical rehabilitation include stretching, exercises or reconditioning (to improve strength, endurance, and flexibility), gait and posture training, and attention to ergonomics and body mechanics. Other non-invasive physical treatments for pain include thermotherapy, cryotherapy, counter-irritation, and electro analgesia (Ray, 2001).

Surgical Approaches

Neurosurgical procedures for managing pain include neurolysis (injection of a chemical or application of heat or cold to destroy neural tissue), neuroaugmentation procedures, and neuroablative surgeries (disruption of neural signals and/or removal of neural structures associated with pain). For example, micro vascular decompression of the trigeminal nerve is sometimes used to manage trigeminal neuralgia (Berry *et al.*, 2001).

2.2.4.2 Pharmacological treatment of pain

This is the mainstay of pain treatment. The World Health Organization (WHO) has published a simple and validated three-step approach to pain management that has been shown to be effective in relieving pain. The basic principles behind the three steps of the ladder include selecting the appropriate analgesic for the pain intensity and individualizing the dose by titration of opioid analgesics (Katz *et al.*, 2008).

Step 1

Treatment of mild pain: this involves the use of non-opioid analgesics with or without adjuvants. Pain can usually be adequately treated with aspirin, acetaminophen, and nonsteroidal anti-inflammatory drugs (NSAIDs). These drugs differ from opioids in two important ways: there is a ceiling effect to the analgesia and they do not produce tolerance or physical dependence. Management of all levels of pain includes a drug from this category, except in case of contraindication. Acetaminophen may be preferable in patients at risk for NSAID side effects such as renal failure, bleeding, gastric ulceration or hepatic dysfunction. NSAIDs may be appropriate for other patients, particularly if there is inflammation.

Step 2

Mild to moderate pain: this involves the use of a weak opioid or low dose opioid, with a non-opioid, with or without an adjuvant. In the initial treatment of moderate pain, low-dose opioid drugs are added to aspirin, acetaminophen, or NSAIDs. For patient convenience, many opioids are marketed as combination products containing one of these agents. It is the daily cumulative acetaminophen dose that limits the dosing of the opioid in combination medications. For this reason separate dosing of the opioid and acetaminophen is preferred ((Katz *et al.*, 2008)).

Step 3

Treating Severe pain: this involves the use of strong opioid analgesics with acetaminophen or aspirin, with or without adjuvant.

Drugs used in treatment of pain

There are three classes of drugs used in the treatment of pain which include;

Non-opioid analgesics (non-opioids)

This group includes drugs such as acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin and other salicylic acid derivatives. The primary mechanism of action of NSAIDs is inhibition of the enzyme cyclooxygenase (COX) which blocks prostaglandin synthesis. Acetaminophen appears to act mostly via a central mechanism. All non-opioids have anti-inflammatory, antipyretic, and analgesic effects, but the anti-inflammatory effect of acetaminophen is essentially negligible. The analgesic effect of NSAIDs is prompt, whereas the anti-inflammatory effect is longer (1-2 weeks or more). This latter effect can indirectly relieve some pain by reducing tissue swelling (Berry *et al.*, 2001).

Opioid analgesics (opioids)

This group include opioid agonists such as morphine-like agonists and agonist-antagonist opioids. Opioids bind to opioid receptors in the central nervous system. They inhibit the transmission of nociceptive input from the periphery to the spinal cord, activate descending inhibitory pathways that modulate transmission in the spinal cord, and alter limbic system activity. Thus, opioids modify sensory and affective aspects of pain (Stein, 1995).

Adjuvant analgesics or co-analgesics

This is a diverse group of drugs, with primary indications for conditions other than pain, with analgesic properties relevant to some conditions. Commonly used adjuvant analgesics include antiepileptic drugs, tricyclic antidepressants, and local anaesthetics.

Antiepileptic drugs such as pregabalin and gabapentin bind to pre-synaptic voltage-gated calcium channels in the dorsal horn, which result in decrease in the release of excitatory neurotransmitters such as glutamate and substance P. These drugs are effective in the treatment of diabetic neuropathy and postherpetic neuralgia. The exact basis for analgesic effect is not known, but is not specifically related to their anticonvulsant activity (Moulin *et al.*, 2007).

Tricyclic anti-depressants provide the best evidence in the management of neuropathic pain. The mechanism of analgesic activity is also unclear. They block the reuptake of serotonin and noradrenaline, and block hyperalgesia induced by N-methyl-D-aspartate agonists. They also have sodium channel blocking properties. Their analgesic property is independent of their antidepressant effects. Examples of such drugs include; amitriptyline and imipramine (Moulin *et al.*, 2007).

Local anaesthetics are another type of adjuvant analgesic. They block sodium channels and inhibit the generation of abnormal impulses by damaged nerves to exert their peripheral analgesic effects. When used systemically, they do not produce conduction block as they do with local injection and topical application but may suppress unusual electrical activity in structures associated with pain. Example of such drugs is topical lidocaine (Berry *et al.*, 2001).

2.3 Inflammation

Inflammation is the body's reaction to injury or chemical irritants. It has been characterised based on visual observation by five cardinal signs. These signs include redness (rubor), swelling (tumour), heat (calor) which is only applicable to the body extremities, pain (dolor) and loss of function (functio laesa). Inflammation is described as the succession of changes which occurs in a living tissue when it is injured provided that the injury is not of such a degree that can destroy its structure and vitality at once (Punchard *et al.*, 2004).

Inflammation is the means by which the body deals with insult and injury. Insult may be caused: mechanically by pressure or foreign bodies; chemically by toxins, acidity, and alkalinity; physically by temperature, by internal processes such as uremia, and by microorganisms such as bacteria, virus, and parasites. Inflammation rids the body of foreign matter, disposes of damaged cells, and initiates wound healing. It is controlled by mast cells that are in close proximity to autonomic nerves. Mast cells are constituents of connective tissues containing large granules that contain heparin, serotonin, bradykinin, and histamine. These substances are released from the mast cell in response

to injury and infection, and, by their degranulation, they control most inflammatory processes (Hotamisligil, 2006).

2.3.1 Acute inflammation

This is a rapid or transient response to injury or irritant. Acute inflammation can be caused by infections such as bacterial or viral infections, immune reactions such as reaction to a bee sting and several other stimuli such as tissue necrosis, trauma, radiation, burns and foreign body. It is an immediate response to trauma, usually within seconds to two hours. It is manifested by vascular changes, oedema, and predominantly neutrophilic infiltration (Kumar *et al.*, 2012).

Acute inflammation has three major components: alterations in vascular quality that lead to an increase in blood flow; structural changes in the microvasculature that permit plasma proteins and leukocytes to leave the circulation; and emigration of the leukocytes from the microcirculation, their accumulation in the focus of injury, and their activation to eliminate the offending agent (Badizadegan, 2003).

In acute inflammation, fluid loss from vessels with increased permeability occurs in different phases: an immediate transient response lasting for less than 30 minutes, mediated mainly by the actions of histamine and leukotrienes on endothelium; a delayed response starting at about 2 hours and lasting for about 8 hours, mediated by kinins, complement products, and other factors; and a prolonged response that is most noticeable after direct endothelial injury, such as after burns (Khan and Solomon, 2007).

2.3.2 Chronic inflammation

Chronic inflammation can occur either by evolving from an acute inflammation or without an acute phase. It is the persistence of inflammation with attempts of repair resulting from persistence of the injurious agent. Its response is prolonged, usually weeks, months and even years. Chronic inflammation is caused by persisting infection or prolonged exposure to irritants, repeated acute inflammation such as otitis and autoimmune diseases such as rheumatoid arthritis (Kumar *et al.*, 2012).

Chronic inflammation is characterized by infiltration with mononuclear cells; which include macrophages, lymphocytes, and plasma cells, tissue destruction; induced by the persistent offending agent or by the inflammatory cells, and healing attempts by connective tissue replacement of damaged tissue, accomplished by proliferation of small blood vessels and, in particular fibrosis. It inevitably causes tissue damage and is accompanied by simultaneous attempts at healing and repair. The exact nature, extent and time course of chronic inflammation is variable and depends on the balance between the causative agent and the attempts of the body to remove it (Wassung, 2012).

2.3.3 Chemical mediators of inflammation

The inflammatory process begins with chemical a series of inflammatory chemicals that are released in the extracellular fluid. Sources of inflammatory mediators include injured tissue cells, phagocytes, lymphocytes, mast cells and blood proteins, the most important of which are histamine, kinins, prostaglandins, complement, and lymphokines. Some of these mediators may have individual inflammatory role, they all promote dilation of the small blood vessels in the vicinity of the injury. As more blood flows into the area congestion with blood occurs which accounts for the redness and the

heat of the inflamed area. They also increase the permeability of local capillaries. As a result, exudates, fluid contain proteins such as clotting factors and antibodies, seeps from the bloodstream into the tissue spaces. This exudate is the cause of the local oedema or swelling which in turn, presses on adjacent nerve endings, contributing to a sensation of pain (Wassung, 2012).

Eicosanoids are short-lived, hormone-like substances present in tissues throughout the body. They function as mediators of a variety of physiological responses such as inflammation, blood clotting, vascular dilation, and immunity. They can be divided into four classes: prostaglandins, leukotrienes, thromboxanes, and prostacyclins. A large part of the inflammatory process is regulated specifically by the prostaglandins and leukotrienes (Percival, 1999; Danesh *et al.*, 2004).

Histamines are chemicals responsible for the itchy nose, watery eyes, or rash that often accompany an allergic reaction. Their function is to help remove the toxin causing the problem by sneezing, coughing, crying, and scratching. They bring more blood and lymphatic fluid to the site of the invasion, which in turn carries white blood cells to the site and toxins away from it. It can act on sensory neurones to produce itching at low concentration and pain at high concentrations (Danesh *et al.*, 2004).

Kinins observe a number of pro-inflammatory effects including the release of prostanoids, cytokines, and free radicals from a variety of cells. They degranulate mast cells to releases histamine and other inflammatory mediators and also cause plasma extravasation by contraction of vascular endothelial cells (Dray, 1995).

Cytokines are immune system modulators produced by cells throughout the body. They communicate with the brain, signalling an alarm when an intruder is detected. A

subclass of cytokines known as leukotrienes or interleukins ensures that the immune response is checked before it begins to attack outlying healthy cells and tissue. They significantly call off the inflammatory response (Wassung, 2012).

2.4 Fever

Fever can be defined as the rise of core body temperature above normal. The normal average oral temperature is 37°C (98.6°F) in adults. In oncology practice, a single temperature of more than 38.3°C (101°F) or three readings at least one hour apart of more than 38°C (100.4° F) are considered significant. Elevations of temperature in the very young or old and in patients receiving steroids or other immunosuppressants are considered abnormal (Dalal and Zhukovsky, 2006). Fever can be prolonged, recurrent, or of an unknown origin.

Prolonged fever: this is referred to a single illness in which duration of fever exceeds that expected for the clinical diagnosis; or a single illness in which fever was an initial major symptom and afterwards is low grade or only a perceived problem (Long, 2005).

Recurrent fever: Refer to repeated unrelated febrile infections of the same organ system such as sinopulmonary and urinary tract; or multiple illnesses occurring at irregular intervals, involving different organ systems in which fever is one, variable component (Long, 2005).

Fever of unknown origin

Fever of unknown origin can be defined as fever that does not resolve spontaneously in the period expected for self-limited infection and whose cause cannot be ascertained in spite of considerable diagnostic effort. It can also be illness of more than three weeks duration, fever higher than 38.3°C (101°F) on several occasions, and diagnosis still undecided after a week of study (Arnow and Flaherty, 1997).

The most common cause of fever of unknown origin is infections such as intra-abdominal abscesses, endocarditis, and tuberculosis. Neoplasms and multisystem or collagen vascular diseases comprise the remaining major causes. 30% of the causes are undiagnosed. The applicability of research literature on fever of unknown origin to everyday medical practice may be limited by several factors such as the geographic location of cases, differences in diagnostic facilities between hospitals and countries, and individual experience of the investigators and the specific sub-populations of patients who had fever of unknown origin (Tal *et al.*, 2007).

The causes of fever of unknown origin conventionally have been divided into five categories: infectious, malignant, non-infectious inflammatory disease (NID), miscellaneous, and undiagnosed. The non-infectious disease group has been designated differently by investigators as autoimmune diseases, systemic diseases, multisystem diseases, collagen vascular disease, or vasculitides. A typical example of disease in the miscellaneous group is Whipple's disease, an infection caused by *Tropheryma whipplei* (Knockaert *et al.*, 2003).

2.4.1 Pathophysiology of fever

Fever is one component of febrile response, it is a complex physiologic reaction to disease involving a cytokine-mediated rise in body temperature, generation of acute-phase reactants, and activation of numerous physiologic, endocrinologic, and immunologic systems. The temperature of the body is dependent on maintaining a balance between the production and indulgence of heat. Under normal circumstances, heat is generated internally during metabolic processes or when external environmental temperatures exceed those of the body. Heat can also be produced by increased skeletal muscle activity, such as that which occurs with shivering. Heat loss occurs predominantly from the skin by means of evaporative losses and also, to some extent, through the lungs (Dalal and Zhukovsky, 2006).

Core body temperature is regulated closely by intricate control mechanisms, involving a complex interaction of autonomic, endocrine, and behavioural responses. The hypothalamus is central to this process, functioning as a thermostat, controlling thermoregulatory mechanisms that balance heat production with heat loss. Central to the process are the heat-sensitive receptors located in the pre-optic area of the anterior hypothalamus. These receptors, which are sensitive to elevations in blood temperature, increase signal output as the temperature rises above a fixed thermal set point (37.1°C average) and decrease output when the temperature drops below the set point. Similar receptors are in the skin, spinal cord, and abdomen, sending impulses to the hypothalamus via the spinal cord (Dalal and Zhukovsky, 2006).

When elevation of core body temperature occurs, the sympathetic system is inhibited, leading to vasodilatation of skin vessels and stimulation of the sweat glands to facilitate

evaporative loss. This process prevails until the body temperature matches the thermal set point, when heat production matches heat loss. Similarly, when body temperature is below the thermal set point, a variety of responses are initiated to conserve and increase production of heat. They include activation of the sympathetic nervous system to induce vasoconstriction of skin blood vessels; inhibition of sweating; activation of the shivering centre in the posterior hypothalamus, thereby increasing muscle heat production; and secretion of neurotransmitters, which increase cell metabolism and, consequently, heat production (Watkins *et al.*, 1995).

2.4.2 Thermoregulatory substances

Fever is considered a characteristic of immune system activation, resulting in a regulated rise in body temperature. This regulation is accomplished by the actions of two types of endogenous immunoregulatory proteins called cytokines, with some functioning as pyrogens and others acting as antipyretics. Lipopolysaccharide is a cell wall product derived from gram-negative bacteria, has been the most extensively studied exogenous pyrogen. Others such as super antigens, peptidoglycans, muramyl dipeptides (derived from gram-positive and gram negative bacteria), and viral products have also been investigated. When these exogenous pyrogens are injected systemically into experimental animals, they induce the production of pro-inflammatory cytokines, such as interleukins 1 β (IL-1 β) and 6 (IL-6), interferon (INF)- α , and tumor necrosis factor (TNF), which enter the hypothalamic circulation and stimulate release of local prostaglandins, resetting the hypothalamic thermal set point. The action of these pyrogenic cytokines may be opposed by other cytokines that have antipyretic properties, such as IL-10, arginine vasopressin, melanocyte-stimulating hormone, and

glucocorticoids, thus limiting the magnitude and duration of fever (Watkins *et al.*, 1995; Elmquist *et al.*, 1997).

2.4.3 Clinical phases of fever

Fever consists of three clinical phases: chill, fever, and flush. In the first phase, also known as the cold or chill phase, core temperature rises to reach the new thermal set point. Cutaneous vasoconstriction and increased muscle activity boost heat production, as manifested by chills and shivering. In the second phase, also termed fever, a balance occurs between production and loss of heat at the elevated set point. The skin becomes warm, flushed, and dry. When the set point returns to normal, the body perceives itself to be too warm. Heat-dissipating mechanisms are then initiated, resulting in cutaneous vasodilatation and diaphoresis, which are clinical manifestations of the third or flush phase (Dalal and Zhukovsky, 2006).

2.4.4 Fever management

In the management of fever, drugs known as antipyretics can be used. In addition, increased fluid intake, removal of excess clothing and linens, and use of tepid sponge with water has shown to be very effective. Antipyretic agents, such as acetaminophen, aspirin, or non-steroidal anti-inflammatory drugs (NSAIDs), act by lowering the elevated thermal set point by inhibiting Cox. Cox inhibitors such as NSAIDs, are potent antipyretics and impair the ultimate transformation of arachidonic acid to prostaglandins. Acetaminophen which is a weak Cox inhibitor in peripheral tissue is oxidized in the brain by the P-450 cytochrome and inhibits Cox activity. Corticosteroids reduce the synthesis of PGE₂ by inhibiting the activity of phospholipase A₂, which is needed to release arachidonic acid from the membrane and also act by blocking the

transcription of mRNA for pyrogenic cytokines. They are also effective in fever control (Mackowiak, 1994; Simmons *et al.*, 2000; Creechan *et al.*, 2001).

2.5 Traditional Medicine

Traditional medicine as identified by the World Health Organization, is the sum total of knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement of treatment of physical and mental illness (WHO, 1999).

Traditional healing can be defined as practices designed to promote mental, physical and spiritual well-being that are based on beliefs which date back to the time before the spread of western scientific bio-medicine. It includes a wide range of activities, from physical cures using herbal medicines and other remedies, to the promotion of psychological and spiritual well-being using ceremony, counselling and the accumulated wisdom of elders. Large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs in many developing countries (Martin, 2003).

The primary source of remedies in Traditional Medicine is botanical, although animal and mineral materials have been used. Of the several thousands of remedies available worldwide, about 500 are in common use. Herbal medicines are used after processing, which may be soaking in water or vinegar, or even wine (Li, 2000). Plants and their secondary metabolites have a long history of use in modern medicine and in certain

systems of traditional medicine, and are the sources of important drugs such as atropine, codeine, digoxin, morphine, quinine and vincristine (WHO, 2002; 2005).

2.5.1 Safety of traditional medicine

The use of herbal medicinal products continues to grow worldwide, as well as a growing concern by the public on the safety, quality, availability, preservation and further development of this type of health care (Corbin, 1998). Many herbal therapies have promising potential, and are increasingly used, but few have been tested and monitored. As a result of this, knowledge of their potential side-effects is limited, which makes the identification of the safest and most effective therapies difficult (Obamsawin, 2008).

Plants commonly used in traditional medicine are assumed to be safe. This safety is based on their long time use in the treatment of diseases according to knowledge accumulated over centuries. Scientific research has shown that many plants used as food or in traditional medicine are potentially toxic, mutagenic and carcinogenic (Fennell *et al.*, 2004). Many herbs or plants in use have been screened for toxicity and considered safe such as;

Artemisia annua (a variety of worm wood) was first used traditionally in the treatment of malaria. This plant have been found to cause rapid, as well as extensive death of cancer cells in both in vitro, and in vivo trials such as retarding fibrosarcoma tumours implanted in animals. In a study, human leukemia cells and white blood cells were exposed to an artemisinin compound, while the leukemia cells quickly died; the white blood cells remained essentially unharmed (Harril, 2005).

Cinchona bark is effective in the treatment of malaria, as well as quinine isolated from it. Parasite causing malaria has become resistant to quinine, but till today, no strain of malaria parasite is resistant to the whole cinchona bark. In addition, unlike the isolated compound quinine that can cause life-threatening side effects, there are no known side effects of the whole bark. This shows that whole plant medicines contain natural checks and balances for their strong bio-chemical alkaloids and other elements, which includes built-in enzymes and protective chemistry that assist the human body in dealing constructively with the actions that they help to precipitate (Obamsawin, 2008).

Moringa oleifera is a source of food and medicine. It contains many nutrients such as essential vitamins, essential minerals, amino acids, beta-carotene, anti-oxidants, anti-inflammatory nutrients, phytochemicals and both omega-3 and omega-6 fatty acids. It is used medicinally in detoxification and purification of water, as antibiotics, in skin treatment, as an anti-inflammatory, in ulcers, in reduction of blood pressure, in diabetes, anaemia and many other uses (Kasolo *et al.*, 2010).

According to world health organization, traditional and complementary or alternative medicine has demonstrated efficacy in areas such as mental health, disease prevention, treatment of non-communicable diseases, and improvement of the quality of life for persons living with chronic diseases as well as for the ageing population. Although further research, clinical trials, and evaluations are needed, traditional and complementary or alternative medicine has shown great potential to meet a broad spectrum of health care needs (WHO, 2001).

WHO Regional Office for the Western Pacific invited a group of experts to develop criteria and general principles to guide research work on evaluating herbal medicines in 1992. They recognized the importance of herbal medicines to the health of many people throughout the world. They stated that, few herbal medicines have withstood scientific testing, but others are used simply for traditional reasons to protect, restore, or improve health. There is an increasing need for most herbal medicines to be studied scientifically, although the experience obtained from their traditional use over the years should not be ignored. This is because of limited evidence produced by common scientific approaches to answer questions of safety and efficacy about most of the herbal medicines now in use, the rational use and further development of herbal medicines will be supported by further appropriate scientific studies of these products, and thus the development of criteria for such studies. The document covers topics such as developing protocols for clinical trials using herbal medicines, evaluating herbal medicine research, guidelines for quality specifications of plant materials and preparations, and guidelines for pharmacodynamic and general pharmacological studies of herbal medicines and for toxicity investigations of herbal medicines (WHO, 1993).

Guidelines for the assessment of herbal medicines have also been issued by WHO. These guidelines defined the basic criteria for the evaluation of quality, safety and efficacy of herbal medicines with the goal of assisting national regulatory authorities, scientific organizations and manufacturers in assessing documentation, submissions and dossiers in respect of such products. It was recommended that such assessments take into account long-term use in the country, any description in the medical and pharmaceutical literature or similar sources or documentation of knowledge on the

application of a herbal medicine, and marketing authorizations for similar products (WHO, 1996).

2.5.2 Specialized fields of practice within traditional medicine

Herbalist; a practice that emphasizes botanical and pharmacology knowledge of the indigenous plants and fauna. These individuals work closely with other indigenous doctors and assist in providing remedies for individuals whom they or others have diagnosed. Their practice can be highly specialized in one field, such as remedies for snakebites, or as diverse as the illnesses themselves (Waldram, 1990).

Medicine man/woman; a practice that may and often does possess all of the above gifts and more. Their work usually engages in ritual, ceremonial activity and prayer. In some societies they are identified as medicine men or women because they possess sacred bundles, sacred pipes, sacred masks, and the rights to rituals, songs and medicines that have been inherited from their parents, grandparents, or that they earned through apprenticeship with a respected medicine man or woman (McWhorten, 1992).

Healer; a gifted individual who may heal in a variety of ways, including all of the above and or a “gift” of touch, or energy work, which means that ritual is not always needed. Healers can be ritualistic, but they may also have an ability to use a variety of therapies to heal people spiritually, emotionally or physically (Coulehan, 1980).

2.6 *Dalbergia Saxatilis*

Dalbergia saxatilis is an African shrub 2-3m high , the flowers are white or pink borne in loose axillary or terminal panicles ,15cm and is widely distributed in northern

Nigeria. It is native to Angola, Cameroon, Gabon, Ghana, Guinea, Guinea Bissau, Ivory Coast, Liberia, Nigeria, Senegal, Sierra Leone and Zaire and is used as decoction for treatment of cough, small pox, skin lesions, bronchial ailments and toothache. Common parts used include the leaves, stem bark and roots (Saha *et al.*, 2013).



Plate 1: *Dalbergia saxatilis* (www.westafricanplants.seckenberg)

Taxonomy

Family: Fabaceae

Sub-Family: Faboideae

Genus: *Dalbergia*

Species: *Dalbergia saxatilis*-Hook.f

Common names: Flatbean (English), *Runhun zaki* (Hausa), *Ogundu* (Yoruba), and *Obunzizi* (Igbo).

Various studies have been carried out on the plant *Dalbergia saxatilis*. The described pharmacological properties of the plant include repellent, insecticidal and antimicrobial effects of dried leaves of the plant (Okwute *et al.*, 2009). The root solvent extract of the plant was shown to have uterine contractile activity, and also contain biologically active principle that act as a competitive inhibitor of the β_2 - selective adrenoreceptor agonist and enhanced α -adrenoreceptor function (Uchendu, 2000, 2003). The root solvent extract has also been shown to exhibit anticonvulsant effect as aqueous decoction of the root is used to treat convulsive disorders traditionally. The results obtained from the study indicate that *Dalbergia saxatilis* may provide protection against absence and partial seizures (Yemitan and Adeyemi, 2005). Another study carried out on the root extract indicated anxiolytic and muscle relaxant activities, as the decoction from the root was used by herbal practitioners to produce sleep (Yemitan and Adeyemi, 2003). The antioxidant activity of *Dalbergia saxatilis* has also been carried out using the radical scavenging method which indicates the presence of phenolic compounds. Antioxidants are relevant in prevention of pathologies such as arthritis, cancer, arteriosclerosis and heart diseases (Sofidiya *et al.*, 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant Materials

The leaves of *Dalbergia saxatilis* was collected in June, 2013 at Galadimawa, Giwa Local Government Area, Kaduna state. The plant was identified and authenticated in the Herbarium of the Biological Science Department of Ahmadu Bello University, Zaria by Mallam Umar S. Gallah and was compared with a voucher specimen number of 717.

3.2 Experimental Animals

Wistar rats and mice of both sexes weighing 100-150g and 20-25g respectively were obtained from the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were allowed free access to standard feed and water *ad libitum*. They were kept in clean cages filled with saw dust, which was replaced every three days. The study was conducted according to ethical guidelines on laboratory animal use and care policy, which is in compliance with Ahmadu Bello University Research Policy (Revised 2010).

3.3 Chemicals

Methanol, normal saline, distilled water, acetic acid, aspirin, carrageenan (Sigma chemical, Germany), and 10% formalin solution. Reagents used for the phytochemical screening were obtained from the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria. All the reagents were of analytical standard grade.

3.4 Materials

Digital weighing balance (AE240 dual range, Mettler instrument corporation, US), digital vernier calliper (Battenfeld Technologies Inc. USA), digital thermometer (Mediklin, China), mortar and pestle, scissors, syringes (1ml, 2ml and 5ml), cannula, EDTA and plain bottles, hand gloves, sample bottles, automated haematological machine (Cell-Dyn™ Abbot, US), commercial kits (Reckon Diagnostics P. Ltd, India), TLC plates and laboratory record book.

3.5 Preparation of Plant Extract

The leaves collected were air-dried for two weeks and size reduced with mortar and pestle. This was extracted by cold maceration using absolute methanol. 200g of the powder was added into Erlmeyer Flask with 1000ml of the solvent and kept for 48 hours and shaken intermittently, then filtered. The extract was evaporated to dryness in an evaporator under reduced pressure and controlled temperature (40-60°C). After evaporation, the extract was preserved in a levelled air tight container for future use.

3.6 Phytochemical Screening

Phytochemical screening was carried out on the dried extract using simple chemical tests to detect the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, anthraquinones, and cardiac glycosides. The following methods were used:

3.6.1 Test for alkaloids

To 0.5 g of the extract, dilute HCl (20 ml) was added in a conical flask, heated on a steam bath and then filtered. The filtrate was made alkaline with dilute NH₃ solution and then extracted with chloroform ($3 \times 10 \text{ cm}^3$). The CHCl₃ extract was concentrated

and treated with equal volume of 1% HCl. Dragendoff's reagent and Mayer's reagent 2 ml each were added to the aqueous portion in two separate test tubes. Occurrence of orange-red and cream precipitate indicated the presence of alkaloids, respectively (Evans, 2002).

3.6.2 Test for tannins (Ferric chloride test)

0.2 g of extract was dissolved in 5 ml of distilled water and then filtered. Few drops of ferric chloride solution was added to the filtrate. Formation of a blue-black precipitate indicated hydrolysable tannins while green precipitate indicated the presence of condensed tannins (Evans, 2002).

3.6.3 Test for free anthraquinones (Bontrager's test)

Small portion of the extract was shaken with 10 ml of benzene and filtered. Five milliliters of dilute NH_3 solution was added to the filtrate and shaken. The production of a pink, red or violet colour indicated the presence of free anthraquinones (Evans, 2002).

3.6.4 Test for saponins (Frothing test)

Small quantity of the extract was dissolved in 10 ml of distilled water. It was then shaken vigorously for 30 seconds and allowed to stand for 30 minutes. Formation of froth that last more than 15 min indicated the presence of saponins (Brain and Turner 1975).

3.6.5 Test for steroids and triterpenes (Liebermann-Burchard's test)

0.5g of the extract was dissolved in 5ml of chloroform and filtered. Equal volume of acetic anhydride was added to the filtrate. One milliliter of concentrated H_2SO_4 was

added downside the tube and the colour change observed immediately and over a period of one hour. Red, pink or purple colour indicates the presence of triterpenes, while blue or blue-green indicated steroids (Evans, 2002).

3.6.6 Test for flavonoids (Shinoda test)

0.5 g of the extract was dissolved in 5 ml of methanol and warmed on steam bath. Magnesium chips and 5 drops of concentrated hydrochloric acid were added. A red or orange colour indicated the presence of flavonoids (Evans, 2002).

3.6.7 Test for cardiac glycosides (Keller Kiliani's test)

3ml glacial acetic acid was added with one drop of ferric chloride to 5 ml of the extract. The mixture was kept for one minute then transferred to a test tube. 1.5 ml of concentrated sulphuric acid was added with a pipette, so that it runs down the wall of the tube and forms a separate layer at the bottom. On standing, a brown colour at the interface indicates the presence of deoxy sugars and a pale green colour in the upper layer indicates the presence of a steroid nucleus (Brain and Turner, 1975).

3.6.8 Thin layer chromatographic studies of extract

The TLC profile of the extract was obtained on pre-coated TLC plate using chloroform: methanol: acetic acid (18:1:1) to determine the separation profile of the extract. The plates were sprayed either with p-anisaldehyde solution, Liebermann- Burchard reagent, or Ferric chloride each followed by heating at temperature of 110°C for a ten minutes.

3.7 Acute Toxicity Studies (LD₅₀)

The LD₅₀ of the extract was determined using Lorke's method (1983). The study was carried out in two phases and animals (both mice and rats) were deprived of food for 16-18h prior to administration of the extract. In phase 1, three groups of three animals per group were used. The extract was administered orally in geometrically increasing doses (10mg/kg, 100mg/kg and 1000mg/kg). The treated animals were observed for four hours post administration for signs of toxicity. After 24 hours, when no death occurred, phase 2 was initiated. In phase 2, four groups of one animal each were given the extract orally in geometrically increasing doses (1200 mg/kg, 1600 mg/kg, 2900 mg/kg and 5000 mg/kg). The animals were then observed for signs of toxicity for the first 4 hours and mortality for 24hours. The arithmetic mean of the lowest dose that killed an animal and the highest dose that did not kill was taken as the mean lethal dose (LD₅₀) of the extract.

3.8 Sub-Chronic Toxicity Studies

The study was carried out in accordance to WHO (1992) and OECD 407(1995) guidelines. Twenty rats of either sex were deprived of food for 24 hours, and divided into four groups of five rats each. Group 1, which served as control received normal saline, while rats in groups 2, 3 and 4 were given graded doses of the extract (250 mg/kg, 500 mg/kg and 1000 mg/kg) body weight respectively daily for 28 days. The rats were allowed free access to food and water throughout the duration of the experiment and were observed daily for general symptoms of toxicity (hyperactivity, sedation, and salivation) and mortality. Rats were weighed twice weekly and the average change in weight calculated. Rats were then sacrificed on the 29th day of the experiment by cervical dislocation, blood samples were collected for estimation of

biochemical and haematological parameter. Organs such as heart, spleen, stomach, liver and kidney were removed for determination of organ weight ratio and histopathology.

3.8.1 Calculation of organ weight ratio

The heart, spleen, stomach, liver, and kidney of the rats were removed and weighed using weighing balance (AE240 dual range, Mettler instrument corporation, US), and the relative organ body weight ratio (ROW) was determined using the formula:

$$\text{ROW} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rats on sacrifice day (g)}}$$

3.8.2 Biochemical studies

Blood samples were collected into plain bottles, allowed to clot and centrifuged at 3500 rpm for 10 minutes. The sera were separated, stored at -4°C, and used for evaluation of biochemical parameters which include, alanine aminotransferase (ALT), aspartate aminotransferase (AST) levels and alkaline phosphatase (ALP) levels, and serum bilirubin, serum urea nitrogen, creatinine, chloride, sodium, potassium, and bicarbonate using commercial kits obtained from Reckon Diagnostics P. Ltd, India.

3.8.3 Haematological studies

Blood samples were collected into EDTA bottles for estimation of Packed cell volume (PCV), haemoglobin concentration (Hb), platelets (PLT), white blood cell count (WBC) and differentials, mean corpuscular haemoglobin concentration (MCHC), using an automated haematological machine (Cell-Dyn™ Abbott, US).

3.8.4 Histopathology

The organs removed from the rats were fixed in 10% formal saline for at least 48 hours. They were then processed routinely, and the tissues were embedded in paraffin wax. Histological sections were cut at 5 - 6 μ m and stained with routine haematoxylin and eosin. These were then examined by a consultant histopathologist. Lesions were observed and assessed. Photomicrographs of representative lesions were taken at various magnifications.

3.9 Efficacy Studies

The studies conducted in efficacy studies included anti-pyretic, analgesic, and anti-inflammatory studies. Sixty albino rats and sixty mice were used for the efficacy studies.

3.9.1 Antipyretic Studies

3.9.1.1 Brewer's yeast induced pyrexia model

The antipyretic activity was evaluated using Brewer's yeast-induced pyrexia in rats as described by (Kumar *et al.*, 2009). Fever was induced by administering 20 ml/kg of 20% aqueous suspension of Brewer's yeast in normal saline subcutaneously 17 hours before treatment. 30 wistar rats of both sexes were divided into five groups, 1 and 2 served as negative control (distilled water 10 ml/kg) and positive control (Aspirin 300 mg/kg), while groups 3, 4, and 5 received 250 mg/kg, 500 mg/kg, and 1000 mg/kg of the extract. All administrations were per oral. Rectal temperatures were taken by the use of digital thermometer (Mediklin, China) before yeast injection, 19 hours after the injection, and at 1, 2, 3, and 4 hours after drug administration.

3.9.2 Analgesic studies

3.9.2.1 *Acetic acid induced writhing in mice*

Acetic acid induced writhing method described by (Koster *et al.*, 1959) was adopted for evaluation of analgesic activity. Writhing is defined as a stretch, tension to one side, extension of hind legs, contraction of the abdomen so that the abdomen of mice touches the floor, turning of trunk (Mishra *et al.*, 2011). 30 Swiss albino mice of both sexes were divided into five groups, 1 and 2 served as negative control (distilled water 10 ml/kg) and positive control (Aspirin 300 mg/kg), while groups 3, 4, and 5 received 250 mg/kg, 500 mg/kg, and 1000 mg/kg of the extract. *All administrations were per oral. Sixty minutes after treatment, the mice received 0.6% acetic acid (10ml/kg) interperitoneally to induce pain. 5minutes after acetic acid injection, the animals were observed and number of writhes by each mouse was counted for 15minutes. Percentage inhibition was calculated using the following formular:*

$$\% \text{ inhibition} = \frac{\text{Average number of writhes (control)} - \text{Average number of writhes (test)}}{\text{Average number of writhes (control)}}$$

3.9.2.2 *Hot plate method*

This method was carried out as described by Eddy and Leimback (1953). The paws of mice and rats are very sensitive to heat at temperatures which are not damaging to the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The temperature of the hot plate was set at $55 \pm 1^{\circ}\text{C}$ (Mishra *et al.*, 2011). 30 Swiss albino mice of both sexes used were fasted 12 hours prior to the experiment, and divided into five groups, 1 and 2 served as negative control (distilled water 10 ml/kg) and positive control (Morphine 10 mg/kg), while groups 3, 4, and 5 received 250 mg/kg, 500 mg/kg, and 1000 mg/kg of the extract. The test and standard drugs were administered orally.

The animals were individually placed on the hot plate and the time until either licking or jumping occurred was recorded by a stop-watch, 30, 60, 90 and 120 minutes after treatment.

3.9.3 *Anti-inflammatory Studies*

3.9.3.1 *Carrageenan induced rat paw oedema model*

The anti-inflammatory study was carried out using the method described by Winter *et al* (1963). 30 wistar rats were divided into five groups, 1 and 2 served as negative control (distilled water 10 ml/kg) and positive control (Aspirin 300 mg/kg), while groups 3, 4, and 5 received 250 mg/kg, 500 mg/kg, and 1000 mg/kg of the extract. Treatments were administered 1 hour before carrageenan injection. Carrageenan was prepared as 1% w/v solution in 0.9 % w/v NaCl and 0.1 ml was injected underneath the planter region. The paw size was then measured with digital vernier calliper at 0, 1, 2, 3, 4, and 5 hours after Carrageenan injection (Sharma *et al.*, 2010).

3.10 Statistical Analysis

Results were expressed as mean $\pm$ standard error of mean and presented as graphs and tables. Data were analysed using one way analysis of variance (ANOVA) followed by Dunnett t-test and repeated measures ANOVA followed by Bonferroni test for multiple comparison. Results were considered significant at $p < 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical Screening

Phytochemical screening of the methanol leaf extract of *Dalbergia saxatilis* revealed the presence of alkaloids, flavonoids, cardiac glycosides, tannins, triterpenes, and saponins. Anthraquinones and steroids were absent (Table 4.1).

Table 4.1: Phytochemical screening of methanol leaf extract of *Dalbergia saxatilis*

Phytochemical constituents	Result
Alkaloids	Present
Flavonoids	Present
Cardiac glycosides	Present
Triterpenes	Present
Saponins	Present
Tannins	Present
Anthraquinones	Absent
Steroids	Absent

4.1.1 Thin layer chromatography

Results of the thin layer chromatographic analysis of the methanol leaf extract of *D. saxatilis* using different spraying reagents also revealed the presence of several phytochemical constituents. P-Anisaldehyde/Sulphuric acid is a general spraying reagent (Plate II). Liebermann Burchard is specific for steroids and triterpenes, where pink, red, or purple colour indicated the presence of triterpenes, steroids are absent in the extract as blue or green colour is not evident on the plate (Plate III). FeCl_3 is specific for tannins, where a black colour indicated the presence of tannins (Plate IV).

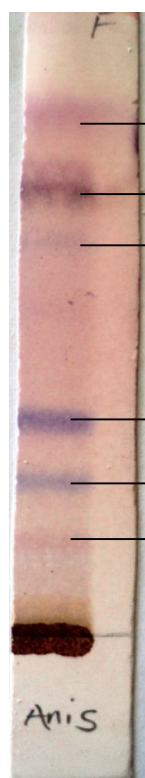


Plate II

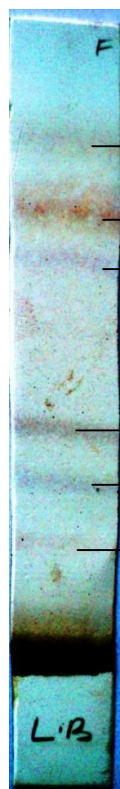


Plate III



Plate IV

II = P- anisaldehyde/Sulphuric acid

III = Liebermann Burchard

IV = Ferric chloride

Table 4.2: R_f values for chromatograms A, B, and C

Spray Reagents	Spots	Colours	R_f values
Anisaldehyde/Sulphuric acid	a	Pink	0.164
	b	Blue	0.284
	c	Violet	0.388
	d	Purple	0.701
	e	Brown	0.821
	f	Violet	0.925
Liebermann Burchard	a	Pink	0.075
	b	Purple	0.224
	c	Grey	0.328
	d	Violet	0.433
	e	Brown	0.836
	f	Brown	0.896
Ferric chloride	a	Grey	0.134

$$R_f \text{ value} = \frac{\text{distance travelled by the spot}}{\text{Distance travelled by the solvent}}$$

4.2 Toxicity studies

4.2.1 Acute toxicity study (LD₅₀ determination)

The mice and rats that received the methanol extract of *Dalbergia saxatilis* orally did not show any sign of toxicity after the first four hours of extract administration. The median oral lethal dose for both mice and rats was found to be greater than 5000mg/kg.

4.2.2 Sub-chronic toxicity studies

(a) Effect of the methanol leaf extract of *D. saxatilis* on average body weight (g)

The effect of sub-chronic oral administration of the methanol extract of *D. saxatilis* for 28 days on animal body weights is shown in table 4.3. There was significant increase in the animal weights at all weeks compared to week zero in the control group at $p < 0.05$. A significant increase was also observed in the 500 mg/kg at the fourth week compared to week zero at $p < 0.05$.

Table 4.3: Effect of the methanol leaf extract of *D. saxatilis* on average body weight (g)

Treatment (mg/kg)	Week 0	Week 1	Week 2	Week 3	Week 4
Distilled water	119.00±7.04	134.10±6.90 ^a	138.60±5.20 ^a	146.50±3.20 ^a	146.10±3.71 ^a
Extract 250	115.80±5.66	117.80±6.44	116.30±5.42	119.63±9.05	128.33±4.80
Extract 500	116.20±5.08	110.10±3.11	118.10±2.85	127.00±6.83	140.25±5.95 ^a
Extract 1000	116.60±4.12	111.30±4.32	109.00±10.1	121.67±8.74	123.17±10.6

Data were analysed using repeated measures ANOVA followed by Bonferroni test. ‘a’ in superscript represent $p < 0.05$ level of significance. Values are $\pm$ SEM, n= 5.

(b) Effect of methanol leaf extract of *D. saxatilis* on haematological indices

The effects of sub-chronic oral administration of the methanol extract of *D. saxatilis* leaves on haematological indices such as packed cell volume (PCV), haemoglobin concentration (Hb), white blood cells (WBC), platelets (PLT), neutrophils (NEUT), lymphocytes (LYMPH) and mean corpuscular haemoglobin concentration (MCHC) for 28 days are shown in table 4.4. There were no significant changes observed in the concentrations of all the haematological parameters except white blood cells. A significant reduction at $p < 0.01$, $p < 0.001$ and $p < 0.01$ was observed in all the groups of 250 mg/kg, 500 mg/kg and 1000 mg/kg of the extract respectively.

Table 4.4: Effect of methanol leaf extract of *D. saxatilis* on haematological indices

	Control	250mg/kg	500mg/kg	1000mg/kg
PCV (%)	33.88 ± 0.93	32.28 ± 0.58	31.04 ± 1.45	34.38 ± 2.53
Hb (g/dL)	130.80 ± 3.77	125.00 ± 2.27	121.00 ± 6.42	131.75 ± 10.38
WBC (x 10 ⁹ /L)	14.04 ± 1.13	8.08 ± 1.51 ^b	6.10 ± 1.42 ^c	6.68 ± 0.34 ^b
MCHC (g/L)	386.00 ± 5.21	386.75 ± 2.50	389.00 ± 3.76	382.25 ± 3.88
PLT (x 10 ⁹)	698.00 ± 63.23	544.00 ± 52.86	597.20 ± 70.53	633.75 ± 83.38
NEUT (%)	19.20 ± 2.74	18.78 ± 2.98	18.28 ± 1.44	25.50 ± 8.57
LYMPH (%)	73.03 ± 3.63	74.25 ± 3.44	75.13 ± 1.60	67.33 ± 9.64

Data were analysed using one way ANOVA followed by Dunnett *t-test*. 'b and c' in superscript represent $p < 0.01$ and $p < 0.001$ level of significance respectively. Values are $\pm$ SEM, $n = 5$.

(c) Effect of methanol leaf extract of *D. saxatilis* on renal indices

The effects of sub-chronic administration of methanol leaf extract of *D. saxatilis* leaves on renal indices such as urea, creatinine, sodium, potassium, chloride and bicarbonate serum concentrations is presented in table 4.5. A significant reduction in serum concentrations of sodium, chloride and bicarbonate at $p < 0.05$ was observed. For sodium and chloride, the significant reduction was observed in the 250 mg/kg and 500 mg/kg groups of the extract. And for bicarbonate, the reduction was only observed in the 250 mg/kg group at $p < 0.05$.

Table 4.5: Effect of methanol leaf extract of *D. saxatilis* on renal indices

Electrolytes	Control	250mg/kg	500mg/kg	1000mg/kg
Urea (mmol/l)	10.38±1.35	15.46 ± 2.66	14.70 ± 1.42	15.80 ± 2.68
Creatinine(μmol/l)	47.69 ± 6.06	65.05 ± 5.04	59.59 ± 2.59	61.13 ± 4.54
Chloride (mmol/l)	122.60±4.06	105.00 ± 4.56 ^a	107.00 ± 4.64 ^a	111.75 ± 2.96
Sodium (mmol/l)	168.80±7.74	139.00 ± 7.15 ^a	141.20 ± 5.98 ^a	146.00 ± 4.51
Potassium(mmol/l)	5.56±0.27	5.95 ± 0.63	5.50 ± 0.32	5.35 ± 0.18
Bicarbonate (mmol/l)	28.80±2.25	18.50 ± 1.56 ^a	20.80 ± 1.63	21.00 ± 1.16

Data were analysed using one way ANOVA followed by Dunnett *t-test*. ‘a’ in superscript represent $p < 0.05$ level of significance respectively. $n = 5$. Values are $\pm$ SEM, $n = 5$.

(d) Effect of methanol extract of *D. saxatilis* on liver function indices

The effects of sub-chronic administration of methanol extract of *D. saxatilis* leaves on liver function indices such as aspartate, alkaline phosphate, alanine transferase and serum bilirubin are shown in table 4.6. Among the indices, there was statistical significance in only aspartate and alanine transferase. A statistically significant increase in alanine transferase was observed in the 250 mg/kg and 500 mg/kg groups of the extract at $p < 0.05$ and for aspartate transferase in the 250 mg/kg group of the extract at $p < 0.05$ respectively.

Table 4.6: Effect of methanol extract of *D. saxatilis* on liver function indices

	Alanine transferase (U/L)	Aspartate transferase (U/L)	Alkaline phosphatase (U/L)	Serum bilirubin (mg/dL)
Control	62.36 ± 11.657	181.22 ± 34.639	225.40 ± 10.605	1.29 ± 0.191
250mg/kg	119.48 ± 20.239 ^a	350.23 ± 29.491 ^a	362.25 ± 50.579	0.94 ± 0.147
500mg/kg	107.00 ± 6.909 ^a	261.38 ± 24.226	311.60 ± 15.667	0.99 ± 0.182
1000mg/kg	95.34 ± 6.585	278.80 ± 51.923	341.25 ± 66.386	0.72 ± 0.107

Data were analysed using one way ANOVA followed by Dunnett *t-test*. ‘a and b’ in superscript represent $p < 0.05$ and $p < 0.01$, level of significance respectively. Values are $\pm$ SEM, $n = 5$.

(e) Effect of the methanol leaf extract of *D. saxatilis* on relative organ weight

The effect of methanol extract of *D. saxatilis* leaves on relative organ body weight is shown in table 4.7. No statistical significance was observed in all the relative organ weights and at all doses of the extract. Though there was no statistical significance, the weights in the 500 mg/kg group were comparable to the control group. The weights in the 250 mg/kg and 1000 mg/kg groups were similar, and reduction in weight compared to control was also observed.

Table 4.7: Effect of the methanol leaf extract of *D. saxatilis* on relative organ weight

Organs	Control (X 10⁻³)	250mg/kg (X 10⁻¹)	500mg/kg (X 10⁻³)	1000mg/kg (X 10⁻¹)
Heart	4.21 ± 0.0004	2.04 ± 0.199	4.04 ± 0.0003	2.04 ± 0.199
Stomach	10.41 ± 0.0006	2.09 ± 0.198	1.10 ± 0.0009	2.09 ± 0.198
Spleen	7.46 ± 0.0009	2.05 ± 0.199	5.17 ± 0.0004	2.05 ± 0.198
Kidney	6.94 ± 0.0005	2.06 ± 0.198	7.28 ± 0.0001	2.06 ± 0.199
Liver	33.6 ± 0.0028	2.28 ± 0.193	34.08 ± 0.0011	2.27 ± 0.193

Data were expressed as mean ± SEM, n = 5, no statistical differences noted in all organs.

4.2.3 Histopathological findings:

(a) *Heart*

The histopathological examination of the heart of the rats administered all doses of the methanol extract of *D. saxatilis* (250 mg/kg, 500 mg/kg and 1000 mg/kg) daily for 28 days and control revealed normal cardiac cells as shown in plate 5.

(b) *Stomach*

The stomach of the rats administered 250 mg/kg, 500 mg/kg of the extract and control revealed normal mucosa, while that of the group administered 1000 mg/kg of the extract revealed damaged mucosa with ulceration as shown in plates 6 and 7 respectively.

(c) *Spleen*

The spleen of the rats administered 500 mg/kg of the extract and control group, revealed normal red and white pulp. The group administered 250 mg/kg of the extract revealed moderate lymphocyte hyperplasia, while the 1000 mg/kg group revealed intense lymphocyte hyperplasia, as shown in plates 8, 9, and 10 respectively.

(d) *Liver*

The liver of the control group showed normal hepatocytes and there was no vascular congestion. The 250 mg/kg group of the extract showed vascular congestion with slight lymphocyte hyperplasia, 500 mg/kg group showed slight lymphocyte hyperplasia, while the 1000 mg/kg group showed slight peri-vascular necrosis, as shown in plates 11, 12, 13, and 14 respectively.

(e) *Kidney*

The kidneys of rats in the control group showed normal tubules and glomerulus, while those of rats in the 250 mg/kg and 500 mg/kg group of the extract showed massive necrosis of the glomerulus with tubular distortion and lymphocyte hyperplasia. The

kidneys of rats in the 1000 mg/kg group of the extract revealed intense glomerular and tubular necrosis, as shown in plates 15, 16, 17, and 18 respectively.

4.3 Antipyretic studies

The effect of methanol extract of *Dalbergia saxatilis* leaves on brewer's yeast induced pyrexia in rats is shown in Figure 4.1. Statistically significant reduction in rectal temperature was observed in the group treated with aspirin 300 mg/kg at $p < 0.05$ from the 21st hour to the 23rd hour compared to the 19th hour. There was reduction in rectal temperature in the groups treated with 250 mg/kg, 500 mg/kg and 1000 mg/kg of the extract at 20th to 23rd hour, though not statistically significant. There was statistical significant reduction in the 500 mg/kg group and 1000 mg/kg groups of the extract at the 23rd hour at $p < 0.05$.

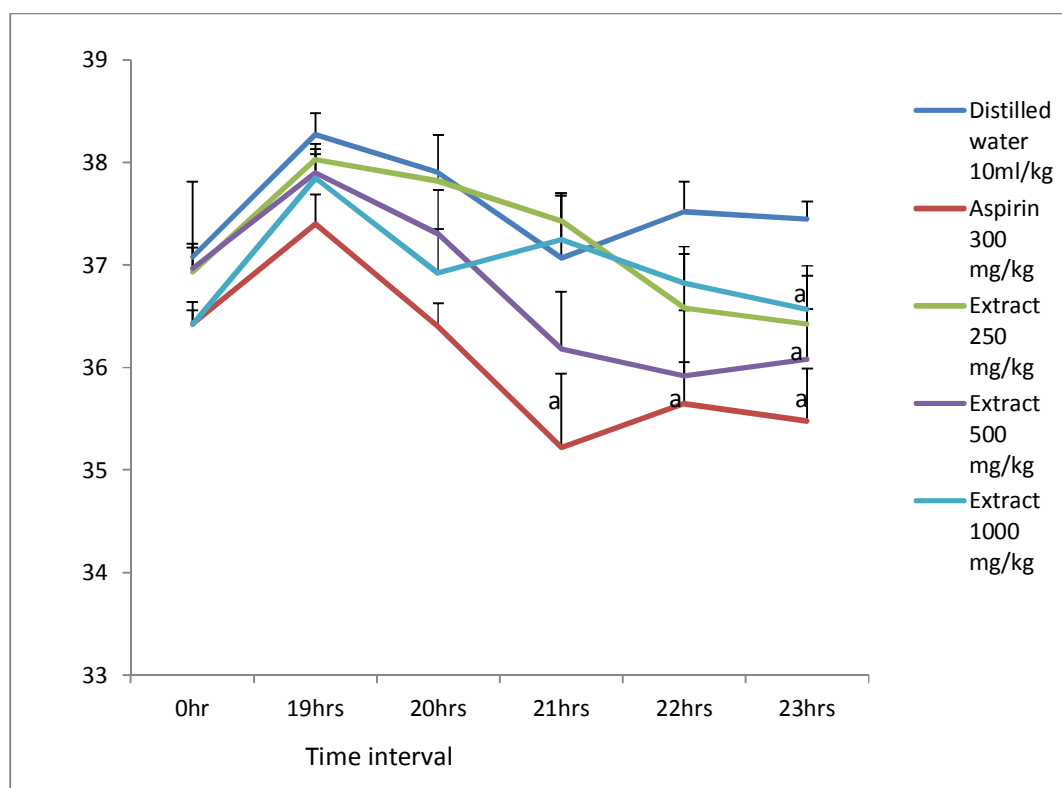


Figure 4.1: Effect of the methanol leaf extract of *D. saxatilis* on Brewer's yeast induced pyrexia in rats.

Data were analysed using repeated measures ANOVA followed by Bonferoni test. 'a' in superscript represent decrease in rectal temperature at $p < 0.05$ level of significance. Values are $\pm$ SEM, $n = 6$.

4.4 Analgesic studies:

4.4.1 Acetic acid induced writhing in mice

The extract significantly decreased the number of writhes caused by acetic acid in a dose independent manner at $p < 0.001$ as shown in table 4.8. The effects observed at 250 mg/kg and 1000 mg/kg were more than that of 500 mg/kg of the extract. The effect observed in 1000 mg/kg group was comparable to that of the standard.

Table 4.8: Effect of methanol leaf extract of *D. saxatilis* on Acetic Acid Induced writhing in mice

Treatment	Dose(mg/kg)	Mean number of writhes $\pm$ SEM	Inhibition (%)
Distilled water	10ml/kg	28.33 $\pm$ 3.844	-
Aspirin	300	4.67 $\pm$ 1.453 ^c	83.5
Extract	250	9.50 $\pm$ 3.128 ^c	66.5
Extract	500	11.50 $\pm$ 3.658 ^c	59.4
Extract	1000	4.67 $\pm$ 1.563 ^c	83.5

Data were analysed using one way ANOVA followed by Dunnett *t*-test. 'c' in superscript represent $p < 0.001$ level of significance respectively.

4.4.2 Hot plate method in Mice

The methanol leaf extract of *D. saxatilis* significantly increased the reaction time at different doses at $p < 0.05$ as shown in Figure 4.2. At 30 minutes, there was significant increase in reaction time in the 500 mg/kg and 1000 mg/kg groups of the extract. At 60, 90, and 120 minutes, there were significant increase in reaction time in both the standard and all doses of the extract as presented in the graph at $p < 0.05$ and $p < 0.01$ respectively.

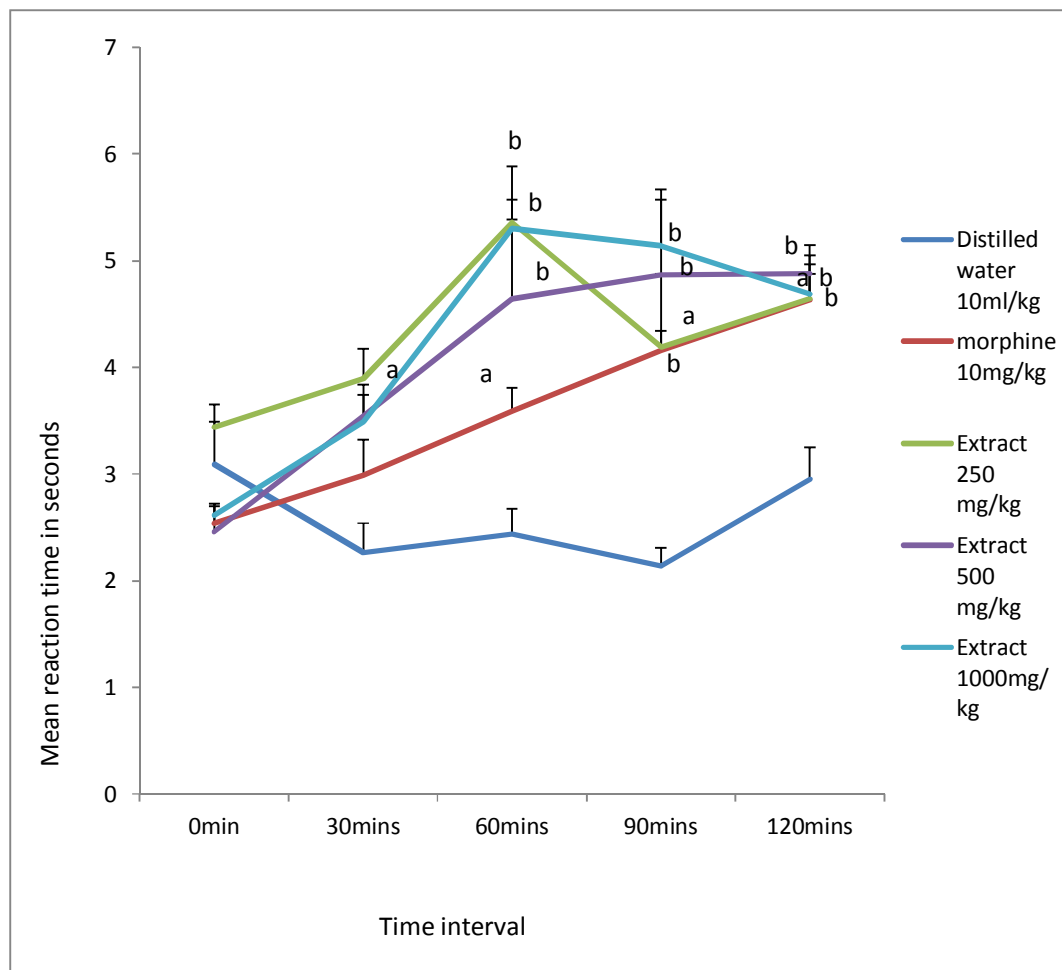


Figure 4.2: Effect of the methanol extract of *Dalbergia saxatilis* on Hot plate induced pain in mice.

Data were analysed using repeated measures ANOVA followed by Bonferoni test. 'a and b' in superscript represent $p < 0.05$ and $p < 0.01$, level of significance respectively. Values are $\pm$ SEM, $n = 6$.

4.5 Anti-inflammatory studies

4.5.1 Carrageenan induced paw oedema in rats

The methanol extract of *D. saxatilis* leaves decreased the rat paw size at different doses though not statistically significant as shown in figure 4.3. Zero hour in each group was taken as control and compared to other times. There was statistical significant increase in paw size at the second hour compared to the first hour, a significant reduction was seen in the fourth and fifth hour compared with the second hour (peak increase) in the distilled water group. There was no statistical significant increase or decrease in paw size when time zero was compared to the other times in the positive control group. In the 250 mg/kg group, the peak increase was observed in the third hour which was also statistically significant. In the 500 mg/kg group, the peak increase in paw size was observed in the first hour at $p < 0.05$, while in the 1000 mg/kg group, there was statistical significant increase in paw size in all the hours when compared to the zero hour.

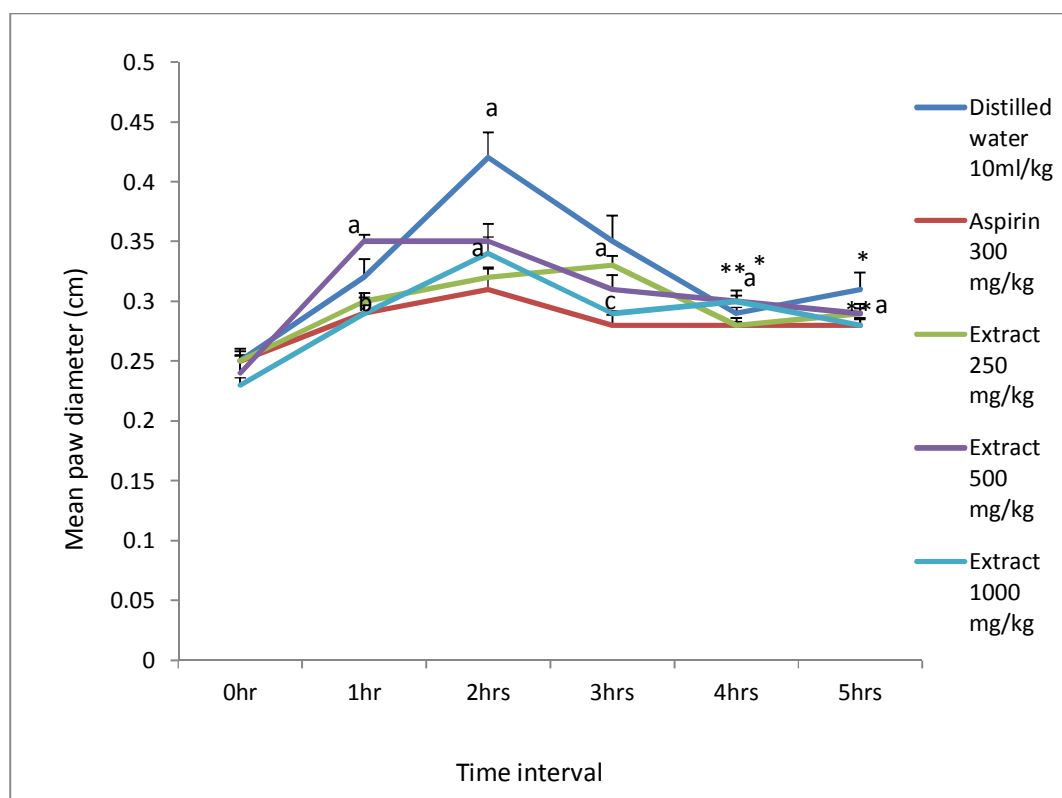


Figure 4.3: Effect of methanol leaf extract of *D. saxatilis* on Carrageenan-induced Rat paw oedema model.

Data were analysed using repeated measures ANOVA followed by Bonferroni test. 'a and b' in superscript represent statistical significant increase in paw diameter, while * and ** represent statistical decrease in paw size at $p < 0.05$, and $p < 0.01$ respectively. Values are $\pm$ SEM, $n = 6$.

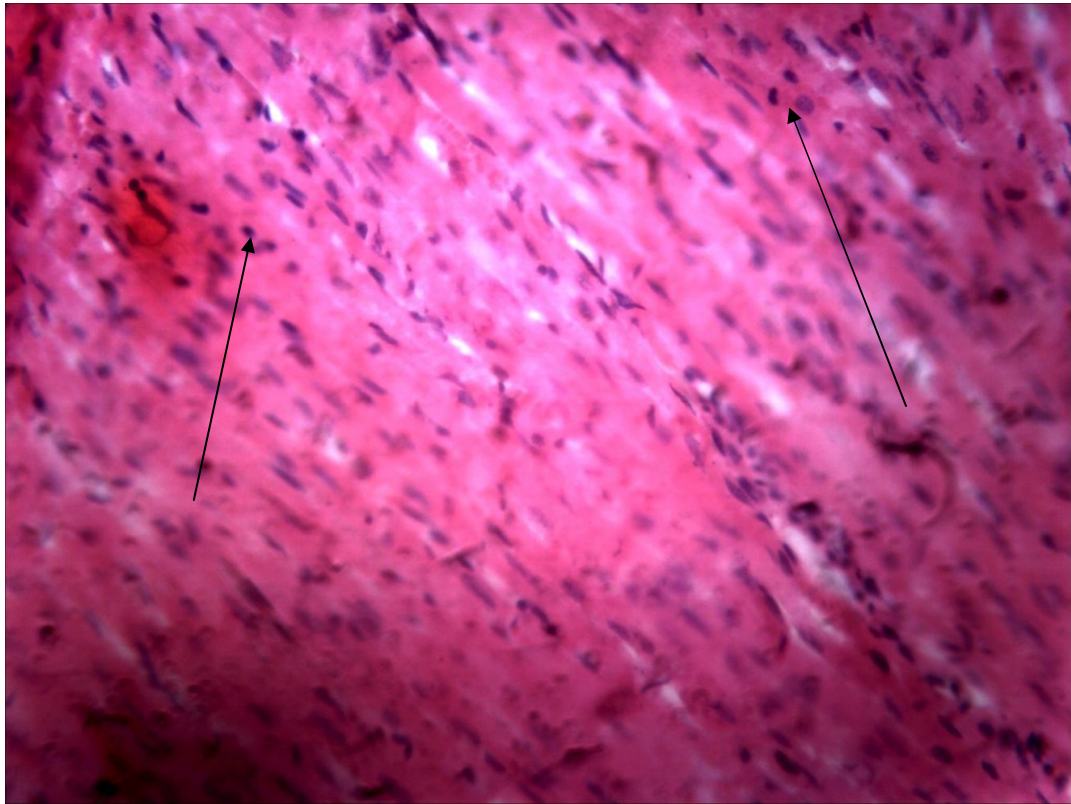


Plate 5: Photomicrograph of the rat heart showing normal cardiac cells, magnification (X 250) after 28days treatment with distilled water 10ml/kg body weight.

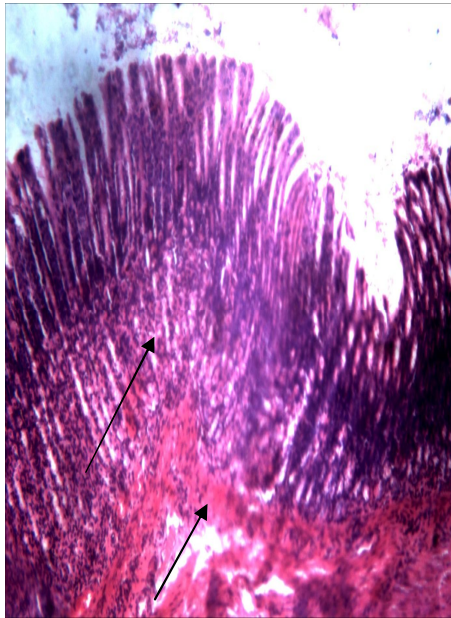


Plate 6

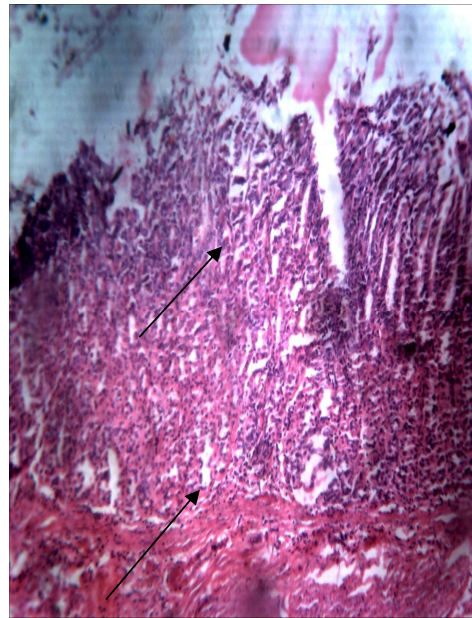


Plate 7

Photomicrographs of the rat stomach showing normal mucosa plate 6; rat stomach showing damaged mucosa with ulceration plate 7, magnification (X 100) after sub-chronic oral administration with distilled water 10ml/kg body weight and 1000mg/kg of methanol leaf extract of *D. saxatilis*.

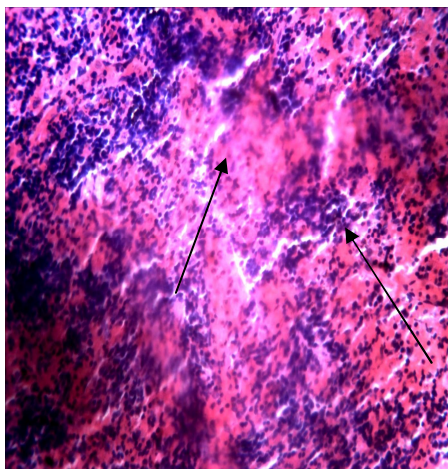


Plate 8

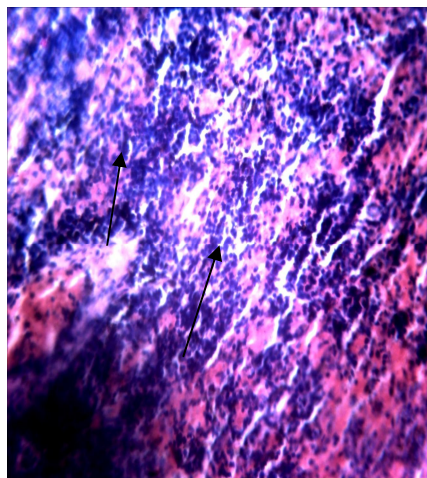


Plate 9

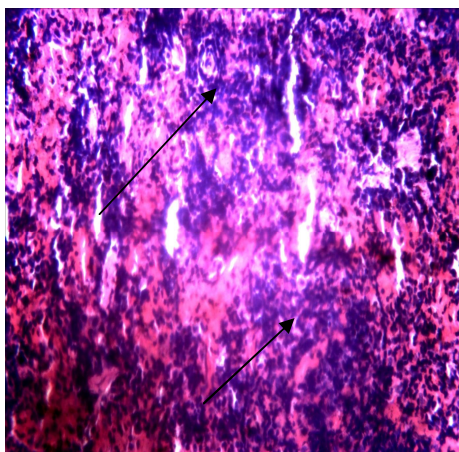


Plate 10

Photomicrographs of the rat spleen showing normal red and white pulp plate 8; rat spleen showing moderate lymphocyte hyperplasia plate 9; rat spleen showing intense lymphocyte hyperplasia plate 10; magnification (X 250) after sub-chronic oral administration with 10ml/kg of distilled water, 250 mg/kg and 1000 mg/kg of methanol leaf extract of *D. saxatilis* respectively.

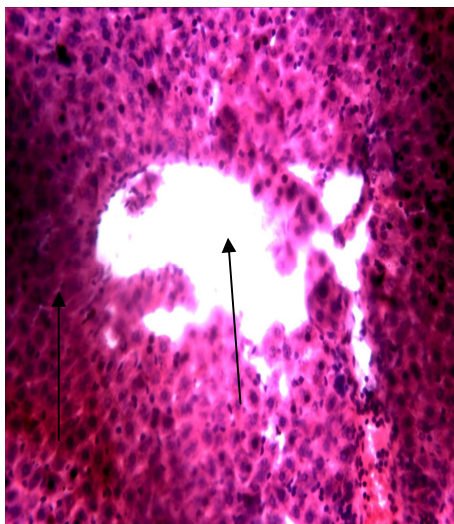


Plate 11

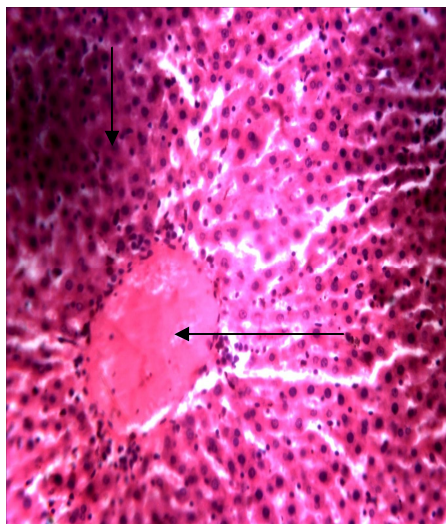


Plate 12

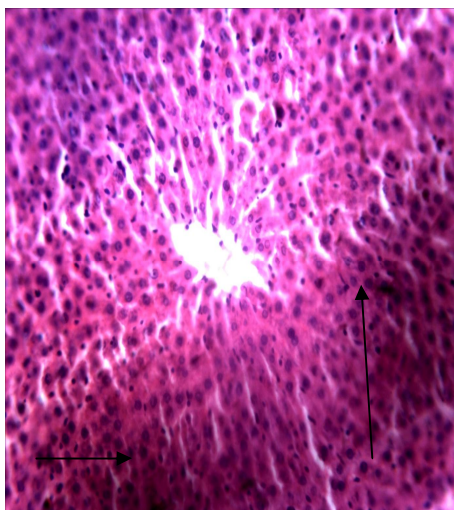


Plate 13

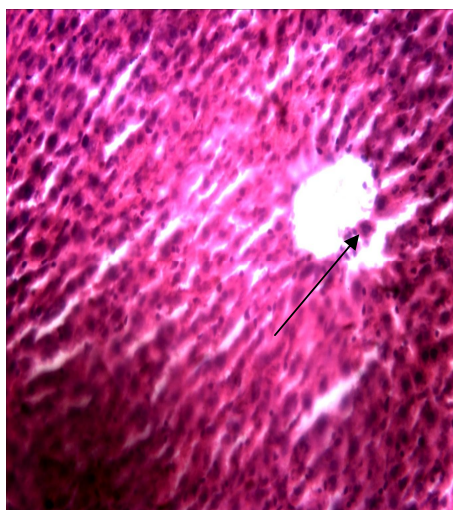


Plate 14

Photomicrographs of the rat liver showing normal hepatocytes plate 11; rat liver showing vascular congestion with slight lymphocyte hyperplasia plate 12; rat liver showing slight lymphocyte hyperplasia plate 13; rat liver showing slight peri-vascular necrosis plate 14; magnification (X 250) after sub-chronic oral administration with distilled water 10ml/kg body weight, 250 mg/kg, 500 mg/kg, and 1000 mg/kg of the methanol leaf extract of *D. saxatilis* respectively.

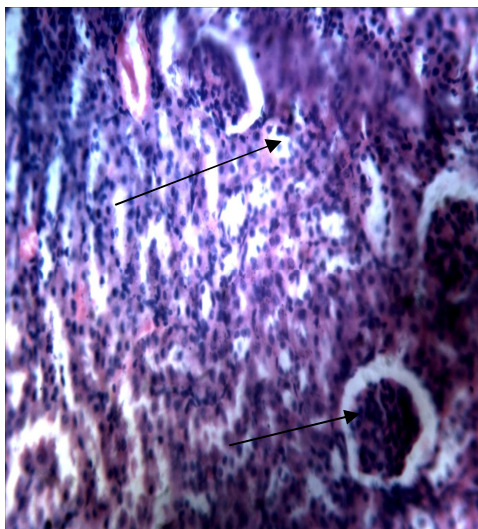


Plate 15

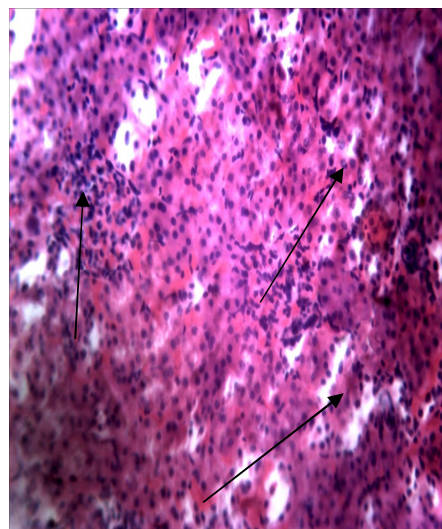


Plate 16

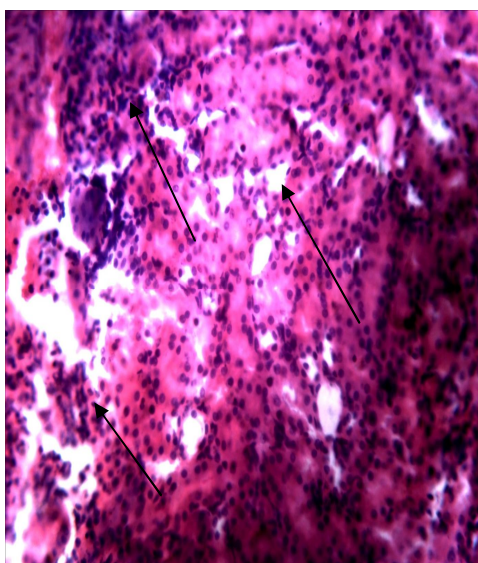


Plate 17

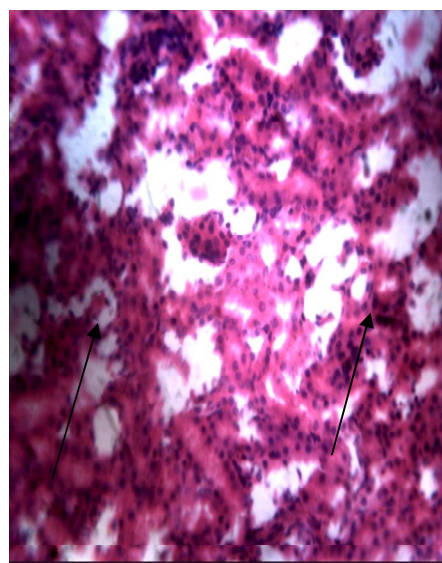


Plate 18

Photomicrographs of the rat kidney showing normal tubules and glomerulus, plate 15; rat kidney showing massive glomerular necrosis with tubular distortion and lymphocyte hyperplasia, plate 16; rat kidney showing glomerular necrosis and slight tubular distortion with lymphocyte hyperplasia, plate 17; rat kidney showing intense necrosis of the tubules and glomerulus, plate 18, magnification (X 250) after sub-chronic administration with distilled water 10ml/kg, 250 mg/kg, 500 mg/kg, and 1000 mg/kg of the methanol leaf extract of *D. saxatilis* respectively.

CHAPTER FIVE

5.0 DISCUSSION

The preliminary phytochemical screening and thin layer chromatography of the methanol leaf extract of *Dalbergia saxatilis* revealed the presence of several constituents as shown in table 4.1. These constituents are known to be responsible for several pharmacological activities. Flavonoids were reported as prostaglandin synthetase inhibitors (Watanebe *et al.*, 2000). Prostaglandins are known to be involved in the pain perception (Helms and Barone, 2008). This suggests that reduced availability of the prostaglandins by flavonoids might have been responsible for their analgesic activity. The presence of tannins and saponins possibly might have given rise to the observed anti-inflammatory property and contributed to the anti-pyretic activity of the plant extract.

Saponins possess a wide range of therapeutic actions in the body including anti-inflammatory, expectorant, diuretic, anti-malarial and haemolytic effects on red blood cells, while tannins are used in compress for cuts and wounds, haemorrhoids, varicose veins and in medicine for diarrhoea, catarrh, heavy menstrual flows and inflammatory conditions of the digestive tract (Evans, 1989). Cardiac glycosides increase the force of myocardial contraction and reduce conductivity within the atrioventricular (AV) node. They are used in the treatment of supraventricular tachycardias, especially for controlling ventricular response in persistent atrial fibrillation (Prassas and Diamandis, 2008).

The oral median lethal dose value for the methanol leaf extract of *D. saxatilis* obtained in both mice and rats was found to be above 5000mg/kg. This suggests that the plant

extract is non-toxic as no death was recorded. Acute toxicity studies are usually carried out to determine the dose that will cause death or serious toxic manifestations when administered singly or severally at few doses in order to establish doses that should be used in subsequent studies (Wanda *et al.*, 2002). The Organization for Economic Cooperation and Development (OECD), Paris, France, recommended chemical labelling and classification of acute systemic toxicity based on oral median lethal dose values as: very toxic if ≤ 5 mg/kg, toxic if > 5 mg/kg but ≤ 50 mg/kg, harmful if > 50 mg/kg but ≤ 500 mg/kg, and non-toxic or not harmful if > 500 mg/kg or ≤ 2000 mg/kg (Walum, 1998). Based on this classification, the oral median lethal dose obtained for both rats and mice found to be above 5000 mg/kg, is relatively safe orally. However, the situation is not the same on repeat dose experiment in sub-chronic toxicity studies.

All rats used for the sub-chronic toxicity studies appeared normal before treatment; there was significant weight reduction during the experiment as shown in table 4.3, especially the first and second week. Reduction in weight was also observed in the fourth week at 250 mg/kg of the methanol leaf extract of *D. saxatilis*. Mortality was recorded from day 18 of the experiment. This data suggests that the extract may have a component or constituent that decreases appetite. This may be as a result of decreased feed consumption observed in all groups compared to control.

The sub-chronic oral administration of the methanol leaf extract of *D. saxatilis* on haematological parameters produced significant decrease in white blood cells count, but had no significant change on other parameters compared to the negative control as shown in table 4.4. White blood cells are a very important component of the blood. They defend the body against offending organisms and injury, and play a very

important role in infections, inflammation and immune responses (Abramson and Melton, 2000). A decrease in white blood cell count also termed leucopenia results from decreased production of total white blood cells in the bone marrow or increased destruction of the cells. Leucopenia indicates bone marrow depression which occurs as a result of viral infection or toxic reactions (Goerge-Gay and Parker, 2003). Disease of the liver or spleen and autoimmune diseases may also cause reduction in white blood cell count. The data presented suggests that the cause of reduction in white blood cells by the extract may be as a result of spleen and liver damage, and also toxic reactions as evidenced in the histopathological findings of the liver and spleen.

The sub-chronic oral administration of the methanol extract of *D. saxatilis* leaves on renal indices produced significant reduction in the concentrations of serum sodium and chloride at 250 mg/kg and 500 mg/kg of the extract, while the significant reduction observed in bicarbonate was at 250 mg/kg of the extract as shown in table 4.5. Electrolytes are molecules that are electrically charged, which help move nutrients into and waste products out of the body's cells. They maintain healthy water balance and help stabilize the body's acid level. Sodium is regulated by the kidneys and adrenal glands. Low sodium levels are caused by kidney disease and adrenal disease, diuretics, and at times conditions that cause fluid to build up in the body. The most common cause of high sodium is dehydration (Halperin and Goldstein, 1994; Briggs *et al.*, 1996). The balance of sodium, chloride, and bicarbonate in the blood is a good indicator of how well the kidneys and heart are functioning. Chloride levels fluctuate with sodium levels. Low chloride levels can occur as a result of chronic lung disease, prolonged vomiting, and metabolic alkalosis. High chloride can be due to kidney disease as well as

dehydration. Change in serum chloride indicates an alteration in fluid status and/or acid-base balance (Koch and Taylor, 1992).

Acid-base balance is partly regulated by renal production and excretion of bicarbonate ions. Carbon dioxide in the form of bicarbonate is excreted and reabsorbed by the kidneys. High or low bicarbonate levels may signify acid/base or electrolyte imbalance often due to dehydration or drinking too much water. The primary regulators of bicarbonate are the proximal tubules (Halperin and Goldstein, 1994). From the data presented, the reduction in serum sodium, chloride and bicarbonate may be as result of tubular damage and necrosis as evidenced in the histopathological findings.

The sub-chronic oral administration of the methanol extract of *D. saxatilis* leaves on liver function indices produced significant increase in alanine transferase at 250mg/kg and 500mg/kg of the extract; while the significant increase observed for aspartate was at 250mg/kg of the extract as shown in table 4.6. Elevated levels of alanine transferase, aspartate, alkaline phosphatase and bilirubin are indicative of abnormalities in liver function. Alanine transferase is a specific marker of hepatocellular injury; and is released due to the use of certain drugs or strenuous exercise. Release of aspartate and alanine transferase is as a result of cellular injury in the liver (Giboney, 2005). An elevated level of alkaline phosphatase is as a result of injury to liver, bone, leukocytes, kidneys, intestine or placenta. Aspartate is present in two isoforms which are not distinguished in blood assays, the cytosolic isoenzyme is present in skeletal muscle, heart muscle, and kidney tissue (Hall and Cash 2012). The data presented suggests that the elevation in alanine transferase and aspartate levels observed at doses of the extract may be as a result of hepatocellular damage as evidenced in the histopathological

findings of the cross section of the liver. Despite the high toxicity exhibited by the plant, it has considerable antipyretic, analgesic and anti-inflammatory properties.

Injection of Brewer's yeast subcutaneously induces pyrexia by increasing the synthesis of prostaglandins. It is a suitable model for the screening of natural or synthetic drugs or compounds for their antipyretic effect (Devi *et al.*, 2003). Yeast-induced pyrexia is called pathogenic fever and its aetiology may possibly be the production of prostaglandins. Therefore, inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action and the inhibition of prostaglandin can be achieved by blocking the cyclooxygenase enzyme activity (Hullati and Sharada, 2007). The reduction in temperature observed from the data presented suggests that the antipyretic effect of the methanol extract of *D. saxatilis* is mild compared to that of the standard. Mechanism by which it causes reduction in rectal temperature may be inhibition of prostaglandins by blockade of cyclooxygenase enzyme activity.

Acetic acid-induced writhing is among the sensitive methods for evaluating potential analgesic drugs or compounds that act peripherally. Writhing is defined as a stretch, tension to one side, extension of hind legs, contraction of the abdomen so that the abdomen of mice touches the floor, or turning of trunk (Mishra *et al.*, 2011). This model of response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathway. The acetic acid induced writhing model has been associated with increased level of prostaglandins (PGE₂ and PGF_{2α}) in peritoneal fluids as well as lipoxygenase products; this enhances inflammatory pain by increasing capillary permeability (Lakshman *et al.*, 2006; Khan *et al.*, 2010). The mechanism, by which any substance that inhibits these writhings causes analgesia, will be preferably by

inhibition of prostaglandin synthesis, which is a peripheral mechanism of pain inhibition (Somachit and Shahid, 2003).

The data presented suggests that the methanol extract of *D. saxatilis* leaves possess peripheral analgesic property. The extract at all doses was shown to have analgesic property as evidenced in the model used. The reduction in the number of writhes caused by the extract, suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and release of prostaglandins and other endogenous substances.

Hot plate method as described by Eddy and Liembach 1953 is the most common thermal nociception model used for evaluating central analgesic efficacy of drugs or compounds. This method is considered to be selective for the centrally acting analgesics; therefore, effect of any drug or compound on this pain model indicates that it could have centrally acting anti-nociceptive activity (Khan *et al.*, 2010). The paws of mice and rats are very sensitive to heat at temperatures which are not damaging to the skin. The responses are usually jumping, withdrawal and licking of the paws (Mishra *et al.*, 2011). In this model, sensory nerves sensitize the nociceptors with minimal involvement of endogenous substances such as prostaglandins (Ezeja *et al.*, 2011).

The data presented suggests that the methanol extract of *D. saxatilis* leaves possess a central analgesic property. The prolongation of the reaction time in all doses of the extract which were comparable to that of morphine, suggests that the extract may have a centrally acting property and mechanism by which it produce analgesia may be via central opioid receptors. Morphine alleviates pain by acting on the pain receptors in the brain or by activating the opioid receptors in the central nervous system (Yousuf *et al.*, 2013).

Carrageenan induced rat paw oedema is a well established animal model for evaluating the anti-oedematous effect of drugs or compounds (Sharma *et al.*, 2010). Formation of oedema caused by carrageenan is said to be in two phases; the first hour after carrageenan injection (first or early phase), involves the release of serotonin, histamine and bradykinin while the second or late phase (2 –5 hours) with increased oedema formation that remains up to the fifth hour involves the release of prostaglandins (Khan *et al.*, 2009). The second phase of swelling not only involves the elevated production of prostaglandins, but has been recently attributed to the induction of inducible cyclooxygenase (COX-2) in the hind paw (Nantel *et al.*, 1999).

From the data presented, oedema was inhibited by both the standard (aspirin) and 250mg/kg group of the methanol leaf extract of *D. saxatilis* at the second hour. There was intense inhibition in the second, third, and fifth hour, this indicates that the extract may not have activity on the early phase of inflammation (1-1.5 hours), thus it may act by inhibiting the release of prostaglandins. Aspirin acts by inhibiting the activity of the enzyme cyclooxygenase (COX) which leads to the formation of prostaglandins (PGs) that cause inflammation, swelling, pain and fever (Vane and Botting, 2003).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The methanol leaf extract of *Dalbergia saxatilis* was found to possess several bioactive constituents including flavonoids, saponins, tannins, cardiac glycosides among others, associated with potent pharmacological activities. The extract was found to possess considerable analgesic and anti-inflammatory, with mild antipyretic properties at doses tested. This partly justifies the claim for the traditional use of the plant in the treatment of toothache.

The oral administration of the extract produced significant effect on white blood cells count, liver and renal indices. This suggests that the extract has adverse effects on these indices. Also, the histopathological findings on the organs revealed normal heart, ulcerative stomach at the highest dose of the extract, moderate spleen damage, and vascular congestion with slight lymphocyte hyperplasia of the liver and massive necrosis of the tubules and glomerulus at all doses of the extract. Thus, it can be suggested that, the extract should only be used with caution in liver disease as the liver is capable of regenerating, but contraindicated in kidney disease due to the severe necrosis observed. For the ulcerative stomach, higher doses of the extract should be avoided for long periods. These findings provide evidence for the relative acute safety of the extract, but toxic on prolonged use.

6.2 Recommendations

- I. A short term (14 days) toxicity studies should be carried on the methanol leaf extract of *D. saxatilis* to see its effects on the heart, spleen, stomach, liver and kidney.
- II. Sub-chronic toxicity studies for 28 days should be carried out using lower doses of the extract.
- III. Further studies should be carried out on the plant using other solvents (non-polar) such as petroleum ether and hexane.
- IV. The bioactive constituents responsible for the pharmacological activities should be isolated and characterised.
- V. Studies should be carried out to ascertain the exact analgesic, anti-inflammatory and antipyretic mechanism of action of the plant.

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APPENDIX A

Table : Effect of the methanol extract of *Dalbergia saxatilis* on Hot plate induced pain in mice.

Treatment	Mean reaction time in seconds $\pm$ SEM				
(mg/kg)	0 min	30 mins	60 mins	90 mins	120 mins
Distilled water 10	3.09 $\pm$ 0.40	2.26 $\pm$ 0.28	2.44 $\pm$ 0.24	2.14 $\pm$ 0.17	2.95 $\pm$ 0.30
Morphine 10	2.54 $\pm$ 0.18	2.99 $\pm$ 0.33	3.59 $\pm$ 0.22 ^a	4.16 $\pm$ 0.18 ^b	4.63 $\pm$ 0.24 ^b
Extract 250	3.44 $\pm$ 0.21	3.89 $\pm$ 0.29	5.36 $\pm$ 0.52 ^b	4.19 $\pm$ 0.69 ^a	4.64 $\pm$ 0.41 ^b
Extract 500	2.46 $\pm$ 0.24	3.54 $\pm$ 0.30 ^a	4.64 $\pm$ 0.75 ^b	4.87 $\pm$ 0.80 ^b	4.88 $\pm$ 0.26 ^b
Extract 1000	2.61 $\pm$ 0.11	3.49 $\pm$ 0.25	5.30 $\pm$ 0.27 ^b	5.14 $\pm$ 0.43 ^b	4.69 $\pm$ 0.28 ^a

Data were calculated using repeated measures ANOVA followed by Bonferoni test. ‘a and b’ in superscript represent $p < 0.05$ and $p < 0.01$, level of significance respectively.

Values are $\pm$ SEM, n= 6.

APPENDIX B

Table : Effect of methanol leaf extract of *Dalbergia saxatilis* on Brewer's yeast induced pyrexia in rats

Treatment (mg/kg)	Rectal temperatures ($^{\circ}$ C)					
	0hr	19hrs	20hrs	21hrs	22hrs	23hrs
Distilled water	37.08 $\pm$ 0.73	38.27 $\pm$ 0.21	37.90 $\pm$ 0.37	37.07 $\pm$ 0.63	37.52 $\pm$ 0.29	37.45 $\pm$ 0.17
Aspirin	36.43 $\pm$ 0.13	37.40 $\pm$ 0.29	36.40 $\pm$ 0.23	35.22 $\pm$ 0.72 ^a	35.65 $\pm$ 0.40	35.48 $\pm$ 0.51 ^a
Extract 250	36.93 $\pm$ 0.24	38.03 $\pm$ 0.15	37.82 $\pm$ 0.45	37.43 $\pm$ 0.25	36.58 $\pm$ 0.53	36.42 $\pm$ 0.48
Extract 500	36.97 $\pm$ 0.24	37.90 $\pm$ 0.18	37.30 $\pm$ 0.43	36.18 $\pm$ 0.56	35.92 $\pm$ 0.64	36.08 $\pm$ 0.49 ^a
Extract 1000	36.42 $\pm$ 0.22	37.85 $\pm$ 0.28	36.92 $\pm$ 0.43	37.25 $\pm$ 0.45	36.82 $\pm$ 0.36	36.57 $\pm$ 0.42 ^a

Data were analysed using repeated measures ANOVA followed by Bonferoni test. 'a' in superscript represent decrease in rectal temperature at $p < 0.05$ level of significance.

Values are $\pm$ SEM, n= 6.

APPENDIX C

Table : Effect of methanol leaf extract of *Dalbergia saxatilis* on Carrageenan induced rat paw oedema.

Treatment (mg/kg)	Mean paw diameter (cm)					
	0hr	1hr	2hrs	3hrs	4hrs	5hrs
Distilled water	0.25± 0.01	0.32±0.02	0.42±0.02 ^a	0.35± 0.02	0.29±0.01*	0.31±0.01*
Aspirin 10	0.25± 0.01	0.29±0.01	0.31±0.02	0.28±0.01	0.28±0.01	0.28±0.01
Aspirin 300	0.25± 0.01	0.29±0.01	0.31±0.02	0.28±0.01	0.28±0.01	0.28±0.01
Extract 250	0.25±0.01	0.30±0.01	0.32±0.01	0.33±0.01 ^a	0.28±0.00	0.29±0.00
Extract 500	0.24± 0.01	0.35±0.01 ^a	0.35±0.02	0.31±0.01	0.30±0.01**	0.29±0.01**
Extract 1000	0.23±0.01	0.29±0.01 ^b	0.34±0.01 ^a	0.29±0.00 ^c	0.30±0.01 ^a	0.28±0.01 ^a

Data were analysed using repeated measures ANOVA followed by Bonferroni test. ‘a and b’ in superscript represent statistical significant increase in paw diameter, while * and ** represent statistical decrease in paw size at $p < 0.05$, and $p < 0.01$ respectively.

Values are ± SEM, n= 6.

APPENDIX D

Extraction of the plant sample

Weight of evaporating dish = 575.40g

Weight of dried extract + evaporating dish = 621g

Weight of extract = 45.6g

Percentage yield = $\frac{\text{weight of dried extract}}{\text{Powdered plant}} \times 100$

$$\begin{aligned} &= \frac{45.6\text{g} \times 100}{200\text{g}} \\ &= 22.8\% \end{aligned}$$

The percentage yield of the plant was found to be 22.8%.