ANALGESIC, ANTI-INFLAMMATORY AND TOXICITYSTUDIES ON THE METHANOL ROOTEXTRACT OF *ERIOSEMA PSORALOIDES* (LAM.) G.DON IN LABORATORY ANIMALS

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SEPTEMBER, 2021

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY ZARIA,
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DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS,
FACULTY OF PHARMACEUTICAL SCIENCES,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

SEPTEMBER, 2021

DECLARATION

I declare that the work in this Dissertation entitled "Analgesic, Anti-Inflammatory and Toxicity Studies onthe Methanol RootExtract of *Eriosema psoraloides* (lam.) G.don in Laboratory Animals" has been performed by me in the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

Michael Oromidayo BAMIKUNLE _____ _____

Signature **Date**

CERTIFICATION

This dissertation entitled "ANALGESIC, ANTI-INFLAMMATORY AND TOXICITY STUDIES ON THE METHANOL ROOT EXTRACT OF *ERIOSEMA PSORALOIDES* (LAM.) G.DON IN LABORATORY ANIMALS'' by Michael Oromidayo BAMIKUNLE, meets the regulations governing the award of the degree of Masters of Science in Pharmacology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Prof. A.U. Zezi	_____	_____
Chairman, Supervisory Committee	Signature	Date
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Member, Supervisory Committee	Signature	Date
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Prof. S. A. Abdullahi	_____	_____
Dean, School of Postgraduate Studies	Signature	Date

DEDICATION

This dissertation is dedicated to Mrs Funmilayo Bamikunle, Dr. Dideoluwa Bamikunle and Late Prof. Aderemi Bamikunle.

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ABSTRACT

The clinical management of pain stillremains a serious challenge due to adverse drug reactions associated with the commonly used analgesic and anti-inflammatory drugs.There is an urgent need to continue the search for alternative means to manage pain, especially from medicinal plants because they are a large reservoir of phytochemicals. Various parts of the *Eriosema psoraloides*planthave been used traditionally over time by indigenous people of Nigeria and Africa in the treatment of pain, rheumatism and infertility to mention a few. This study was aimed at establishing the toxicity profile, anti-nociceptive and anti-inflammatory potentials of the methanol root extract of *E. psoraloides*(EPE); and elucidating possible mechanisms of these actions.Preliminary phytochemical screening was done to determine the phytochemicals and secondary metabolites that might be present in the extract. An acute toxicity study was also carried out to ascertain the toxicity potential of the extract. Sub-acute toxicity studies were also conducted in rats to ascertain the toxicological profile of the methanol root extract.The extract was evaluated for analgesic activities using acetic acid-induced writhing, and hot plate tests in mice, while it was evaluated for anti-inflammatory activities using the carrageenan-induced hind paw oedema test. The roles of cyclooxygenase (COX) pathways in the analgesic and anti-inflammatory activities of the methanol root extract at graded doses (250, 500and 1,000mg/kg*p.o*) were also investigated. Results of the preliminary phytochemical screening of the methanol root extract revealed the presence of carbohydrates, glycosides (cardiac glycosides), triterpenes, saponins, tannins, flavonoids, steroids and alkaloids. The oral LD_{50} of the methanol root extract was estimated to be greater than 5,000 mg/kg in both rats and mice.In the sub-acute toxicity study, a statistically significant (p <0.05) increase in weight of the rats was observed at the 1,000 mg/kg dose compared to the control group.

Increase in the body weights of the rats was observed across all the treatment groups. A statistically significant(p <0.05) decrease in Alanine transaminase (ALT) was observed at the 250 mg/kg dose as compared to the normal saline group (1 ml/kg). Aspartate transaminase (AST) also decreased significantly (p <0.05) when compared to control after animals were treated for 28 days. Similarly, total protein and albumin were also decreased significantly (p< 0.01). A statistically significant (p <0.05) decrease in serum urea levels was also observedin all extract treated groups compared to the negative control. Serum sodium levels were also decreased significantly (p <0.05) in the group treated with EPE at the 1,000mg/kg dose. The extract also affected the liver and kidneys of the treated animals adversely from histological examinations. A significant(p< 0.05) dose dependent reduction in acetic acid-induced writhes was demonstrated by the administration of methanol root extract of *Eriosema psoraloides* at 250, 500 and 1,000 mg/kg. All doses inhibited writhing which was comparable to piroxicam (20 mg/kg).Oral administration of the extract increased the mean reaction time of the mice to thermal stimulus, though these increases were not statisticallysignificant.The methanol root extract also significantly (p < 0.05) inhibited carrageenan-induced hind paw oedema in the fourth hour of the test at the 250mg/kg extract dose as compared to normal saline (1 ml/kg). Other doses (500 and 1,000 mg/kg) inhibited carrageenan-induced hind paw oedema the third and fourth hour, but not significantly. The extract also demonstrated an ability to down regulate serum concentration of both COX 1 and COX 2, though not to a statisticallysignificant level. From the findings of this study, it was evident that the methanol root extract of *Eriosema psoraloides* possesses analgesic and anti-inflammatory activity and is moderately toxic to laboratory animals at sub-acute doses.

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ABBREVIATIONS, DEFINITIONS AND SYMBOLS

ABBREVIATION	DEFINITION
5-HT	Serotonin
ABU	Ahmadu Bello University
ABUCAUC	Ahmadu Bello University Committee on Animal Use and Care
AD	Alzheimer's Disease
AIDS	Acquired Immunodeficiency Syndrome
ALB	Albumin
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
ANOVA	Analysis of Variance
AST	Aspartate Transaminase or Aspartate Aminotransferase
ATP	Adenosine triphosphate
BDH	American Company
BUN	Blood Urea Nitrogen
CBD	Cannabidiol
CBL	Cannabinol
CD	Crohn's Disease
CDC	Centre for Disease Control
CKD	Chronic Kidney Disease
CNS	Central Nervous System
COPD	Chronic Obstructive Pulmonary Disease
COX	Cyclooxygenase
DA	Dalton
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediamine tetra acetic acid
ELISA	Enzyme Linked Immunosorbent Assay
EPE	Methanol Root Extract of *Eriosema psoraloides*
GIT	Gastrointestinal Tract
GMP	Guanosine Monophosphate
H	Hepatocyte
Hb	Haemoglobin
HBV	Hepatitis B Virus

HCV	Hepatitis C Virus
HN	Hepatic Necrosis
IASP	International Association for the Study of Pain
IBD	Inflammatory Bowel Disease
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
LD_{50}	Lethal Median Dose
LOT	Batch Number
LOX	Lipoxygenase
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
NA	Noradrenaline
NMDA	N-methyl-D-aspartate Receptor
NO	Nitrous Oxide
NOS	Nitrous Oxide Synthase
NSAID	Non-Steroidal Anti-inflammatory Drug
OECD	Organisation for Economic Co-operation and Development
OTC	Over the Counter
PAF	Platelet Activating Factor
PCV	Packed Cell Volume
PD	Parkinson's Disease
PG	Prostaglandin
PYK	Hyperplasia of Pyknotic Cells
QT	QT Interval
QTc	Corrected QT Interval
ROW	Relative Organ Weight
SC	Sinusoidal Congestion
SEM	Standard Error of Mean
SIRS	Systemic Inflammatory Response Syndrome
TA	Tubular Adhesion
TCA	Tricyclic Antidepressant
TENS	Transcutaneous Electrical Nerve Stimulation

TFPI	Tissue Factor Pathway Inhibitor
THC	Tetrahydrocannabinol
TN	Tubular Necrosis
TNF	Tumour Necrosis Factor
TP	Total Protein
UC	Ulcerative Colitis
UK	United Kingdom
USA	United States of America
VC	Vacuolation
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Pain is defined as an unpleasant sensory and emotional experience associated with or resembling that associated with actual orpotential tissue damage or described in terms of such damage"(IASP, 2020).The experience of pain makes the organismaware of a potential danger or injurious material in close proximity, with the option of fleeing or removing the injurious stimuli. When the ability to sense pain is lost, it results in self-mutilation, corneal scarring, bone fractures, multiple scars, joint deformities, amputations, and early death.This underscores the significance of theprotective role of nociceptive pain (Schon *et al*., 2018).

Pain can severely diminish an individual'squality of life(Hadi*et al.*, 2019). It is one of the chief symptoms of most diseases reported by patients.Pain is a sensual and perceptual phenomenon,which causes suffering and emotional state of risks connected with anxiety.The word "pain" for the patient is associated with disease and suffering, for the physician it is a symptom, and for the physiologist it is a kind of feeling that has its own anatomical and physiological system which begins with the receptors and ends up in the brain cortex (Świeboda *et al*., 2013).Pain perception varies by individual and also varies at different times for the same individual. The perception of pain depends on factors such as arousal, attention, distraction and expectation (Chayadi and McConnell, 2019).

Pain can be categorized based on;the part of the body involved, the system whose dysfunction may be causing the pain, duration and pattern of occurrence, intensity and time since onset and the cause(IASP, 2020).

Inflammation is an immune system reaction to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation. It eradicates the injurious stimuli and initiates the healing process(Chen *et al*., 2017).

During the acuteinflammatory responses, cellular and molecular events and interactions efficiently minimize impending injury or infection. This mitigation process contributes to restoration of tissue homeostasis and resolution of acute inflammation.Acute inflammation has therefore been considered as a component of innate immunity, the first line of host defence againstforeign invaders and danger molecules(Hannoodee and Nasuruddin, 2020). However,unmitigated acute inflammation may become chronic, contributing to a variety of chronic inflammatory diseases(Zhou *et al*., 2016).

At the tissue level, inflammation is characterized by redness, swelling, heat, pain, and loss of tissue function, which result from local immune, vascular and inflammatory cell responses to infection or injury. Important microcirculatory events that occur during the inflammatory process include vascular permeability changes, leukocyte recruitment and accumulation and inflammatory mediator release(Chen *et al*., 2018). In some pathologies, the inflammatory process which is normally self-limiting, becomes perpetual and this steers towards the development of some chronic inflammatory diseases. Acute inflammation is characterized by the infiltration of immune cells like neutrophils and macrophages, while chronic inflammation is characterized by the infiltration of T lymphocytes and plasma cells. The final result of chronic inflammation is loss of tissue function and fibrosis (Gieseck *et al*., 2018).

Pain and inflammation, even with their boundless beneficial effects, still cause a lot of suffering globally. Inflammation and pain may be linked by cyclooxygenase (also known as COX) enzymes most especially COX 2, which helps in the synthesis of prostaglandins (PGs)precisely PGE_2 and PGF_{2a}, implicated in high concentrationat the

inflammatory site(Chen *et al.*, 2018). The released PGs can either stimulate pain receptors or sensitize pain receptors to other pain producing substances. Use of conventional medicines for the management of pain and inflammation have been linked with a plethora of undesired side effects(Bharti, 2019).

Since prehistoric times, herbal preparations have been used for the treatment of a wide range of diseases, this is because they are natural products, which contain bioactive molecules and are important for the development of new drugs.The development of new drugs relying purely on modern technology appears to be reaching something of a limit. Over the decades, increasing attention has accordingly been paid to natural products in the search for novel drugs in combination with new technology, such as high-throughput selection. Natural products will undergo continual use toward meeting the urgent need to develop effective drugs, and they will play a leading role in the discovery of drugs for treating human diseases(Yuan*et al*., 2016).

1.2 Statement of Research Problem

Pain is a major indicator of infirmity. It has served an evolutionary role, helping animals to be able to detect and avoid noxious stimuli.Furthermore, potentially painful events result in behavioural and physiological changes such as reduced activity, guarding behaviour, suspension of usual behaviour, increased ventilation rate and abnormal behaviours which are all counteracted by the use of pain-relieving drugs(Sneddon, 2019).

Inflammation, despite the great protective effect it offers, poses a great threat to bodily tissues if left uncontrolled. When there is no resolution of inflammation, acute inflammation evolves into chronic inflammation and other inflammatory diseases (Chen *et al*., 2018).

Conventional analgesics are widely used globally for the treatment of pain, inflammation and even the prevention of cardiovascular disease.However, Non-steroidal anti-inflammatory drugs (NSAIDS) have been associated with gastrointestinal bleeding(McEvoy *et al*., 2021),even NSAIDS that are COX-2 selective have now been shown to have some cardiorenal side effects(Kirkby *et al*., 2018). Paracetamol, one of the most common analgesics in use today, causes liver damage with prolonged use at high doses(Ramachandranand and Jaeschke, 2017). From 1999 to 2008, more overdose deaths involved opioid analgesics than heroin and cocaine combined, an indication of the opioid epidermic (Mattson *et al*., 2021).

1.3 Justification of the Study

Analgesics are one of the most widely used medicines in the world and the primary drugs used to ameliorate pain. They mainly include non-opioids (paracetamol, non-steroidal anti-inflammatory drugs), and opioids (tramadol, codeine, morphine, oxycodone, meperidine, fentanyl etc.).

Despite extensive research, the majority of analgesics available to prescribers and patients are based on mechanistic classes of compounds that have been known for many years. This alludes to the fact that newer, safer and more efficacious analgesic molecules have not been discovered(Burgess and Williams, 2010). Even though there is wide availability of generic and over the counter (OTC) analgesics based on NSAIDs, paracetamol, and “weak” opiates that provide many individuals with an accessible source of relief from mild to moderate pain, many patients with chronic conditions such as osteoarthritis remain poorly treated(Hajat and Stein, 2018).

Even with the vast amounts of agents available, the management of pain still poses a lot of problems mainly due to preventable adverse reactions still being detected

clinically.Gastrointestinal complications, renal toxicity and hypersensitivity reactions have been associated with NSAIDs.Opioids, on the other hand, have been associated with dependence, tolerance, constipation, sedation, respiratory depression and a whole lot of other adverse effects(Machelska and Celik, 2018). The immunosuppressive effects of steroidal anti-inflammatory drugs have also been well documented(Williams, 2018).

These problems encountered with conventional analgesics and anti-inflammatory agents have prompted researchers to seek alternative agents that can be employed in the management of pain.These alternative medicines have to be validated for safety and efficacy.

Some plants have been shown to possess significant analgesic activities. According to Danjuma *et al*.,(2007), the stem bark of *Ziziphus mucronata*, a plant found locally in many parts of northern Nigeria, possesses significant analgesic activity. Similarly, Sani *et al*.,(2010)worked on the leaves of *Adenodolichos paniculatus* and came to a similarconclusion.

Herbal preparations contain compounds that occur naturally and can be said to be more "friendly" to the human body, producing less adverse reactions when used in the treatment of symptoms and diseases(Bhuiyan *et al*., 2020). A lot of herbs have shown promising potent analgesic and anti-inflammatory activities, one of such herbs is *Eriosema psoraloides*. Little research has been carried outto validate folkloric claims made on the herb. Hence, the safety and efficacy of this herb needs to be validated and standardized.

1.4 Aim and Objectives

1.4.1 Aim

The aim of the study was to investigate the analgesic, anti-inflammatory activity and toxicityprofile of the methanol root extract of *Eriosema psoraloides* in mice and rats.

1.4.2 Specific objectives

The specific objectives of the study were:

i. To determine the phytochemical constituents present in the methanol root extract of *E. psoraloides.*
ii. To determine the acute toxicity profile (LD_{50}) of the methanol root extract of *E. psoraloides.*
iii. To determine the subacute toxicity profileof the methanol root extract of *E. psoraloides.*
iv. To evaluate the analgesicactivity of the methanol root extract of *E. psoraloides.*
v. To evaluate the anti-inflammatory activity of the methanol root extract of *E. psoraloides.*
vi. To evaluate the possible mechanism of analgesic and anti-inflammatory activity of the methanol root extract of *E. psoraloides*.

1.5 Research Hypothesis

The methanol root extract of *Eriosema psoraloides* possessesanalgesic, anti-inflammatory activities and has no toxic effect on vital organs and systems of the body.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Pain

Due tothenature of its qualities, a consensus on the definition of pain has not been realistically obtainable. In Greek, the word “Pain” implies penalty. The Great Greek philosopher, Plato believed that pain arises from within the body and indicated that pain is more of an emotional experience(Hanoch and Elavarasi, 2016).

In recent times, the idea of pain has evolved from one-dimensional to a multi-dimensional entity touching sensory, cognitive, motivational and affective qualities(Talbot *et al*., 2019). Pain is always subjective and every individual uses this word based on their previous experience related to the injury. Over time, various definitions have been given to describe and understand pain in medical literature(Kumar and Elavarasi, 2016).Pain can be defined in a number of ways. Pain is an “an unpleasant sensory and emotional experience associated with or resembling that associated with actual or potential tissue damage or described in terms of such damage”(IASP, 2020).It can also be definedas an unpleasant sensory and emotional feelingaccompanying existing or impending tissue damage orreferenced to such damage(Świeboda *et al*., 2013).

Pain perception varies by individual and also varies at different times for the same individual(Fillingim, 2017). The perception of pain depends on such factors as arousal, attention, distraction and expectation(Chayadi and McConnell, 2019).

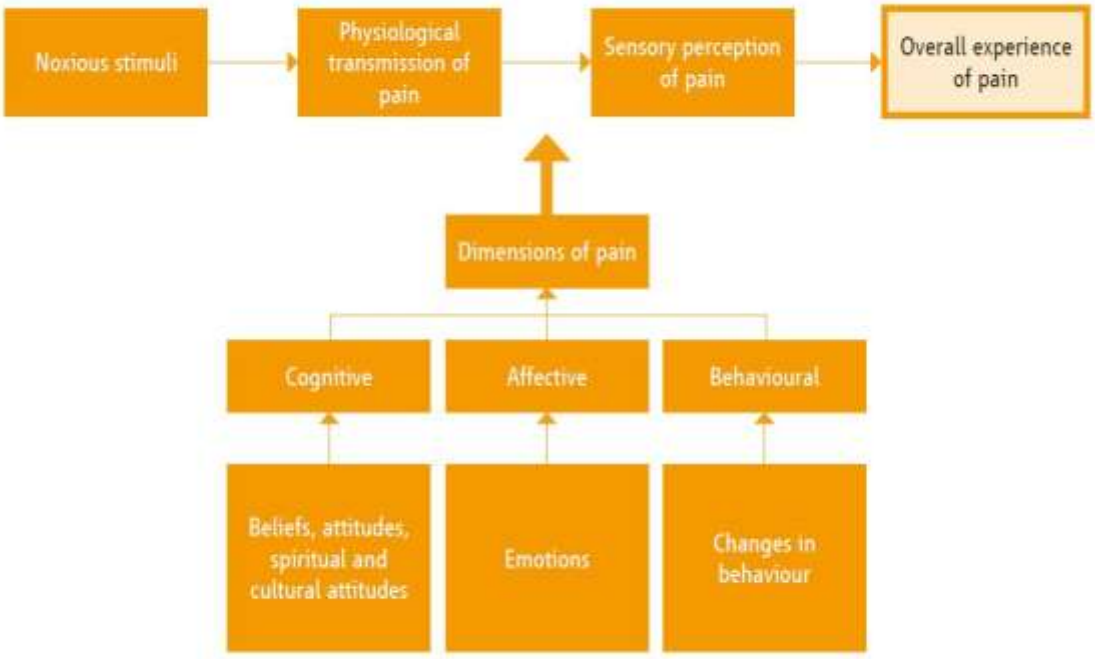


Figure 2.1: The Dimensions of Pain (WHO, 2012)

Table 2.1: Characteristics of Receptor and Non-receptor Pain (WHO, 2012)

Receptor pain	**Non-receptor pain**
From irritating the receptors of integuments, muscle, joints and internal organs	Nerve or central nervous system damage
No changes in nervous system	Damaged nervous system
Skin, osteo-articular, muscular, and organ pain	Neuropathic pain – from the nerves, central pain – from the spinal cord

Paincan also be classified based on; the region of the body involved, the system whose dysfunction may be causing the pain, duration and pattern of occurrence, intensity and time since onset and the cause(IASP, 2020).

2.1.1 Classification of pain

Pain, being a multifaceted entity can be classified along many lines. Basically, it can be classified based on pathophysiology, duration, aetiology and anatomical location(WHO, 2012).

2.1.1.1 Classification based on pathophysiology

There are two major classes of pain based on pathophysiology; nociceptive and neuropathic.

Nociceptive pain:This type of pain is caused when tissue injury activates specific pain receptors called nociceptors. Nociceptors are sensitive to injurious stimuli. They can react to heat, cold, vibration, stretch stimuli and chemical substances released from tissues in response to oxygen deprivation, tissue disruption or inflammation. Nociceptive pain can further be subdivided into *somatic* and *visceral* pain depending on the location of activated nociceptors.

1. Somatic pain is caused by the activation of nociceptors in either surface tissues (skin, mucosa ofmouth, nose, urethra, anus, etc.) or deep tissues such as bone, joint, muscle or connective tissue.For example, cuts and sprains causing tissue disruption produce surface somatic pain while musclecramps due to poor oxygen supply produce deep somatic pain.
2. Visceral pain is caused by the activation of nociceptors located in the viscera. Viscera constitutes the internal organs in the main cavities of the body.Visceral pain can occur due to infection, distension from fluid or gas, stretching orcompression, usually from solid tumours. Visceral pain can also be called referred pain and correlates with internal organ tissues and can be felt indirectly.

Neuropathic pain:Neuropathic pain arises from abnormal neural activitycaused by disease, injury or dysfunction of the nervous system(IASP, 2020).Neuropathicpain results from a lesion that affects the central or peripheral nervous system(Spring, 2016).It can be further divided into three subcategories: sympathetically mediated, peripheralor central(IASP, 2020).Symptoms of neuropathic pain include altered sensations, includingnumbness, tingling, burning and shooting pains(Spring, 2016).Common diseasesassociated with neuropathic pain include diabetic neuropathy, human immunodeficiencyvirus and phantom limb pain(WHO, 2012).Some sensory dysfunctions related to neuropathic pain include; hypoesthesia, hyperesthesia, dysesthesia, paraesthesia, hypoalgesia, hyperalgesia and Allodynia.

Mixed pain occurs when neuropathic pain coexists with nociceptive pain. In certain diseases, mixed pain can consist of somatic, visceral and neuropathic pain all at the same period, or each separately at different times. Examples include trauma that damages tissue and nerves, burns (affecting skin and nerve endings) and cancer that causes external nerve compression and nerve damages by infiltration(Freynhagen *et al*., 2019).

2.1.1.2 Classification based on pain duration

Under this classification, pain is either acute or chronic in nature. A conventional definition of acute pain is a pain that persists for less than 30 days, while chronic pain is any pain that last longer than 3 months. Symptoms and causes of the two types of pain may intersect and pathophysiological factors can be independent of duration, thus making this classification problematic and unclear.

Acute pain:is of immediate onset and is perceived immediately following injury. It is severe in intensity but is normally short-lasting in duration. It arises as a result of acute tissue injury stimulating nociceptors and generally disappears when the injury heals.

Chronic pain:is a perpetual or recurrent pain that persists beyond the expected normal time ofhealing(Cohen, 2021). Chronic pain may begin as acute pain and persist for long periods or may recur due topersistence of noxious stimuli or repeated exacerbation of an injury. It may also occur andpersist in the absence of identifiable pathophysiology or medical illness. Chronic pain can adversely affect all aspects of daily life, including physical activities, school attendance, sleep patterns, familyinteractions and social relationships. It can also lead to distress, anxiety, depression, insomnia, fatigueor mood changes, such as irritability and negative coping behaviour(Cohen, 2021).

Episodic or recurrent pain: occurs intermittently over a long period of time. Painful episodes can often fluctuate in intensity, quality andfrequency over time and are consequently unpredictable. Examples of this type of pain include migraine, episodic sickle cell diseasepain, recurrent abdominal pain. Persisting and recurrent pain can coexist, especially in conditions suchas in sickle cell disease.

Incident pain or pain due to movement:has a definite cause. The pain can be induced bysimple movements, such as walking, or by physical movements that exacerbate pain, such as weightbearing, coughing or urination(Berninger and Smith, 2021).

End of dose pain:occurs when the blood level of the medicine falls below the minimumeffectiveanalgesic level towards the end of dosing interval (Zimmermann and Richarz, 2014).

2.1.1.3 Classification based on the aetiology of pain

This classification is commonly based on the underlying disease being malignant or non-malignant. This classification has little relevance to treatment and mechanism of pain.

2.1.1.4 Classification based on the anatomy of pain

Pain can be categorised based on the anatomical location (example head, back or neck) or anatomical function of the affected tissue (example; myofascial, rheumatic, skeletal, neurological and vascular).This classification can be useful for diagnosis but not for the clinical management of pain(Breivik, *et al.*, 2013).

2.1.2 Pathways of pain

Pain is caused, primarily, by the irritation of nociceptors, which are free nerve endings that respond to painful stimuli. These nociceptors are found in the skin, eyes, organs of motion etc. There is a plethora of them in the meninges, organ walls and peritoneum. When they are stimulated by biological, electrical, thermal, mechanical and/or chemical stimuli, they transmit such information to the brain. Pain perception occurs when stimulitransmittedvia the spinal cord gets to the central areas of the brain. Impulses run to the dorsal horn of the spine, where they synapse with dorsal horn neurons in the substantia gelatinosa, and then enter the brain. The basic sensation of pain occurs at the thalamus. It perpetuates to the limbic system which is the emotional centre and the cerebral cortex, where pain is perceived and interpreted.Nociceptors are simple structures because they are at the end of the nerve fibres(Helms and Barone, 2008). There are two types of fibres: Aδ and C, involved in pain transmission. The larger Aδ fibres, produce sharp, well-defined pain, which is typically stimulated by a cut, an electrical shock, or a physical blow. They are myelinated and can allow an action potential to travel at a rate of about 20 meters/second towards the central nervous

system. Transmission through Aδ fibres is so fast that the body responds faster than the pain stimulus. This causes retraction of the affected body part before the person perceives the pain. This allows a quick response: “escape” or preparation for “fight”. These fibres have practically no opioid receptors, while pain receptors located at the ends are always on standby. There are limited possibilities for pharmacological modification of these receptors. In practice, it is easy to inhibit chronic, “slow” pain, using analgesic drugs and difficult to block “sharp”, “fast” pain. After the so-called first pain, the smaller C fibres transmit dull burning or aching sensations, known as a “second pain.”(Helms and Barone, 2008).

C fibres are very thin and susceptible to damage. They do not get stimulated by a cut, an electrical shock, or a physical blow. At the ends of these nerve fibres there are different receptors, the most important of which are the opioid receptors. The proteins thatform part of these receptors are synthesized in ganglioncells and transported inside the axons, both into the synapsein the corners of the spinal cord and towards nerveendingsin peripheral tissues. Inactive forms of receptors or “sleepingreceptors” areincorporated in the cell membrane of nerveendings(Stein, 1995). They may be “awakened” by inflammation.Various cytokines produced by inflammatory cells are ableto penetrate the damaged perineurium and activate thereceptors. In this way, the opioid receptors are activatedand after sensitization are able to react to endogenous andexogenous opioids. C-fibre nerve endings are also “sensitized”by prostaglandin and other mediators. Inhibition ofprostaglandin synthesis by a non-steroidal anti-inflammatorydrug, and inhibition of inflammation by corticosteroidsreduces the fibres nerve sensitivity and increase the painthreshold. This basic defence mechanism is based on thecooperation between the immune and nervous systems. Asa result, pain comes in two phases. The first phase is mediatedby the fast-conducting Aδ fibres and the second part due toC

fibres. Physiological pain has significant importance as awarning sign that ensures human safety(Helms and Barone, 2008).

2.1.3 Regulators of pain

Prostaglandins induce inflammation and other inflammatory mediators. In the incidenceof tissue damage, chemical substances that modulate the transmission of pain are liberated into the extracellular tissues. Chemical mediators responsible for pain activation include histamine, substance P, bradykinin, acetylcholine, leukotrienes and prostaglandins.At the location of tissue destruction, mediators can generate other reactions, such as constriction, vasodilatation, or altered capillary permeability (Abdulkhaleq *et al*., 2018).

Fibres in the dorsal horn, brain stem, and peripheral tissues emancipate neuromodulators and endogenous opioids, that inhibit the action of neurons transmitting the pain impulses. Endorphins are natural opioid-like substances responsible for pain relief. Endorphin levels differ between individuals; resulting in different perceptions of pain(Chaudhry and Gossman, 2021).

2.1.4 Clinical characteristics of pain

Clinical features of pain include; location, quality, duration and intensity.These qualities are assessed mainly subjectively.

The location of pain allows the prediction of a probable cause of the pain. The site of a pain does not always correspond tothe site of injury or disease process. Deep organ pains areparticularly hard to locate. This is clinically important asit can act as a stumbling block in the location of the disease. Pain often occurs as a phenomenon called reflected pain (projected or referred). This occurs due to fact that the internal organsdo not have pain receptors(Świeboda *et al*., 2013).

The intensityof painexperienced by the patient is subjective and is the mostdifficult feature of pain to assess. The advocate of the intensity ofpain is its tolerance.To evaluate intensity, visual or analogue scales are used tocompare pain with the strongest pain which the patient has ever endured. In practice, most popular scale divides pain intovery strong, strong, moderate, weak and no pain(Świeboda *et al*., 2013).

The duration of pain is a quantifiable quality thatallows differentiation between acute and chronic pain. Pain canbe continuous and paroxysmal, as in headaches or neuralgia.Considering the durations of symptoms, pain can bedivided into the following groups:

*Acute pain***:**Has aduration of less than 3 months.

*Chronic pain***:** Has duration of more than 3 months.

2.1.5 Management of pain

In order to relieve pain, there are pharmacological and non-pharmacological methods utilised. For the pharmacological therapy, analgesics and some other adjuvant drugs are used. An analgesic drug is any drug thatrelieves pains selectively without blocking the conduction of nerve impulses, markedly altering sensory perceptions or affecting consciousness(Bloom, 2017). This selectivity gives an important differentiation between an anaesthetic and an analgesic drug(Bharti, 2019).

Analgesics have different ways they elicit their therapeutic effects. Some act by inhibiting the activity of cyclooxygenase (COX), while others bind to specific receptors in the central nervous system (CNS) to elicit their action.Some examples of analgesics include: paracetamol, NSAIDs such as the salicylates and opioid drugs such as morphine and oxycodone. While NSAIDs inhibit the COX enzyme to elicit their effect, opioids interact with the neurotransmitters and modulators of the pain system to alleviate and control pain(Osafo*et al*., 2017; Oldham, 2020).

2.1.5.1 Guidelines for the management of pain

Many decades ago, inthe year 1986, the World Health Organization (WHO) developedand introduced guidelines for pain management, called theWHO scheme or three-stage analgesic ladder. It can be summarised as follows;

1. A non-opioid plus optional adjuvant analgesic for mild pain.
2. Weak opioid plus non-opioid and adjuvant analgesics for mild to moderate pain
3. Strong opioid plus non-opioid and adjuvant analgesics for moderate to severe pain.

There is little surprise that the WHO analgesic ladder has becomethe universal standard for painmanagement. More comprehensively,if pain occurs,there should be quick oral administration of drugs in thefollowing sequence: non-opioids (e.g.,aspirin); then,as necessary, mild opioids (codeine); followed strong opioids,such as morphine, until the patient is free of pain. To calmfears and anxiety, complimentary drugs (adjuvants)shouldbe used. This three-step approach of administering theproper drug in the right dose at the right time is inexpensive. According to a study conducted in Poland, about 85–90% of patients can besuccessfully treated using this guideline. If the drugs are not wholly effective,surgery on the appropriate nerves may providefurther pain relief(WHO, 1986).This analgesic pathway, established following the recommendations of an international group of professionals, has undergone several modifications over the years and is currently applied for managing not only cancer pain but also acute and chronic non-cancer painful conditions due to a broader spectrum of diseases such as degenerative disorders, musculoskeletal diseases, neuropathic pain disorders and other types of chronic pain. The efficiency of the strategy is debatable and yet to be proven through large-scale studies. Nevertheless, it still provides a simple, palliative approach towards reducing morbidity due to pain in 70% to 80% of the patients (Anekar and Cascella, 2021).

2.1.5.2 Opioid analgesics

Opioid analgesicsare indicated for the management of pain in patients with moderate, severe or chronic pain,although the appropriate indication for the use of opioid analgesics is still a highly debated issue(Cohen*et al*., 2021). TheCentre for Disease Control and Prevention's 2016 guidelines for prescribing opioids for chronic pain(Dowell *et al*., 2016) states that “clinicians should consider opioid therapy only if expected benefits for both pain and function are anticipated to outweigh risks to the patient. If opioids are used, they should be combined with non-pharmacologic therapy and non-opioid pharmacologic therapy, as appropriate”. In the same guidelines, the CDC defines the indication of opioid use for acute pain, stating that “when opioids are used for acute pain, clinicians should prescribe the lowest effective dose of immediate-release opioids and should prescribe no greater quantity than needed for the expected duration of pain severe enough to require opioids. Three days or less will often be sufficient; more than seven days will rarely be needed.”(Dowell*et al*., 2016; Guy*et al*., 2017; Aiyer *et al*., 2018; Chakote and Guggenheimer, 2019).

Opioids work both presynaptically and postsynaptically to create an analgesic effect. Presynaptically, opioids block calcium channels on nociceptive afferent nerves to inhibit release of neurotransmitters such as substance P and glutamate which contribute to nociception. Postsynaptically, opioids open potassium channels which hyperpolarize cell membranes, increasing the required action potential to generate nociceptive transmission. The *mu*, *kappa* and *delta* opioid receptors mediate analgesia spinally and supraspinally(Aubrun *et al*., 2019).

Furthermore, some opioid agents can affect serotonin kinetics in the presence of other serotonergic agents. The proposed mechanism for this is either through weak serotonin reuptake inhibition orthrough increased release of intrasynaptic serotonin via inhibition

of gamma-aminobutyric acidergic presynaptic inhibitory neuron on serotonin neurons. Opioids acting through this mechanism include tramadol, oxycodone, fentanyl, methadone, dextromethorphan, meperidine, codeine and buprenorphine. These opioids have the potential to cause serotonin syndrome and should be used cautiously with other agents with serotonergic activity(Aubrun*et al*., 2019; Baldo and Rose, 2020).

Opioids such as methadone also possess activity at the N-methyl-D-aspartate (NMDA) receptor. Methadone binds to NMDA receptors and antagonizes the effect of glutamate, which is theorized to explain why methadone has efficacy in the treatment of neuropathic pain above other opioids(Crockett *et al*., 2019).

Opioids are contraindicated when there is a risk of substance abuse (Cohen*et al*., 2021). Opioids that possess serotonergic activity (fentanyl, methadone, pethidine, tramadol etc.) have the potential to lower seizure threshold and should therefore be used cautiously or avoided in patients with a history of seizure disorder. Opioids such as methadone which have the potential to prolong QTc interval should be used cautiously or avoided entirely in patients with Long QT syndrome(Cohen*et al*., 2021).

Opioids are the most potent analgesic agents available for the management of chronic pain. However, they have the potential to cause fatal overdose through respiratory depression, especially when combined with CNS depressant medication(Schiller*et al*., 2020). Patients with altered mental status, depressed respiration and constricted pupils should be suspected of suffering from an acute opioid-related overdose which can be fatal if untreated(Kuczyńska *et al*., 2018). Overdose can be reversed by agents such as naloxone which can be given intravenously, intramuscularly, or intranasally. Naloxone is a centrally-acting pure opioid antagonist with a high affinity which rapidly counteracts opioid action (Cohen *et al.*, 2021).

2.1.5.3 Non-opioid analgesics

These include analgesic agents that produce pain relief without interaction with opioid receptors.

Paracetamol:Paracetamol is the most widely used analgesic in the world(Lau*et al*., 2016). It is the drug of choice for treatment of pain in children, pregnant women and the elderly, an indicator of its safety.Paracetamol is indicated for all three steps of pain treatment intensity on the WHO analgesic ladder.It finds use as a first-line drug to manage moderate pain. For lingering moderate to severe pain, when used in tandem with other agents including NSAIDs, caffeine, weak or strong opioids, it improvesanalgesic efficacy while decreasing the side effects of the adjunctagent. It is the drug of choice in patients in whom NSAIDs arecontraindicated(Bharti, 2019).

Even in all these, till this very moment in time, the mechanism of action of paracetamol ispoorly understood.It possesses analgesic and antipyretic properties just like Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) butdoes not possess any anti-inflammatory activity. Consequently, it isnot considered a member of the NSAIDs family(Bharti, 2019).

Paracetamol is metabolized in the liver by the cytochrome p450enzyme system (CYP2E1 and CYP3A4).In the United States and Great Britain, paracetamol intoxication is the most known cause of acute liver failure(Ardid, 2009).Sudden hepatic failure has also been noted with iatrogenic overdose.Paracetamol dose reduction is advocated in certain patientgroups; the elderly, infants, in starvation or malabsorption,severe renal impairment, or hepatic failure whereglutathione stores may be low(Bharti, 2019).

Non-steroidal anti-inflammatory drugs:NSAIDsare commonly used for their anti-inflammatory and analgesic effects.NSAIDs represent the foundation of pain management worldwide, for the treatment of inflammatory, acute and chronic pain, alone or in association with other analgesic-antipyretics or opioids. NSAIDs act by inhibiting prostaglandin synthesis, a mechanism of action that explains their analgesic, antipyretic and anti-inflammatory properties. Central inhibition of COX is also involved in their analgesic activity(Buvanendran *et al*., 2007).They are effectively used in the management of a variety of disorders including but not limited to rheumatoidarthritis, osteoarthritis, ankylosing spondylitis, gout,headache disorders, dental pain and dysmenorrhea(Bharti, 2019).

The COX enzyme exists in three isoforms: COX-1and COX-2, COX-3.COX-1 is expressed constitutively and in quiescent conditions;it performs perpetual regulatory functions including gastro andrenal protection, macrophage differentiation, platelet aggregationand mucus production, with a limited role in inflammatoryprocess(Bharti, 2019).

COX-2 is an inducible enzyme that is unregulated by tissueinjury and other stimuli including interleukin-1, tumour necrosisfactor alpha (TNFa). It is active at injury sites and in a variety oftissues, mediating inflammation, pain, fever and carcinogenicresponses. It also has a regulatory role in reproduction, renalphysiology, bone resorption and neurotransmission.COX-3 enzymes have remained depicted as being fully expressed in the brain, spinal cord, and heart. The mainrole of COX-3 is to regulate pain responses and fever. It doesnot affect inflammation. COX-3 has been postulated to be the target of acetaminophen(Resnik, 2018).

Because of the regulatoryeffects of these enzymes, their inhibition by NSAIDs is associated with important side effects ranging from gastric irritation and bleeding to

deranged renalautoregulation and impaired wound healing. COX-2 specific, inhibitors while decreasing the gastric side effects are associatedwith greater incidence of cardiovascular adverse effects andare no longer recommended for long-term use or in patients withrisk factors for such effects(Cazacu *et al*., 2015).

As a group, NSAIDs are excellent pain-relieving agents and are equally asefficaciousas intramuscularmorphine for purposes of acute painrelief(Bharti, 2019; Sobieraj *et al*., 2019). Their use for chronic pain conditions, over long periods oftime is not recommended and is associated with significant sideeffects such as gastrointestinal bleeding, nephrotoxicity etc. Some commonly used NSAIDs include; ibuprofen, mefenamic acid and diclofenac.

Antidepressants:Tricyclic antidepressants (TCAs) are considered as first-line analgesics for neuropathic pain. Doses required to produce analgesic effects are lower than the usual antidepressant doses.Their mechanism of action is through blockadeof noradrenaline (NA) and serotonin (5-HT) at the dorsal hornsynapses(Bharti, 2019).TCAs are also useful in multi-mechanistic chronic pain with aneuropathic component and as adjuvant analgesics in nociceptiveand inflammatory pain. Examples of TCAs include amitriptyline andnortriptyline.

Duloxetine (a serotoninnorepinephrine reuptake inhibitor) iseffective in treating painful diabetic peripheral neuropathy.Venlafaxine blocks reuptake of noradrenaline and 5-HT and isrelatively free of muscarinic cholinergic, histaminic, and alpha-adrenergicreceptor activity(Bharti, 2019).

Anticonvulsants:Anticonvulsants reduce ectopic neuronal activity and stabilizeneuronal cell membranes through modulation of thevoltage-gated sodium or calcium ion channels. They mayinhibit sodium channels (phenytoin, lamotrigine and others) orinhibit calcium channels (gabapentinoids, that is, gabapentin andpregabalin). The

gabapentinoids have been recommended by the National Institute for Health and Care Excellence for use in neuropathic pain conditions including postamputation pain(Bharti, 2019).

Cannabinoids:Cannabinoids are naturally occurring substances. Some cannabinoids are; tetra hydro cannabinol (THC), cannabidiol(CBD) and cannabinol (CBL). The CBD (Cannabidiol) receptors CB1 andCB2, located extensively in the brain and peripheral tissues, areG-proteincoupled receptors that are linked to the $G_{i/o}$ system inthe same manner as opioid receptors and result in a reduction inafferent neuronal transmission(Zou and Kumar, 2018).The current public opinion is moving toward more widespreadacceptance despite the continuing regulatory limbo inwhich the drug resides(Bridgeman and Abazia, 2017).

2.1.5.4 Neuromodulation

Neuromodulating therapies are targeted at stimulating the pain systems. Currently, several neuromodulation methods are used:

- percutaneous nerve electrostimulation (TENS)
- peripheral nerve stimulation
- acupuncture and vibration

Neuromodulation supports pain treatment methods and by activating the pain inhibitory mechanisms can reduce pain and improve the quality of life of the patient with chronic pain (Świeboda *et al*., 2013).

2.1.5.5 Non-pharmacological treatment of pain

Physical therapy and rehabilitation:Theseare supportive methods used in the management of pain. The most popular methods of physical treatment are: thermotherapy (heat), cryotherapy(cold), laser therapy, electrotherapy, manual technics

andkinesitherapy. These methods when utilized properly, may improve life and mobility ofsome patients(Świeboda *et al*., 2013).

Psychological treatment:Psychologyhas a biginfluence on the perception of pain, as well as the effectivenessof the treatment. Due to this fact, all patients with chronic painshould be able to take advantage of professional psychological therapy, which can affect the emotional aspect of pain. Amongthe psychological methods that can be effective as a techniquesupporting the treatment of chronic pain, the most commonlyused are: cognitive therapy, behavioural therapy, relaxationtechniques and hypnotherapy(Świeboda *et al*., 2013).

Invasive methods:Invasive methods of pain management should be implemented and enforced by experienced specialists in specific cases. Methods include: individual nerve blocks by intrathecal administration of drugs (e.g., epidural anaesthesia during childbirth), neuro-destructive methods (thermolesion, neurolysis) and neurosurgery(Świeboda*et al*., 2013).

2.2 Inflammation

Inflammation is an immune system reaction to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation. It removes the injurious stimuli and initiates the healing process (Chen *et al*., 2018).

Usually, during acute inflammatory responses, cellular and molecular events and interactions efficiently minimize impending injury or infection. This mitigation process contributes to restoration of tissue homeostasis and resolution of the acute inflammation. Acute inflammation has therefore been considered as a part of innate immunity, the first line of host defence againstforeign invaders and danger molecules

(Marshall *et al*., 2018). However, uncontrolled acute inflammation may become chronic, contributing to a variety of chronic inflammatory diseases (Zhou *et al*., 2016).

Important microcirculatory events that occur during the inflammatory process include vascular permeability changes, leukocyte recruitment and accumulation, and inflammatory mediator release. At the tissue level, inflammation is characterized by redness, swelling, heat, pain, and loss of tissue function, which result from local immune, vascular and inflammatory cell responses to infection or injury (Chen *et al*., 2017).

2.2.1 Resolution of inflammation

The inflammatory response must be suppressed to prevent extensive tissue damage. Inflammation resolution is a well-controlled sequence of events involving the spatially and temporally-controlled production of mediators, during which chemokine gradients are diluted over time. Circulating white blood cells eventually fail to sense these gradients and are not recruited to sites of injury. Dysregulation of this process can lead to uncontrolled chronic inflammation(Fullerton and Gilroy, 2016).

Resolution of inflammation involves extremely coordinated actions of different types immune and non-immune cells. Clearance of damaged cells and pro-inflammatory immune cells normally takes place through coordinated processes of cell death such as apoptosis and cell removal such as efferocytosis. Supplementary repair mechanisms may possibly then allow full or at least partial reconstitution of tissue integrity and function. These sequences require the tightly controlled interaction between various cell types and immune cells such as granulocytes, tissue resident macrophages, innate lymphoid cells, and lymphocytes, and all these cell types have all been suggested to play an important role in controlling resolution of inflammation under some

circumstances. Such vital interaction is of pivotal importance to foster resolution of inflammation and to achieve tissue homeostasis(Neurath, 2019).

2.2.2 Inflammatory diseases

2.2.2.1 Heart

Cardiovascular disease, and its underlying pathology, atherosclerosis, is the major cause of death and disability worldwide(Sofi *et al.*, 2016). Inflammatory mediators play key roles in atherosclerosis, from initial leukocyte recruitment through rupture of the atherosclerotic plaque(Soehnlein and Libby, 2021).

2.2.2.2 Pancreas

Pancreatitis, caused by pancreatic duct obstruction, trypsinogen gene mutation, or alcoholism, is an inflammatory disease of the pancreas(Manohar *et al.*, 2017). Pancreatitis is characterized by acinar cell destruction and activation of inflammatory cells, including macrophages, neutrophils, and granulocytes, which secrete inflammatory cytokines (Manohar *et al.*, 2017).

2.2.2.3 Liver

The liver is the biggest solid organ in the body and is a target of both infectious and non-infectious inflammatory pathologies. Infectious inflammation of the liver is mainly caused by microorganisms, such as bacterial products, hepatitis B virus (HBV), or hepatitis C virus (HCV)(Schaefer and John, 2020).

2.2.2.4 Lungs

Lung inflammation occurs predominantly from tissue exposure to bacterial and viral pathogens and/or environmental pollutants. Excessive acute inflammation and subsequent lung injury can cause pulmonary fibrosis and impair gas exchange. Unresolved lung injury and chronic inflammation are frequently observed in acute

respiratory distress syndrome, cystic fibrosis, chronic obstructive pulmonary disease (COPD) and asthma (Wong *et al*., 2016).

2.2.2.5 Kidney

Kidney inflammation sums up to progressive renal injury, which may lead to glomerulonephritis, end-stage renal disease, or acute or chronic kidney disease (CKD) (Ernandez and Mayadas, 2016).

2.2.2.6 Intestinal tract

The complex, polygenetic inflammatory bowel diseases (IBDs) are characterized by an excessive inflammatory response to gut lumen microbial flora.IBDs mainly include ulcerative colitis (UC) and Crohn disease (CD), but also non-infectious inflammation of the bowel (Seyedian *et al*., 2019).

2.2.2.7 Brain

Inflammatory responses also occur in the brain (CNS) to produce CNS diseases like; Alzheimer's (AD), Parkinson's disease (PD), and epilepsy.

2.2.3 Drugs used in the treatment of inflammation

2.2.3.1 Non-steroidal anti-inflammatory drugs

NSAIDs represent the foundation of pain management worldwide, mostly being used for the treatment of inflammatory, acute and chronic pain, alone or in association with other analgesicantipyretics or opioids. NSAIDs act by impeding prostaglandin synthesis, a mechanism of action that explains their analgesic, antipyretic and anti-inflammatory properties. NSAIDs are considered nonspecific analgesic drugs, used mainly for their anti-inflammatory effect. But the coexisting analgesic effect makes them indispensable in the management of inflammatory pain in rheumatic diseases, such as osteoarthritis or rheumatoid arthritis (Sofat and Kuttapitiya, 2014).

2.2.3.2 Corticosteroids

Steroids were first identified in 1935. Since then, they have been employed in a vast amount of uses.Presently, many of the clinical roles of steroids are related to their potent anti-inflammatory and immune-modulatingproperties((Lucena and Rangel, 2018;Quatrini and Ugolini, 2021).

2.3 Toxicology

Toxicology is the study of poisons, or a little more accurately, the study of how chemicals interfere with the normal function of a biological system. The 15th century physician and father of toxicology,Paracelsus (1493-1541), said, "All substances are poisons; there is none whichis not a poison. The right dose differentiates a poison from a remedy"(Grandjean, 2016).Toxicology can also be defined as a multidisciplinary science that investigatesthe adverse effects of chemical substances on biological systems(Eaton and Gallagher, 2010).

Today, toxicology is an applied science that is built on other medical sciences including physiology, biochemistry, pathology, pharmacology, medicine and epidemiology(Schrager, 2006). The specialtiesof toxicology fall into three distinct categories: descriptive toxicology,research/mechanistic toxicology, and applied toxicology, although newer fields like forensic and environmental toxicology have developed over the past few decades(Costa and Teixeira, 2014).

2.3.1 Duration of exposure

Two maintypes of exposure conditions exist for toxiccompounds: acute and chronic. Acute exposureoccurs where a particular amountof a compound enters the organism, it is a single episode. While acuteexposure usually refers to a single doseof a chemical (within less than 14 days),repeated exposures may be given within a brief periodof time

(typically less than 24 hours) for less toxic chemicals and still be considered acute exposure(Sullivan *et al*., 2005).

Repeated exposures for greater than 24 hours are consideredchronic, which may then cause a cumulative toxiceffect. However, the frequency of repeated exposurein laboratory animal studies is often subdivided intothree categories: subacute, sub-chronic, and chronic(Sullivan *et al*., 2005).

Subacute exposure refers to repeated exposure to achemical for 1 month or less, sub-chronic for 1–3months, and chronic for more than 3 months.For many chemicals, the toxic effects resultingfrom acute exposure are far different from thoseresulting from chronic exposure(Eaton and Gallagher, 2010)

2.3.2 Types of toxic effect

Toxic effects vary greatly as regards to nature, scope, target tissue, and mechanism of action. Toxic effects are a result ofbiochemical interactions which occur between toxicantsand certain target structures of the exposedorganism(Gupta, 2020). Typically, these target structures may bea specific cell type or subcellular organelle within atissue. However, the target structure may also benonspecific, such as any tissue or organ whichcomes into direct contact with the toxicant. The variety of toxic effects observed can beclassified according to the duration, target organ,and mechanism of action. In addition, reversibleeffects of toxicant exposure are those that disappearfollowing cessation of exposure. Irreversible effects,in contrast, will persist or worsen after exposure isdiscontinued. Examples of irreversible effects of toxicantexposure include cirrhosis of the liver andcancer(Gupta, 2020).

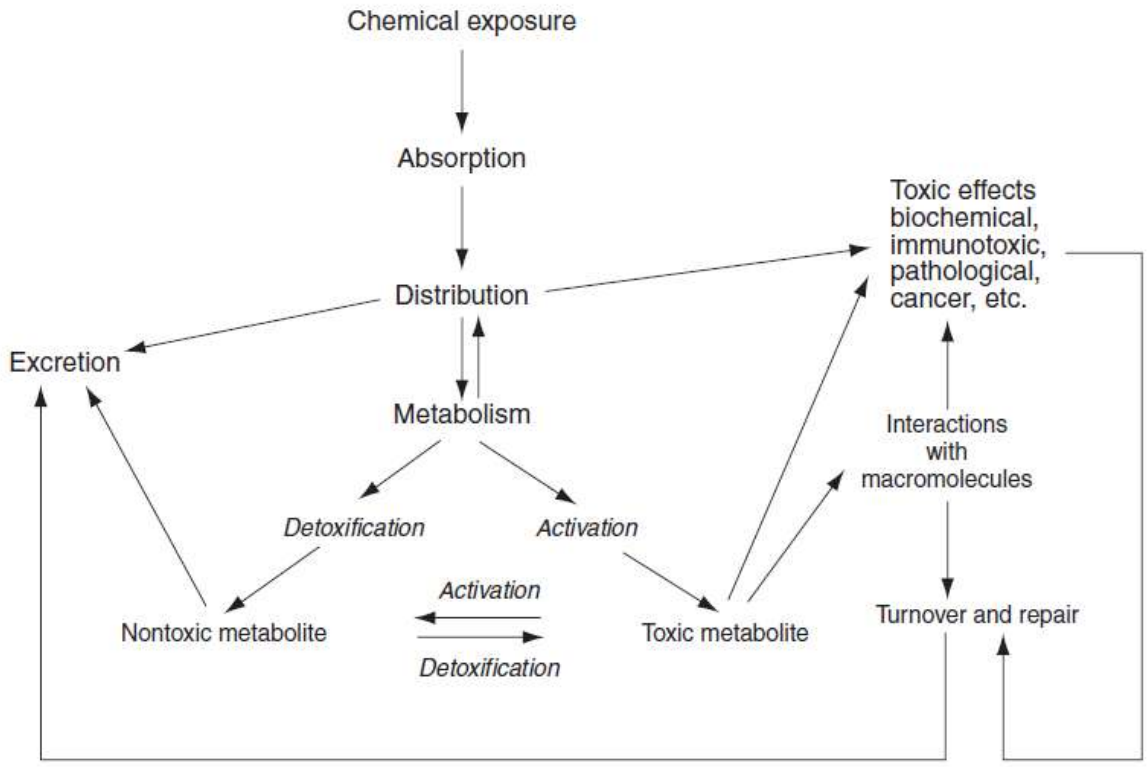


Figure 2.2: The Sequence of Events Following Exposure to Toxic Chemicals (Eaton and Gallagher, 2010)

2.3.2.1 Idiosyncratic and allergic reactions

Humans will normally respond to toxic chemicals in a way similar to laboratory animals, and usually in doses that are relatively comparable on a body weight basis. However, there are often individuals in a population that have some genetic variation that causes them to react at a dose far below the anticipated dose. This type of hypersensitivity is often referred to as an idiosyncratic reaction and, if it occurs at all, is usually seen only in a very small percentage ofthe population(Böhm *et al*., 2018).

2.3.2.2 Biological and morphological changes

Cyanide alters or stops the electron transport chain in the mitochondria of cells, therebyinterfering with the processing of oxygen to generate energy (ATP). This can lead to the death of cells in vital organs, ultimately causing death to the organism (Graham and Traylor, 2021).

2.3.2.3 Systemic toxicology

Systemic toxicology refers to the toxic effects xenobiotics cause to the bodily systems of living organisms. It covers toxic responses of the liver, toxic responses of the kidney, pulmonary toxicology, neurotoxicology and toxic responses of other organ systems(Eaton and Gallagher, 2010).

2.3.2.4 Mutagenesis

One of the most crucial types of toxic response a xenobiotic can produce is the production of mutations. Mutations arise when the DNA in a cell is damagedin such a way that the information contained in the genetic code is altered(Durland and Ahmadian-Moghadam, 2020).

2.3.2.5 Carcinogenesis

It has been stated, that weare in an epidemic of cancer and that this epidemic isdue in large part to our unprecedented exposure toenvironmental pollutants associated with the start of the industrial revolutionwhich increased environmental pollution(Manisalidis*et al*., 2020).

2.3.2.6 Teratogenesis

Concern over the potential for occupational and/or environmental chemical exposure to produce birth defects has heightened in the last few decades. As the developing embryo is often highly sensitive to toxic chemicals, it is necessary to consider women of childbearing age to beat particularly high risk from toxic chemical exposures(Hall, 2016).

2.3.3 Toxicity testing in experimental animals

There are two main principles that govern all descriptive animal toxicity testing. The first is that the effects of chemicals in laboratory animals, when properly quantified, are

applicable to humans. In general, doses are determined based on a body weight, and doses in bothhumans and laboratory animals are usually presented in units of milligram of chemical per kilogram of body weight (mg/kg). However, extrapolation of dosage across species often correlates better when dose is expressed on the basis of dose per unit of body surface area (Eaton and Gallagher, 2010).

The second main principle is that exposure of experimental animals to toxic substances in large doses is a necessary and valid method of identifying possible hazards to humans. Practical considerations require that the number of animals used in toxicology experiments will be small compared with the size of human populations at risk (Eaton and Gallagher, 2010).

2.3.4 Extrapolation of animal data to humans

Complicating these extrapolations are differences in lifespan, genetics, body size, routes of metabolism, and rates of exposure. Animal studies nearly always utilize high doses because of the statistical limitations inherent in determining a low-probability outcome. Obviously, if one wanted to estimate reliably a tumour incidenceof one additional cancer per thousand exposed animals,many more than 1,000 animals would berequired(Eaton and Gallagher, 2010).

If practical, ethical, and economic constraints preventthe use of thousands of animals per test, then thenext best thing is to assume that the response obtainedat doses sufficient to give a measurable response in areasonable-sized population (e.g., 50 animals per dose)is proportional to dose even at doses several orders ofmagnitude lower than the doses used in the experiment.Thus, it is necessary to invoke mathematicalmodels to predict the shape of the dose–responsecurve at doses 3–6 orders of magnitude below theactual doses used(Eaton and Gallagher, 2010).

2.4 *Eriosema psoraloides* (Lam.) G.Don

2.4.1 Description of *E. psoraloides*

E. psoraloides is plant from the legume family (Fabaceae) which grows naturally in northern Nigeria and part of southern Nigeria. The herb is common throughout tropical Africa, extending to itssouthern tip. It is an erect perennial plant which produces more or less woody stemsand has golden yellow flowers which are odorous. It usually develops a single main stem with branches spreading upward, growing up to 2meters tall(Burkill, 1985).

2.4.1.1 Detailed description of the herb

Erect, branched subshrub or woody herb, 0.75–2.4(3.6) m tall. Branches strongly ribbed, shortly densely brownish hairy, often velvety and covered with small orange-red glands. Leaflets 3 or occasionally some lower leaves 1-foliolate, paler beneath, 2.3–9.5 × 0.8–3.5(4) cm, narrowly elliptic, elliptic-oblong or obovate-elliptic, rounded and mucronulate, emarginate or rarely acute at the apex, cuneate, finely to densely velvety pubescent above, velvety beneath, the venation prominent and usually with buff hairs, the rest of the leaflet surface with silvery or ferruginous tomentum or slightly coarser indumentum; petiole 1–5 mm long; rhachis 1–8 mm long; petiolules 1–3 mm long. Racemes many-flowered, terminal and axillary; rhachis (2)6–12 cm long; peduncle 1–3 cm long; pedicels 2 mm long. Calyx appressed pilose or pubescent, usually glandular, 3.5–7 mm long; lobes triangular or narrowly triangular, equalling the tube. Corolla deep golden-yellow; standard 7–14 mm long, obovate, glabrous or rarely with a few hairs outside, glandular; keel paler yellow, glandular. Pods 1.1–1.8 × 0.9–1.1 cm, subcircular, ovate or elliptic-oblong, often oblique, covered with long ferruginous hairs. Seeds dark reddish-brown or pinkish-buff with blue-black mottling, shiny, 4.5–5.2 × 2.5–3.5 × 1–1.5 mm; rim aril creamy-brown(Mackinder *et al*., 2001).

2.4.2 Taxonomy of *E. psoraloides*

The genealogy of the plant is as follows;

Kingdom: Plantae

Order: Fabales

Family: Fabaceae

Subfamily: Papilionoideae

Genus:*Eriosema*

Species:*Eriosema psoraloides* (GBIF, 2021)

Common names: Canary Pea, Shrubby yellow Eriosema

Local names:*Bii raánár* (Hausa), *òpá àwon funfun*, *roro*, *isapa iluju* (Yoruba), *agwa cana* (Igbo).(Burkill, 1985).

2.4.3 Ethnobotanical uses of *E. psoraloides*

E. psoraloides has been claimed to possess so many medicinal activities by natives of Africa. The roots are considered aid in healing. The roots are also considered to possess diuretic, emetic, febrifuge activities and have a positive influence on female fertility and health. It is used in the treatment of conditions such as nasopharyngeal infections, stomach troubles, diarrhoea, dysentery, kidney problems etc. Itis seen as a genital stimulant, regulator of the menstrual cycleand is also used to cause abortion (Burkill, 1985).

The roots, flowers and fruits are used to relieve pain. The flowers and fruits are used as sedative. The leaves are used in the treatment of eye problems, skin problems and cutaneous and subcutaneous parasitic infection.The leaves and root are abortifacientand vermifuge. It is also used in the treatment of venereal diseases (Burkill, 1985).The twigs areused as chewing sticks to clean teeth and maintain good oral hygiene(Khan *et al*.,

2000). The leaves are rubbed into the fur of dogs to treat or prevent lice infestation (McIndoo, 1945).

2.4.4 Previous studies on *E. psoraloides*

Researchers have reported on the antibacterial and antifungal properties of *E. psoraloides.* It was found to be effective against the growth of gentamicin resistant *Staphylococcus aureus*and *Candida albicans*(Elechi and Igboh, 2016).In another study, the plant was subjected to phytochemical screening andvarious fractions of *E. psoraloides* were discovered to contain flavonoids, tannins, carbohydrates and steroids(Ateba *et al*., 2021).

Furthermore, the herb has been shown to have significant antidiabetic activity. This was confirmed by its ability to significantly reduce blood glucose level in alloxan-induced diabetic Wistar rats (Nduka *et al*., 2018; Elechi and Ewelike, 2019; Nduka *et al*., 2019).

Plate I: Photograph of *Eriosema psoraloides*Growing in its Natural Habitat, Shika, Giwa Local Government Area, Kaduna State, December, 2019. Photo Credit: Bamikunle, M. O.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection and Identification of Plant Material

The whole plant of *Eriosema psoraloides*was collected from Shika village,Giwa Local Government Area, Kaduna State in November, 2019. On the day of collection, it was identified by its conspicuous flowers and leaves.The collected plant was identified and

authenticated, by comparing with an existing specimen by a taxonomist, Mr Sanusi Namadi of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria. A voucher specimen number of 03763 was collected for further reference.

3.2 Animals

Adult Wistar rats (150- 200 g) and Swiss albino mice (18-25 g) of both sexes were used for the purpose of this study. The animals were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. They were housed in clean propylene cages were saw dust was utilized as beddings, each cagehousedfive animals. The beddings were replaced every three days. The animals were fed with standard feed (Vital Feed) and given access to water *ad libitum* until the end of the study.Ethical clearance for the use of laboratory animals was sought from the ABU Committee on Animal Use and Care (ABUCAUC).

3.3 Drugs, Chemicals and Reagents

Chemicals and drugs that were used for this study included; piroxicam (Pfizer Laboratories, Pakistan, NAFDAC number: 04-0710 LOT: BK12513); carrageenan;acetic acid anhydride; morphine sulphate (*Martindale Pharma, UK*,LOT: 0119123); chloroform (Sigma Chemical Co. USA).The reagents used were freshly prepared and of analytical grade.

3.4 Equipment and Other Materials

Equipment and materials that were used for this study included; animal cages, pestle and mortar, pair of scissors,digital Vernier caliper 52 (Battenfeld Technologies Inc. USA),Mettler balance AG Type p162 NO: 633741, hot plate (MR 2002), dissecting kits, measuring cylinders,stop watch, crucible, water bath (Gallenkamp Cat No:H1054),

rat COX-1 ELISA kit (Shanghai Koon Biotech Co., Ltd, LOT: 202102)rat COX-2 ELISA kit (Shanghai Koon Biotech Co., Ltd, LOT: 202002).

3.5 Preparation of the Extract

The roots of *Eriosema psoraloides* were cleaned, air-dried under shade, with intermittent weighing until a constant weight was obtained, and thenpulverised into powder with the aid of a pestle and mortar. A portion of the powdered plant (1,000g) was macerated with 1.5 litres of 70% methanol to exhaustion with occasional shaking for 72 hours. The solvent was removed by placing the extract on water bath set at 50± 5°C. The extract wasthereafter stored in a desiccator until it was required for the study. The percentage yield of the extract was calculated using the formula below;

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered material}} \times 100\ \%$$

3.6 Phytochemical Screening of theMethanol RootExtract of *Eriosema psoraloides*

Qualitative phytochemical analysis was conducted according to standard procedures.

3.6.1 Test for carbohydrates and sugar

Molisch test: The methanol root extract (0.5 g) was dissolved in 3 ml of water in a test tube. Three drops of Molisch reagent were added then followed by 5 drops of concentrated sulphuric acid along the test tube wall and observed. A purple to violet colour at interface was observed (Evans, 1996).

3.6.2 Test for flavonoids

a. *Shinoda's reduction test*: Four pieces ofmagnesium metal were added to themethanol root extract solution (3 ml) followed by a few drops concentrated hydrochloric acid

(HCl). The solution was observed for the formation of a reddish colour which indicated the presence of flavonoids (Sofowora, 1993).

b. *Sodium hydroxide test*: Twomillilitres of filtered methanol root extract was dissolved in 2 ml of 10% aqueous sodium hydroxide solution which gave a yellow solution to which dilute HCl was added for formation of colourless solution (Evans, 1996).

3.6.3 Test for cardiac glycosides

Keller Killiani's test: Glacial acetic acid (1 ml) was added to about 3 ml of the solution containing 2 mg of extract in a test tube held at 45º angle; then two drops of concentrated H_2SO_4were added along the side of the test tube. Formation of purple ring colour at the interface was observed for the presence of cardiac glycosides (Evans, 1996).

3.6.4 Test for saponins

Methanol root extract (0.5 g) was shaken with 3 ml of water for 30 seconds and allowed to stand for 30 minutes. Formation of honey comb which persisted for more than 30 minutes was observed for the presence of saponins (Sofowora**,** 1993).

3.6.5 Test for tannins

Lead sub-acetate test: Methanol root extract (0.5g) was dissolved in 2 ml of water; 3 drops of lead sub-acetate solution were then added and observed for formation of black-green coloured precipitate which indicated the presence of tannins(Evans, 1996).

3.6.6 Test for steroids and triterpenes

Liebermann-Burchard's test: Plant material (0.5 g) was extracted with 5 ml of methanol and then filtered. The filtrate was evaporated to dryness on a water bath at 100ºC. The residue obtained was shaken with 2 ml chloroform and then filtered into a cleaned and dried test tube. Twomillilitres of acetic acid anhydride were added to the filtrate and shaken, and then 1 ml of concentrated sulphuric acid was also added carefully. A

brownish-red colour was observed immediately at interphase and violet blue-green at the upper layer later. Red colour observed indicated the presence of triterpenes while blue-green colour indicated steroids (Evans, 1996).

3.6.7 Test for alkaloids

Methanol root extract (0.5 g) was treated with 5 ml of 1% aqueous HCl and then heated. It was filtered and the filtrate was divided into four portions in four test tubes **(**Sofowora, 1993).

a. Dragendoff's reagent: To test tube 1, two drops of dragendoff's reagent were added and observed for the formation of arose red precipitate which indicated the presence of alkaloids.

b. Wagner's reagent: Totest tube 2, two drops of Wagner's reagent was added and the formation a brownish precipitate indicated the presence of alkaloids.

c. Mayer's reagent: To test tube 3, two drops of Mayer's reagent was added and observation of a creamy precipitate indicated the presence of alkaloids.

d. Picric acid reagent: To test tube 4, two drops of picric acid was added and the appearance of a yellowish precipitate indicated the presence of alkaloids(Evans, 1996).

3.7 Acute Toxicity Study

3.7.1 Selection of animals

Female rats and mice nulliparous and non-pregnant of average weights 150-200g and 18-25g respectively were used because of their higher sensitivity to the effect of chemicals. Animals were kept and maintained under natural day and night cycle. Animals were housed in cages and fed with conventional rodent laboratory diet with access to drinking water.

3.7.2 Fixed dose method of acute toxicity study of the methanol root extract of *Eriosema psoraloides* in rats and mice

Median lethal dose (LD_{50}) determination for the methanol root extract of *Eriosema psoraloides*(EPE) was conducted using Organization for Economic Co-operation and Development (OECD) 425 (2008) guidelines in rats and mice. In this method, a limit test was conducted. Two groups each of three animals were fasted prior to dosing (food but not water was withheld overnight for the rats and for 3 hours for mice). The body weight was determined for each animal and the dose was then calculated according to the body weight. Food was then further withheld for 3-4 hours in rats and 1-2 hours in mice after the extract administration *per oral*. The extract was administered in a single oral dose using an oral cannula. A start dose of 5,000 mg/kgwas used for one animal.On survival of the first animal, two more animals were dosed.

Animals were observed individually at least once during the first 30 minutes after dosing and periodically during the first 24 hours with careful observation during the first 4 hours and then daily for 14 days. Observations included changes in skin and fur, eyes and mucous membranes, somatosensory activity and behavioural pattern, autonomic and central nervous systems etc. Animals were also observed for tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Time of onset of toxic symptoms and disappearance were also noted.Individual weights of animals were determined daily. At the end of the test, surviving animals were weighed and then sacrificed using chloroform. Organs of the animals such as liver, kidney, spleen, lungs, heart and gastrointestinal system were freshly harvested and fixed in 10% v/v formalin for histopathological studies.

3.8 Sub-Acute Toxicity Studies on the Methanol Root Extract of *Eriosema psoraloides* in Rats

This was carried out in accordance with the Organization for Economic Co-operation and Development (OECD) 407 guidelines (2008). Twenty male rats were divided into four treatment groups of five rats each. The rats were treated orally with the extract (250, 500 and 1,000 mg/kg) and normal saline (1 ml/kg) daily for a period of 28 days. The animals were given water and food throughout the duration of the study and their food and water intake were recorded on a daily basis. They were also observed daily for general symptoms of toxicity. Weekly body weights of the animals were recorded for the 28 days of treatment and on the 29th day under slight chloroform anaesthesia, the rats were euthanized. Blood samples were collected into plain and EDTA bottles for biochemical and haematological investigations respectively. The relative organ weight ratio (ROW) of the various isolated organs were calculated as:

$$\text{ROW} = \frac{\text{Absolute organ weight (g)}}{\text{Weight of rats on day of sacrifice (g)}} \times 100$$

The tissue slices of the liver, kidney, stomach and heart of the animals were subsequently fixed in 10% formalin and taken for histopathological investigation.

3.8.1 Biochemicalstudies

Blood samples (2ml) were collected from the jugular veins of the animals into plain bottles, allowed to clot and centrifuged at 3,500 rpm for 10 minutes. The separated sera were stored at -4°C and used for evaluating the following biochemical parameters thereafter: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphate (ALP), serum, chloride, creatinine, potassium, sodium and total protein.

3.8.2 Haematological studies

Blood samples of the animals were used to conduct haematological studies. Haematological parameters like red blood cell count, white blood cell count, packed cell volume, haematocrit, etc. were assayed.

3.8.3 Histopathology of organs

Tissue samples from the selected organs (liver and kidney, GIT) of both control and extract treated rats were removed and fixed in 10% buffered formalin (pH 7.4). Following fixation, tissue specimens were dehydrated in a graded series of ethanol (70–100%), cleared in toluene and finally enclosed in paraffin. Thereafter, 5-µm thin sections were prepared using a microtome (Leica RM2235) and stained with haematoxylin and eosin (H and E) prior to microscopic examination. The microscopic features of the organ of treated groups were compared with the control group, and photomicrographs were recorded.

3.9 Pharmacological Studies

3.9.1 Acetic acid-induced writhes test in mice

The acetic acid-induced writhes test in mice as described by Koster*et al.*(1959) was employed. Thirty Swissalbino mice were divided into five groups of six mice. Group I (negative control) and group V (positive control) were treated with distilled water (10 ml/kg) and Piroxicam (20 mg/kg) per body weight respectively. Group II, III and IV were pretreated with graded oral doses (*p.o*) of EPE(250, 500 and 1,000 mg/kg respectively).Sixty minutes posttreatment, mice in all groups were administered acetic acid (0.6% $^{v}/_{v,}$ 10 ml/kg body weight *i.p*). After a five-minute lag period, the number of writhes (abdominal contractions accompanied with backward stretching of hind limbs) were counted within a period of 10 minutes. Percentage (%) inhibition of writhes was calculated as follows:

$$\text{Percentage Inhibition} = \frac{\text{No of writhes (control)-No of writhes(test)}}{\text{mean number of writhes(control)}} \times 100\ \%$$

3.9.2 Hot plate test in mice

The method described by Eddy and Leimbach (1953)was employed for the study. Randomly selected Swiss albino mice were individually exposed to the hot plate before any form of treatment commenced (0 reading). This was done in order to ensure adequate responsiveness of each animal within the cut-off time (20 seconds). Thirty albino mice were divided into five groups of six mice each. Group I (negative control) and group V (positive control) were treated with distilled water (10mg/kg *p.o*) and morphine (5 mg/kg *p.o*) body weight respectively. Group II, III and IV were pretreated with graded oral doses (250, 500 and 1,000 mg/kg) of EPE.

Each mouse was then placed on a hot plate at controlled temperature of 55ºC ± 1ºC for a maximum time of 20 seconds. Reaction time was then recorded using a stop watch (when animals licked their hind paw or jumped off the hot plate). This process was repeated four more times with (60, 90, 120 and 150 min).

3.9.3 Carrageenan-induced paw oedema

The acute anti-inflammatory study was carried out using the carrageenan-induced hind paw oedema test in rats as previously described by Winter *et al*. (1962). Five randomly selected groups of rats (n=5) were orally administered normal saline (1 ml/kg) and the extract, (250, 500 and 1,000 mg/kg) and piroxicam (20 mg/kg). Sixty minutes post treatment, 0.1 ml of 1% carrageenan in normal salinewas injected into plantar surface of the right hind paw of each rat. The hind paw oedema was measured and recorded at times 0, 1, 2, 3, 4 and 5 hours using Verniercalliper to determine the diameter of the oedema. The increase in paw diameter (oedema index) for each rat was calculated as the

difference in paw diameter before carrageenan injection and after carrageenan injection at each time interval, while the percent inhibition of oedema was calculated for each group with respect to its vehicle-treated control group using the following relationship:

$$\text{Percentage Inhibition} = \frac{\text{Mean increase in paw size of control-Mean increase in paw size of treated}}{\text{Mean increase in paw size ofcontrol}} \times 100\ \%$$

Blood samples were then collected for each group after euthanizing the animals from the jugular vein. These samples were used toanalyse COX-1 and COX-2 serum levels.

3.9.4 Analysis of cyclooxygenase 1 (COX1) and cyclooxygenase 2 (COX 2) proteins by ELISA

Serum COX1 and COX 2 levels were measured by solid phase sandwich ELISA kit specified for COX 1 and COX 2 proteins (Shanghai Coon Koon Biotech Co. Ltd, Shanghai, China) with a lower detection limit of 0.4 ng/ml. Blood samplesfrom different groups were centrifuged at 2,500 rpm/20 min and the serum was collected and frozen at -70℃. The analysis of COX 1and COX 2 protein expression were done according to the manufacturer's instruction. An antibody specific for rat COX 1 and COX 2 coated onto the wells of the microtiter strips provided was used.A biotinylated antibody specific for rat COX 1 and COX 2, and Streptavidin-Peroxidase (enzyme) was also used to bind to the biotinylated antibody to complete the fourth member sandwich. The plates were then read by a microplate reader at 450 nm. COX 1 and COX 2 protein concentration were obtained from a standard curve. Concentrations were expressed in picogram per millilitre and nanogram per millilitre.

3.10 Data Presentation and Analysis

The results were presented as mean ±standard error of the mean (SEM) in tables, figures and photomicrograph as appropriate. The statistical analysis of the data was done using SPSS version 25. One-way analysis of variance (ANOVA) and split-plot analysis of variance were employed followed by Dunnett's and Bonferroni post hoc tests followed by Levine's test of homogeneity of variances. $p \leq 0.05$ was considered statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Extractive Yield of *Eriosema psoraloides* Roots

Extraction of one thousand grams (1,000 g) of the powdered root of *Eriosema psoraloides*afforded a percentage yield of 5.23% $^{w}/_{w}$. The extract obtained was of a dark brown colouration, had a glossy appearance, was hard and sticky.

4.2 Phytochemical Constituents

Preliminary phytochemical screening of *Eriosema psoraloides* root extract showed the presence of carbohydrates, glycosides (cardiac glycosides), triterpenes, saponins, tannins, flavonoids, steroids and alkaloids, while anthraquinones were absent (Table 4.1).

4.3 Acute Toxicity Studies (Median Lethal Dose)

Acute oral administration of the root extract of *Eriosema psoraloides* did not produce any observable behavioural signs of toxicity in both rats and mice. No deaths were recorded as doses were increased up to 5,000 mg/kg. The median lethal dose (LD_{50}) was as thus estimated to be greater than 5,000 mg/kg body in both mice and rats.

Table 4.1: Phytochemical Constituents of the Methanol Root Extract of *Eriosema psoraloides*

Phytoconstituents	**Result**
Alkaloids	+
Carbohydrates	+
Flavonoids	+
Glycosides	+

Cardiac glycosides	+
Anthraquinones	-
Saponins	+
Steroids	+
Triterpenes	+
Tannins	+

Key: (-) = Absent, (+) = Present

4.4 Sub-acute Toxicity Study of the Methanol Root Extract of *EriosemaPsoraloides* Administrations in Rats

4.4.1 Effect of themethanol root extract of *Eriosema psoraloides* on body weights of rats

Weekly body weight recordings for the subacute toxicity study revealed that in all the groups, there was a progressive increase in average weight of the rats. However, the animals that were treated with the extract showed greater weight gain when compared to the control group (distilled water 1 ml/kg). After subjecting the data to statistical analysis, on the fourth week, a statistically significant increase ($p< 0.05$) in weight was observed at the 1,000mg/kg dose compared to the control group (Figure 4.1).

4.4.2 Effect of the methanol root extract of *Eriosema psoraloides* on serum biochemical parameters of liver function

A significant decrease in ALT and AST ($p< 0.05$) was observed in animals treated daily for 28 days with the extract. However, an insignificant ($p >0.05$) decrease in serum ALP level occurred. The total protein and albumin were also decreased statistically significantly ($p < 0.05$), (Table 4.2).

4.4.3 Effect of the methanol root extract of *Eriosema psoraloides* on serum biochemical parameters of kidney excretory functions and electrolytes

A decrease in serum urea level that was statistically significant ($p <0.05$) at all dose levels of the extract treated groups were observed compared to the normal saline (1 ml/kg). A significant ($p <0.05$) decrease in serum sodium level was observed at the 1,000 mg/kg dose. All other parameters showed inconsistent differences that werenot significant (Table 4.3).

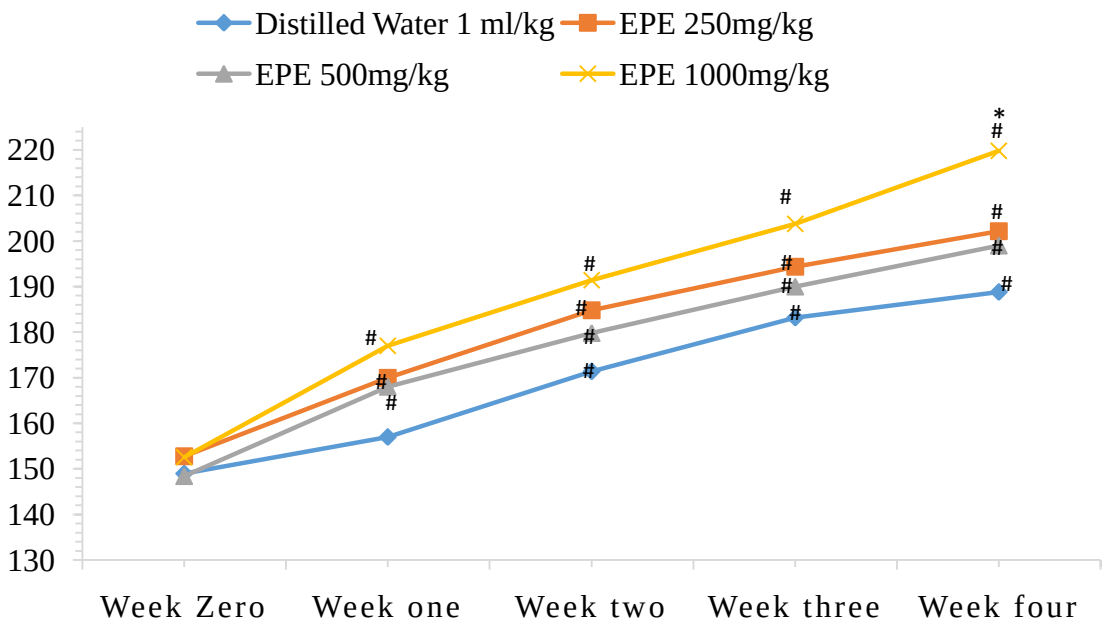


Figure 4.1: Weekly Body Weight Changes of Rats After 28 Days of Oral Administration of *Eriosema psoraloides* Methanol Root Extract

Data analysed using SPANOVA and Bonferroni post hoc test, data presented as Mean ± SEM, n=5, * indicates significance at $p< 0.05$ compared to the control group,# indicates significance at $p< 0.05$ compared toweek zero. EPE = Extract of *Eriosema psoraloides*.

Table 4.2: Liver Function Biomarkers in Rats TreatedDaily for 28 Days with theMethanol Root Extract of *Eriosema psoraloides*

Liver biomarkers	Treatment groups (per kg)			
	N/S (1ml)	**EPE250 mg**	**EPE 500 mg**	**EPE1,000 mg**
ALT (IU/L)	29.0 ± 2.21	16.0 ± 3.19*	20.6 ± 1.36	21.0 ± 2.35
AST (IU/L)	44.2 ± 6.15	24.4 ± 3.06*	32.2 ± 3.32	19.8 ± 4.12*
ALP (IU/L)	36.44 ± 4.23	27.64 ± 3.64	25.42 ± 3.87	34.8 ± 4.14
Total Protein (g/dL)	4.88 ±0.37	3.74 ± 0.21*	3.52 ±0.23*	3.78 ± 0.16*
Albumin (g/dL)	3.26 ± 0.16	2.68 ±0.16*	2.80 ± 0.11	2.56 ±0.11*

Data was analysed using one-way ANOVA and Dunnett *post hoc* test, data is expressed asMean ±SEM; * indicates significance at $p<0.05$. compared to the N/S groupn=5; N/S = normal saline; ALT= alanine aminotransferase; AST; aspartate aminotransferase; ALP; alkaline phosphatase, EPE= *Eriosema psoraloides* extract

Table 4.3: Kidney Function Biomarkers in Rats Treated Daily for 28 Days with theMethanol Root Extract of *Eriosema psoraloides*

Kidney biomarkers	Treatment groups (per kg)			
	N/S (1 ml)	**EPE250 mg**	**EPE500 mg**	**EPE1,000 mg**
Urea (mg/dL)	20.04 ± 1.53	14.48 ± 1.89*	11.14 ± 0.52*	12.46.40±1.23*
Creatinine (mEq/L)	0.76 ±0.06	0.72 ±0.10	0.94 ±0.12	0.92 ± 0.44
K^+ (mmol/L)	9.30 ± 0.64	7.34 ± 0.43	6.50 ± 0.21	10.56 ± 1.49
Na^+ (mmol/L)	148.72 ± 3.15	134.12 ± 6.63	133.24 ± 3.40	127.74 ± 7.53*
Cl^- (mg/dL)	93.80 ± 6.01	85.00 ± 6.29	86.00 ± 2.93	82.60 ± 2.16
HCO_3^- (mg/dL)	26.6 ±2.69	26.4 ± 2.48	26.6 ± 2.93	23.60 ± 3.17

Data was analysed using one-way ANOVA and Dunnett *post hoc* test,data expressed asmean ±SEM; statistics; * indicates significance at $p<0.05$ compared to the N/S group, n = 5. N/S=normal saline; K^+=potassium ion; Na^+=sodium ion; Cl^-=chloride ion; HCO_3^-=bicarbonate ion, EPE= *Eriosema psoraloides* Extract

4.4.4 Effect of 28-day oral administration of the methanol root extract of *Eriosema psoraloides* on haematological parameters

There was an increase in packed cell volume and Haemoglobin levels which was not significant compared to the control.Inconsistent changes were also observed in the levels of lymphocytes, platelets and granulocytes. (Table 4.4).

4.4.5 Effect of 28-day administration of the methanol root extract of *Eriosema psoraloides* on relative organ weight of rats

Oraladministration of the extract did not produce any significant changes in relative organ weight of the isolated organs at all tested doses (Table 4.5).

4.4.6 Histopathological effects of the methanol root extract of *Eriosema psoraloides* on the kidney of wistar rats treated daily for 28 days

Histology of the kidney of the normal saline group showed normal tubules (T) and glomerulus (G) in the kidney (Plate II_A). At the 250mg/kg EPE dose, the kidneys showed slight tubular necrosis (TN), (Plate II_B). At the 500mg/kg dose of EPE, moderate tubular adhesion (TA) was observed (Plate II_C), with moderate tubular necrosis recorded at 1,000mg/kg of EPE (Plate II_D).

4.4.7 Histopathological effects of the methanol root extract of *Eriosema psoraloides* on the liver of wistar rats

In the normal saline group, normal histology of the liver cell was maintained (Plate III_A). At the dose of 250mg/kg of EPE, slight hyperplasia of pyknotic (PYK) cells was observed (Plate $III_{\mathbf{B}}$). At the dose of 500mg/kg (Plate III_C) and 1,000mg/kg of EPE (Plate III_D), slight hepatic necrosis (HN), vascular congestion (VC) and sinusoidal congestion (SC) was observed.

Table 4.4: Haematological Parameters in rats Treatedfor 28 Days with the Methanol Root Extract of *Eriosema psoraloides*

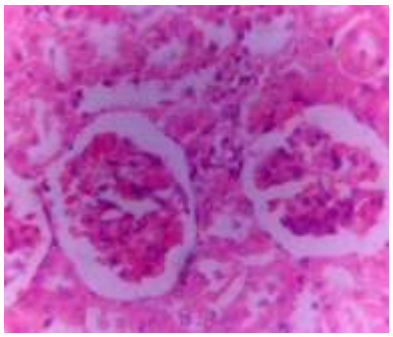
Haematological indices	Treatment groups (per kg)			
	N/S (1 ml)	**EPE 250 mg**	**EPE 500 mg**	**EPE 1,000 mg**
PCV (%)	39.40 ±1.57	38.40 ±1.91	39.00 ±1.10	44.8 ± 3.69
Hb (g/dL)	13.16 ± 0.57	12.72 ±0.48	12.96 ±0.38	15.80 ± 2.09
WBC ($\times 10^3$ /µL)	4.74 ±0.08	4.30 ±0.19	4.76 ±0.32	5.08 ±0.21
RBC ($\times 10^6$/µL)	5.76 ±0.17	5.88 ±0.24	6.04 ±0.07	5.96 ±0.12
Platelet ($\times 10^3$ /µL)	172.02 ± 6.26	219.40±22.85	177.80 ± 8.96	175.60 ±7.14
Lymphocytes (10^3/µL)	5.62 ± 0.18	5.88 ± 0.28	5.64 ± 0.37	5.26 ±0.18
Granulocytes (10^3/ µL)	2.06 ± 0.20	2.74 ±0.18	2.66 ± 0.22	1.88 ± 0.24

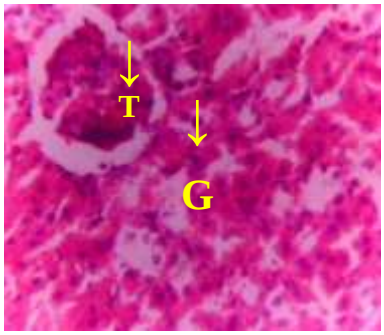
Data expressed asmean ± SEM; statistics: Data was analysed using one-way ANOVA;n = 5; N/S=normal saline; PCV=packed cell volume; Hb=haemoglobin; WBC=white blood cell; RBC=red blood cell, EPE= *Eriosema psoraloides* extract

Table 4.5: Relative Organ Weights in Rats Treated for 28 Days with the Methanol Root Extract of *Eriosema psoraloides*

Treatment groups (mg/kg)	Relative Organ weight (g)			
	Liver	**Kidney**	**Stomach**	**Heart**
NS (1ml)	3.83 ±0.71	0.61 ±0.05	1.85 ±0.23	0.33 ± 0.01
EPE (250)	3.62 ±0.08	0.60 ±0.02	1.85 ±0.35	0.35 ± 0.02
EPE (500)	3.74 ±0.15	0.61 ±0.01	1.52 ±0.14	0.31 ± 0.04
EPE (1,000)	3.84 ±0.06	0.58 ±0.03	1.61 ±0.07	0.32 ± 0.03

Data expressed asmean ±SEM, n = 5; EPE= *Eriosema psoraloides* Extract, NS = Normal Saline.n = 5

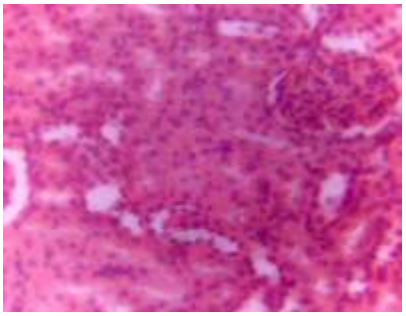


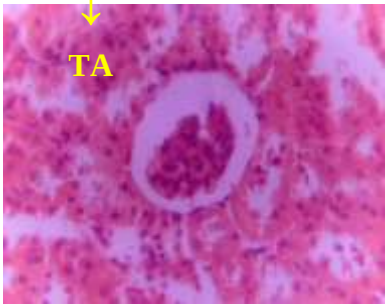


TN

Plate II_A: 1ml/kg Normal Saline

Plate II_B: 250mg/kg EPE





TN

Plate II_C: 500mg/kg EPE

Plate II_D: 1,000mg/kg EPE

Plate II: Photomicrographs of Sections of the Kidney Tissues of Control and Treated Rats (×400), Stain H/E

II_A: Section showing normal histological appearance of kidney with normal glomerulus (G) and normal renal tubules (T)

II_B: Section showing slight tubular necrosis (TN) of a tubular cell

II_C: Section showing moderate tubular adhesion (TA) of distorted tubular cells

II_D: Section showing moderate tubular necrosis (TN) of tubular cells

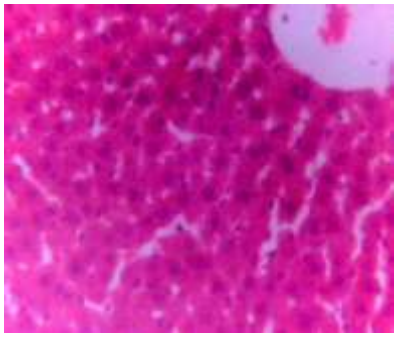


Plate III_A: Normal Saline 1ml/kg

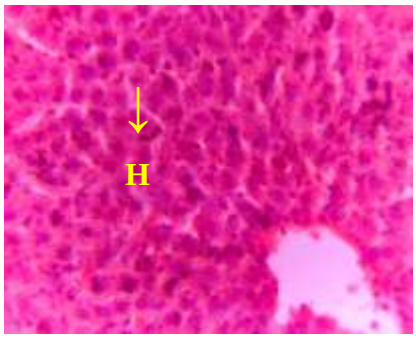


Plate III_B: EPE 250mg/kg

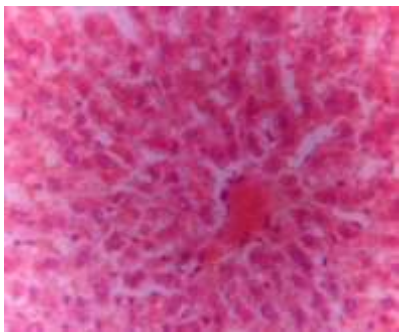


Plate III_C: EPE 500mg/kg

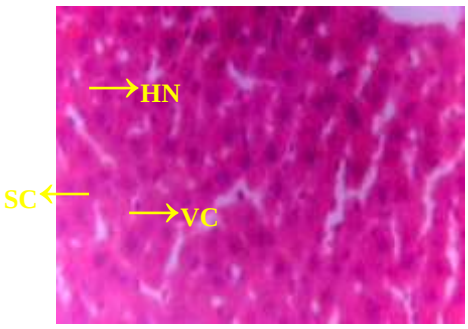


Plate III_D: EPE 1,000mg/kg

Plate III: Photomicrographs of Sections of the Liver Tissues of Control and Treated Rats (×400), Stain H/E

III_A: Section showing normal histological appearance of liver with normal hepatocytes (H)

III_B: Section showing slight hyperplasia of pyknotic cells (PYK)

III_C: Section showing slight hepatic necrosis (HN),vacuolation (VC) and sinusoidal congestion (SC)

III_D: Section showing slight vacuolation (VC)

4.4.8 Histopathological effects of the methanol root extract of *Eriosema psoraloides* on the stomach of wistar rats

The histopathological effects of EPE on the stomach at 250mg/kg and 500mg/kg, showed normal mucosa epithelium (M), (Plate IV_A, Plate IV_BPlate IV_C). Slight hyperplasia of inflammatory cells (LH) at dose of 1,000mg/kg when compared with the control group administered 10ml/mg of normal saline was observed (Plate IV_D).

4.4.9 Histopathological effects of the methanol root extract of *Eriosema psoraloides* on the heart of wistar rats

Twenty-eight day oral administration of EPE had no effect on the heart at 250mg/kg and 500mg/kg doses when compared to control (Plate V_A), displaying normal myocardial epithelium (M) (Plate V_B and V_C). Slight hyperplasia of inflammatory cells (LH) at dose of 1,000mg/kg was observed (Plate V_D).

4.5 Effect of Methanol Root Extract of *Eriosema Psoraloides* on Acetic Acid-Induced Writhes Test in Mice

The methanol root extract of *Eriosema psoraloides* significantly ($p < 0.05$) decreased the number of acetic acid-induced writhes in mice at all the tested doses (250, 500 and 1,000mg/kg). It demonstrated this in a dose dependent manner comparable to Piroxicam 20mg/kg (Figure 4.2).

4.6 Effect of the Methanol Root Extract of *Eriosema psoraloides* on Hot Plate (thermally-induced) Pain in Mice

The methanol root extract of *Eriosema psoraloides* increased the mean reaction time of the mice, however, not to ac statistically significant level (Figure 4.3).

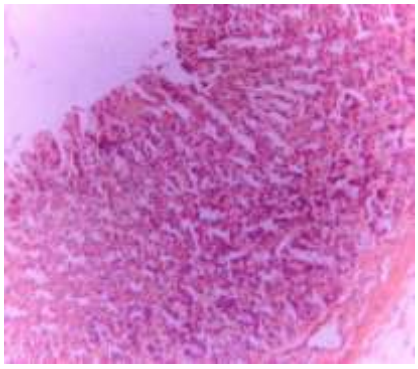


Plate IV_A: Normal Saline 1ml/kg

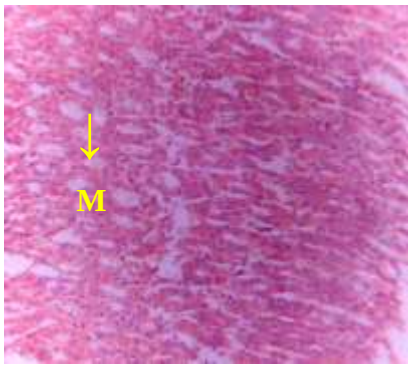


Plate IV_B: EPE 250mg/kg

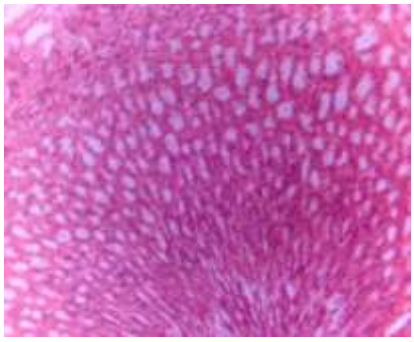


Plate IV_C: EPE 500mg/kg

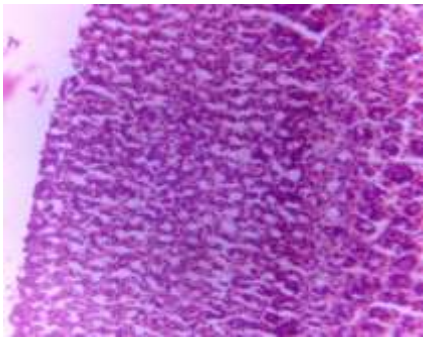
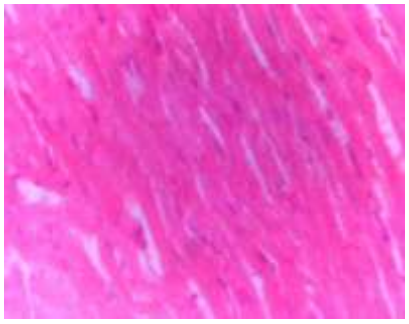


Plate IV_D: EPE 1,000mg/kg

Plate IV: Photomicrographs of Sections of Stomach Tissues of Control and Treated Rats (×400), Stain H/E

IV_A: Section showing normal mucosa epithelium of the stomach (M)
IV_B: Section showing normal epithelium of the stomach (M)
IV_C: Section showing normal epithelium of the stomach (M)
IV_D: Section showing Slight hyperplasia of inflammatory cellsof the stomach (M)



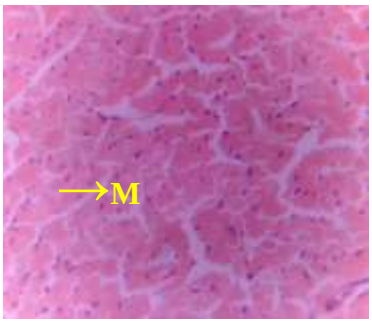
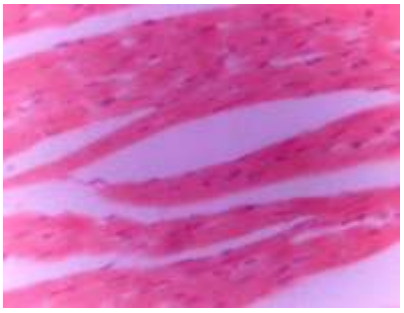


Plate V_A: Normal Saline 1ml/kg

Plate V_B: EPE 250mg/kg



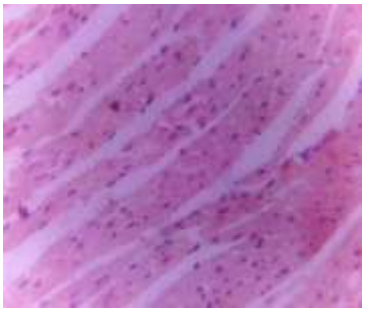


Plate V_C: EPE 500mg/kg

Plate V_D: EPE 1,000mg/kg

Plate V: Photomicrographs of Sections of Heart Tissues of Control and Treated Rats (×400), Stain H/E

IV_A: Section showing normal myocardial epithelium of the heart (M)
IV_B: Section showing normal myocardial epithelium of the heart (M)
IV_C: Section showing normal myocardial epithelium of the heart (M)
IV_D: Section showing Slight hyperplasia of inflammatory cells of the heart (M)

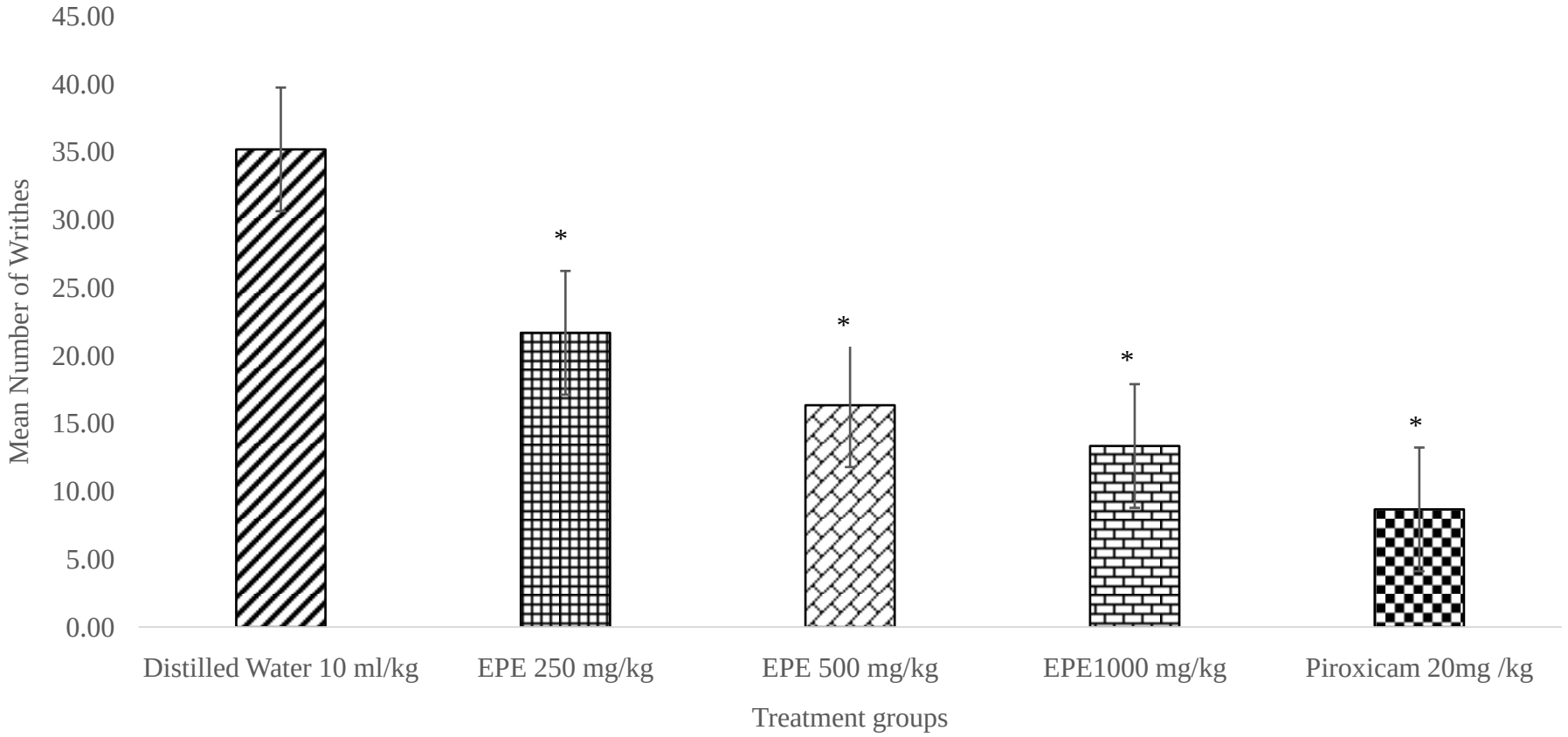


Figure 4.2: Effect of Methanol Root Extract of *Eriosema psoraloides* on Acetic Acid-Induced Writhes Test in Mice
Data was analysed using one-way ANOVA followed by Dunnett's Post Hoc test and presented as Mean ± SEM, * =p<0.05, significant statistical difference as compared with Distilled water group, n=6, EPE= Methanol root extract of *E. psoraloides*

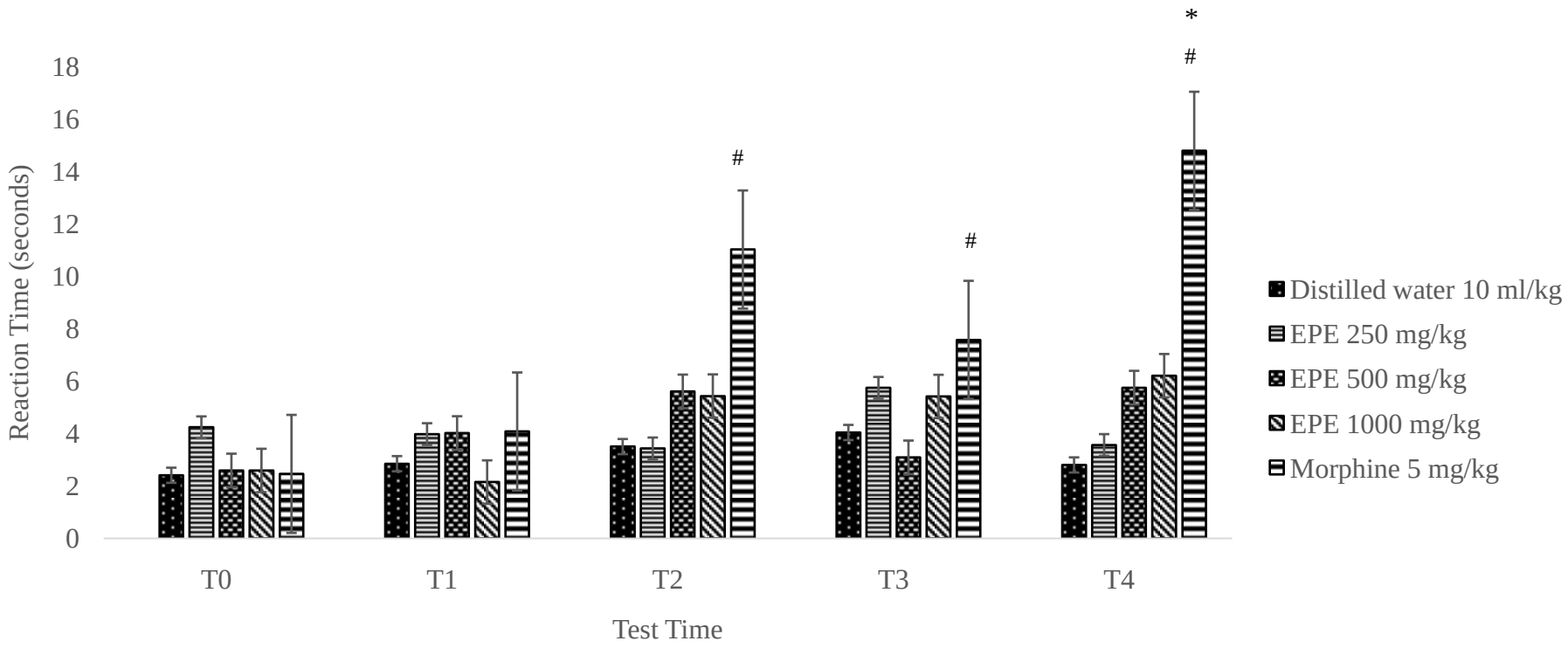


Figure 4.3:Mean Reaction Time of Rats Treated with the Methanol Root Extract of *Eriosema psoraloides*
Data was analysed using Split Plot analysis of variance (SPANOVA) followed by Bonferroni post-hoc Test.* $p< 0.05$ compared to Normal Saline, # $p< 0.05$ compared to T0. EPE = Extract of *Eriosema psoraloides;* S.E.M. = Standard Error of Mean. T= Test time where T0 is baseline, T1, T2, T3 and T4 represent 60 min, 90 min, 120 min and 150 min after treatment administration; n = 6.

4.7 Effect of the Methanol RootExtract of *Eriosema psoraloides* on Carrageenan-Induced Paw Inflammation in Rats

There was a decrease in carrageenan-induced paw oedema observed in all the tested doses starting from the third hour of the test as compared to the distilled watergroup.However, a statistically significant decrease in paw oedema was observed at thefourth hour in the group treated with 250mg/kg of the extract (p <0.05), (Table 4.6).

4.8 Effect of the Methanol Root Extract of *Eriosema psoraloides* on Cyclooxygenase 1 (COX-1) Serum Levels in Rats

It was observed that EPE reduced serum COX-1 levels, although not to a statistically significant level. COX-1 inhibition of 16.39 % at the 500mg/kg dose level and 11.06% at the 1,000mg/kg dose level were observed. COX-1 inhibition of 13.02% for the positive control (piroxicam 20mg/kg) was observed (Table 4.7).

4.9 Effect of the Methanol Root Extract of *Eriosema psoraloides* on Cyclooxygenase 2 (COX-2) Serum Levels in Rats

It was observed that EPE reduced serum COX-2 levels, however, not to a statistically significant level (p> 0.05). COX-2 inhibition of 9.55 % at the 250mg/kg dose level and 11.75 % at the 500mg/kg dose level were observed. COX-2 inhibition of 20.75% at the 1,000mg/kg dose level and 10.74% for the positive control (piroxicam 20mg/kg) were also observed (Table 4.8).

Table 4.6: Effect of the Methanol Root Extract of *Eriosema psoraloides* on Carrageenan-Induced Paw Oedema in Rats

	Mean Paw Diameter in Millimetre (% Inhibition)					
Treatment/Time	**0 hrs**	**1 hr**	**2 hrs**	**3 hrs**	**4 hrs**	**5 hrs**
N/saline 1ml/kg	2.63 ± 0.07	3.746 ± 0.198	4.602 ± 0.208	5.322 ± 0.179	5.686 ± 0.182	6.146 ± 0.173
EPE 250 mg/kg	2.90 ± 0.21	3.886± 0.099 **(-3.74%)**	4.380 ± 0.228 **(4.82%)**	4.504 ± 0.356 **(15.35%)**	4.290±0.452 **(24.55%)** *	5.162 ± 0.276 **(16.01%)**
EPE 500mg/kg	2.81 ± 0.08	3.478 ± 0.069 **(7.15%)**	4.308 ± 0.257 **(6.39%)**	4.556 ± 0.225 **(14.39%)**	4.502 ± 0.345 **(20.82%)**	5.598 ± 0.262 **(8.92%)**
EPE1,000mg/kg	2.67 ± 0.09	3.726 ± 0.054 **(0.53%)**	4.236 ± 0.274 **(7.95%)**	4.448 ± 0.215 **(16.42%)**	4.416 ± 0.303 **(22.34%)**	5.200 ± 0.442 **(15.39%)**
Piroxicam 20mg/kg	2.50 ± 0.08	3.170 ± 0.083 **(15.38%)** *	3.052 ± 0.188 **(33.68%)** *	3.070 ± 0.118 **(42.31%)** *	2.790 ± 0.090 **(50.93%)** *	3.234 ± 0.103 **(47.38%)** *

Data was analysed using SPANOVA followed by Bonferroni Post Hoc test, *= $p<0.05$, significant statistical decrease in mean paw oedema size. n= 5. Values are Mean ± SEM. EPE= *Eriosema psoraloides*extract, hrs= hours. Figures in parentheses (bold) are percentage inhibition of inflammation

Table 4.7: Effect of the Methanol Root Extract of *Eriosema psoraloides* on Cyclooxygenase 1 (COX-1) Serum Levels in Rats

Treatment (mg/kg)	Mean COX-1 serum concentration (pg/mL)	Percentage inhibition of COX-1 (%)
D/W 1ml/kg	69.42 ± 6.55	
EPE 250	70.62 ± 8.26	-1.73
EPE 500	58.04± 3.22	16.39
EPE 1,000	61.74 ± 4.67	11.06
Piroxicam 20	60.38 ± 3.99	13.02

Data was analysed using one-way ANOVA and presented as Mean ± SEM, n=5, COX-1= Cyclooxygenase 1, EPE= Methanol root extract of *E. psoraloides*,D/W =Distilled Water

Table 4.8: Effect of the Methanol Root Extract of *Eriosema psoraloides* on Cyclooxygenase 2 (COX-2) Serum Levels in Rats

Treatment (mg/kg)	Mean COX-2 serum concentration (ηg/mL)	Percentage inhibition of COX-2 (%)
D/W 1 ml/kg	21.7 ± 3.22	
EPE 250	19.70 ± 0.84	9.55
EPE 500	19.22± 1.08	11.75
EPE 1,000	17.26 ± 0.59	20.75
Piroxicam 20	19.44 ± 1.32	10.74

Data was analysed using one-way ANOVA and presented as Mean ± SEM, n=5,COX-2= Cyclooxygenase 2, EPE= Methanol root extract of *E. psoraloides*, D/W= Distilled Water

CHAPTER FIVE

5.0 DISCUSSION

The use of herbs is growing globally, this is precipitated by the fact that a large proportion of the world's population find them to be cheap, readily available and easy to use(Bhuiyan *et al*., 2020). However, there are concerns about the safety and efficacy of various herbs, though these concernsare shared particularly by the scientific community. *Eriosema psoraloides*is a plant native to Africa as a whole. It has been used locally to manage a number of illnesses, nevertheless, many of these claims have not been validated.

Preliminary qualitative phytochemical studies done on the methanol root extract of *Eriosema psoraloides* unveiled the presence of saponins, alkaloids, tannins, triterpenes, cardiac glycosides and flavonoids. Many studies have shown the anti-inflammatory activities of alkaloids(Sicong*et al*., 2020). Flavonoids have also shown diverse activities which include anti-inflammatory and analgesic activities. They have been reported to demonstrate these activities through mechanisms by which they inhibit various steps of the inflammatory process and interfer with the synthesis and the release of inflammatory and pain mediators(Verri *et al*., 2012).

The oral median lethal dose (LD_{50}) of the methanol root extract of *Eriosema psoraloides* in mice and rats was estimated using the OECD 425 guideline. The $LD_{50}$was discovered to be greater than 5,000 mg/kg which suggests that the extract of *Eriosema psora*loides is practically innocuous (OECD, 2008). The assessment of the safety of chemicals, pharmaceuticals, natural products and industrial products is very pivotal prior to their approval for human use and consumption(Erhirhie *et al.,* 2018).In toxicity testing of xenobiotics, there is no doubt that the most critical candidate species for humans are

humans, since accurate extrapolation of animal data directly to humans may not be definite due to interspecies variation in anatomy, physiology and biochemistry.Nevertheless, due to ethical reasons, such chemicals are tested using animal models prior tobeing subjected to trials in humans (Parasuraman, 2011).

The sub-acute daily administration of the methanol root extract of *Eriosema psoraloides*for 28 days showeda statistically significant increase in mean body weights of the animals over the duration of the study.This may be dueto the presence of steroids and saponins in the plant and from literature, it has been reportedthat saponins can be transformed to aglycone sapogenin (a steroid or triterpene)which may probably act centrally and have stimulatory effects on feedingcentres in the brain of experimental rats(Shamaki *et al*., 2017). However, body weight alterations can also occur through alteration of the activity of a growth hormone, somatostatinor neurotransmitters that affect food consumption(Bailey *et al*., 2004).There was no significant increase in food intake in the first week of the study, this pattern remained relatively constant on all the following weeks, indicating that the weight gain was not as a result of appetite stimulatory effects of the extract, but rather was triggered through other mechanisms. Also, the administration of the extract did not inhibit water consumption of the animals.

The extract did not alter the relative organ weights of the rats and could hence be considered non-toxic since reductions in organ weights are sensitive indicators of toxicity (Lazic, *et al*., 2020). Organ weight also is an important indicator of physiological and pathological status of animals. The relative organ weight is vital to confirm if an organ was exposed to injury or not. Some of the target organs normally exposed to toxic effects of drugs include lungs, liver and kidney (Gupta, 2018).

The liver plays a key role in transforming and removing chemicals and is vulnerable to the toxicity from these agents.Unusual liver enzyme levels might signal liver damageor alteration in bile flow(Wen *et al*., 2017).

Liver function tests involveevaluating serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, bilirubin and albumin levels. Common indicators used to assess the integrity of the liver include; the ALT and AST. These enzymes are typically located in the liver cells and become free and released from the cells and into the blood stream following hepatic damage. These liver enzymes have their levels become raised in the blood in disease conditions such as liver cirrhosis, hepatitis and hepato-biliary obstruction; they have been known to provide a quantitative assessment of the degree of damage sustained by the liver (Wen *et al*., 2017).

In this study, the result of the liver function test revealed a significant decrease in the levels of ALT caused by the extract of *Eriosema psoraloides*in the 250 mg/kg treated group as compared to control. This is often suggestive of normal activities of the liver enzyme because even though there was a statistically significant decrease in ALT levels, the levels still fall within the normal acceptable range (7-56 IU/L). AST levels were also significantly decreased ($p < 0.05$) by the 28-day administration of the extract.

The main medical relevance of measuring serum alkaline phosphatase lies in the diagnosis of cholestatic liver disease due to the fact that some of the highest elevations in alkaline phosphatases is found in patients with cholestasis.Normally, four times of the upper limit of normal or greater elevation presents in up to 75% of the patients with cholestasis, either intrahepatic or extrahepatic(Shamban *et al*., 2014). Comparable increases happen in biliary obstruction due to cancer, choledocholithiasis, biliary

stricture, sclerosing cholangitis or causes of intrahepatic cholestasis such as primary biliary cholangitis, drug-induced liver injury, chronic rejection of liver allografts, infiltrative liver disease (sarcoidosis, amyloidosis, tuberculosis, and liver metastasis) and severe alcoholic hepatitis causing steatonecrosis(Shamban *et al*., 2014). There was no statistical change in ALP levels across the various groups hence no indication of any bile duct pathology in the extract treated groups or liver disease.

The serum total protein and albumin levels of the extract treated groups showed a statistically significantdecreasein both TP and ALB compared to the those of the control group. This decrease may be attributed to the extract showing a potential to inhibit the liver's protein generating ability(Carvalho and Machado, 2018).

Both urea and creatinine remain nitrogenous end products of metabolism. Urea is the main metabolite gotten from dietary protein and tissue protein turnover. Creatinine is the product of muscle creatine catabolism (Delanaye*et al*., 2017). The BUN and creatinine tests are screening tests used to assess renal function. Therefore, elevated levels of serum creatinine and urea are indicators of possible malfunction of the kidneys. In the present study, BUN showed a significantdeclinein the extract treated groups compared to the control group, though the urea levels still remained within the acceptable normal range (5-20 mg/dl). This can be attributed to the lower levels of total protein and albumin observed in the liver function test. Serum sodium levels also decreased statistically significantly as compared to the control group. This suggests the extract may have a diuretic property and may have potential for use as an antihypertensive agent, though, further studies need to be conducted. All other measured kidney parameters showed no observable changes.

Toxicological studies on the hematopoietic systemarecriticaltoassessing both preclinical and clinical safety assessment of substances. Reasons for this may be the high replication rate and exposure of the tissue to allsubstances administered systematically. Blood cells are exposed to any xenobiotic that are introduced into the body either orally or by injection(Bloom, 1993).

In the present study, there was no significant change in the haematological parameters in all the extract treated groups in comparison with the control group. This suggests that the extract did not adversely affect erythropoiesis, morphology or osmotic fragility of the red blood cells and also did not stimulate an immune response or affect other blood cells adversely.

Histopathological examination of the kidney showed slight to moderate tubular necrosis and slight tubular adhesion. Thisagrees with the slight changes in kidney biochemical parameters measured.Histopathological examination of the liver showed slight hyperplasia of pyknotic cells, slight hepatic necrosis, slight vascular congestion and sinusoidal congestion.This is a distinguishing effect of chemicals on the liver and can be validated with the changes in the levels of the liver metabolizing enzymes. Due to the regenerating capability of the liver, necrotic lesions are not necessarily critical (Kholodenko and Yarygin, 2017).

All histipathological features of the stomach and the heart remained normal, showing only slight changes at the highest dose of the extact used. This shows the that extract doesn’t have any significant impact on the physiology of both tissues.The most interesting feature of *Eriosema psoraloides* is a lack of gastrointestinal side effects despite having an anti-inflammatory activity. *Eriosema psoraloides* may be considered

as a natural alternative to anti-inflammatory agents for the treatment of inflammatory pain.

The acetic acid–induced abdominal writhing test has been used asan assessmenttool forexamining analgesic or anti-inflammatory agents(Dzoyem *et al.*, 2017).When animals are intraperitoneally injected with acetic acid, a painful response and acute inflammation arises in the peritoneal area. Writhingstimulated by acetic acid is consideredto be a nonselective antinociceptive model, as acetic acid works indirectly by inducingthe release of endogenous mediators which stimulate the nociceptive neurons that areresponsive to nonsteroidal anti-inflammatory drugs, to narcotics, and to other centrally activedrugs(Bighetti *et al.*, 1999).Writhing is described as a stretch, tension to a side, lengthening ofhind legs, or contraction of the abdomen so that the abdomen of the mice touches the ground, or turning of the trunk (twist). Any writhing is considered a positive response(Mishra *et al*., 2011).

The ability of all the graded doses of methanol root extract of *Eriosema psoraloides*to attenuate the acetic acid-induced abdominal constriction significantly indicates that the extract's anti-nociceptive activity possibly involves its ability to inhibit COX in the peripheral tissues which results in the decrease in PGEs synthesis and the impairment of pain transduction in primary afferent nociceptors. The acetic acid-induced writhing model has been deemed as a highly sensitive nociceptive test due to its ability to detect anti-nociceptive activities at dose levels that will not be effective in other test models. This sensitivity is linked mainly to the direct interaction of the extracts/compounds with the peripheral receptors within the peritoneal cavity(Dzoyem *et al*., 2017).Conversely, the acetic acid-induced abdominal contraction model is limited by its non-specificity in the illumination of the involvement of peripheral or central mechanisms inthe antinociceptive activity of drug molecules (Chen *et al*., 1995). Consequently, in an attempt

to establish whether the extract mitigated either the peripheral or central, or both levels of pain sensation, the thermal-induced nociceptive model was used in this study. Non-steroidal anti-inflammatory drugs, including aspirin, ibuprofen, etc. are less active in this test compared to more powerful analgesics such as opioids (Castagné *et al.*, 2014).

Animals exposed to the hot plate apparatus respond by jumping, withdrawal of the paws and or licking of the paws. The period of latency is usually prolonged following the administration of central acting analgesics, whereas peripheral analgesics do not elicit any significant activity that is capable of ameliorating these responses (Castagné *et al.*, 2014). The methanol root extract of *Eriosema psoraloides*at 250, 500 and 1,000 mg/kg increased the reaction time of the mice to the thermal stimulus at 60, 90, and 150 minutes compared to the negative control group, though not significantly.This suggests that the extract may have centrally mediated activity, although not as potent as its peripheral antinociceptive activity.

Carrageenan-induced paw oedema is the most commonly used experimental model for evaluating the anti-inflammatory potency of compounds or natural products.Fundamental signals of inflammation, such as oedema, pain, and redness, develop immediately following sub-plantar injection of carrageenan into a hind paw, as a result of the action of proinflammatory mediators (Vazquez *et al*., 2015).In the initial phase (0-1 hour), histamine, serotonin, and bradykinin are the first mediators implicated, while prostaglandins and various cytokines such asIL-1β, IL-6, IL-10, and TNF-α are concerned in the second phase(Dzoyem *et al.*, 2017).The anti-inflammatory response of EPE was more pronounced at the fourth and fifth hour after sub-plantar injection of 1% carrageenan, one study from literature also had similar findings (Adedayo*et al*., 2019). This occurred possibly due to the potency of the carrageenan used for this study. It was also observed that the percentage inhibition of inflammation gradually increased from 0 hour up till the fifth hour being highest at the fourth and fifth

hour. This indicates that the extract possesses a significant effect against acute inflammation. Though there is inhibition at the first and second hour, the effect from the third hour can possibly be due to the inhibition of COX responsible for prostaglandin biosynthesis, since these prostaglandins, being major inflammatory mediators,are implicated in the second phase of carrageenan-induced paw oedema (Dzoyem *et al.*, 2017). The ability of the extract to inhibit carrageenan-induced paw oedema suggests that it possesses a significant effect against acute inflammation.

Three isoforms of COX exist, also knownas prostaglandin G/H synthase. COX-1, is constitutivelyproduced in most tissues and creates prostaglandins (PGs) in minutequantities, and has thus been implicated to function primarily in themaintenance of physiological functions. COX-2, isextremely inducible in reaction to pro-inflammatory stimuli,cytokines, and mitogens, and it has thus been generally theorized to function in the inflammatory liberation of PGs.Finally, COX-3, a splice variantof COX-1, is inhibited by paracetamol and is thus thought tobe responsible for the antipyretic and analgesic effects of this drug. COX converts arachidonic acid to prostaglandins (Rajakariar *et al*., 2006). At orthodox therapeutic doses, common NSAIDs exert their analgesic and anti-inflammatory effectsvia COX-2 inhibition(Osafo *et al*., 2017). Investigational indication suggests that PGE_2 is strongly implicated via its actions during the inflammatory cascades (Simon, 1999).It was observed thatEPE reduced serum COX-1 levels, although not to a statistically significant level.It was also observed that EPE reduced serum COX-2 levels, however, notstatistically significantly. This shows that EPE downregulated both COX 1 and COX 2 serum levels, but these observed downregulations were not statistically significant.However, the extract showed a significant decrease in pain intensity in acetic acid test. Thus, it may be said that the extract did not statistically reduce the serum levels of COX 1 and COX 2 for a

number of reasons. Firstly, the test utilised was an acute test. Secondly, the test measured both COX-1 and COX-2 serum concentrations, but not their activities.

It has been indicated that flavonoids impede the peroxidase active site of COX-1, COX-2, and 5-lipoxygenase (5-LO) triggering an inhibition of prostanoids (prostaglandins and thromboxanes) and leukotrienes production. As a matter of fact, flavonoids prevented the production of PG_{E2}, which was consistent with the inhibition of COX activity (Verri *et al*., 2012).Therefore, it may be said, the analgesic and anti-inflammatory activity of *Eriosema psoraloides* may be attributed to its various phytochemical constituents which include flavonoids, alkaloids, tannins or a proposed combinatory effect.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The methanol root extract of *Eriosema psoraloides* afforded a yield of 5.23% $^{w}/_{w}$. Preliminary phytochemical screening of *Eriosema psoraloides* root extract showed the presence of carbohydrates, glycosides (cardiac glycosides), triterpenes, saponins, tannins, flavonoids, steroids and alkaloids. The acute toxicity study showed that the extract is relatively safe orally, given that the LD_{50} was greater than 5,000 mg/kg.

The continuous oral daily dosing of the methanol root extract of *Eriosema psoraloides* for 28 days showed variations in the biochemical parameters; owing to the slight toxic response observed in the histopathological examination of the relative organs. This was mostly observed in the histopathology of the liver and kidney, with heart and stomach tissue remaining largely unchanged, showing the extract may be used without there being concerns of it producing gastrointestinal side effects, which is a major drawback of most NSAIDS.

This study also demonstrated that the methanol root extract of *Eriosema psoraloides* has significant analgesic and anti-inflammatory activity mediated via peripheral and central mechanisms (to a lesser extent) which further supports the ethnomedical use of *Eriosema psoraloides* in the management of pain and inflammatory conditions. The mechanistic studies showed that the analgesic and anti-inflammatory activity of *Eriosema psoraloides*

involves a possible interaction with the COX 1 and COX 2 iso enzymes, though further studies in this regard need to be conducted.

6.2 Conclusion

From the findings of this study, it is evident that the methanol root extract of *Eriosema psoraloides* possesses analgesic and anti-inflammatory activity that is potentiallymediated through primarily peripheral mechanisms through the inhibition of COX 1 and COX 2. However, long term administration of the extract should be with cautionsince mild alterations in liver and kidney histology were observed

6.3 Recommendations

I. The bioactive constituents responsible for the observed pharmacological activities in the methanol root extract of *Eriosema psoraloides* should be quantified, isolated, purified and characterized through bioassay guided fractionations in further studies.

II. Pharmacodynamics and pharmacokinetics studies should be carried out to establish the exact mechanism of action of the extract and its fractions.

III. The diuretic properties of the extract should be further investigated. This is because the extract statistically significantly decreased serum sodium (Na^+) levels.

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APPENDICES

Appendix I:Effect of 28 Days Daily Administration of Methanol Root Extract of *Eriosema psoraloides* on Body Weights of Rats

Treatment (*p.o*)	Weekly Body weights (g) Week 0	Week 1	Week 2	Week 3	Week 4
D/W 1ml/kg	149.00 ± 4.68	157.00 ± 6.38	171.40 ± 6.95	183.20 ± 6.40	188.80 ± 5.62
EPE 250mg/kg	152.80 ± 6.04	170.00 ± 5.96	184.80 ± 5.61	194.40 ± 6.57	202.20±5.70
EPE 500mg/kg	148.40 ± 6.90	168.00 ± 7.17	179.80 ± 7.45	190.00 ± 7.92	199.00 ± 7.70
EPE 1,000mg/kg	152.60 ± 7.11	177.60 ± 8.36	191.40 ± 8.21	203.80 ± 7.65	219.80 ± 8.65 *

Data are expressed as Mean ± S.E.M; *= $p<0.05$ compared to control – Repeated measure ANOVA followed by Bonferroni post hoc test, n=5, D/W = Distilled water, EPE = Methanol Root Extract of *Eriosema psoraloides*, n = 5

Appendix II: Effect of Methanol Root Extract of *Eriosema psoraloides* on Acetic Acid-Induced Writhes in Mice

Treatment (mg/kg)	Mean number of Writhings
D/W 10	35.16 ± 3.67
EPE 250	21.67 ± 3.19 *
EPE 500	16.33± 2.59 *
EPE 1,000	13.33 ± 2.63 *
Piroxicam 20	8.67 ± 4.13 *

Data was analysed using one-way ANOVA followed by Dunnett's Post Hoc test and presented as mean ± SEM, * indicates significance at $p<0.05$ as compared with Control group, n=6, EPE= Methanol root extract of *E. psoraloides*,D/W = Distilled water group,n = 6

Appendix III: Effect of Methanol Root Extract of *Eriosema psoraloides*on Thermally-Induced Pain in Mice

Groups (mg/kg)	Mean reaction time ± SEM (secs)				
	0 min	60 mins	90 mins	120 mins	150 mins
Normal Saline (10)	2.41 ±0.12	2.85 ±0.57	3.50 ±0.45	4.04 ±0.67	2.80 ±0.30
EPE (250)	4.24 ±1.88	3.98 ±0.48	3.43 ±0.88	5.74 ±1.05	3.56 ±0.76
EPE (500)	2.59 ±0.39	4.02 ±0.34	5.61 ±1.92	3.09 ±0.70	5.75± 1.12
EPE (1,000)	2.59 ±0.65	2.15 ±0.46	5.43 ± 1.91	5.41 ±1.07	6.21 ±2.09
Morphine (5)	2.46 ±0.32	4.08 ±0.59	11.02 ±3.03#	7.57 ±2.05#	14.78 ± 1.57*#

Data was analysed using Split Plot analysis of variance (SPANOVA) followed by Bonferroni Post-hoc Test. Data is presented as Mean ± Standard Error of Mean, * indicates significance at$p < 0.05$ compared to Normal Saline, # indicates significance at$p < 0.05$ compared to 0 min. EPE = Extract of *Eriosema psoraloides*, min = minutes*;* S.E.M. = Standard Error of Mean.

Appendix IV: Ethical Approval

Committee On Animal Use And Care
Directorate of Academic Planning & Monitoring
Ahmadu Bello University, Zaria

Appl No: ABUCAUC/2021/Pharmacology & therapeutics/027 13th April, 2021
Approval No: ABUCAUC/2021/027

Prof. A.U. Zezi,
Department of Pharmacology & therapeutics,
Ahmadu Bello University,
Zaria.

Dear Sir,

APPROVAL OF RESEARCH STUDY 'ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF ERIOSEMA PSORALOIDES (LAM.) G.DON IN LABORATORY ANIMALS'

This is to convey the approval of the ABUCAUC to you for the aforestated study domiciled in the Department of Pharmacology and Therapeutics. The approval is predicated on the assumption that you shall maintain and care for the Experimental Animals as approved after the visitation of the Committee.

Monitoring of the Research by spot checks, invitations or any other means the Committee deems fit shall be undertaken at the convenience of the Committee.

This approval can and shall be revoked should a significant breach in the terms and condition of the approval occur. It is hence your responsibility to ensure that the agreed terms are maintained to the end of the Study.

The said approval shall be posted on the ABUCAUC Page on the University's website.
Note upon completion of the research, ethical clearance certificate will be issued.

A.B. Abdulkadir
For: Chairman, ABUCAUC.

CC. Director, IAIICT
Dean, Faculty of Pharmaceutical Sciences
HOD, Pharmacology and Therapeutics
Prof. C. A. Kudi, Chairman, ABUCAUC

Director: [illegible] Tel: [illegible]
Chairman: Prof C. A. Kudi DVM, MSc, PhD (ABU), PhD (Lec.) FCDE (Pcn) 08065978003 [illegible]