PHARMACOLOGICAL EVALUATION OF THE EXTRACT FROM *Tephrosia bracteolata* LEAVES IN LABORATORY ANIMALS

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DECLARATION

I declare that this work is wholly the author's, and has not been part o any presentation for higher degree.



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CERTIFICATION

This thesis entitled "Pharmacological Evaluation of the Extract from *Tephrosia bracteolata* Leaves in Laboratory Animals" by **Onaolapo, Mary Aderoju Olalonpe** meets the regulation governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

Dedicated to the glory and honour of the great God of heaven who determines the times and the seasons.

Quote for thought.

"***Let not the wise man glory in his wisdom neither let the mighty man glory in his might, let not the rich man glory in his riches; But let him that glorieth, glory in this: That he understandeth and knoweth me --------***

----------"

(*Jer. 9:23&24*)

BASHIR IBRAHIM L

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ABSTRACT

Tephrosia bracteolata is a legume widely spread in the Northern and Southern states of Nigeria and commonly known as "Kimi or Shege" in Hausa and "Roro" in Yoruba. The plant is employed as a fodder for animals and for various medicinal purposes, especially as a pain killer. Hence, the present pharmacological studies were carried out on the plant leaves. The crude extract of the leaves of T*ephrosia bracteolata,* which was found to be non-toxic (LD_{50} > 5,000), induced a dose-dependent contractile response in guinea pig ileum. It possesses no significant antimicrobial activity, but contains a potent analgesic principle. The most effective dose for the analgesic activity was 400 mg/kg. The crude extract was also found to possess a potent antipyretic activity at a dose of 600 mg/kg, which compared very favourably with that of the standard drug, aspirin. The anti-inflammatory studies revealed that the crude extract inhibited inflammation. At a dose of 800 mg/kg body weight, the inhibition of a carrageenan induced oedema two (2) hours post-induction was 57 percent compared to the 40 percent inhibition by the standard anti-inflammatory drug, indomethacin. The plant extract did not potentiate barbiturate sleeping time, thus; the plant extract contains principles possessing a potent non-narcotic analgesic, with both antipyretic and anti-inflammatory, properties.

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CHAPTER ONE

1.0 INTRODUCTION

Plants are ubiquitous in nature and many animals depend largely or entirely on plants and water for their sustenance. The health, growth and performance of animals especially livestock are affected by the chemical composition of the plants they consume. However plants are generally studied either for their beneficial or toxicological effects, while some are beneficial and edible, others are undesirable and even toxic (Dalziel, 1957; Nwude, 1979).

In Africa, it is a common knowledge that plant extracts are employed to treat many ailments. Hence, some plants serve as sources of drugs in orthodox and especially in traditional human and veterinary pharmacopiea. A large number of plants have been employed for various purposes in traditional medicine, including the following:

(i) Mahogany bark is used as remedy for anaemia, amenorrhroea and other blood diseases;

(ii) The tobacco plant (*Nicotiana tabacum*), is employed as a good remedy for convulsion, giddiness and other nervous diseases;

(iii) *Cinchona* bark, which contains quinine is used in the treatment of malaria;

(iv) Red raspbery, has been found to be active in reducing labour pain, giving strength after birth and increasing flow of breast milk; and

(v) *Clansena anisata*, is used for the relief of pains, lumbago, rheumatism and angina pectoris (Sofowora, 1984).

Many other plants have been named in several publications (Dalziel 1957; Watt and Breyer-Bandwijk, 1962; Trease and Evans, 1978) as sources of traditional medicine.

1.1.0 *TEPHROSIA*

The plant genus *Tephrosia* is a legume with many species, some of which have been used for many beneficial purposes. Although many members of this plant genus have been used for fish poisoning (James, 1987), a few other members have other uses. The bark of *Tephrosia acquilata* is employed by the Hausas for the relief of abdominal pain (Watt and Breyer-Brandwijk, 1962). *Tephrosia vogelli* is said to have a numbing effect on the feet of those that enter into the river where the plant has been used to poison fishes (Nwude, 1979). *Tephrosia macropoda* has been explored for its insecticidal potentials, while *Tephrosia atroviolacea* is administered to women after childbirth to ease stomach pain or discomfort (Sofowora, 1984).

1.1.1 *Tephrosia bracteolata*

The plant *Tephrosia bracteolata* grows in the open country (Dalziel, 1957, Hutchinson 1958), and has a wide spread habitat in Northern and Southern Nigeria. It is employed for different purposes among the local people. In Hausa, the plant is known as "Kimi" or "Shege", while among Yorubas it is referred to as "Roro". It is an erect branched under-shrub, usually two to three feet high but may reach eight feet. The plant has long straight thinly silky branches and bright pink or purple flowers. The fruits are narrowly linear, five to six centimetres long and four millimetre broad, erect and usually curved upwards. The seeds are contained in pods. The leaflets are four and half to eight centimetres long, three to five centimetres broad, linear, silky pubescent beneath, glaborous above with bracteoles broadly ovate, five millimetres enclosing the buds (Hutchinson, 1958). It grows in the



Fig. 1.0 ***Tephrosia bracteolata*** **Plant**

open country and is widespread in tropical Africa (Dalziel, 1957; Hutchinson, 1958). It is common during the rains, but scantly distributed during the dry season. Herbal preparations of *Tephrosia bracteolata* are used as analgesics in ethnomedical practices of several tribes in Northern Nigeria (Dalziel, 1957).

According to Dalziel (1957), the plant is used as fodder for horses, while the leaves and roots serve as ingredients of many herbal preparations. Information obtained through personal communications, and interviews conducted in villages around Malumfashi Local Government Area of Katsina State, Nigeria in 1997 confirmed that *Tephrosia bracteolata* leave-preparation is used for the treatment of whitlow, tooth-ache, ear-ache, and open wound. It is also freely fed to animals. Although these observations remain unconfirmed in the laboratory, it was reported by Jibril (1989) that extract from *Tephrosia bracteolata* leaves was poisonous.

Based on this background, an investigation into the crude extract of *Tephrosia bracteolata* leaves was carried out to determine the toxicity and some medicinal properties of the plant. If toxic, the dose at which toxicity occurs need to be established, since it is being consumed by both man and animals. It is believed that this work could lay a good foundation for future studies on the active chemical components of *Tephrosia bracteolata* leaves. The experiment was, therefore, designed to include the following:

1. Determine the acute and subchronic toxicity of the crude extract.
2. Monitor the effects of *Tephrosia bracteolata* crude extract on isolated smooth muscle preparations.
3. Assess the antimicrobial activity of the crude extract.
4. Determine analgesic effects of the crude extract.
5. Study the antipyretic effect of the crude extract.

6. Assess the anti-inflammatory effect of *Tephrosia bracteolata* crude extract, and
7. Study the effect of the extract on barbiturate sleeping – time.

The work is expected to improve on the basic scientific phytochemical data on *Tephrosia bracteolata* leaves.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.10 MEDICINAL PLANTS

In Africa, many plants are in common use, most of which grow near houses and are easily overlooked by urban dwellers. However, a professional herbalist grows many of them in his compound, knowing that these plants are frequently required on their own or in combination with other herbs for very common ailments in Africa (Sofowora, 1984). In many cases, the whole plant is not used but certain parts (like the leaves, roots, bark etc) are normally employed.

It is of interest to note that some of the plants employed in traditional medicine are toxic (Dalziel, 1957). However, the dosage at which the constituent is utilized in herbal medicine may be insufficient to cause toxicity.

In addition, many of these plants are used in combinations with other agents, which may have ingredients that attenuate or even counteract the toxic constituents. The important fact is that some of these plants do have toxic side effects, which may be mild or strong depending on the idiosyncratic reaction of different species and individual users (Sofowora, 1984). Studies on toxicity of herbal preparations are essential public health requirements.

Farnsworth and Bingel (1977) who investigated the active plant principles of 325 plants found that the majority of the biologically active plant principles include alkaloids (22%), sesquiterpenes (14%), diterpenes (8%), triterpene saponins (7%), triterpene aglycones (4%), flavonoids (6%), sterols (5%), coumarins (5%), quinines (5%) and monoterpenes (4%). Hence, the extraction of bioactive agents from plants is one of the most intensive areas of natural product research today (Sofowora, 1984).

These biologically active plant principles can be mildly toxic or non-toxic depending on their chemical composition and dosages. Therefore some of these bioactive agents have been extensively studied for their physiological, pharmacological and toxicological effects and for their potential values as sources for future drugs (Farnsworth and Bingel, 1977; Culvenor, 1980; Sofowora, 1984). Generally in plants, these bioactive agents tend to accumulate in:

(i) Very active tissues;

(ii) Epidermal and hypodermal tissues;

(iii) Vascular sheaths, and

(iv) Latex vessels (Robinson, 1968)

The screening of plants for bioactive substances, in addition to its use for drug development, is to demonstrate that the reported physiological activity of the plant has scientific basis.

According to Farnsworth and Bingel (1977) and Sofowora (1984), the mode of action of the whole plant or plant part producing the biological effect can also be better investigated if the active principle is characterized. Very often, such studies can lead to a better application of the drug through better formulation into appropriate dosage forms and may also lead to better understanding of the disease itself. Broadly speaking, studies on bioactive agents from plants involve two fundamental procedures:

(i) Biological screening for the physiological effects which a plant or the extract produces, and

(ii) Phytochemical screening for the active compounds involved.

Each of the above procedures involves various steps. For example, the biological screening normally require animal mode studies to test for toxicity and physiological effects such as analgesia and some others. The

phytochemical screening involves testing the plant extract for various possible chemical constituents like alkaloids, saponins, tannins and others (Sofowora, 1984).

2.2.0 THE CONCEPT OF PAIN

Plants or their extracts are employed for various medicinal purposes, especially for the relief of pain. According to Ruth (1983), pain is the most intrusive of all symptoms that commonly lead a patient to consult a physician or a dentist. Pain has a useful aspect of serving as an indication of the presence and the location of a disease, and for alerting us of threats to bodily injury (Barttal, 1984). However when pain exceeds its protective functions, it becomes destructive. Therefore, relief of pain is one of the greatest objectives in medical practices.

According to Webster's dictionary, pain is "a basic bodily sensation induced by a noxious stimulus received by naked nerve endings, characterized by physical discomfort (as pricking, throbbing or aching) and which typically leads to evasive action". However, according to Vickers *et al.* (1978), pain is difficult to assess, and drugs which will relieve experimentally produced pain are not necessarily equally effective in pain of pathological origin and vice versa. In line with previous works, (Melzak and Wall., 1965; Ruth, 1983; Barttal, 1984), three basic concepts on pain will be considered here and these are:

(i) The Concept of Pain Receptors

There are specific receptors for pain which are simple, unencapsulated nerve endings present in skin mucous membranes, skeletal muscles, blood vessels, visceral organs, etc. These pain receptors respond to a variety of

stimuli including mechanical (pressure or stretching), thermal, electric and chemical. It is important to note that the specific pain receptors in different areas are frequently more sensitive to one type of stimulus than another (Ruth,1983; Barttal, 1984).

(ii) **The Concept of Perception of Pain**

The physiology of pain perception is complex. It involves discriminative capacity to identify the location, the onset, the intensity and the duration, as well as the physical characteristics of the eliciting stimulus. Yet, the physiology of the perception of pain may be almost the same in all animals (Melzak and Wall, 1965; Ruth, 1983).

(iii) **The Concept of the Reaction to Pain**

The reaction to pain is highly individualized in humans. It is determined by the social mores, the psychic state of the individual, the emotions and the interpretation of the painful stimulus in terms of past and present events. The complete pattern of response to painful stimuli is, to some extent, a learned behaviour, unlike pain perception which is innate. The reaction to pain is also influenced by the significance of the pain to the individual (Melzak and Wall, 1965; Ruth, 1983; Barttal 1984).

2.2.1 Theoretical Concepts of the Mechanisms of Pain

The theory of specificity quoted by Bragin (1991) is the most orthodox in the explanations of the mechanisms of the origin of pain. According to this theory, pain is a specific modality formed under the influence of pain stimuli only. It is suggested that there is a direct correlation between the intensity of the stimulus and sensation of pain. This is because there are undifferentiated

free nerve endings localized in skins, tendons, muscles, vessels of internal organs, serious membranes, etc. which are pain receptors, responsible for pain impulse. This pain impulse is relayed to the posterior horns of the spinal cord along specific fibres. Pain signals are relayed via the lateral spinal thalamic tract to the specific pain center such as the thalamus and thereafter to the cerebrum where a sensation referred to as pain is produced. The receptors are activated by strong extraneous and deleterious stimuli such as mechanical injury, high thermal and electrical influences. These receptors are called pain receptors or nociceptors.

However pain excitation may occur during the stimulation of not only specific nociceptors, but also polymodal or even non-nociceptive receptors. Besides, pain sensation may occur as a result of action of a non-painful stimuli. According to Beecher (1957), the peripheral mechanism of pain originates mainly as a result of the ratio between afferent impulses and summation of sensitive stimuli (even non-painful), which have attained the critical level, causing the origin of pain sensation.

Recently, the gate - control theory of pain (Bragin, 1991), has gained wide popularity. This theory explains the origin of pain based on a neurophysiological modulation of pain signals at the spinal cord level. The theory suggests a model in which nerve impulses evoked by pain stimulus are controlled in the posterior horn of the spinal cord, within which are specialized mechanisms, involved in the modification of the ascending of the stimulus (nociceptive flow) along the peripheral nerve fibres. Whenever the gates are opened, the nociceptive information reaching the brain induces the formation of psychophysiological state called pain. Whenever the gates are partially or completely closed, the transmission of nociceptive afferent flow into the brain markedly decreases or ceases completely. The functional status of the gates,

and consequently the ability of nociceptive afferent flow to pass through them into the supraspinal formations, depend on (i) the ratio of functional activity between the thick and thin afferent fibres and (ii) their interactions with the activity of the ascending fibres from higher brain centres. The gate control theory of pain is of value not only in explaining the mechanisms of organization of pain sensitivity and its suppression as a result of various influences, such as electro-stimulation, acupuncture, pharmacological analgesics and other compounds but also in the practical aspect. For example, electro-analgesia which is a new method of anaesthesia has been suggested based on this concept.

Kryzhanovsky (1980) proposed the theory of generator mechanisms of central pain syndromes. According to this theory, the cause of pain syndrome is likened to the origin of generator of extraneous excitation as a new functional formation at different levels of the central nervous system. It is not as a result of changes in the nociceptive flow from the periphery due to impairment of control of flow of nerve impulses at the spinal and supraspinal level. This concept proposes that one of the major factors in the genesis of central pain is the depression of inhibiting influence, leading to intensive excitation of neurons in different brain structures.

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2.2.2 The **Treatment of Pain**

Pain is generally treated by a class of drug known as analgesics, (pain killers). According to Vickers *et al.* (1978) and Agada (1991), any chemical agent used to produce loss of pain with or without loss of touch can be regarded as analgesic. These agents are normally applied to various parts of the body depending on the location of the pain. They can be injected into the spinal theca where all nerve roots in the vicinity are affected, or simply

injected into the subcutaneous tissues where mainly the sensory fibres from the skin are affected. The various local analgesics in use today differ in toxicity, potency and duration of action which are strictly linked to their chemical compositions (Vickers *et al.*, 1978; Barttal, 1984; Agada, 1991). There are two main groups of analgesics.

(i) the local and non - narcotic analgesics, and

(ii) the general (opium) or narcotic analgesics.

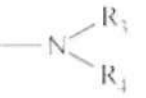
2.2.3 The Non-narcotic Analgesics - Antipyretics

These are agents which, when applied to nervous tissue, can prevent conduction of the nerve impulse in any part of the neurone (Vickers *et al.*, 1978; Ruth, 1983; Barttal, 1984). In most cases, when applied, they produce loss of pain without producing unconsciousness or sleep. According to Vickers *et al.* (1978), most synthetic non-narcotic analgesics are either tertiary amino esters or amides of aromatic acids with the basic formula:

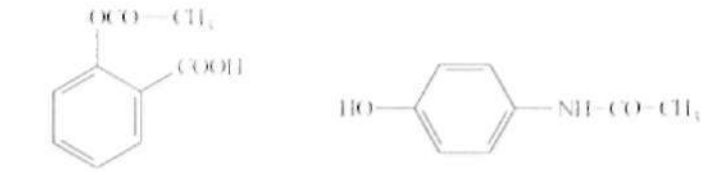


Fig. 2.0: Basic and General Formula of Simple Analgesics

These molecules consist of three parts:

- acidic - R_1CO
- alcoholic $-OR_2$
- terminal amine 

Examples of non - narcotic analgesics are acetylsalicyclic acid (ASA), popularly called aspirin and paracetamol.



ASA
o-acetoxybenzoic acid

p-acetamidophenol

Fig 3.0: Chemical Structure of Aspirin and Paracetamol

2.2.4 **Mode of Action**

2.2.4.1 **Mechanism of Action of the Analgesics**

According to Winter (1965) and Vickers *et al.* (1978), it is only the free base compounds, which have high lipid solubility that are effective. They act by preventing the depolarization of the axon, which accompanies transmission of the action potential in the nerve. They do this by preventing the migration of ions across the nerve membrane, and are believed to compete with calcium ions at some receptor sites that control permeability. Thus, they reduce resting membrane permeability, without producing any charge on the resting membrane potential. Fine fibres are blocked before larger ones. Therefore pain, temperature, touch and motor functions are lost in that progressive order. In a medullated nerve, the block occurs only at the nodes of Ranvier.

Generally speaking, a non-narcotic analgesic may possess three principal qualities of action; they may act as antipyretic, analgesic and anti - inflammatory agents (Ose, 1993). However, these properties are not correlated quantitatively; in one an antipyretic action may be dominant, while in another excellent anti-inflammatory properties may exist without marked analgesia or antipyretic action. Yet in others, analgesia may be present at dose levels at which no anti-inflammatory or anti-pyretic action can be demonstrated (Vickers *et al.*, 1978; Barttal, 1984).

2.2.4.2 **Mechanism of Action of Antipyretic Drugs**

Normal body temperature is maintained within limits by a balance between the heat producing and heat dissipating mechanisms of the body. The peripheral mechanisms of production and loss of body heat are regulated by certain nuclei in the hypothalamus (Winter, 1965; Ruth, 1983). The action of the antipyretic drugs is mainly on the hypothalamus and not on the peripheral blood vessels or sweat glands. The antipyretic action is usually rapid and effective in febrile patients by causing an increase in heat loss in the following ways:

(i) increasing peripheral blood flow through peripheral vasodilation;

(ii) producing a shift in water to the bloodstream with a corresponding dilution of the blood; and

(iii) increasing perspiration (Ruth, 1983).

2.2.4.3 **Mechanism of Action of Anti-inflammatory Drugs**

Inflammation is a reaction of the local micro-circulation to tissue injury or more simply a defence reaction to injury caused by mechanical trauma, exposure to chemicals and antibody-antigen reactions (Ose, 1993). Inflammation reaction is characterized by transient arteriolar constriction followed by a prolonged dilation of the arterioles, capillaries and venules. Inflammation can either be acute (self-limiting), in which the stimulus is severe but transient, or it can be chronic if the stimulus is persistence, as in rheumatoid arthritis (rheumatism). However, the cardinal features of inflammation are:-

(i) dilation of blood vessels in the inflammed area,

(ii) increased capillary permeability and leakage of large molecular compounds,

(iii) swelling or oedema formation with fluid in the extravascular site.

(iv) pain and tenderness in the inflammed area, and

(v) migration of phagocytic cells, e.g. polymorphonuclear cells into the inflammed area (Green, 1978; Vickers *et al.*, 1978; Ose, 1993;).

The major effects of anti-inflammatory agents are mediated peripherally at the site of inflammation. They are effective in preventing the oedema and pain of experimental inflammation (Vickers *et al.*, 1978; Ruth, 1983; Agada, 1991). According to recent scientific observations (Moncada and Vane, 1980; Barttal, 1984; Agada 1991), prostaglandins have been markedly implicated in potentiating the effects of chemical mediators of inflammation such as 5-hydroxytriptamine (5-HT, also known as serotonin) and bradykinin. Anti-inflammatory drugs are believed to block the early stage in the synthesis of prostaglandins.

2.2.5 The **Narcotic (Sleep - Inducing) Analgesics**

These groups of drugs induce narcosis or sleep. All the members of this group are complex chemical compounds. Some occur naturally, but others are prepared synthetically. They include all the alkaloids of opium, especially morphine and its derivatives (George, 1965; Vickers *et al.*, 1978) (Fig. 4.0).

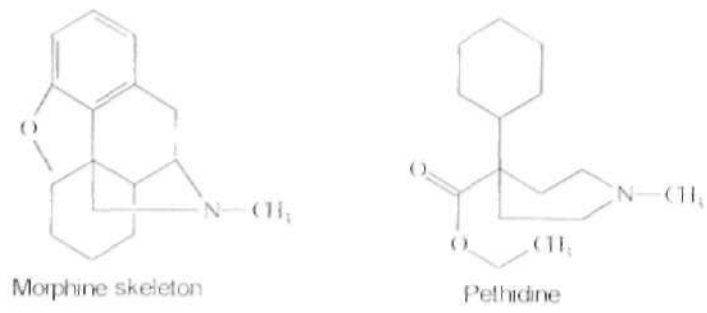


Fig 4.0: Chemical Structure of Morphine and Pethidine

The members of this group are the most potent analgesics. These drugs are effective against pain arising from superficial structures such as skin and mucous membranes and against the more deep seated pain, arising from joints, periosteum and muscles, and also in visceral pain due to distension of hollow viscera. Both the naturally occurring and the synthetic drugs of this group appear to have a fundamentally similar mode of action (Ruth, 1983; Barttal, 1984; Agada, 1991). The morphine-like analgesics are often referred to as narcotic analgesic, and their actions have three basic components. These are:

(i) elevation of pain threshold,

(ii) dissociation of pain perception from reaction to pain, in such a way as to free pain from its implications, and

(iii) production of lethargy and sleep.

Their precise site of action is, however, not known yet.

2.3.0 METHODS OF EVALUATING MEDICINAL PROPERTIES OF PLANT MATERIALS.

To evaluate a whole plant or its part (for example, leaves, seed, root, bark etc.) for its medicinal properties, a number of laboratory techniques are normally employed. From results obtained through such studies, a pharmacological profile of the plant can be deduced. The basis for some of these methods are discussed below.

2.3.1 Toxicological Evaluation of Potential Herbal Source of Drugs

Before a new drug is accepted for human use, the agent is subjected to toxicity studies in laboratory animals, since the toxic effects produced in animals when properly quantified apply to toxic effects in humans. The first

toxic test is the estimation of the LD_{50} in two animal species by two routes of administration, one of which is the expected route of usage in humans. The next test for toxicity is by sub-acute exposure usually for 90 days. This is also done in two species and in at least three doses. A variety of parameters are monitored during this test and organs as well as tissues are examined for pathologic lesions (Culvenor, 1980).

The long-term or chronic studies are done for drugs undergoing clinical trials. An exposure time of 6 months or more is required, but usually up to 2 years. These studies are used to determine the carcinogenic potential of the chemical or drug. Additional toxicologic studies that may be performed include teratogenicity, mutagenicity as well as embrotoxicity studies. Various other toxicity studies may be recommended, depending on the nature and intended use of the drugs (Rall, 1991; Van Gelder *et al.*, 1976).

2.3.2 Measurement of Analgesic Activity

Most tests for the determination of analgesia depend on the application of an external stimulus to an animal and the observation of typical changes in behaviour as the animal attempts to escape from the situation. In many tests the animals' response is a squeak, in others it moves the hurt part of its body and where heat is applied to the feet, it may move its fore-paws and blows on them. In all these tests, it is not possible to say that the sensations in the animal are similar to those experienced by man or that the animals feel pain in exactly the same degree as man does. However, results obtained in animal tests usually provide indication on the mode of activity of drugs in man (Anonymous, 1970; Mafwalal, 1993).

The following methods of evaluating noxious stimuli may be used, and the effects of drugs can be assessed as graded response.

(i) **Thermal Methods**

These methods are the most widely used techniques for screening new compounds for analgesic activity (Ramadan *et al.*, 1973; Vohora *et al.*, 1997). Heat is a frequently employed measurable stimulus. In man when the skin temperature reaches about 44 °C, the sensation of heat changes to that of pain. The heat stimulus can be directed to the skin of animals by means of a focused lamp (radiant heat) or applied directly by means of a heat conducting system (hot plate). It is convenient to employ a stimulus of constant intensity and vary its duration of application. The time for reaction by the animal is taken as a measure of the pain threshold.

(ii) **Mechanical Methods**

The use of pressure on the skin or tail, or the distension in the hollow viscera to produce pain are among the oldest techniques used to assay analgesic activity. These methods have the disadvantages of producing tissue injury and lacking in precision of end point for the nociceptive stimulus (Aliyu and Nwude, 1985; Vohora *et al.*, 1997).

(iii) **Chemical Methods**

The major chemical assay procedure is the writhing test or the mouse peritoneal test. This technique and its modifications are suitable for general screening of all types of analgesics. The chemical compounds often used for the studies include: 2 - phenyl - 4 - benzoquinone, acetic acid, bradykinin, sodium iodomethamate and hydrochloric acid.

Most non-narcotic analgesic do show activity when tested in this assay procedure, but many other classes of compounds including the antihistaminics and local anaesthetics are also active in this assay (Anonymous, 1970; Vohora *et al.*, 1997).

(iv) **Electrical and Behavioural Methods**.

This method employs electric current as the noxious stimulus. However, the use of electric shock as a nociceptive stimulus is froth with difficulties and gives rise to inconsistent results despite the simplicity with which it can be applied (Aliyu and Nwude, 1985; Vohora *et al.*, 1997). Those methods which combine a nociceptive stimulus produced by electric stimulation with any other method is usually followed by a response more complex than, for instance, the squeak, turning of the head or lifting of a stimulated limb, and have been designated behavioural (Anonymous, 1970).

However, it is important to measure not only the intensity but also the duration of effect of drugs to avoid producing permanent damage to the tissue since this will prevent repeated observations. Preferably the best method should provide a graded relation between intensity of pain stimulus and dose of analgesic needed to suppress the pain. In this way, a quantitative assessment of analgesic activity may be obtained. Appendix IA is a table adapted from Parkhouse *et al.* (1979), showing analgesic testing methods in experimental animals. According to Parkhouse *et al.* (1979), the subjective nature of pain makes its evaluation especially in animals difficult.

2.3.3 Physiological Studies (Electromyographic Methods)

The effect of various analgesics or compounds possessing analgesic activity can be tested on isolated smooth muscles. This is because it is known that analgesics are blockers of nerve impulses in any part of the neurone (Vickers *et al.*, 1978).

According to Aliyu and Nwude (1985), smooth muscle is the tissue of visceral organs and many sphincter membranes. It is distinguished anatomically from skeletal and cardiac muscles because it lacks striations, but both syncytia and discrete fibres are found. The distinctive characteristics of the smooth muscle are to a large degree found in the nature of its contractions. These contractions are slower than those of either cardiac or skeletal muscles. Smooth muscle is essentially always contracted to some degree, thus maintaining a basal tone.

Probably, the most distinctive feature of the smooth muscle is its plasticity. That is its ability to contract, relax, or maintain the same amount of tone. The control of smooth muscle is performed by the automatic nervous system. Sympathetic stimulation causes, among other things contraction of the smooth arteries and veins. Parasympathetic stimulation increase the rate and strength of smooth muscle contractions in the wall of the gastro - intestinal tract (Aliyu and Nwude, 1985; Anchola *et al.*, 1993; Aguiyi *et al.*, 1997).

2.3.4 Potentiation of Barbiturate Sleeping Time

Many drugs depress the central nervous system and produce unconsciousness or sleep in animals such as rats and mice. The righting reflex can be used to assess whether or not animals are "asleep" (Vohora *et al.*, 1997). In addition the potentiation of barbiturate sleeping time can be

used to assess the effect of drugs on the central nervous system (Anonymous, 1970; Aliyu and Nwude, 1985).

2.3.5 Antimicrobial studies

The whole basis of antibacterial therapy depends upon the action of a drug either to kill or to prevent the growth of bacteria without harming the host.

According to Bauer *et al.* (1966) and Cruickshank *et al.* (1975), a crucial factor in antibacterial chemotherapy is the minimal inhibitory concentration (MIC) of the drug against a specified bacterium. The MIC for a given culture system is that concentration of agent below which bacterial growth is not prevented. Plainly the lower this concentration, the more potent the antibiotic. The clinical value of the drug then depends upon the relationship between the MIC and the plasma concentration, at which adverse effects occur.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 SAMPLE COLLECTION AND IDENTIFICATION

The plant material was collected from the open lands around the Ramat Hall and between the Institute of Agricultural Research (IAR) and the residential Area F of the Ahmadu Bello University (ABU), Zaria. The sample was identified at the Herbarium of the Department of Biological Sciences, the Department of Soil Science of IAR and the herbarium of the National Animal Production Research Institute (NAPRI) ABU, Zaria. A sample of the identified plant has been deposited at the herbarium of the Department of Physiology and Pharmacology of Faculty of Veterinary Medicine, ABU, Zaria. Fresh leaves of *Tephrosia bracteolata* were harvested from the plants without regard to the age of the leaves between July and August, 1997 and dried openly in the sun in the above Departmental laboratory.

3.1.1 LABORATORY ANIMALS USED

Mature Wistar rats (60 – 300 g) and mice (10 – 30 g) of both sexes were purchased from the animal house of the Department of Veterinary Pharmacology and Physiology, ABU, Zaria. The rats were of about 8 – 12 weeks old, while the mice were 8 – 10 weeks old. The rats and mice were fed on growers mash (feed) purchased from Pfizer Nigeria Limited. The feed was made into balls by adding cassava flour (flour) as a binding agent in ratio 5:1 (feed: flour).

New Zealand white rabbits (lapine) were purchased from Samaru market. The rabbits weighed between 1.5 - 1.7 kg and were aged 12 –16 weeks. The rabbits were maintained on vegetables (carrots, lettuce and cabbage leaves). The rats and mice were kept separately in plastic cages while the rabbits were kept in metal cages.

All the animals were acclimatized for two weeks in the laboratory before they were used for each of the experiment.

3.2.0 CHEMICALS / REAGENTS

The following chemicals were used at various stages of the experiment.

(i) 95% Methanol

(ii) Ammonium hydroxide

(iii) Hydrochloric acid

(iv) Sodium chloride

(v) Magnesium chloride (hydrated)

(vi) Calcium chloride (hydrated)

(vii) Sodium bicarbonate

(viii) Potassium chloride

(ix) Sodium phosphate

All the reagents used are of analytical grade and products of the British Drug House (BDH), Poole, England.

3.3 EQUIPMENT

Below is the list of the various equipment used.

(i) Physiography four – A model V100KG. E and M instrument Co. INC. Houston Texas.

(ii) Fisher serological Bath. Fisher Scientific Co. New York, USA.

(iii) Harvard Trip Balance. Ohaus Scale Corp. Union New York, USA.

(iv) Mettler P161. Mettler instrument AG CH – 8606 Greifensee – Zurich, Switzerland.

(v) *Soxhlet Extractor. Pyrex® brand, USA. Friedrich's type with Coil* (Corning 3880).

(vi) Constant Temperature Circulator. Ealing, UK.

(vii) Transducer Physiograph. MK III Ser no 1770. E and M instrument Co. INC. Houston Texas.

(viii) Preamplifier physiograph MK III Ser no 1772. E and M instrument Co. INC. Houston Texas

(ix) Amplifier physiograph MK VII. E and M instrument CO. INC. Houston Texas.

(x) Stimulator physiograph. MK V. E and M instrument CO. INC. Houston, Texas.

(xi) Paper Control. MK II. E and M instrument CO. INC. Houston, Texas.

(xii) Büchi Rotatory Evaporator with spiral condenser Glass and Teflon® construction Buchler PTFE IGN Pyrex® brand, USA

(xiii) Plethysmometer 7150. Ugo Basile Biological Research Apparatus 21025 Comerio- Vares. Italy

(xiv) Christy Milling Machine. Laboratory Mill Ser no. 49814. Christy and Norris Limited Process Engineers Chemsford, England.

3.4 EXTRACTION

The dried leaves were pulverized at NAPRI,ABU, Zaria using Christy laboratory milling machine with a 1mm filter. About 35.5 g of the pulverized leaves was extracted continuously with 95% methanol using Soxhlet extractor for 12 hours (Awe *et al.*, 1997). The methanolic extract was filtered using glass wood and filtrate was concentrated using Buchi rotatory evaporator, connected to an automatic thermo-regulator adjustable from – 25^{0}C to 90^{0}C. The crude extract was then kept in a dessicator and weighed daily until the last three consecutive weights were recorded to be constant.

The percentage yield was calculated and the crude extract was kept for use. When required, the extract was taken and dissolved in a known volume of distilled water to obtain the required concentration.

3.4.1 General Screening for Alkaloids

General Test: 1 ml of the crude extract suspension in distilled water was tested with litmus paper and dissolved in 0.2% HCl and 2 - 3 drops of Dragendoff's reagent was added.

3.5 EVALUATION

3.5.1 Toxicity studies

3.5.1.1 Acute Toxicity.

The method of Lorke (1983) was employed in which three different doses (10, 100 and 1000 mg/kg) of the crude extract were administered to adult mice and rats respectively, by using a curved needle to which a catheter has been fixed. Three animals were chosen for each dose level. A forth group containing 3 rats and 3 mice respectively, was administered 0.2 ml of distilled water, to serve as the control. The animals were closely monitored every 30 minutes, for the first 3h after administration of the crude extract and hourly for the next 6h, observing to see any adverse effects and the time of occurrence. The animals were then observed for another 72h.

The second stage of 1,600, 2,900 and 5,000 mg / kg dose levels of the crude extract were administered to three groups of fresh adult rats and mice respectively, by using a curved needle to which a catheter has been fixed. In each group only one animal was required for the test. The forth group also had one animal of rat and mice respectively and was administered 0.2 ml distilled water to serve as control. The animals were observed every 30 minutes for first 3h, then hourly for another 6h and further observations for 72h (Lorke, 1983).

All the animals were sacrificed and post mortem examination conducted at the termination of the studies.

For all the animals used, food and water were withdrawn 12 hours before the administration of the crude extract of *Tephrosia bracteolata* leaves.

3.5.2 PHYSIOLOGICAL STUDIES

3.5.2.1 Effect of *Tephrosia bracteolata* Extract on Rabbit ileum

The animals were killed by a blow behind the head, exsanguinated and the abdomen opened. Clean isolated rabbit ileum, devoid of mesenteric tissue, was cut into pieces of about 2 - 3 centimetres, and were suspended (one at a time) with a tension of 2 grams in 2 baths of 100 ml and 25 ml capacities, maintained at 34 ^{o}C with a constant temperature circulator and containing Tyrode's solution of the following composition (μm/L): NaCl (137), KCl (2.68), $CaCl_2.2H_2O$ (1.84), $MgCl_2.6H_2O$ (1.03), $NaHCO_3$ (11.9) $Na_2H_2PO_4$ (0.45), glucose (5.5). Extract concentrations of 1, 5, 10, 20, 30, 40 and 50 mg/ml were tested on the ileum strips. The effect of acetylcholine 10^{-5} g/ml and atropine sulphate (10^{-8} g/ml) were also evaluated (Aguiyi *et al.*, 1997). The organ bath was connected via a myograph transducer to a physiograph for recording the rhythmic contractions. The chart speed was set at 0.2 cm/sec. After obtaining a control tracing, extracts of *Tephrosia bracteolata* at various concentrations of 1, 5, 10, 20, 30, 40 and 50 mg/ml were tested on the strips of the isolated smooth muscle. After testing with a known piece of tissue, the tissue set up was always washed 2 - 3 times before the addition of a new concentration.

3.5.3. Antimicrobial Studies

For the antimicrobial studies, both The Gram negative and Gram positive bacteria, were used.

For the Gram negative bacteria, *Escheria coli* NCTC 10418 and EC 189 a clinical isolate were used, while *Staphylococcus aureus* ATTC 13709 and S 40 were used for Gram negative bacterial. All cultures were obtained from the Department of Pharmaceutical Microbiology, Faculty of Pharmaceutics and Pharmaceutical Sciences, ABU, Zaria, Nigeria.

Nutrient agar medium (Cruickshank *et al.*, 1975; Williams and Popoola, 1983; Ummu-Kuthume, 1995) was used to prepare seeded plates. The bacterial were kept on agar slant at 4^0C until when needed.

To prepare subcultures, inoculums from the slant cultures were aseptically transferred to 5 ml of nutrient broth and incubated at 37 ^{0}C for 24h. One ml of each culture of the selected organism was transferred to 20ml of molten nutrient agar medium each and transferred to sterile petridishes and allowed to set. After solidification cups were evenly made with a sterile cork borer (internal diameter 7 mm) on the plates.

Each cup was filled with 0.1 ml each of the crude extract (2000 μg / ml) and the standard inoculums only as the control. The plates were left at room temperature for 1h for diffusion to take place, and incubated at 37 ^{0}C for 18h. The diameter of the zones of inhibition were measured using Thermonik antibiotic zone reader. The results of test organism was compared with that of the standard organisms.

3.5.4. **Analgesic Studies**

3.5.4.1. **Analgesic Triple Test**

(i) ***Electrical Stimulation***

Thirty mice were used and they were placed in six different groups of five animals each. Group one served as the negative control and received only the vehicle, (0.2 ml distilled water), group 2, received standard analgesic, aspirin (ASA) at 50 mk/kg body weight to serve as the positive control. Groups 3, 4, 5 and 6 received 200, 400, 600 and 800 mg/kg body weight of *Tephrosia bracteolata* crude extract respectively.

The route of administration for all the animals was interaperioneal (i.p.)

Electrodes were inserted into the skin of the animal and electric shocks of 3, 5, and 10 volts were respectively delivered continuously for 0.5 milliseconds at a frequency of 2 per second. A squeak, or a movement away from the source of the electrode, was regarded as positive (+ve), while a non - response was taken as negative (-ve), (Anonymous, 1970; Aliyu and Nwude, 1985).

This was repeated every 30 minutes for three hours and the mice were observed each time for responses.

(ii) ***Thermal Stimulation***

Six groups of mice were used consisting of five animals in each group. Group one was administered 0.2 ml distilled water, (negative control) while group two received 50 mg /kg body weight of a standard analgesic, aspirin, (positive control). Groups 3, 4, 5 and 6 were respectively administered 200,

400, 600 and 800 mg / kg body weight of the *Tephrosia bracteolata* crude extract. All the animals were administered intraperitoneally.

The thirty animals were subjected to thermal stimulation, one at a time and group by group, using Eddy's constant temperatures of 55 ± 1^0 C (serological bath). A restraining cylinder was used to keep the animal in place and the duration of stay of the animal before jumping up was recorded in seconds.

This was repeated every 30 minutes for 3 hours (Anonymus, 1970; Aliyu and Nwude, 1985).

(iii) ***Mechanical Stimulation***

Tail clip experiment of Aliyu and Nwude (1985) was employed. Thirty adult mice of five animals per group were used. Groups one and two served as negative and positive controls respectively. The mice in these groups were administered intraperitoneally with 0. 2 ml of distilled water and 50 mg / kg body weight of aspirin respectively. The mice in groups 3, 4, 5 and 6 were given intraperitoneally, 200, 400, 600 and 800 mg / kg body weight of the *Tephrosia bracteolata* crude extract.

A constant pressure was applied to the tail of each mice and when the animal squeaked or attempted to move its tail away from the source of pressure, it was regarded as a positive response (+ve) while the failure to do any of the above was taken as negative (-ve) response. This was repeated at interval of 30 minutes for 3 hours.

3.5.5 **Antipyretic Studies**

Procedure

The methods of Ramadan *et al.* (1994) and Awe *et al.* (1997) in which thirty five albino Wistar rats in seven groups of five animals each were used. Six groups were made hyperthermic by injecting subcutaneously 12 % yeast suspension at a dosage of 1 ml/kg body weight. The seventh group was not given yeast at all. The animals were left for 15 hours after which their temperatures were recorded and the groups were treated as follows:-

Group 1: Control 1, received 0.2 ml of distilled water.

Group 2: 50 mg/kg body weight of aspirin was administered to this group, i.p.

Groups 3, 4, 5 and 6: Each group was administered by intraperitoneal inoculation with 200, 400, 600 and 800 mg/kg body weight respectively with *Tephrosia bracteolata* crude extract.

Group 7: The animals in this group served as untreated control II and were neither administered yeast nor any drug.

After drug administration, the rectal temperature of each animal was measured hourly for six hours with a clinical thermometer calibrated in degree Celsius.

3.5.6 **Sleeping Time Potentiation Studies**

Procedure:

The methods of Awe *et al.* (1997) and Vohora *et. al.* (1997), were used as follows:

Group 1: Control group which was given 0.2 ml normal saline

Group 2: Chlorpromazine was administered i.p at 10 mg/kg body weight.

Groups 3, 4, 5, and 6: The animals in these groups were administered 200, 400, 600 and 800 mg/kg body weight of the *Tephrosia bracteolata* crude extract intraperitoneal respectively.

After thirty minutes, all animals were administered with 20 mg/kg body weight sodium thiopentone i.p. and positioned on their backs. The duration between the onset of the loss and recovery of righting reflex was taken as a measure of sleeping time.

3.5.7. **Anti - Inflammatory Studies**

The anti-inflammatory effect of the crude *Tephrosia bracteolata* extract was studied using thirty rats, divided into six groups of five animals per group. At the beginning of the experiment the thickness of the rat paw was measured for all the animals using plethysmometer, which is based on fluid displacement technique (Ramadan *et al.*, 1993; Awe *et al.*, 1997).

Thereafter, the groups were treated as follows:

Group 1 served as a non-treated control and was administered normal saline.

Group 2 was administered with 10 mg/kg indomethacin i.p as a positive control of a standard anti - inflammatory drug.

Groups 3, 4, 5, and 6 were respectively administered 200, 400, 600 and 800 mg/kg of the *Tephrosia bracteolata* extract, i.p.

After thirty minutes 0.1 ml of carrageenan was injected into the hind paw of each rat, and the paw thickness measured hourly for the first four hours, and at 6, 18, and 24 hours, post-induction of oedema. The mean response for each animal in each group was calculated as an index of oedema.

3.6 STATISTICAL ANALYSIS

The results were statistically analysed using Students' *t* – test correlation analysis and analysis of variance (ANOVA), and the appropriate graphs were drawn. Values of $P< 0.001$, $P< 0.01$ and $P< 0.05$ are considered statistically significant.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 EXTRACTION AND PERCENTAGE YIELD

The crude extract was a greenish solid, partially soluble in distilled water, with a percentage yield of 21.3.

4.1.1 Test for Alkaloids

The general test for alkaloids using Dragendoff's reagent was positive with immediate development of turbidity.

4.2 TOXICITY STUDIES

Table 1: Evaluation of Crude Extract *of Tephrosia bracteolata* Leaves **Toxicity in Rats and Mice Administered Intraperitoneally.**

Dose (mg/kg)	No of Deaths	No of Animals per Test	Survival	Mortality Ratio
10	0	3	3	0/3
100	0	3	3	0/3
1000	0	3	3	0/3
1600	0	1	1	0/1
2900	0	1	1	0/1
5000	0	1	1	0/1
Control	0	3	3	0/3

For all the animals no visible adverse reactions were observed up to 72 hours post - administration. In all cases, no death was recorded, even at a dose of 5,000 mg/kg body weight. When the animals, which were apparently healthy, were sacrificed and posted, no gross and histologic lesions were

seen in the organs. The restlessness observed in the rats when higher doses of 1,600 - 5,000 mg/kg were given may be attributed to the high volume of aqueous extract administered to the animals (> 0.8 ml) since they all recovered within a short time (about 5 minutes) and resumed eating.

The observation that a dose greater than 5,000 mg/kg body weight did not produce adverse effects implies that *Tephrosia bracteolata* is not toxic, and is safe for human and animal consumption. The high safety level is favourable for the widespread use of the plant among the local people as livestock feeds and as a pain reliever.

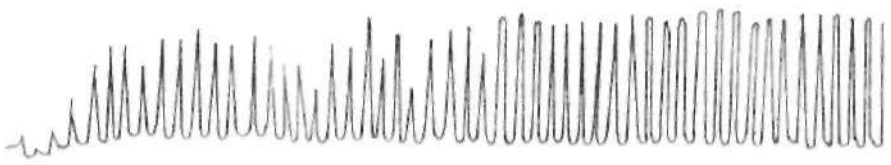
The fact that no mortality occurred in all experimental animals administered 5000 mg/kg of the extract during the acute toxicity studies indicated that the crude methanolic extract of *Tephrosia bracteolata* leaves was not toxic in rats and mice. This shows that the crude extract is safe for human and animal consumption (Lorke, 1983). Indeed according to the results of nutritional investigation conducted by Adeloye (1994), the plant *Tephrosia bracteolata* is good as sole feed for goats.

4.3 PHYSIOLOGICAL STUDIES

In rabbits pig ileum studies, the *Tephrosia bracteolata* crude extract induced concentration - dependent contractile responses at concentrations of 5, 10, 20, 30 and 50 mg/ml (Figs. 5 - 8). There was a complete blockage of the effect of the crude extract by atropine, a muscarinic receptor (Fig. 9).

NORMAL CONTROL

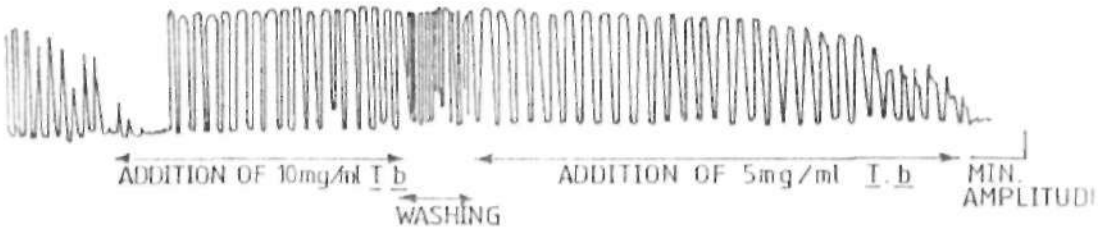
Figure 5



NORMAL

Normal contractile activity of smooth muscle of ileum of rabbit.

Figure 6



NOTE:

T.b = Tephrosia bracteolata

Figure 7

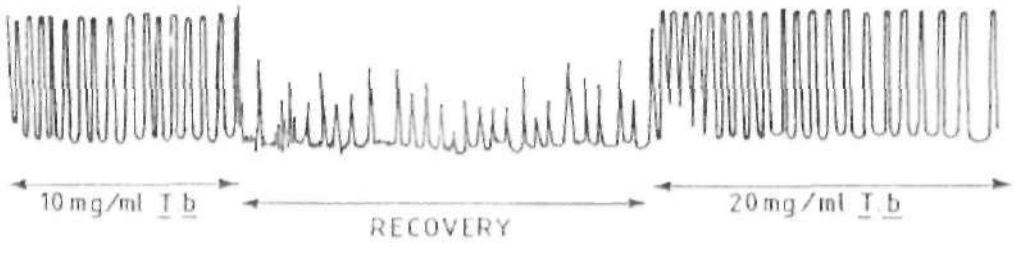


Figure 8

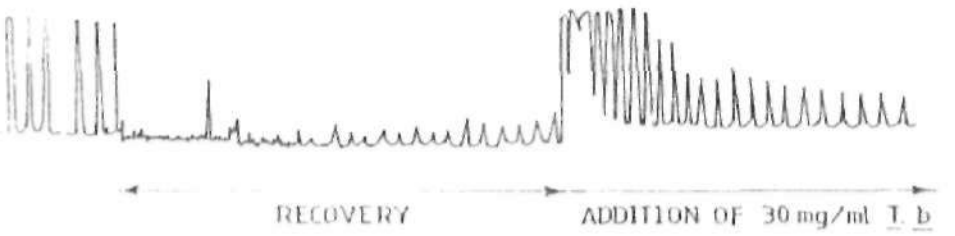
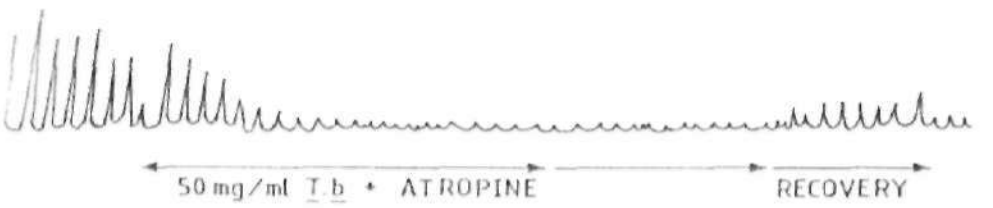


Figure 9



NOTE :

T. b = Tephrosia bracteolata

4.4 ANTI - MICROBIAL STUDIES

Table 2: Antimicrobial activity of Crude Extract of *Tephrosia bracteolata* Leaves against *Escheria coli* and *Staphylococcus aureus*

Organisms	Mean zone of inhibition ± S.E. (mm)
Escheria coli NCTC 10418	13 ± 0.816*
Escheria coli 189	13 ± 0.816
Staphylococcus aureus ATCC 13709	13 ± 0.471
Staphylococcus aureus S 40	13 ± 0.50

E.C. 189 and S. 40 are clinical isolates.

* The figures are averages of four experiments.

4.41 Interpretation of results

The result in Table 2, was interpreted using the modified Kirby – Bauer method (Bauer *et al.*, 1966) which defined three degrees of sensitivity.

(a) Sensitive, when the zone diameter of the test sample is equal to or larger than that of the control.

(b) Intermediately sensitive, when the diameter of the test is over 6 mm smaller than that of the control and

(c) Resistant, when the test produces no zone or when the zone diameter is below that of the intermediate result.

From the results and using the above interpretation, the extract seems not to have strong antimicrobial activity, inspite of the traditional uses

for whitlow and wounds. This may be due to the fact that the major active ingredient of the plant is alkaloids (Farnsworth and Bingel 1977) and may not possess any antimicrobial activity. Saponins, that are known to exhibit antimicrobial activity may be present at a very low concentration.

4.5 ANALGESIC STUDIES

The methanolic extract of *Tephrosia bracteolata* leaves was found to block both the electrical and mechanical stimulation in mice (Tables 3 & 4). The blockage occurred for all the doses (200, 400, 600 and 800 mg/kg body weight), of the crude extract used. Effective blocking of both electrical and mechanical stimulations in mice occurred 60 minutes post administrations. In both electrical and mechanical stimulations in mice, inhibition by the crude extract of *Tephrosia bracteolata* was very apparent even up to 180 minutes post administration. These observations tallied with the inhibition effect of a standard analgesic, aspirin in timing (at 60 minutes) and duration (more than 180 minutes). Even at the lowest dose of 200 mg /kg body weight, the crude extract of *Tephrosia bracteolata* was effective against both electrical and mechanical stimulations.

The ability of the extract to block thermal induced pain sensation increased dose dependently. The highest values for the inhibition was recorded at 150 minutes post-administration and at the dose of 400 mg/kg of the extract (Table 5.0). When the values of the inhibition times obtained for 400 mg/kg were correlated with those for ASA, a standard analgesic used in the experiment, the correlation coefficient was 0.918 ($P < 0.001$). However, correlation of inhibition time of aspirin with that of any of the dose levels was insignificant ($P > 0.05$). It thus appears that the optimum dose level for the extract against thermal stimulation was 400 mg/kg (Table 5.1).

TABLE 3: The Inhibition Induced by the Crude Extract of *Tephrosia bracteolata* Leaves Against Electrical Stimulation at 3 Volts (n = 5), using mice.

GROUPS / TIME IN MINUTES	1 CONTROL	2 50 mg/kg ASA	3 200 mg/kg (T.b) Extract	4 400 mg/kg (T.b) Extract	5 600 mg/kg (T.b) Extract	6 800 mg/kg (T.b) Extract
0	++	++	++	++	++	++
30	++	+	+	+	+	+
60	++	-	-	-	-	-
90	++	-	-	-	-	-
120	++	-	-	-	-	-
150	++	-	-	-	-	-
180	++	-	-	-	-	-

ASA = Acetylsalicylic acid (Aspirin), T. b Extract = *Tephrosia bracteolata* crude extract ++ = Very responsive, + = Responsive, - = Not responsive.

TABLE 4: Inhibition Induced by the Crude Extract of *Tephrosia bracteolata* against Mechanical Stimulation (n = 5), using mice.

GROUPS / TIME IN MINUTES	1 Control	2 50 mg/kg ASA	3 200 mg/kg (T.b) Extract	4 400 mg/kg (T.b) Extract	5 600 mg/kg (T.b) Extract	6 800 mg/kg (T.b) Extract
0	++	++	++	++	++	++
30	++	+	+	+	+	+
60	++	-	-	-	-	-
90	++	-	-	-	-	-
120	++	-	-	-	-	-
150	++	+	-	-	-	-
180	++	+	-	-	-	-

ASA = Acetylsalicylic acid (Aspirin), T.b. Extract = *Tephrosia bracteolata* crude extract ++ Very responsive, + = Responsive, - = Not responsive.

TABLE 5.0: Response of Mice treated with Crude Extract of *Tephrosia bracteolata* Leaves to Thermal Stimulation at 55 ^{o}C (Mean ± S.E.M.)

Groups	1	2	3	4	5	6
	Inhibition Time in Seconds					
	n = 5	n = 5	n = 5	n = 5	n = 3	n = 5
Time in Minutes						
0	11.8 ± 0.84	11.8 ± 0.84	11.8 ± 0.84	11.8 ± 0.84	11.8 ± 0.84	11.8 ± 0.84
30	11.8 ± 0.84	15.4 ± 0.75	22.3 ± 1.45	12.4 ± 0.40	22.67 ± 1.45	12.5 ± 0.96
60	11.8 ± 0.84	15.8 ± 0.74	24.7 ± 0.33	23.0 ± 4.72	24.3 ± 0.67	18.75± 3.75
90	11.8 ± 0.84	24.2 ± 1.71	40.7 ± 8.82	30.0 ± 8.37	54.0 ± 0.58	56.25 ± 2.1
120	11.8 ± 0.84	28.2 ± 0.74	53.7 ± 3.18	60.0 ± 18.97	59.3 ± 0.67	53.75 ± 13.75
150	11.8 ± 0.84	30.0 ± 0	24.7 ± 0.33	63.25 ± 6.24	34.0 ± 1.00	26.67 ± 3.33
180	11.8 ± 0.84	37.5 ± 4.33	21.3 ± 0.88	52.5 ± 7.50	16.7 ± 0.88	-

ASA = Acetylsalicylic acid (Aspirin)

T.b Extract = *Tephrosia bracteolata* crude extract

TABLE 5.1: Statistical Comparison between Time of Administration of Crude Extract of *Tephrosia bracteolata* Leaves and Duration of the Stay of Mice on Hot Plate

Dose	r	P	Remarks
50 mg /kg ASA	0.959	< 0.001	Very significant
200 mg/kg Extract	0.064	< 0.05	Significant
400 mg/kg Extract	0.918	< 0.001	Very significant
600 mg/kg Extract.	0.008	> 0.05	Not significant
800 mg/kg Extract	0.704	> 0.05	Not significant

ASA = Acetylsalicylic acid (Aspirin)

T. b Extract = *Tephrosia bracteolata* crude extract.

r = correlation coefficient, P = level of statistical significance.

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A plot of the response time against thermal stimulation of mice treated with *Tephrosia bracteolata* crude extract showed that the activity was almost twice higher than that of the standard analgesic (ASA) used in the study (Fig. 10).

Results obtained from the present studies indicated that *Tephrosia bracteolata* has a potent analgesic activity against mechanically stimulated pain. Thus, the study provides experimental evidence in support of the local use of the plant in northern Nigeria for the relief of pain.

The analgesic activity of the extract was broad, 200 to 800 mg/kg. This indicates a relatively wide safety margin for the extract. The highest blockade of mechanically-induced pain sensitivity was recorded for all doses following 90 minutes (1½ hours) of the extract administration.

The crude extract compares favourably with analgesic activity of aspirin for virtually all the doses tried in the experiment; (200, 400, 600 and 800 mg/kg). However based on the onset of response, the value obtained for both extract and aspirin were the same, 60 minutes. Further studies is, however, needed on the plant extract to elucidate the mechanism underlying the actions of the extract and to isolate active fractions. Data obtained from such studies could be of value in developing the plant extract as an analgesic agent.

4.6 ANTIPYRETIC STUDIES

Table 6.0 shows the summary of antipyretic studies on *Tephrosia bracteolata*. The results obtained for the doses (200, 400, 600, and 800 mg/kg) of the extract compared very well in efficacy with that of the standard

drug, aspirin. The results suggest strongly that the extract has antipyretic activity. When Students' *t*-test was applied to analyse the temperature values obtained for the extract and aspirin treated groups with the untreated control, it was highly significant (Table 6.1) for the plant extract.

However, there was no significant difference between the aspirin-treated (group 2) and the untreated control (group 1). Plot of temperature (°C) against time in hours (Fig. 11) showed that 600 mg/kg of the extract was the most potent antipyretic dose. The antipyretic effect of the *Tephrosia bracteolata* extract was most effective within the first 2 hours post-induction. It appears, therefore, that the methanolic extract of *Tephrosia bracteolata* is a potential antipyretic agent. The work strongly suggests that the extract may contain compounds possessing both analgesic and antipyretic activities.

The result of this study has estabilised the scientific basis for the traditional use of *Tephrosia bracteolata* leaves in Northern Nigeria.

TABLE 6.0: Temperature Response with Time in Rats treated with Crude Extract of *Tephrosia bracteolata* Leaves (Mean ± S.E.M., n = 5).

Time (Hr.)	Temperature in °C for each group						
	1 Control (Yeast only)	2 50 mg/kg ASA	3 200 mg/kg T.b.	4 400 mg/kg T.b.	5 600 mg/kg T.b.	6 800 mg/kg T.b.	7 Control II (No Yeast)
0	38.40 ± 0.032	38.26 ± 0.09	38.32 ± 0.08	38.34 ± 0.04	38.20 ± 0.15	38.10 ± 0.28	38.10 ± 0.00
1	38.54 ± 0.09	38.38 ± 0.34	37.58 ± 0.16	37.44 ± 0.35	37.56 ± 0.17	37.76 ± 0.13	38.10 ± 0.00
2	38.74 ± 0.15	38.50 ± 0.19	38.10 ± 0.25	37.64 ± 0.33	37.30 ± 0.12	37.66 ± 0.21	38.10 ± 0.00
3	38.72 ± 0.15	38.76 ± 0.19	38.54 ± 0.24	37.80 ± 0.29	37.40 ± 0.13	37.68 ± 0.27	38.10 ± 0.00
4	38.88 ± 0.19	38.40 ± 0.21	38.38 ± 0.18	38.24 ± 0.15	37.38 ± 0.05	37.66 ± 0.25	38.10 ± 0.00
5	38.48 ± 0.19	38.50 ± 0.10	38.42 ± 0.15	38.14 ± 0.22	37.32 ± 0.06	37.48 ± 0.13	38.10 ± 0.00
6	38.08 ± 0.37	38.52 ± 0.14	34.44 ± 0.20	38.16 ± 0.22	37.30 ± 0.10	37.32 ± 0.07	38.10 ± 0.00

ASA = Acetylsalicylic acid (Aspirin)

b. = *Tephrosia bracteolata* extract.

Table 6.1: **Statistical Comparison of Temperature Values obtained for the Crude Extract of *Tephrosia bracteolata* Treated Rats, and those treated with the Standard Drug (aspirin) and the Untreated Control**

Groups	*t*-value	P-value	Remarks
1 vs 2	0.640	> 0.05	Not significant
2 vs 5	7.432	< 0.001	Very significant
6 vs 3	3.826	< 0.01	Very significant
5 vs 1	6.660	< 0.001	Very significant
5 vs 4	3.850	< 0.01	Very significant
4 vs 6	2.660	< 0.05	Significant

Group 1 - Untreated control

2 - Aspirin treated

3 - 200 mg/kg Extract treated

4 - 400 mg/kg Extract treated

5 - 600 mg/kg Extract treated

6 - 800 mg/kg Extract treated

Extract - *Tephrosia bracteolata* extract

t -value - Calculated Students' t – test value

p -value - Level of statistical significance.

4.7 ANTI-INFLAMMATORY STUDIES

The results in Table 7.0 show that carrageenan induced oedema in both the control and experimental rats. The methanolic extract of the *Tephrosia bracteolata* exerted a significant inhibitory effect on the carrageenan-induced oedema (CIO) at different time intervals in a dose dependent pattern. The CIO decreased significantly from the highest value, for all animals including the untreated control, to the minimum value at 24 hour post induction. The percentage inhibitions of the extract at different doses 2 hour post-induction of oedema are shown in Table 7.1. The methanolic extract of *Tephrosia bracteolata* did not have any significant inhibitory effect on the development of CIO until 2 hour post-induction. The highest reduction in the CIO was recorded in rats treated with the extract 2 hours after injection. There was a significant difference ($P < 0.05$) between the inhibition of CIO in control (group 1) and that of experimental animals (groups 3 - 5), especially in the animals treated with 800 mg/kg (group 6) of the extract ($P < 0.001$). There was a significant correlation between the values of percent inhibition of the oedema in the control and those obtained following the administration of a standard drug indomethacin. The inhibition of CIO in rats treated with 400 and 800 mg/kg compared well with that induced by indomethacin (Table 7.0) ($r = 0.951$ and 0.927, respectively). In both cases, the correlation coefficients were highly significant ($P < 0.01$). Also, the results of single factorial ANOVA showed a significant difference ($P < 0.05$) between the various treatment groups and the readings at different hours.

The effect of the extract at different doses was almost the same as that of indomethacin 18 and 24 hour post-induction of oedema. The percentage

suppression at 2 hour post-induction of oedema varied depending on the dose and the highest value of 57% was obtained in rats treated with 800 mg/kg of the extract (Table 7.1). At 2 hour post-induction, there was a positive correlation ($r = 0.660$, $P > 0.05$) between the dose of the extract and the percent inhibition of CIO.

Also, the effect of the extract on suppression of CIO increased as the post induction time increased, especially for animals treated with 800 mg/kg of the extract ($r = 0.927$, $P < 0.001$).

The CIO of the rat foot, used widely as a working model of acute inflammation in the search for new anti-inflammatory agents (Winter, *et al.*, 1962; Van Arman, *et al.*, 1969; Disilvestro, 1995), was found to be a good model of inflammation in the present study. This finding is in agreement with that of Di Rosa *et al.* (1971b) who observed that the model is a universal system which follows pattern similar to those in other types of inflammation.

The results obtained in the present study showed that when rats were treated with the methanolic extract of *Tephrosia bracteolata*, the treatment led to a marked suppression of the CIO. Thus, for the first time, an experimental evidence is provided in support of the extensive use of the plant *Tephrosia bracteolata* in the traditional treatment of inflammatory processes including whitlow. The results of the experiments showed that the extract did not have any effect on the early development of CIO; i.e., before 2 hour post-induction.

The mechanism of induction of oedema by carregeenan has been shown to be bi-phasic (Di Rossa *et al.*, 1971a; 1971b). The first phase involves the release of histamine and serotonin, while the second phase is due to the release of bradykinin, protease, prostaglandin and lysosome

(Crunckhon and Meacock, 1971). According to Di Rossa *et al.* (1971a), the time-course of mediators involved in the CIO showed that histamine and hydroxytryptamine (5H-I) were released within the 1½ hours post-induction, while kinins and prostaglandins were involved in mediating the inflammatory activity 1 - 2 hours and 2 - 6 hours post-induction, respectively. Therefore, the fact that maximum CIO suppression occurred 2 hour post-induction suggested that the extract may be acting during the last phase of inflammation involving prostaglandins as mediators.

The inhibition of CIO in rats by the crude methanolic extract of *Tephrosia bracteolata* was comparable to that of indomethacin. Indomethacin is a standard non-steroidal anti-inflammatory drug that ultimately decreases the release of prostaglandins (Di Rosa *et al.*, 1971b), predominantly E and F types (Velo *et al.*, 1973).

The results obtained from this studies thus indicated strong similarities of action between the extract of *Tephrosia bracteolata* and indomethacin. The experimental evidence provided by the present study on anti-inflammatory actions of the crude extract of *Tephrosia bracteolata* strongly suggests that the plant may contain a non-steroidal anti-inflammatory agent (s).

Further studies are still required to elucidate the mechanism of action of the plant extract, and to further isolate and characterise the compounds responsible for the anti-inflammatory activity.

Table 7.0: Inflammatory Measurements of Carrageenan-Induced Rat Paw Oedema with Time (Mean ± S.E.M., n = 3)

Group Time	(1) Untreated Control	(2) Indomethacin treated	(3) 200 mg/kg Extract	(4) 400 mg/kg Extract	(5) 600 mg/kg Extract	(6) 800 mg/kg Extract
Before Oedema induction	0.22 ± 0.01	0.23 ± 0.02	0.23 ± 0.01	0.27 ± 0.05	0.28 ± 0.03	0.23 ± 0.03
0 (At induction)	0.39 ± 0.02	0.36 ± 0.01	0.31 ± 0.03	0.34 ± 0.01	0.36 ± 0.02	0.35 ± 0.01
1	0.46 ± 0.01	0.43 ± 0.04	0.39 ± 0.02	0.45 ± 0.02	0.40 ± 0.02	0.38 ± 0.003
2	0.64 ± 0.02	0.48 ± 0.02	0.54 ± 0.03	0.56 ± 0.02	0.55 ± 0.02	0.41 ± 0.02
3	0.52 ± 0.01	0.47 ± 0.01	0.54 ± 0.03	0.55 ± 0.03	0.53 ± 0.01	0.39 ± 0.02
4	0.47 ± 0.03	0.36 ± 0.03	0.42 ± 0.01	0.44 ± 0.01	0.52 ± 0.01	0.36 ± 0.03
6	0.47 ± 0.03	0.35 ± 0.02	0.41 ± 0.01	0.41 ± 0.01	0.49 ± 0.01	0.33 ± 0.03
18	0.40 ± 0.04	0.27 ± 0.01	0.28 ± 0.01	0.30 ± 0.02	0.30 ± 0.03	0.30 ± 0.03
24	0.32 ± 0.03	0.26 ± 0.01	0.23 ± 0.03	0.28 ± 0.01	0.28 ± 0.01	0.23 ± 0.003

Extract = *Tephrosia bracteolata* crude extract.

Table 7.1: Effect of Methanolic Crude Extract of *Tephrosia bracteolata* Leaves on Carrageenan-Induced Rat Paw Oedema, Measured Two hour Post-Induction (n = 3)

Group	Paw Volume (ml) (Mean ± S.E.M.)	Percent Inhibition
Control, 0.2 ml Distilled water	0.64 ± 0.02	-
Indomethacin 10 mg/kg	0.48 ± 0.02	**40**
Extract, 200 mg/kg	0.54 ± 0.03	**26**
Extract, 400 mg/kg	0.56 ± 0.02	**31**
Extract, 600 mg/kg	0.55 ± 0.02	**36**
Extract, 800 mg/kg	0.41 ± 0.02	**57**

Extract = *Tephrosia bracteolata* crude extract

Table 7.2: Statistical Comparison of Oedema Inhibition between the Crude Extract of *Tephrosia bracteolata*-treated, Indomethacin-treated and Untreated Control Rats(n = 3)

Groups	*t*-value	P-value	Remarks
1 vs 2	5.66	< 0.01	Very significant
1 vs 3	2.77	< 0.05	Significant
1 vs 4	2.83	< 0.05	Significant
1 vs 5	3.18	< 0.05	Significant
1 vs 6	8.13	< 0.01	Very significant

Group 1 - Untreated control

2 - Indomethacin (10 mg/kg) administered.

3 - 200 mg/kg Extract administered

4 - 400 mg/kg Extract administered

5 - 600 mg/kg Extract administered

6 - 800 mg/kg Extract administered

Extract - *Tephrosia bracteolata* extract

t-value-Calculated Students' t – test value

p-value-Level of statistical significance

4.8 POTENTIATION OF BARBITURATE (SODIUM THIOPENTONE) SLEEPING TIME

All the animals slept from the effect of sodium thiopentone but the group that received chlorpromazine in addition slept for a longer time than all others (Table 8.0). The results in groups 3 - 6 were very similar to those obtained in group 1, the control group. From the values in Table 8.1, there was no significant difference between the mean sleeping time of the animals in the control group and those in groups 3 - 6 treated with different doses (200, 400, 600 and 800 mg/kg) of the methanolic crude extract of *Tephrosia bracteolata*.

Table 8.0: Effect of Crude Extract of *Tephrosia bracteolata* Leaves on Barbiturate (Sodium Thiopentone) Sleeping Time
(Mean ± S.E.M, n = 3)

Group	Dose (mg/kg) i. p.	Mean Sleeping Time (± S.E.M) (minutes)
1. Control, untreated	0.2 ml Normal Saline	11.0 ± 0.58
2. Chlorpromazine treated	10	21.0 ± 0.58
3. Extract treated	200	10.7 ± 0.88
4. Extract treated	400	11.3 ± 0.88
5. Extract treated	600	10.3 ± 0.33
6. Extract treated	800	10.7 ± 0.88

Extract = *Tephrosia bracteolata* crude extract.

Table 8.1: Statistical Comparison of Mean Sleeping Time of Chlorpromazine-treated and Crude Extract of *Tephrosia bracteolata* -treated Groups with the Non-treated Group (n = 3)

Groups	*t*-value	P-value	Remarks
1 vs 2	2.19	< 0.001	Very significant
1 vs 3	0.215	> 0.05	Not significant
1 vs 4	0.285	> 0.05	Not significant
1 vs 5	1.05	> 0.05	Not significant
1 vs 6	0.285	> 0.05	Not significant

Group 1 - Untreated control

2 - Chlorpromazine-treated

3 - 200 mg/kg Extract-treated

4 - 400 mg/kg Extract-treated

5 - 600 mg/kg Extract-treated

6 - 800 mg/kg Extract-treated

Extract - *Tephrosia bracteolata* methanolic extract

t-value -Calculated students' t- test

p-value -Level of statistical significance.

The inability of the plant extract to potentiate barbiturate sleeping time suggests that the extract probably exerts specific analgesic, antipyretic, and anti-inflammatory effects without inducing a central depressant effect or sedation or general anaesthesia. Thus, the results obtained from the present study on the extract demonstrate a good prospect for the development of the extract as a potent local analgesic agent with non-central activity.

CHAPTER FIVE

5.0 CONCLUSION AND SUGGESTION

5.1 CONCLUSION

From the present investigations carried out on the methanolic extract of *Tephrosia bracteolata* leaves, the following general conclusions can be made:

1. The leaf of the plant is not toxic.
2. It does not possess a significant anti-microbial activity.
3. It induces a concentration-dependent contractile responses in guinea pig ileum.
4. The plant extract inhibited very significantly the response to mechanically, electrically and thermally induced pains.
5. The extract induced antipyretic effect.
6. The crude extract has anti-inflammatory effect.
7. The extract did not show ability to induce sleep nor drowsiness.
8. The activity of the crude extract of the leaves of *Tephrosia bracteolata*, is similar to that of standard analgesic agent and the standard non-steriodal anti-inflammatory drug, indomethacin.
9. The plant leaf apparently contains analgesic principle which does not induce depression of the central nervous system.

5.2 SUGGESTION FOR FURTHER WORK

The present investigations have established that *Tephrosia bracteolata* leaf is not toxic, and that it contains potent analgesic, antipyretic and anti-inflammatory properties. However, this work is preliminary and more work will be

needed to establish the exact mode of action of the extract and to determine the active component responsible for its activities, and possibly to establish the chemical composition and structure of the active compounds.

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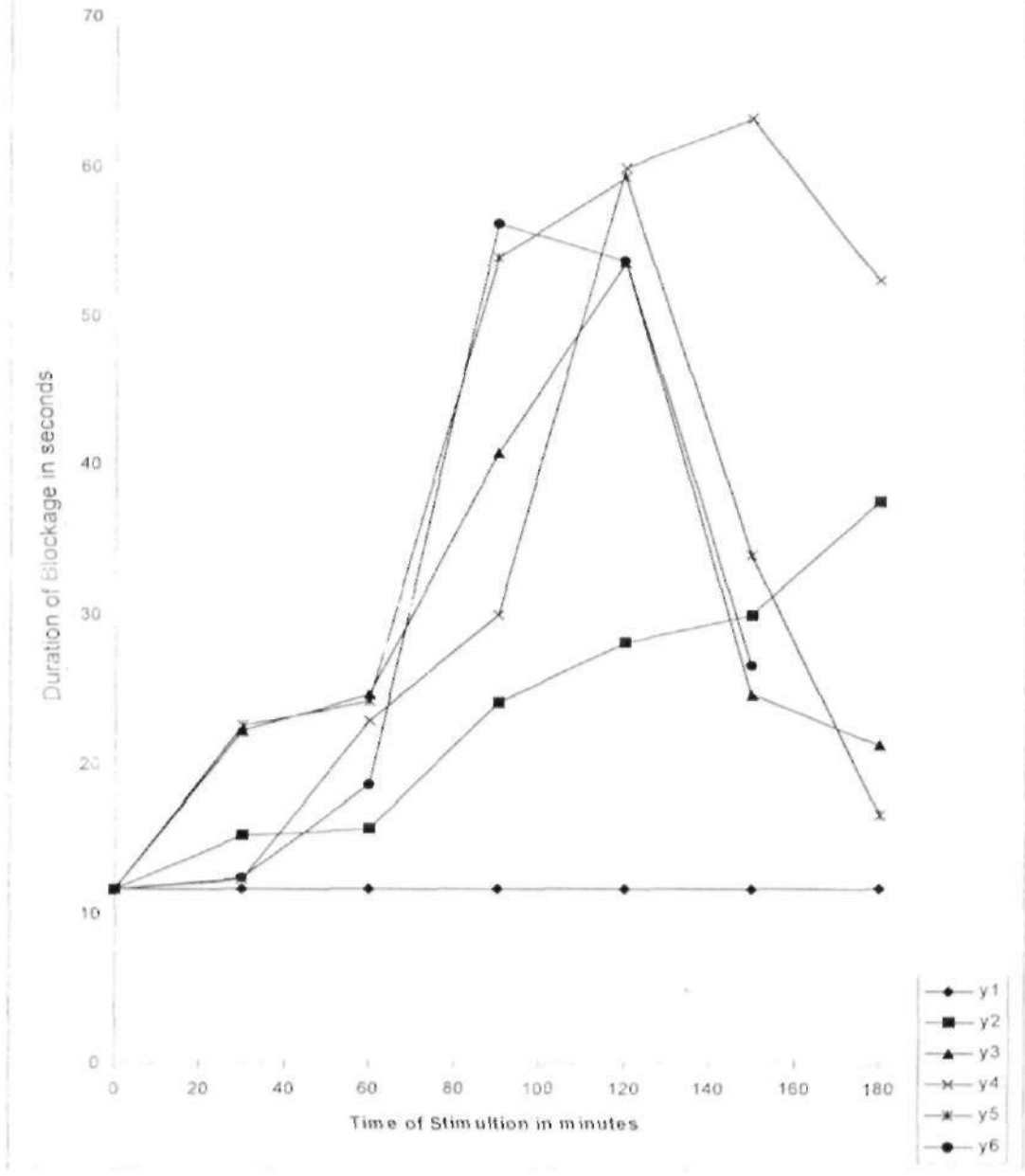
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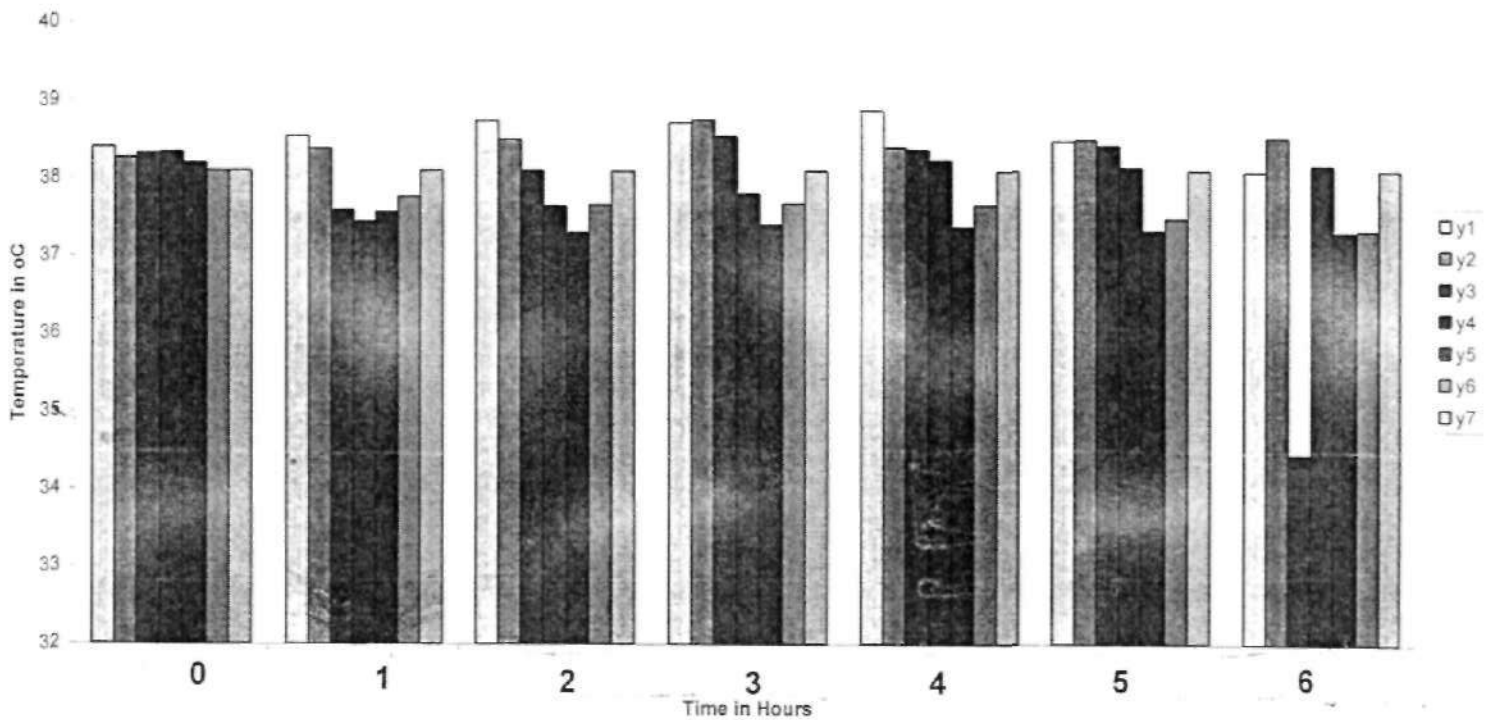
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Fig. 10 A plot of of the response to Themal stimulation at 55^{0}C of Mice treated with Crude Extract of ***Tephrosia bracteolata*** Leaves.



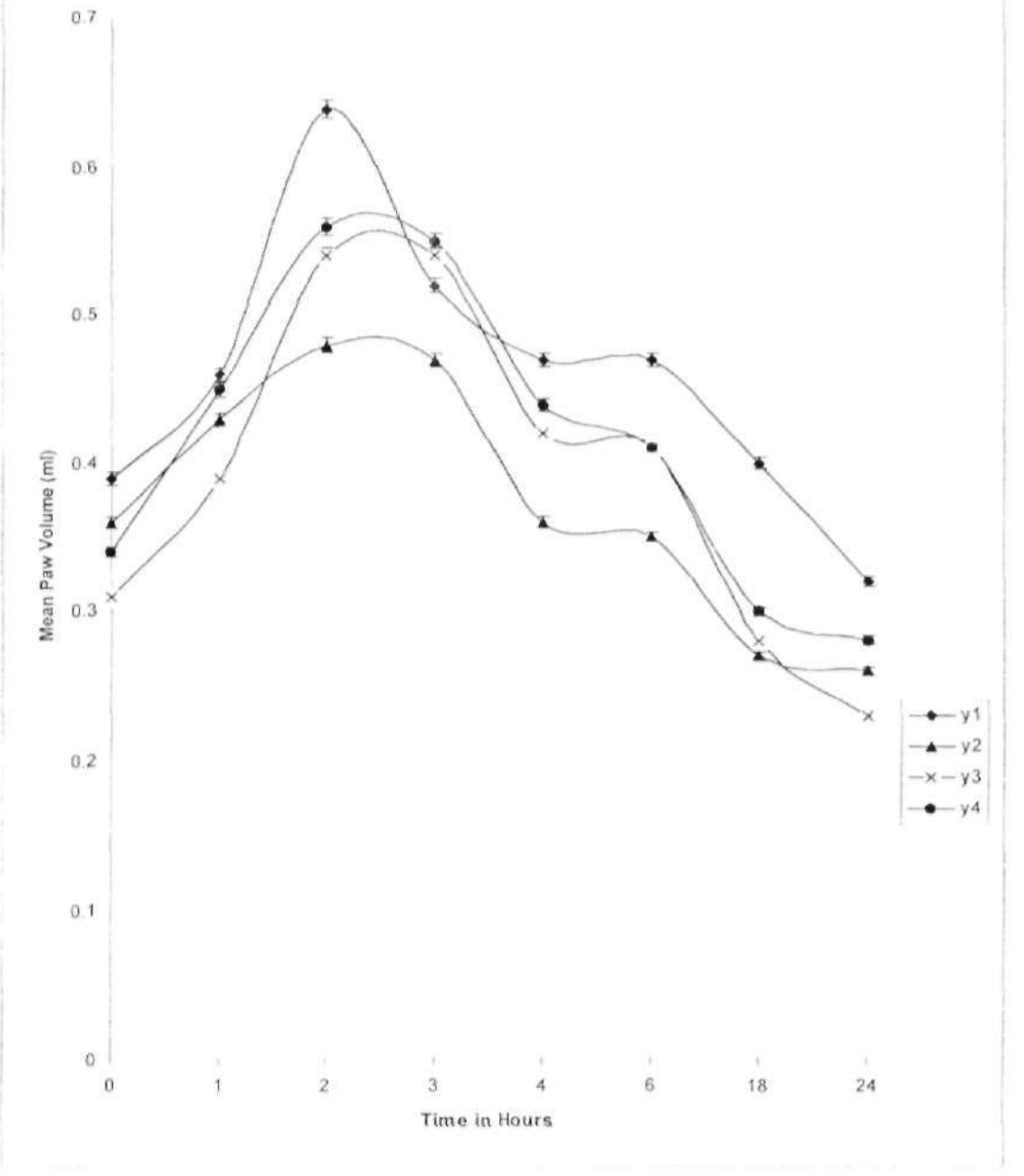
y 1 = Control
y 2 = 50 mg/kg ASA
y 3 = 200 mg/kg extract of *Tephrosia bracteolata*
y 4 = 400 mg/kg extract of *Tephrosia bracteolata*
y 5 = 600 mg/kg extract of *Tephrosia bracteolata*
y 6 = 800 mg/kg extract of *Tephrosia bracteolata*

Fig. 11 A Plot of Temperature Response with Time for Rats treated with Crude Extract of ***Tephrosia bracteolata*** Leaves



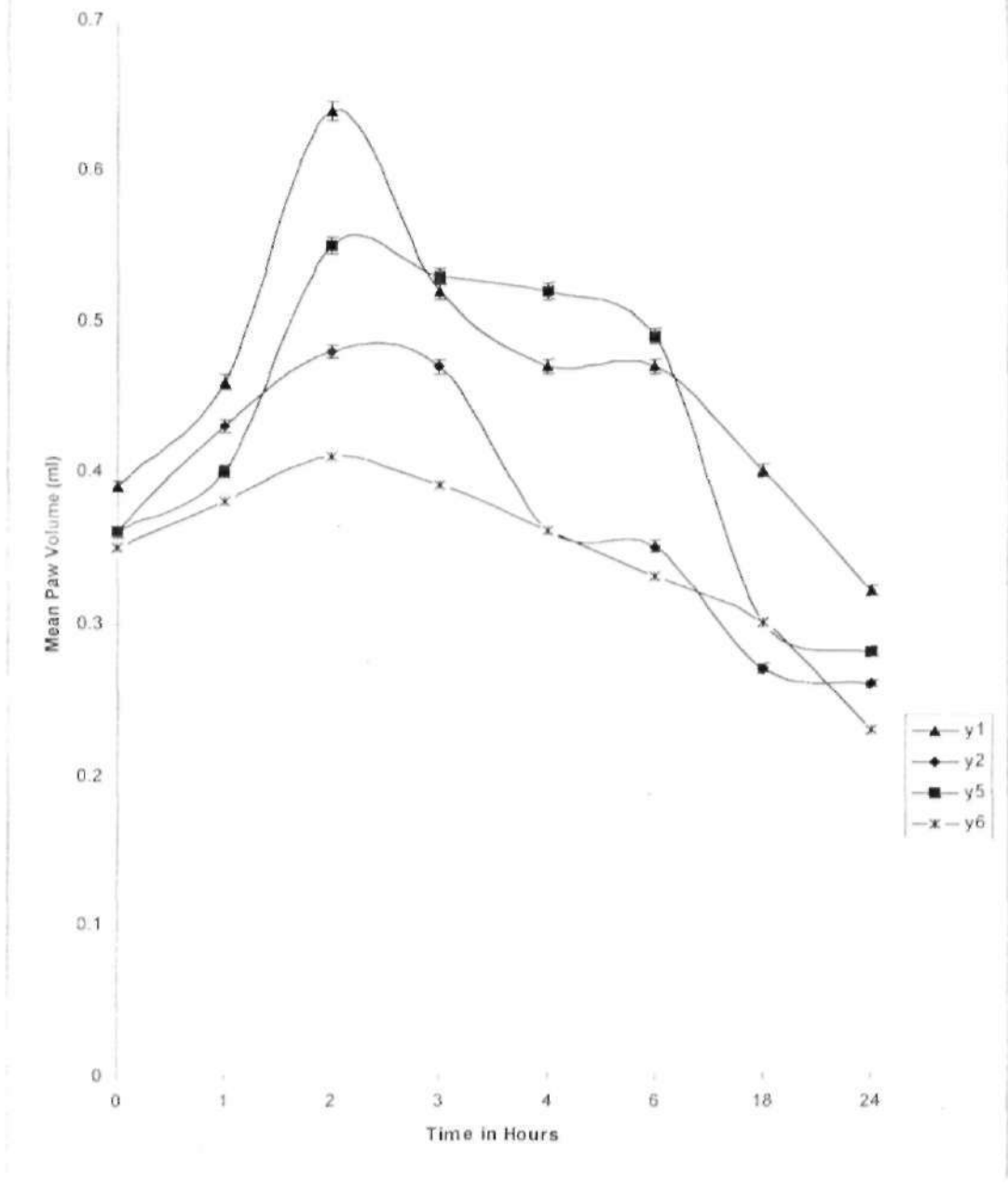
y1 = Control 1; y2 = 50 mg/kg ASA; y3 = 200 mg/kg extract; y4 = 400 mg/kg extract, y5 = 600 mg/kg extract; y6 = 800 mg/kg extract; y7 = Control 2.

Fig. **12.0** A plot of Mean Paw Volume against Time for Control, Indomethacin-treated and 2 doses of Crude Extract of ***Tephrosia bracteolata***



y1 = Untreated Control; y2 = Indomethacin treated
y3 = 200 mg/kg Extract treated; y4 = 400 mg/kg Extract treated

Fig. 12.1 A plot of Mean Paw Volume against Time for Control, Indomethacin-treated and 2 doses of Crude Extract of ***Tephrosia bracteolata***



y1 = Untreated Control; y2 = Indomethacin treated
y5 = 600 mg/kg Extract treated; y6 = 800 mg/kg Extract treated

APPENDIX

APPENDIX 1a: Exploratory Test for Acute Toxicity Studies of the Crude Extract of *Tephrosia bracteolata* Leaves in mice.

Animal groups	Weight (g) and Sex	Dosage (mg/kg)	Conc. mg/ml	Amount administered (mg)	Volume given (ml)	Observation
Group I						
1	27.1 F	10	0.6	0.271	0.45	No Visible reaction
2	27.2 F	10	0.6	0.272	0.45	"
3	27.4 M	10	0.6	0.274	0.46	"
Group 2						
1	11.6 F	100	6.0	1.16	0.19	"
2	14.7 M	100	6.0	1.75	0.29	"
3	17.5 F	100	6.0	1.47	0.25	"
Group 3						
1	21.1 F	1,000	60	25.5	0.43	"
2	23.5 F	1,000	60	23.1	0.39	"
3	25.5 F	1,000	60	25.5	0.43	"
Group 4						
1	22.0 M	Control	-	-	0.2 ml H_2O	"
2	28.3 M	Control	-	-	0.2 ml H_2O	"
3	31.9 M	Control	-	-	0.2 ml H_20	"

APPENDIX 1b:

Exploratory Test for Toxicity Studies of the Crude Extract of *Tephrosia bracteolata* leaves in rats.

Animal group	Weight (g) and Sex	Dosage (mg/kg)	Amount Adm.	Conc. (mg/ml)	Volume given (ml)	Observation
Group I						
1	54.6 F	10	0.546	1.5	0.36	No Visible reaction
2	55.8 F	10	0.558	1.5	0.37	"
3.	57.5 F	10	0.575	1.5	0.38	"
Group 2						
1	68.2 M	100	6.81	15	0.45	
2	78.2 F	100	7.87	15	0.53	"
3.	78.6 M	100	7.86	15	0.52	"
Group 3						
1	62.9 M	1,000	62.9	150	0.45	"
2.	64.3 M	"	64.3	150	0.45	"
3.	68.3 F	"	68.3	150	0.46	"
Group 4						
1	49.4 F	Control	-	-	0.45	water
2.	59.5 M	"	-	-	.45	"
3.	68.1 M	"	-	-	.45	"

Appendix 2a:

Acute Toxicity Studies of the Crude Extract of *Tephrosia bracteolata* leaves in mice.

Animal groups	sex	weight (g)	Dosage (mg/kg)	Conc. Required mg/ml	Amount Adm. (mg/ml)	Volume given (ml)	Observation
Group 1							
1.	F	24.2	10	0.6	0.242	0.40	No Visible Reaction
2.	F	24.5	10	"	0.245	0.41	"
3.	F	25.0	10	"	0.250	0.42	"
Group2							
1	F	18.5	100	6.0	1.85	0.31	No visible Reaction
2.	F	18.8	100	6.0	1.88	0.32	"
3.	M	20.1	100	6.0	2.01	0.33	"
Group 3							
1	M	20.5	1,000	60	20.5	0.34	"
2.	F	22.5	1,000	60	22.5	0.38	"
3.	M	25.0	1,000	60	25.0	0.42	"
Group 4							
1	M	27.2	Control	-	-	H_20	"
2	M	27.5	Control	-	-	H_20	"
3.	M	28.0	Control	-	-	H_20	"

Appendix 2b

Acute Toxicity Studies of the Crude Extract of *Tephrosia bracteolata* leaves in Rat.

Animal groups	weight (g) and sex	Dosage (mg / kg)	Conc. Mg/ml	Amount Adm.(mg)	Volume given (ml)	Observation
Group 1						
1	56.5 F	10	1.5	0.565	0.38	No visible reaction
2.	58.5 F	10	1.5	0.585	0.39	"
3.	62.4 F	10	1.5	0.624	0.42	"
Group 2						
1	74.5 M	100	15	7.45	0.50	"
2.	78.7 F	100	15	7.87	0.52	"
3	81.5 M	100	15	8.15	0.54	"
Group 3						
1	69.4 F	1 000	150	69.4	0.46	"
2	70.3 F	1,000	150	70.3	0.47	"
3	71.5 F	1,000	150	71.5	0.48	"
Group 4						
1	61.6 F	-	-	-	0.48	Water
2	64.5 F	-	-	-	0.48	Water
3	64.5 F	-	-	-		Water

APPENDIX 3

Second Investigation in the Determination of Acute Toxicity Studies of the Crude Extract *of Tephrosia bracteolata* leaves using rats*.

Animal Group	Weight (g) and Sex	Dosage (mg/kg)	Conc. (mg/ml)	Amount given (mg)	Volume Adm. (ml)	Observation
Group 1						
1.	63.0 M	1,600	125	100.80	0.80	Restless for about 3 minutes but resume eating almost immediately
Group 2						
1.	71.6 F	2,900	250	207.64	0.84	"
Group 3						
1.	85.2 M	5,000	500	426.0	0.86	"
Group 4						
1	115.9 M	10,000	2,000	1,159	0.58	No visible reaction

* Method of Lorke, D (1983). Archives of Toxicology 54: 275 - 287

APPENDIX 4

Analgesic Evaluation of Crude Extract of *Tephrosia bracteolata* Leaves

Analgesic Evaluation of *Tephrosia Bracteolata* against electrical stimulations in mice at 3, 5 and 10 volts (n = 3).

						3 VOLTS TIME IN MINUTES							5 VOLTS TIME IN MINUTES							10 VOLTS TIME IN MINUTES						
Animal Group	Weight (g) and Sex	Dosage (mg/kg)	Conc. (mg/ml)	Amt Adm (mg)	Volume Given (ml)	0	30	60	90	120	150	180	0	30	60	90	120	150	180	0	30	60	90	120	150	180
Group 1																										
1	20.0 F	Control	-	-	0.20 water	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
2	22.5 F	Control	-	-	0.20 water	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
3	25.3 M	Control	-	-	0.20 water	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Group 2																										
1	19.8 F	50. ASA	5	0.99	0.20	++	+	-	-	-	-	+	++	+	+	+	+	-	+	++	+	+	-	+	+	+
2	20.1 M	50. ASA	5	1.01	0.20	++	+	-	-	+	-	+	++	+	+	-	+	+	+	++	+	+	-	+	+	+
3	25.0 M	50. ASA	5	1.25	0.25	++	+	-	-	-	-	+	++	+	+	+	+	+	+	++	+	+	-	+	+	+
Group 3																										
1	21.0 M	200. T.b	30	4.20	0.14	++	+	-	-	-	+	++	+	+	+	+	+	+	+	++	+	+	-	+	+	+
2	22.8 M	200. T.b	30	4.56	0.15	++	+	-	+	+	-	++	+	+	+	+	+	+	+	++	+	+	-	+	+	+
3	22.8 F	200. T.b	30	4.56	0.15	++	-	-	-	+	-	++	+	+	+	+	+	+	+	++	+	+	-	+	+	+
Group 4																										
1	16.2 F	400. T.b	60	6.48	0.11	++	+	+	-	-	-	-	++	+	-	-	-	+	+	++	+	+	-	+	+	+
2	18.6 F	400. T.b	60	7.44	0.12	++	+	-	-	-	-	-	++	+	+	+	+	+	+	++	+	+	-	+	+	+
3	22.5 M	400. T.b	60	9.00	0.15	++	+	+	+	-	-	-	++	+	+	+	+	+	+	++	+	+	-	+	+	+
Group 5																										
1	18.3 F	600. T.b	60	10.98	0.18	++	+	+	+	+	-	-	++	+	+	-	-	+	+	++	+	+	-	+	+	+
2	19.5 M	600. T.b	60	11.70	0.20	++	+	+	+	+	-	-	++	+	+	+	+	+	+	++	+	+	-	+	+	+
3	19.8 F	600. T.b	60	11.88	0.20	++	+	+	-	-	-	-	++	+	+	+	+	+	+	++	+	+	-	+	+	+
Group 6																										
1	14.5 M	800. T.b	60	11.60	0.20	++	+	+	-	-	-	-	++	+	+	+	+	+	+	++	+	+	-	+	+	+
2	19.2 M	800. T.b	60	15.36	0.26	++	+	+	-	-	-	-	++	+	+	+	+	+	+	++	+	+	-	+	+	+
3	25.0 F	800. T.b	60	20.00	0.33	++	-	+	-	-	+	+	++	+	+	+	+	+	+	++	+	+	-	+	+	+

++ = Very responsive. + = Responsive — = Not responsive

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APPENDIX 5

Analgesic Evaluation of Crude Extract of *Tephrosia bracteolata* Leaves against Mechanical Stimulation in Mice

Animal Group	Weights (g) and Sex	Dosage (mg/kg)	Conc. (mg/ml)	Amount Adm. (mg)	Volume Given (ml)	TIME IN MINUTES 0	30	60	90	120	150	180
Group 1												
1	19.9 F	Control	-	-	0.20 Dist. water	++	++	++	++	++	++	++
2	22.3 F	Control	-	-	0.20 Dist. water	++	++	++	++	++	++	++
3	25.3 M	Control	-	-	0.20 Dist. water	++	++	++	++	++	++	++
Group 2												
1	19.6 M	50, ASA	5	0.98	0.20	++	+	-	-	-	+	+
2	19.7 F	50, ASA	5	0.99	0.20	++	+	-	-	-	+	+
3	24.9 M	50, ASA	5	1.25	0.25	++	+	-	-	-	+	+
Group 3												
1	20.5 M	200, T.b	30	4.10	0.14	++	+	-	-	-	-	-
2	22.3 M	200, T.b	30	4.46	0.15	++	+	-	+	-	-	-
3	22.8 F	200, T.b	30	4.56	0.15	++	+	-	+	-	-	-
Group 4												
1	15.5 M	400, T.b	60	6.20	0.10	++	+	-	-	-	-	-
2	18.3 F	400, T.b	60	7.32	0.12	++	+	-	-	-	-	-
3	22.1 F	400, T.b	60	8.84	0.15	++	+	-	-	-	-	-
Group 5												
1	17.9 M	600, T.b	60	10.74	0.18	++	+	-	-	-	-	-
2	18.0 M	600, T.b	60	10.80	0.18	++	+	-	-	-	-	-
3	18.5 F	600, T.b	60	11.10	0.19							
Group 6												
1	13.8 F	800, T.b	60	11.04	0.18							
2	18.9 F	800, T.b	60	15.12	0.25							
3	31.2 M	800, T.b	60	18.72	0.31							

++ = Very responsive
\+ = Responsive
\- = Not responsive.

APPENDIX 6

Analgesic Evaluation Of Crude Extract *of Tephrosia bracteolata* Leaves against Thermal Stimulation at 55 ± 1 °c in Mice

Animal Group	Weight (g) and Sex	Dosage (mg/kg)	Conc. (mg/ml)	Amount given (mg)	Volume given (ml)	TIME IN MINUTES 0	30	60	90	120	150	180
Group 1												
1	9.20 F	Control	-	-	0.2 ml Dist. Water	11	11	11	11	11	11	11
2	10.09 F	"	-	-	"	12	12	12	12	12	12	12
3	10.60 M	"	-	-	"	11	11	11	11	11	11	11
4	10.70 M		-	-	"	13	13	13	13	13	13	13
5	15.70 M		-	-	"	12	12	12	12	12	12	12
Group 2												
1	10.60 M	50, ASA	50	8.48	0.17	11	13	14	20	27	30	45
2	11.50 F	50, ASA	50	9.20	0.18	12	15	15	20	27	30	45
3	12.10 M	50, ASA	50	9.68	0.19	11	15	15	28	30	30	45
4	12.40 F	50, ASA	50	9.92	0.20	13	17	18	27	27	30	30
5	15.00 M	50, ASA	50	12.00	0.24	12	17	17	27	30	30	30
Group 3												
1	18.20 F	200,T.b.	25	3.64	0.15	11	22	24	52	51	54	24
2	20.50 F	200,T.b	25	4.10	0.16	12	20	25	54	50	25	20
3	29.50 M	200,T.b	25	5.90	0.24	13	25	25	44	60	25	23
Group 4												
1	10.09 F	400,T.b	25	4.04	0.16	11	13	35	60	120	80	60
2.	10.50 M	400,T.b	25	4.20	0.17	12	12	34	35	90	60	60
3	10.70 M	400,T.b	25	4.28	0.17	11	13	17	25	30	50	30
4	12.50 F	400,T.b	25	5.00	0.20	13	13	15	15	30	63	60
5	15.70 M	400,T.b	25	6.28	0.25	12	11	14	15	30	09	13
Group 5												
1	16.50 M	600,T.b.	50	9.90	0.20	11	25	25	54	60	35	18
2	18.50 M	600,T.b	50	11.10	0.22	12	20	23	55	60	32	15
3	24.40 F	600,T.b	50	14.64	0.29	13	23	25	53	58	34	16
Group 6												
1	17.8 F	800,T.b	50	14.24	0.28	11	15	30	120	90	120	
2	18.7 M	"	"	14.96	0.30	12	11	15	30	35	30	
3	18.8 F	"	"	15.04	0.30	11	11	15	30	60	30	
4	18.9 F	"	"	15.12	0.30	13	13	15	45	30	20	
5	27.6 M	"	"	22.08	0.44	12	13	10	10	9	10	

APPENDIX 7

Antipyretic Effect of Crude Extract of *Tephrosia bracteolata* Leaves in Rats.

RECTAL TEMPERATURES IN ^{0}C

	1	2	3	4	5	6	7
Time (Hrs)	Control +	50 mg/kg	200mg/kg	400mg/kg	600mg/kg	800mg/kg	Control II
After (15 hrs)	yeast	ASA	extract	extract	extract	Extract	No yeast
Group 1							
1	38.40	38.10	38.00	38.30	37.60	37.00	38.10
2	38.30	38.00	38.40	38.20	38.20	38.20	38.10
3	38.40	38.40	38.40	38.40	38.40	38.40	38.10
4	38.50	38.40	38.40	38.40	38.80	38.50	38.10
5	38.40	38.40	38.40	38.40	38.40	38.40	38.10
Group 2							
1	38,40	38.20	37.60	38.50	36.90	37,50	38.10
2	38.30	38.30	37.60	37.70	37.90	38.20	38.10
3	38.60	38.40	37.70	37.60	37.70	37.90	38.10
4	38.60	38.70	37.00	37.00	37.60	37.70	38.10
5	38.80	38.30	38.00	36.40	37.70	37.50	38.10
Group 3							
1	38.60	38.60	38.50	38.70	36.90	38.00	38.10
2	38.10	37.90	37.60	37.80	37.20	38.30	38.10
3	39.20	39.00	37.20	37.80	37.40	37.40	38.10
4	39.00	38.70	37.20	37.00	37.60	37.20	38.10
5	38.80	38.30	38.00	36.90	37.40	37.40	38.1-

Appendix 7 Continued

Group 4							
1	38.20	38.20	38.30	38,80	37.80	37.60	38.00
2	39.00	38.40	39.20	37.60	37.00	38.70	38.10
3	38.60	39.00	38.80	37.80	37.50	37.60	38.10
4	38.80	39.20	37.80	37.80	37.40	37.20	38.10
5	39.00	39.00	38.50	37.00	37.30	37.30	38.10
Group 5							
1	38.30	38.00	38.10	38.70	37.20	38.40	38.10
2	38.80	38.20	38.30	37.80	37.40	38.10	38.10
3	39.50	39.20	38.50	38.10	37.40	37.50	38.10
4	39.00	38.40	38.00	38.30	37.50	37.20	38.10
5	38.80	38.20	39.00	38.30	37.40	37.10	38.10
Group 6							
1	37,90	38.20	38.50	38.50	37.20	37.70	38.10
2	38.30	38.50	38.60	37.30	37.50	37.70	38.10
3	39.00	38.60	38.30	38.20	37.40	37.60	38.10
4	38.5	38.80	37.90	38.30	37.30	37.40	38.10
5	38.70	38.40	38.80	38.40	37.20	37.00	38.10
Group 7							
1	36.80	38.70	38.50	38.60	37.00	37.30	38.10
2	37.80	38.80	38.70	37.40	37.50	37.40	38.10
3	38.90	38.70	38.00	38.00	37.50	37.50	38.10
4	38.40	38.30	38.00	38.20	37.40	37.30	38.10
5	38.50	38.10	39.00	38.60	37.10	37.10	38.10

APPENDIX 8a

Anti-Inflammatory Studies of Crude Extract *of Tephrosia bracteolata* Leaves in Rats.

Group	Sex	Weight (g)	Concn. Received (mg)	Vol. Adm. (ml)	Dosage (mg/kg)
1	F	202.10	-	0.2 ml	-
I. 2	M	182.20	-	Normal	-
3	M	337.00	-	saline	-
1	M	142.60	1.44	0.29	10
2. 2	F	172.20	1.72	0.34	10
3	F	210.58	2.10	0.42	10
1	F	185.40	37.08	0.19	200
3. 2	M	186.18	37.28	0.19	200
3	M	257.18	51.44	0.26	200
1	F	126.60	50.64	0.13	400
4. 2	M	163.60	65.44	0.16	400
3	M	280.00	112.00	0.28	400
1	F	12060	72.36	0.12	600
5. 2	M	187.30	112.38	0.19	600
3	M	280.00	168.00	0.28	600
1	F	147.50	117.60	0.15	800
6. 2	F	154.40	122.72	0.15	800
3	M	217.30	173.84	0.22	800

APPENDIX 8b

Rat Paw Volumes (cm) as a Measure of Inflammation in Rats Administered Crude Extract *of Tephrosia bracteolata* Leaves and indomethacin.

Time (Hrs.)	Untreated Control 1	Indomethacin (10 mg/kg) 2	Crude Extract *of Tephrosia bracteolata* Leaves 200 mg/kg 3	400 mg/kg 4	600 mg/kg 5	800 mg/kg 6
Before	0.23	0.22	0.21	0.32	0.23	0.24
oedema	0.20	0.27	0.23	0.18	0.30	0.17
induction	0.23	0.20	0.26	0.31	0.31	0.29
	0.35	0.34	0.25	0.35	0.39	0.32
0	0.42	0.38	0.35	0.34	0.37	0.36
	0.14	0.36	0.32	0.33	0.32	0.35
	0.45	0.41	0.35	0.43	0.43	0.37
1	0.47	0.51	0.42	0.43	0.38	0.38
	0.47	0.38	0.40	0.49	0.38	0.38
	0.67	0.44	0.51	0.50	0.52	0.38
2	0.61	0.50	0.51	0.59	0.58	0.42
	0.63	0.49	0.59	0.58	0.54	0.43
	0.52	0.48	0.51	0.61	0.51	0.38
3	0.50	0.47	0.60	0.53	0.54	0.38
	0.55	0.45	0.51	0.51	0.55	0.42
	0.51	0.30	0.43	0.42	0.50	0.32
4	0.41	0.38	0.39	0.44	0.53	0.34
	0.50	0.40	0.43	0.45	0.53	0.41
	0.50	0.30	0.41	0.40	0.47	0.31
6	0.41	0.36	0.39	0.40	0.51	0.30
	0.50	0.38	0.42	0.42	0.50	0.38
	0.46	0.28	0.30	0.33	0.28	0.29
18	0.40	0.27	0.27	0.25	0.26	0.21
	0.33	0.26	0.26	0.31	0.36	0.36
	0.29	0.24	0.21	0.30	0.24	0.22
24	0.37	0.27	0.29	0.25	0.30	0.23
	0.29	0.27	0.20	0.28	0.30	0.23

Appendix 9

Evaluation of Potentiation of Barbiturate Sleeping Time in Mice Treated with Crude Extract of *Tephrosia bracteolata* Leaves, Chlorpromazine and Sodium Thiopentone.

Group	Sex	Weight (g)	Concn. Received (mg)	Volume Adm. (ml)	Dosage (mg/kg)	Onset of sleep	Wake-up time	Duration (min.)	Mean ± SEM (min.)	Dosage of sodium thiopentone (mg/kg)
1	F	30.1	-	0.2 ml	-	12^{58}	1^{10}	12		40
A 2	F	30.7	-	normal	-	1^{16}	1^{16}	11	11±0.577	40
3	M	32.1	-	saline	-	1^{20}	1^{20}	10		40
1	F	30.4	0.304	0.30	10	12^{50}	1^{11}	21		40
B 2	M	31.8	0.318	0.32	10	12^{51}	1^{13}	22	21±0.577	40
3	F	37.5	0.375	0.38	10	12^{53}	1^{15}	20		40
1	M	24.3	4.86	0.30	200	1^{12}	1^{23}	11		40
C_1 2	F	27.1	5.42	0.34	200	1^{15}	1^{24}	09	10.7±0.882	40
3	M	37.1	7.44	0.47	200	1^{13}	1^{25}	12		40
1	M	20.8	8.32	0.26	400	1^{18}	1^{28}	10		40
C_2 2	F	27.3	10.92	0.34	400	1^{19}	1^{30}	11	11.3±0.880	40
3	F	38.8	15.32	0.49	400	1^{22}	1^{25}	13		40
1	M	24.8	14.88	0.19	600	1^{25}	1^{36}	11		40
$C_3$2	F	25.1	15.06	0.19	600	1^{30}	1^{40}	10	10.3±0.330	40
3	M	31.4	18.84	0.24	600	1^{28}	1^{37}	10		40
1	M	25.3	20.24	0.32	800	1^{30}	1^{42}	12		40
$C_4$2	F	25.8	20.24	0.32	800	1^{32}	1^{41}	09	10.7±0.882	40
3	F	30.0	24.00	0.38	800	1^{34}	1^{45}	11		40

A is the untreated control group, received only 0.2 ml normal saline.

B received chlorpromazine. C_1 - C_4 received 200, 400, 600, and 800 mg / kg *Tephrosia bracteolata* crude extract, respectively.