ETHNOPHARMACOLOGICAL AND PHYTOCHEMICAL PROPERTIES OF SOME PLANTS USED IN THE MANAGEMENT OF PAIN

OTIMENYIN ORITSETIMENYIN SUNDAY
(B. Pharm. M.Sc.)
(PGPH/UJ/12325/00)

A Thesis in the DEPARTMENT OF PHARMACOLOGY, Faculty of Pharmaceutical Sciences, UNIVERSITY OF JOS.
Submitted to the School of Postgraduate Studies, University of Jos, in partial fulfillment of the requirements for the award of PhD. (DOCTOR OF PHILOSOPHY) of the UNIVERSITY OF JOS.

MAY, 2008

DECLARATION

I hereby declare that this work is the product of my own research efforts: under the supervision of Prof. (Mrs.) M. O. Uguru., and has not been presented elsewhere for the award of a degree or certificate. All sources have been duly distinguished and appropriately acknowledged.

Otimenyin Oritsetimenyin Sunday

(PGPH/UJ/12325/00)

CERTIFICATION

This is to certify that the research work for this thesis and the subsequent preparation of this progress report by OTIMENYIN ORITSETIMENYIN SUNDAY (PGPH/UJ/12325/00) were carried out under my supervision.

_____

Prof. M. O. Uguru. PhD**.**

(Supervisor)

_____

Prof. J. C. Aguiyi. PhD.

(Head of Department)

ACKNOWLEDGEMENTS

"To God be the glory, great things He has done". The ever-faithful God has been so good and is still good to me. His miraculous power has never ceased in my life. I am indebted to Him for the greatest miracle (Salvation) He gave me and for seeing me through the battles of life, which were toughest during my pursuit of this programme. He despite the raging battles gave me the strength to continue with this work to this level. To Him alone be all the glory, adoration and praises.

I also thank God for sparing my life and granting me the strength, wisdom and grace to complete this work.

I am greatly indebted to my supervisor Prof. (Mrs.) M. O. Uguru, who despite her busy schedule, made out time to supervise this work, I thank God for making her my supervisor and for the attention, kindness, patience she exhibited during my working relationship with her. My gratitude also goes to God for the support (research materials) and advice she gave me during my academics pursuit and life generally. This work would not have been possible without her kind assistance and patience.

I am also heavily indebted to my children who were helpful during my research work. They spent boring time with me in the laboratory even at late hours. Their sacrifice is highly appreciated in letting go some legitimate things to enable me achieve this goal. Their advice during stressful times can never be forgotten. Though young they endowed with great wisdom. To God be the glory for their spiritual support in prayer.

My mother and my late father were very understanding even when I have to use some of their entitlements for my research. I thank God on their behalf for their patience, love and care during this programme.

I also thank all the staff in Pharmacology Department and Animal House for their support, attention, assistance and contribution to the work.

The staff of the Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, University of Jos, were also very co-operative. To God be the glory for their assistance and contribution to this work.

I also thank the staff of the Department of Pharmacology, Ahmadu Bello University, Zaria , Nigeria, for their assistance and provision of facilities for part of this work. My thanks go especially to Prof. H. Kwanashie who was of assistance during my research visit to the University.

I also thank Dr. P. Manaseh and the staff of the Department of Pathology, College of Medical Sciences, University of Jos, for interpreting the histological results.

Many thanks go to Prof. F. K. Okwuasaba, who has been a daddy and mentor, for his advice and support during the course of this study.

Once again, I wish to appreciate God for all the innumerable assistance He has rendered to me during my study. He granted me favor, wisdom, knowledge and good health.

DEDICATION

This work is dedicated to the Almighty God and my Lord and Savior Jesus Christ.

To my beloved children: Emmanuel, Faith, and Precious.

TABLES OF CONTENT

CHAPTER THREE
MATERIALS AND METHODS

CHAPTER FOUR
RESULTS

CHAPTER FIVE
DISCUSSION

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Pain is a worldwide problem and is associated with most disease conditions. The properties of herbs used to treat pain were investigated with the aim of confirming their use and finding out some pharmacological properties that may augment their analgesic activity. A total of eighteen plants (*Erythrina senegalesis, Nauclea latifollia, Kizelia africana, Pseudocedrela kotchyi, Crotalaris spp., Boswellia dalzielli, Khaya senegalensis, Annona senegalensis, Xylopia aethiopica, Ficus thonningii, Cassia goratensis, Prosopis Africana, Stachytapheta indica, Crinum glaucum, Holerrhena floribunda, Momordica balsamina, Enantia Chlorantha and Sarcocephalus esculentus*) were collected and identified out of which nine plants (*S. esculentus*, *C. goratensis*, *F. thonningii*, *P. kotschyi*, *E. chlorantha*, *N. latifolia*, *M. balsamina*, *S. indica*, *P. africana*) were selected for study for possible analgesic and anti-inflammatory activities after preliminary investigations (using writhing reflex test). Analgesic activities were studied using acetic acid induced writhing reflex and hot plate method. Anti-inflammatory activities were conducted using egg albumin induced right paw oedema method. The study revealed that all the nine plants studied had peripheral and / or central analgesic activities. They also poses anti-inflammatory activities. Phytochemical analysis showed that majority of these plants contains flavonoids, tannins and saponins. The intraperitoneal LD_{50} revealed that the plants are safe for consumption except *N. latifolia* and *S. indica* with LD_{50} 0.80 and 0.15 g /Kg respectively. Oral LD_{50} (LD_{50} greater than 7.00 g /Kg) revealed that *S. indica* is safe for oral consumption. Sub acute toxicity test showed that *M. balsamina* has no significant ($P>0.05$) effect on vital organs, haematological and biochemical parameter. *M. balsamina* was also effective in tail flick and formalin test models of analgesia, this indicates that it is effective in the management of peripheral and centrally induced pain. Further study also showed that *M. balsamina* has hypotensive, anti-diarrhoeal (castor oil and guinea pig ileum models), shorting of onset of Urethane induced sleep, prolongation of urethane induced sleeping time, but not anti-convulsive effects. *S. indica* was shown to be effective in castor oil induced diarrhoea but not in isolated guinea pig ileum diarrhoea model. *M. balsamina* was of particular interest because it had most of the claimed properties and is the most potent of the nine plants studied. These results support some of the uses of these plants in folk medicine.

CHAPTER ONE
INTRODUCTION

1.1 THE ORIGIN OF TRADITIONAL MEDICINE

It is interesting that even "primitive people" could discover relationships between drugs, food and diseases. The inborn craving for food and medicine has led to their discoveries. Man recognized their dependence on nature in both health and illness. They used plants, animal parts and minerals as sources of food and to control and treat different types of ailments. Led by instinct, taste, and experience, "primitive" people treated illness using plants, animal parts, and minerals that were not part of their usual diet.

All cultures have a history of one folk medicine or the other. These often include the use of plants and in some cases animal parts. In ancient cultures, people methodically and systematically collected information on herbs and developed well-defined herbal pharmacopoeias. Much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native peoples. About 70-80% of the populations of the developing countries still use traditional medicines to meet their health care needs (Farnsworth *et al*., 1985). Modern pharmacopoeias contain at least 25% of drugs derived from plants and many others, are synthetic analogues, built on prototype compounds isolated from plants (Farnsworth *et al*., 1985).

1.2 FACTORS AFFECTING THE DEVELOPMENT OF TRADITIONAL MEDICINE

The development of traditional medicine has been hampered in the past by the attitude of the public and government on its use. The bias arose from the belief that alternative medicine has a strong link with the practice of spiriticism, which

negates practice of orthodox medicine. Another reason for government`s attitude to traditional medicine is lack of scientific basis for the claimed actions of plants and traditional methods of healing. Despite the bias against traditional medicine, there has been in the last decades a dramatic increase in the use of herbs and herbal remedies worldwide (Brevoort, 1998; Blumenthal, 1999). Efforts have been made to eliminate this bias by restructuring the requirements for proof of efficacy and concentrating on safety, and by removing the need for extensive analyses of chemically complex natural product medicine (NIH, 1992; NFAM, 2004). The recognized growing dependence on the use of alternative medicine systems for achieving cost reduction and health care services goals has prompted governments worldwide to increase investment in complementary and alternative medicine (NIH, 1992; NFAM, 2004).

In Nigeria, low income earners and the unemployed depend on herbal medicinal products for their medicinal needs. Most of these herbal products are thought to relieve pain and cure several diseases. Disease conditions are normally associated with pain, head ache and inflammation, and the relief of these symptoms is taken as a cure of the ailment by traditional doctors and patients. Craftsmen (mechanics, painters etc.) take some of these herbs after a day's work in the belief that it will relieve their pain and enhance their performance the next day. Some of these herbal products are not, in some cases, effective. A number of death has been related to the toxic effects of some of these herbs (personal communications). The dependence on herbal medicines is due mainly to the fact that most people are poor and cannot afford orthodox medicines or due to their ready availability and belief that herbal medicines are more effective than

orthodox medicines. These herbs are taken in most cases without proper dosage instructions and are claimed to have many activities.

Pain is a symptom of vitually all diseases, and it is a world-wide problem because it is associated with most ailments. This makes the aleviation of pain an important venture while the cause of pain is being treated. Pain associated with terminal diseases (like cancer), are of great concern. Since these diseases cannot be cured, drugs that relieve the patient of the symptoms (pain) of the disease are normally used to reduce the suffering of the patient.

Potent pharmacological substances are discovered scientifically through pharmacological screening of herbal products. Examples are quinine, strychnine (convulsant), and curare (muscle relaxant), (Sandberg *et al.*, 1971; Verpoorte and Bohlin, 1976). Such search is normaly preceeded by ethnobotanical survey of the medicinal plants used in communities. Identification of such plants is crucial to their use in development and western medicine. Documentation of the habitat of such plants is also necessary because of the difference in the constituents of plants from different geographical locations.

1.3 OBJECTIVES OF THE STUDY

This research seeks

First, to ascertain the claims of traditional healers on the uses of the collected plants

Second, to collect and identify plants used to treat pain in Jos North Local Government Area (LGA) of Plateau State, Nigeria.

Third, to evaluate the analgesic, anti-inflammatory properties of these plants.

Fourth, to evaluate other pharmacological properties that may augument the analgesic activity and

Fifth, to evaluate the analgesic properties of fraction(s) of the plant, if found to have analgesic properties.

1.4 RELEVANCE OF THE RESEARCH

The fact that these plants have been in use for a very long time may be a proof that they have some sort of activity and are safe for consumption. Plants without activity and those that are toxic are normally removed from the list of plants used in folk medicine. Confirmation of the claimed activity is necessary to ascertain the pharmacological properties of these herbs.

Pain is a universal problem and people go for pain relievers to suppress its traumatic and emotional effects. People living with terminal diseases (like Cancer, sickle cell anaemia, etc) are always in pain. A strong, safe and reliable analgesic with minimal side effects is needed to help these individuals live quality lives. Death has been reported after ingestion of some herbal medicines (personal communication). It is therefore necessary for reseach to be carried out in this area to acertain the safety of these herbs. Some herbs, may appear safe, but may have silent negative effect(s) on the body system. For example, high dose of willow bark decoction has eroding effect on the gastro-intestinal tract mucosa. This effect may be fatal, if necessary precautions (ensuring proper dosage) are not taken. It is

hoped that after this work the general public will be better informed on the danger and/or use of the selected herbal medicines. It is also hoped that the traditional healers will stop prescribing herbs that may pose a danger to patient. They will also be advised on a better and more effective way to prepare and use the selected plants.

CHAPTER TWO
LITERATURE REVIEW

2.1 TRADITIONAL MEDICINE

Traditional Medicine is defined as the total combination of knowledge and practices (whether explicable or not) used in diagnosing, preventing or eliminating any physical,mental or social disease and which may rely exclusively on past experience and observation passed from generation to generation verbally or in writing (Sofowora, 1982).

2.1.1 History of Medicinal Plants

According to the World Health Organization (WHO), about three-quarters of the world population relies upon traditional remedies (mainly herbs) for the health care of its citizens. In fact, herbs/plants are the oldest friends of mankind. They not only provide food and shelter but also serve as medicines for different ailments. Herbal medicines also called, traditional or natural medicine has existed in one way or another in different cultures/civilizations, such as Egyptians, Western, African, Chinese, Kampo (Japan) and Greco-Arab or Unani/Tibb (south Asia).

Historians from all around the world have produced evidence to show that apparently all primitive peoples used herbs often in a sophisticated way. Quinine from Cinchona bark was used to treat the symptoms of malaria long before the disease was identified and the raw ingredients of a common garden aspirin tablet have been a popular painkiller for far longer than we have had access to tablet-making machinery.

By the middle of the nineteenth century at least 70 - 80% of all medicines were derived from herbs. This era was followed by the revolution inspired by the development of the pharmaceutical industry and synthetic drugs dominated, though herbal medicine has never been out of the scene. Even today most pharmacies in the Western part of the world, stock at least 25% plant-derived drugs as supplements. Indeed today many pharmacological classes of drugs include a natural product prototype (Gilani *et al.*, 1992). Aspirin, atropine, artimesinin, colchicine, digoxin, ephedrine, morphine, physostigmine, pilocarpine, quinine, quinidine, reserpine, taxol, tubocurarine, vincristine, and vinblastine are a few examples of what medicinal plants have given us in the past. Most of these plant-derived drugs were originally discovered through the study of traditional cures and folk knowledge of indigenous people and some of these could not be substituted despite the enormous advancement in synthetic chemistry.

Morphine, isolated from the opium poppy (*Papaver somniferum*) is one of the early molecules that entered into conventional medicine and is the humanity's finest painkiller. Only the cancer patients suffering from terminal pain can appreciate the value of morphine, which remains drug of choice today despite its abuse potential. Indeed, the isolation of morphine from crude opium by Serturner in 1806 (Jurna, 2003) stimulated so much wide-spread research on vegetable drugs. This discovery led to the synthesis of morphine and morphine related compounds.

One of the important areas in which compounds from plant sources have contributed successfully is cardiovascular research (Gilani, 1998). Digitalis and other cardiac glycosides derived from the foxglove (*Digitalis purpurea* Linn.) are

perhaps the classic examples. They represent a widely used group of clinically effective compounds which produce positive inotropic effect on the failing heart as well as having value in the treatment of atrial fibrillation. As a group they are superior to date to any synthetic or semi-synthetic substitutes even though they are among the most toxic group of clinically useful drugs and have unique mode of action with selective cardiotonic activity, without accompanying tachycardia (Rietbrock and Woodcock, 1985).

2.1.2 The Practice of Traditional Medicine

The practices of Traditional Medicine involve the use of herbs and manipulation techniques. This practice is found in all countries and in every tribe in the world. Africa's traditional medicine practice is not well developed like the Chinese and Indian traditional medicine. Some well developed practices include:

i. Traditional Chinese Medicine (TCM)

Traditional Chinese medicine is the most extensive and best documented source of materia medica that existed in ancient times. The materia medica is unavailable to the western world by language barrier, which has made the interpretation and application exclusive to the Chinese people. It is the traditional medicine practised by the Chinese people. Although Chinese materia medica may not have as long a history as those of Egypt or India, it is well-documented and is still in use today. The majority of Chinese depend on it regularly for their health care and so far little of it has been "rationalized" out of existence by modern science, a contrast to traditional medical practice of some other cultures. It is extremely tempting to rationalize the effects of an herb or herbal preparation

based on the often limited chemical or biological data on it. Such rationalization led to the loss of valuable medicinal plants knowledge. Examples of TCM include rhubarb, aconite, and cinnabar. Rhubarb is one of the most used medicines for upper gastrointestinal bleeding (Sun, 1986) in China. Processed aconite is an ingredient in some Chinese tonics; and cinnabar is a commonly used sedative.

Chinese herbal medicine is empirically based. It is the accumulated knowledge of more than four thousand years of practical experience. Based on ancient literary records, it is now known that back in 1100 BC during the West Zhou era, Chinese medicine had already developed into different branches, including disease therapy, ulcer therapy, diet therapy and veterinary medicine.

ii. Ayurveda

Ayurveda (Ayur: Life; Veda: Science) means science of life in Sanskrit. It aims at holistic management of health and disease. It remains one of the most ancient medical systems widely practised in the Indian subcontinent and has a sound philosophical, experiential and experimental basis. A considerable amount of research on pharmacognosy, chemistry, pharmacology and clinical therapeutics has been carried out on Ayurvedic medicinal plants (Patwardhan *et al.*, 2004). Charak Samhita and Sushrut Samhita (100–500 B.C.) are main Ayurvedic classics, which describe over 700 plants along with their classification, pharmacological and therapeutic properties. Rasayana therapy is one of the eight branches of Ayurveda and generally consists of nourishing and rejuvenating drugs with multiple applications for longevity, memory enhancement, immunomodulation and adaptogenicity (Patwardhan, 2005a). Many researchers

have proposed the neuro-endocrine immune axis theory to explain Rasayana activity and they have considered it to be an innovative source of immuno-drugs (Patwardhan, 2005c, b).

2.1.3 Western Medicine System

This is practised in European and Africa countries. It is classifies plants according to their pharmacological activities e.g. analgesic, etc. Whereas the traditional healers believe in the efficacy of herbs as passed on to them by their fore fathers, western medicine believes in the confirmation of the properties of herbs by subjecting them to thorough screening before putting them into use. These objectives have led to the development of sophisticated equiptments for the evaluation of drugs and the development of complex clinical trial methods for screening drugs which include;

i. Hippocratic Screening

The use of whole animals to test the effect of crude extract has been the practice; this is known as the Hippocratic screening method (Malone and Robichaud, 1962). Crude plant extracts are administered by chosen route of administration, after which a whole set of observations are made during a given time period. The test is validated by injecting a series of known drugs with similar effect. By comparing the observations made for these compounds with those obtained with the extracts and control, an indication about possible activities is obtained. (Sandberg *et al.*, 1971; Verpoorte and Bohlin, 1976). Subsequently, bioassay guided fractionation to identify the active compounds is carried out. In the last decade, attention has shifted from the use of in-vivo models to the use of

in-vitro models of assaying crude drugs. The reason being advanced for the use of in-vitro models have been the protection of animal rights.

ii. System Biology Approach

In recent times, system biology has been re-embraced. Systems biology approach involves a method that allow better understanding of the mode of action of crude extract by comparing the changes in the transcriptome, proteome and metabolome patterns with that of known drugs. Crude extract is administered to whole animals, through specific route of administration; blood samples, urine samples and lumber puncture are withdrawn from the animal. The samples obtained are then subjected to biochemical analysis to detect variations in standard parameters and possibly the involvement of a pro-drug in its actions. It is of advantage over the use of isolated organs and tissues because pro-drug can be detected. It is also possible to screen at molecular level, or in some cases at cellular level.

iii. Holistic Approach

Our ancestors did not only discover active plants, but they also developed a holistic approach in their medical systems, e.g. the Ayurvedic medicine and traditional Chinese medicine (Chan, 2005). This is among others reflected in a tailor-made prescription for each individual patient after an extensive diagnosis. A concept that now is also considered to be of interest for the pharmaceutical industry. One may expect that with the advent of pharmacogenomics in the coming years in Western medicine a more individualized pharmacotherapy will develop, long after the already mentioned traditional medical systems.

Good examples of this approach are the methods developed for urine analysis that allow the diagnosis of a variety of diseases, as well as measuring possible liver or kidney toxicity. Analysis of large numbers of urine samples of healthy people and patients made by NMR has resulted in the identification of certain biomarkers for diseases (Holmes *et al.*, 2000). By analysis of rat`s urine possible toxic properties of novel compounds can be detected (Beckonert *et al.*, 2003; Lindon *et al.*, 2004).

The methods currently used in western medicine can basically be classified into three groups:

I. Chromatography based methods (GC, HPLC);

II. Molecular weight based methods (mass spectrometry);

III. Physical characteristics based methods (NMR spectrometry).

Latest technology (for measuring as many different parameters as possible) can be used to measure as many parameters as possible to discover possible leads to the mode of action. This method of measuring as many parameters as possible may have great impact on future drug development. Pro-drugs, synergistic drugs can be discovered. This can lead to the possibly discovery of new modes of actions, new targets and new lead compounds.

2.1.4 Development of Drugs from Plants

Globally, there is a positive trend in favor of traditional and integrative health sciences both in research and practice. There are common approaches to drug discovery including use of chemical biology, serendipity, chemical synthesis, combinatorial chemistry and genomics. However, the innovative approaches

involve ethno-pharmacology, reverse pharmacology, holistic, systems biology and personalized medicine, (Patwardhan, 2005d).

There are clear trends to show that the mainstream in pharmaceutical research is moving away from single molecule or single target approach to combinations and multiple target approaches. The ethno-pharmacology knowledge and experiential base allows drug research from 'Clinics to Laboratories' a true Reverse Pharmacology Approach (Vaidya, 2005). This opens room for researches into medicinal plants that have been in use for decades. In this process, 'safety' remains the most important starting point and the efficacy becomes a matter of validation. The first step into the research involves the determination of the LD_{50}, to ascertain the level of safety of the crude plant extract. Thereafter, acute toxicity studies are carried out on experimental animals. If safe for administration, researches into the claimed effects are carried out on experimental animals by subjecting the herbal medicines to pharmacological screening and the active principle isolated. If toxic, the toxic principle is isolated, and the other isolated principle tested for the claimed effects and other pharmacological effects. The toxic principle is also assayed for possible pharmacological activity at very low doses. A golden triangle consisting of Traditional Knowledge, Modern Medicine and Modern Science with systems orientation will converge to form an innovative discovery engine for newer, safer, affordable and effective therapies (Mashelkar, 2005). In Nigeria, efforts in these directions are in progress to establish pharmaco-epidemiological and experimental evidence-base for new chemical/molecular entities and development of standardized herbal formulations.

2.1.5 The Practice of Traditional Medicine in Nigeria

In contrast to the two well developed systems of traditional medicine found in Indian Ayurvedic and Chinese traditional medicine, which have been extensively documented over centuries (Cragg and Newman, 2001), there is very little recorded documentation for most of African traditional medicine.

The fact that African traditional knowledge systems are largely oral, and not written, accentuates the fragility of this type of indigenous knowledge. As a result of urbanization and strong cultural influences from other regions of the world, there is now an ever-increasing loss of traditional knowledge in Nigeria, and Africa in general. There are few written materials on the identification and use of plants in Nigeria and West Africa. Examples are the work of Burkhil, (1985) and Dalziel, (1987).

A major emphasis of ethno-pharmacological research in Nigeria has been establishing the scientific rationale for traditional medicines and validating their use. This is largely due to the current trend which is moving towards the integration of traditional herbal medicine with primary healthcare. In Nigeria, the prescription and use of traditional medicine is currently not regulated. As a result, there is always the danger of misadministration, especially of toxic plants. Furthermore, the potential geno-toxic effects that follow prolonged use of some of the more popular remedies are also cause for concern (Fennell *et al.*, 2004a,b).

Many plants from Nigeria have been screened for antibacterial, antifungal, anthelmintic, antiamoebic, antischistosomal, antimalarial, anti-inflammatory and analgesic activity, as well as psychotropic and neurotropic activity using appropriate *in-vitro* an *in-vivo* tests.

2.1.6 Plant Parts Used and Methods of Preparation

Leaves are the most widely used plant parts, followed by roots, flowers, fruits and seeds, above ground parts and the whole plant. Some of the remedies are prepared by boiling the freshly collected plant parts in water and the filtrate stored in bottles for subsequent administration. The solution is normally discarded after five days and fresh ones prepared if there is need to continue the treatment. Method of preparation varies for different medication and depends on subsequent use of the medicine. Some of the methods of preparation are;

i. **Hot infusion:** The leaves, root barks, grass, stem, flowers or as such as may be required is put in an earthen pot with enough quantity of water to cover the herbs and then allowed to boil for some minutes. The resultant fluid is strained and drunk while hot or cold. When the rest in the pot is exhausted, the patient goes back to the herbalist for refill or stops if he gets well.

ii. **Cold infusion:** This requires that the herbs are covered in enough quantity of water to soak and then left over night to properly infuse. This soaking is done in earthen vessels or half-calabash. The following day the infusion is strained and drunk or used to bathe.

iii. **Standard infusion:** This method is like making a cup of tea. Here, the herbs are put in the required container and then water is boiled in a separate container and poured into the container containing the herbs. This is allowed to stand for about 20-30minutes before it is strained and drunk.

iv. Another way is by extracting the juice out of the plant. This is done by washing the plant part(s) and then putting it in a calabash container with

enough quantity of water to cover it. The plant parts are squeezed with clean hands until all the juice pressed is out. This is strained and drunk.

v. For those plants used as external medication, the plant part is ground or pounded with the addition of small quantity of vehicle (water, oil etc). The resultant mash is applied to the affected part of the body.

vi. Plants for medication used by instillation into, body cavities are ground or pounded and then wrapped in another leaf which acts as a container from which the material is squeezed into the part of the body. The leaves most commonly used are those that of the "Ogirichi" plant (*Newbouldia laevis* (P. Beauv.) Seeman ex Bureau), then the medication is held above the part of the body to be instilled and then squeezed out as drops into such cavity for example eye, ear and mouth.

2.1.7 Advantages of Traditional Medicine over Orthodox Medicine

Traditional medicine has some strength that orthodox medicine is lacking, namely the holistic view of the patient's situation. In traditional practice, the psychological, spiritual and social aspects play a large role, and this holistic treatment can, to some extent, make up for the often weaker aspect, the medicinal treatment, when compared to orthodox biomedicine.

This situation means that traditional medicine, which is closely linked with peoples' cultures, is not going to disappear if and when othodox medicines become available to them. A study from Kenya showed that patients had a clear sense of which diseases they would go to a western clinic for, and when they would visit a traditional healer (Van der Geest, 1997). In Nigeria, traditional

healers are flourishing in urban areas where western health care is available (Mander *et al.*, 1997). Patients seem to have a clear sense of which diseases they would go to a western clinic for, and when they would visit a traditional healer.

2.1.8 The Development of Phytotherapies (Phytomedicines)

This area is currently receiving much attention in Nigeria with the establishment of Nigerian Institute of Pharmaceutical Research and improved funding, much is being done to develop Nigerian phytomedicines. The academic community and the pharmaceutical companies are also contributing their quota to the development of phytomedicines. The basic idea is to use medicinal plants to develop standardized phytomedicines (phytotherapics or herbal medicine) with proven efficacy (assessed by both pre-clinical and clinical studies), safety and high quality.

2.2 PAIN

Pain, though usually perceived as an unpleasant feeling, is important for survival. It is the sense of pain felt that alerts of danger and warns of impending danger. The sense of pain helps us to know when to withdraw our hand from a hot surface or to seek medical attention for broken bones. Pain affects us physically, mentally and socially. It affects the quality of life we live and is one of the determinants of our joy. Understanding the mechanisms of pain, how pain is perceived, and what reduces pain, is important for the development of potent pain relieving drugs.

2.2.1 Nociception

The term nociception was coined from the Latin word “nocere” which means “to harm” by Sherrington (1910) at the beginning of the twentieth century. It is commonly referred to as pain. Nociception/pain has at least three functions:

- To warn the individual of the existence of real tissue damage
- To warn the individual of the probability that tissue damage is about to occur by realizing that a stimulus has the potential to cause such damage.
- To warn a social group of danger as soon as it exists for any of one its members (Dennis and Melzack, 1983).

Behaviours resulting from pain can facilitate other fundamental biological functions, such as the maintenance of tissue “trophicity” and regeneration (notably in the processes of inflammation and healing). The importance of these behaviours is well illustrated in humans through pathological cases of congenital insensitivity to painful stimuli, in which truly natural experiences can have catastrophic consequences.

The nociceptive system is complex in humans and mammals, with the overall effect of producing pain. This complexity increases from lower animals to the most developed animals. The aim mainly is to avoid organic lesions or their aggravation (Walters, 1994).

2.2.2 Mechanisms of Pain

Pain is detected by special types of sensory receptors known as nociceptors. Nociceptors are networks of free nerve endings found in the skin (Carlson 2004). These free nerve endings are primary afferent nerves that respond differently to noxious stimuli. There are four types of nociceptors.

- Thermonocicptors: They respond to temperatures lower than 18°C and higher than 45°C (Davies, 2001). Thermonociceptors contain the vanilloid receptor (VR1 protein) that detects heat, especially heat associated with inflammation (Stucky, *et al.*, 2001).

- Mechanoceptors: These receptors respond to mechanical stimuli that cause physical distortion in the skin, especially from sharp objects (Davies, 2001).

- Chemonociceptors: They respond to increased levels of endogenous or exogenous chemicals.

- Polymodal nociceptor: These receptors respond to multiple types of noxious stimuli (Davies, 2001).

2.2.3 Perception of Pain

The perception of pain can be affected by emotional and psychological state of the person in pain, memories of past experiences, person's upbringings,

attitudes, expectations, age, sex, and social and cultural influences. There are three different perceptual and behavioral effects of pain. These include:

- The first is a sensory perception, which is responsible for the perception of how intense the level of pain is. This type of perception is mediated by the section of the spinothalamic tract which projects from the ventral post lateral thalamus to the primary and secondary sensory cortex.

- The second effect is the emotional consequences, such as the discomfort people feel when they are in pain. This perception is caused by a pathway that ends in the insular cortex and the anterior cingulate cortex.

- The third perception of pain is the long term emotions that result from chronic pain which are caused by pathways that reach the prefrontal cortex (Carlson, 2004).

Since the perception of pain is affected by many factors, the assessment of pain is based on the response of the patients to questions relating to pain, e.g. McGill Pain Questionnaire

2.2.4 Models of Nociception

The hot plate, tail flick test, and the formalin test are three popular models of nociception. These models are useful in testing drugs for analgesic activity.

i. Hot Plate Model

The hot plate test uses heat as the noxious stimuli. This test produces significant analgesic effects for acetylcholine agonist, NMDA agonist, opioid

compounds such as morphine, and tricyclic anti-depressents such as amitriptyline and imipramine. It is a model of central pain.

ii. Tail Flick Test

The tail flick test also uses heat as the noxious stimulus. With this test, the stimulus causes a simple nociceptive reflex response in which the rat or mouse flicks its tail away from the heat source.

iii Formalin Test

This test uses 2 - 5% formalin solution as a chemical noxious stimulus. Injection of formalin solution into the paw of a rat or a mouse causes persistent pain caused by peripheral tissue injuries and inflammation of the cells. The formalin injected into the paw causes spontaneous behaviours in mice and rats. The mouse licks, flicks and bites its paws and elevates its foot from the floor. This model produces biphasic nociceptive responses. The first phase lasts approximately 6-10 minutes and begins immediately after the injection. The C pain fibres are believed to be responsible for this phase. The second phase occurs 12-20 minutes after the first formalin injection for rats and 12-40 minutes after the first injection for mice. This phase is mediated by peripheral inflammation and by central sensitization due to the prolonged afferent input to the spinal cord after the injection of formalin. Clinical symptoms such as hyperalgesia associated with tissue injury are believed to be caused by peripheral inflammation and central sensitization. Therefore, drugs with anti-inflammatory and/or analgesic properties can be effectively tested using this model.

iv. Mechanical Stimuli Model

These tests involve the application of pressure of increasing intensity to a punctiform area on the hind paw or, far less commonly, on the tail. The paw or tail of the test animal is jammed between a plane surface and a blunt point mounted on top of a system of cogwheels with a cursor that can be displaced along the length of a graduated beam (Green *et al.*, 1951). These devices permit the application of increasing measurable pressures and the interruption of the test when the threshold is reached. The measured parameter is the threshold (weight in grams) for the appearance of a given behaviour. When the pressure increases, there is reflex withdrawal of the paw, withdrawal movement (whereby the animal tries to release its trapped limb), followed by a sort of struggle, and finally a vocal reaction. The first of these reactions is a spinal reflex (peripheral pain), while the last two clearly involve supraspinal structures (central pain).

v. Electrical Models

a. Electrical Stimulation of the Tail. Electrical stimuli of gradually increasing intensities are delivered through subcutaneous electrodes inserted into the tail of the rat or the mouse (Carroll and Lim, 1960). Reaction of the animals to electrical stimulation include a reflex movement of the tail, vocalization at the time of stimulation, and then vocalization continuing beyond the period of stimulation. These responses are organized on a hierarchical basis; they depend on the different levels of integration of the nociceptive signal in the central nervous system: the spinal cord, the brainstem, and the thalamus (Le Bar *et al.,* 2001*).*

b. Electrical Stimulation of the Paw. In this test, electrical stimuli of increasing intensities are delivered through the floor of the cage in which the animal is

housed and free to move (Evans, 1961). Responses observed after stimulation include: movement of the paw, vocalization during stimulation, and vocalization outlasting the period of stimulation ("vocalization after-discharge"). It is also possible to measure the thresholds for various behaviors: the animal twitching, squeaking or attempting to escape by jumping (the "flinch-jump" test). The vocal response can be recorded, measured and analyzed objectively (Eschalier *et al.*, 1988).

2.2.5 Types of Pain

There are different types of pain. They include;

i. **Arthritis:** Arthritis, or joint inflammation, can be the result of an injury, inactivity, genetics, or naturally with age. Osteoarthritis and rheumatoid arthritis are the two most common forms of arthritis. Osteoarthritis is when the cartilage in joints, especially in the hips, knees, lower back, neck, hands and feet, deteriorates, the bones in the joints rub against each other, causing pain (Rome, 2002).

ii. **Back pain:** Back pain affects large number of the population. Since the lower back or lumbar region is the area of the back that bears the most weight and is also the area which allows one to bend forwards and backwards, and twist sideways, lower back pain is the most common. Overuse or injuries cause acute back pain. Although the cause of chronic back pain is still not fully understood, muscle pain or spasms, sciatica (a condition where the sciatic nerve is either compressed or inflamed) or

herniated disks (in which the disk in between vertebrae slips out of place) may be related to chronic back pain (Rome, 2002).

iii. **Headaches:** Headaches are also one of the most common types of chronic pain. The most common type of headache is the tension-type headache, which is brought on by stressful situations, starring at a computer screen for too long, or assuming stressful postures for extended periods of time. Contracting muscles around the skull and/or enlarged blood vessels in the scalp may be the physiological causes of this type of pain. The other type of headache is migraine. The symptoms of migraine include throbbing pain usually located in the forehead or temple on one side of the head, and the pain is intensified by bright lights and loud noises. Reduction in the brain levels of serotonin are believed to be the cause of migraine headaches (Rome, 2002).

iv. **Cancer Pain:** Poor circulation due to blocked blood vessels, bone fractures due to metastasis, infections, inflammation, side effects form radiation and chemotherapy, a tumor pressing on a nerve, or psychological and emotional problems associated with cancer can cause cancer pain. Two common types of cancer pain are; 1. Persistent pain and 2. Breakthrough, or incident pain (Charlton, 2005). Breakthrough pain is a very severe pain. It lasts up to an hour, and occurs several times a day.

Other causes of chronic pain include: endometriosis, fibromyalgia, interstitial cystitis, irritable bowel syndrome, burning mouth syndrome, trigeminal neuralgia, temporomandibular disorders, neck pain, overuse strain injuries, chronic pelvic pain, peripheral neuropathy, and postherpetic neuralgia (Rome, 2002).

2.2.6 Management of Pain

Pain can be managed in several ways, including:

a. **Medications:** There is a wide range of medications used to treat pain. These include:

   i. **Nonsteroidal anti-inflammatory drugs (NSAIDs).** These are used for the management of mild to moderate pain. Such pain may be associated with edema and inflammation. These types of drugs are often used to treat arthritis, and pain associated with muscle sprains and strains, back and neck injuries, and cramps (Rome, 2002). NSAIDs inhibit the action of cyclooxygenase (COX-1 or COX-2). These enzymes (cyclooxygenases) catalyze the synthesis of prostaglandin from arachidonic acid (Charlton, 2005). Aspirin, Ibuprofen, Ketoprofen, and Naproxen sodium (Aleve) are common types of NSAIDs. They are commonly referred to as OTC drugs (because they can be purchased over-the-counter). Some are however dispensed as prescription drugs. Common side effects for NSAIDs, especially when more than the recommended dose is taken, includes: nausea, stomach aches, gastrointestinal bleeding, ulcers, and kidney damage. Celebrex, Vioxx, and Bextra are newer types of NSAIDs. They inhibit only COX-2 form of cyclooxygenase, and are called COX-2 inhibitors. Acetaminophen, also known as Tylenol or paracetamol is often used to treat mild to moderate pain, and

should not be used for inflammation. Frequent use or taking more than the recommended dose of paracetamol can potentially lead to liver or kidney damage (Rome, 2002).

ii. **Opiates:** Opiates can be used to treat chronic pain. They include; codeine, methadone, morphine, and many others. Opiates bind to opioid receptors to produce analgesia.

iii. **Topical medications.** These are used to treat patients with chronic pain, especially nerve pain (neuralgia) and inflammatory pain. Topical medications may contain local anesthetics, analgesics, or counter-irritant products. Lidnocaine patches are a common form of local anesthetics, which are used to treat chronic pain in patients suffering from post-herpetic neuralgia and nerve pain. A widely used topical analgesic useful for chronic pain associated with arthritis, and post-herpetic neuralgia is capsaicin. It decreases the production of substance P in injured tissues. Counter-irritant products include popular over-the-counter brands such as ArthriCare and Icy Hot, and are used for muscle aches. These counter-irritant products stimulate both hot and cold receptors and therefore suppress pain transmission. Antidepressants can also be given to patients suffering from chronic pain, not only for the depression which may arise because of their pain, but because the antidepressants also influence the release of neurotransmitters, such as serotonin,

which are released during the transmission of pain as well (Rome, 2002).

iv. **Technologies.** Injections and nerve stimulators are types of technologies used to help manage chronic pain. Injections are used to treat chronic pain located in joints, muscles, or nerve pain (e.g. triamcinolone- as intra-articular injection). Since these injections are used at the specific area of pain, the amount of medication needed is not as high as with medications and the side effects are not as severe as when they are administered systemically. Nerve stimulation is another type of technology used in the management of pain. There are three types of nerve stimulators: 1). trans-cutaneous electronic stimulators (TENS), which stimulates the release of endorphins, 2). spinal cord stimulators, and 3). peripheral nerve stimulators, which stimulates the release of neurotransmitters (Rome, 2002).

b. **Pain clinics**. Chronic pain sufferers also sometimes benefit from going to pain centers. "A pain center is a facility with a multidisciplinary group of physicians whose collective expertise allows for the management of a wide variety of pain problems" (Rome, 2002).

2.2.7 Disease Conditions related to Pain

Pain rarely occurs as a pathological condition. In most cases it is the symptoms of other disease conditions. This explains why treatment of the underlining cause of pain may sometimes relieve pain. Management of pain

without paying attention to the underlining cause can be fatal. It is therefore important to treat pain and its underlining cause(s). Some of the disease conditions that manifest as pain include;

i. Diarrhoea

Diarrhoea is defined as the passage of abnormal liquid or unformed stool at an increased frequency (Ahlquist *et al*., 2001). Causes of diarrhoea include infectious agents, certain medications, plant and animal toxins, GIT (gastro-intestinal) disorders, and substances that increase gastrointestinal tract secretions. It can also be caused by the ingestion of poorly absorbable materials, or inflammatory and dysmotility problems of the gastro-intestinal tract (Ahlquist *et al.,* 2001). Diarrhoea is sometimes accompanied by colic and epigastric pain. Traditional herbal practitioners often use medicinal herbs for the management of diarrhoea and other related health problems. Some herbal medicines (e.g. *Xylocarpus granatum* Koeg. and *Guiera senegalensis* J. F. Gmel.) have been scientifically reported to be effective in the management of diarrhoea.

In Nigeria, diarrhoea resulting from infection is one of the known killer diseases among children under 5 years (Audu, *et al,* 2000). This may not be unconnected to the fact that toddlers are at times left to play in unhygienic environment. Such exposure can lead to bacterial and viral infections, which can cause diarrhoea. Diarrhoea is also associated with some terminal illness like AIDS, and if untreated, can lead to demise of the patient. Diarrhoea accounts for about 4-5 million deaths annually, of these 8% have been reported to be from developing countries, putting a heavy burden on the country's health budget (Syder, and

Merson, 1982). Patients who cannot afford the cost of treatment with orthodox medicines often resort to the use of herbs for the management of this disorder.

ii. Microbial Infection

Bacteria and fungi resistance to antimicrobial drugs have continued to grow in the last decades (Cohen, 1992; Nascimento *et al*; 2000). Such infection is often associated with pain and inflammatory responses. The increased prevalence of their resistance is due to extensive use and misuse of antimicrobials. This has rendered the current available antimicrobial agents inadequate to control microbial infections (Cowan, 1999) and thus creates a major public health problem (Bax *et al.*, 2000; Alade, *et al.*, 1993). This development has led to increased search to unfold new, broad spectrum, potent antimicrobial agents. Resistance to antibiotics due to bacteria such as *Staphylococcus aureus* and Pseudomonas species is of great concern (Bax *et al.*, 2000; Chopra *et al.*, 1992). Antimicrobial resistance to antimicrobial agents has lead to treatment failure and the shift of medical care from orthodox to herbal medicine. Most of the herbal medicines in use await validation of their claimed effects and possibly the development of novel antimicrobial drugs from them.

iii. Diabetes

Diabetes mellitus is a hereditary disease, a homozygous recessive trait with multiple environmental factors influencing the clinical expression of the genetic pattern. These factors can be emotional, physical (infection, trauma) and chemical such as drugs (diuretics and steroids), stress, diabetes processes (pancreatic tumor)

and caloric intake. Body weight may also increase the demand for endogenous insulin.

Diabetes is another disease that is on the increase because of modern technology and change in eating habits. Most diabetics are faced with the problem of getting solution to their diabetic pain and sore, which in most cases becomes infected. Their problem is further compounded by the side effects of the existing analgesics. A drug with antidiabetic, antibacterial and analgesic activity will be of great advantage.

iv. Convulsion

Epilepsy refers to a group of disorders characterized by recurrent seizure activity that have in common sudden excessive electrical discharge of cerebral neurons.. There are various animal models of status epilepticus which include; the pentetrazole induced convulsion (Macdonard and Barker 1977), induces seizures by blocking CL^- channel of $GABA_A$ receptors (Corda MG *et al.,* 1990), the amphetamine-induced hypermotility model (Vasquez-Freire, 1994), the bicuculline-induced convulsion and electrically induced models. The development of epilepsy is a plastic-adaptive process, which can be reproduced in animal experiments using the phenomenon known as kindling. Chemical kindling is characterized by an increased susceptibility to seizure following injection of initially subconvulsive doses of convulsants such as pentylenetetrazole (PTZ). After several injections, treated animals develop a behavioral pattern that is very similar to chronic epilepsy with secondary generalized seizures. Seizures involving the cerebral cortex result in convulsions.

Drug therapy has been found very useful in the management of epilepsy and associated seizures. Anticonvulsants are the drug of choice in recurrent seizures. Ethosuxinide is one of the first-line drugs used in the management of seizures. Primidone, the benzodiapines, lonazepam and clobazam, and acetazolamide are examples of second-line drugs (Kumar and Clark, 2005).

Significant advances are being made in recent years to treat epilepsy using second-generation drugs (Sabers and Gram, 1996). Polypharmacy is often advocated to 30% of all epileptic patients for refractory partial or generalized tonic-clonic seizures (Pellock, 1995). However, none of the new drugs fulfills the ultimate goal of drug treatment of epilepsy, namely complete control of seizures (Loscher, 1998). Despite the beneficial effect of the currently available drugs, there is still a need for broadly acting anticonvulsant drugs possessing multiple mechanisms of action with decreased adverse effect, preferably originating from natural products. A number of herbs have been shown to be beneficial in the management of epilepsy. An example is *Nardostachys jatamansi* DC. (Vidya *et al., 2005*). *Nardostachys jatamansi* demonstrated a dose dependent increase in the seizure threshold (decrease in the extension/flexion ratio), suggesting that the extract might possess anticonvulsant property against the Maximal electrical stimulation model. However, it failed to abolish the tonic extensor phase completely (Vidya *et. al., 2005*). Most of the drugs and herbs used for the treatment of epilepsy have one side effect or the other. There is therefore need for further investigation of herbal products with the aim of discovering safe and potent anticonvulsants.

v. Aneamia

Hemolysis is the lysis of the red blood cell's (RBCs) membrane, resulting in the release of cell content into the surrounding fluid. Hemolysis is visually detected by a pink to red tinge in serum, or plasma.

Types of Haemolysis

- In-vivo hemolysis which may be due to pathological conditions, such as autoimmune hemolytic anemia or transfusion reaction.
- In-vitro hemolysis which may be due to improper specimen collection, specimen processing, or specimen transport.

Haemolysis occurs when the pH of the external environment of the cell is altered, such that it is lower than the intracellular pH. When RBCs are placed in a hypotonic solution an osmotic gradient is created which causes an inflow of water into the cytoplasm of the erythrocytes resulting in hemolysis. Human cells have the same features all over the body. They possess a fragile outer cell membrane thin layer. Lysis of this layer results in cell death and the release of the content of the cell. Drugs that inhibit the lysis of the cell will be of advantage in several ways, ranging from prevention of anaemia, to the prevention of anaphylactic shock, which may be life treathening. Herbs with stabilizing effect on the cell will be of advantage in the management of inflammation (it will prevent the lysis of mast cells and thus prevent the release of inflammatory mediators) and aneamia related diseases.

2.3. INFLAMMATION

Inflammation is a protective response of tissues to injury. It is intended to eliminate the initial cause of cell injury as well as necrotic cells and tissue resulting from the original insult, (Mitchell and Cotran, 2000).

2.3.1 Causes of Inflammation

Inflammation can be elicited by numerous stimuli; viral infection, persistent microbial infection from bacteria and fungi, exposure to potentially toxic and irritant chemicals, and auto-immune diseases in which an individual develops an immune response to self-antigens for example rheumatoid arthritis, multiple sclerosis, ischemia, thermal or other physical injury.

2.3.2 Significance of Inflammation

The ability to mount an inflammation response is essential for survival in the face of environmental pathogens and injury; although in some situations and diseases the inflammatory response may be exaggerated and sustained for no apparent reason that is beneficial. These exaggerated effects are the basis of life threatening anaphylactic reactions to insect bite or drugs as well as of certain chronic diseases such as rheumatoid arthritis and atherosclerosis.

2.4. MEDICINAL PLANTS INVESTIGATED

1. *Sarcocephalus esculentus* Afzelius (Rubiaceae)
2. *Cassia goratensis* Fresen, (Caesalpinioideae)

3. *Ficus thonningii* Blume (Moraceae)
4. *Pseudocedrela kotschyi* Harm (Meliacea)
5. *Enantia chlorantha* Oliv., (Annonaceae)
6. *Nauclea latifolia* Smith, (Rubiaceae)
7. *Momordica balsamina* Linn, (Cucurbitaceae)
8. *Stachytarpheta indica* Vahl, (Verbenaceae)
9. *Prosopis africana* (Guillemin & Perrottet)Taubert, (Fabaceae)

2.4.1 ***Sarcocephalus esculentus*** Afzelius (Rubiaceae) is used as a medicinal plant in Northern Nigeria. Often called African peach or Country fig, it is found in Nigeria, Sierra Leone, Liberia, Togo, Gambia and Ivory Coast. In Northern Nigeria it is called tabashiya (Hausa), while in Southern Nigeria it is called egbesi (Yoruba) and uburu (Ibo). S. *esculentus* often grows as a shrub in the desert, and as a medium tree with short bole or a large tree in the forest or flooded land. The bark is bitter and can be used as a cold infusion for indigestion and vomiting. The yellow-colored infusion is taken as a tonic and a febrifuge. The natives chew the plant to relieve halitosis due to indigestion, and as a remedy for toothache, tooth caries and septic conditions of the mouth. The stem back and root are pulverized and applied topically to wounds. The infusion of *Sarcocephalus esculentus* is also used both as a lotion and internally for fever, especially in children (Dalziel, 1987).

2.4.2 ***Cassia goratensis*** Fresen, (Caesalpinioideae) is called runfu in Hausa. An infusion of the leaves and pods has been used for many years by traditional healers in the management of fever and general body pain. Infusions can also be used internally and as a wash in fever, and by pregnant and postpartum women.

The root fibre, blackened by immersion in damp marsh earth, is formed into a pad for women`s coiffure. The plant has been employed as charms against witchcraft, and to bring an enemy into contempt (Dalziel, 1987). *Cassia goratensis* stem bark is often mixed with the stem bark of other plants and water. The resultant infusion is used as an "after-work medicine" by craftsmen to relieve pain and effect body relaxation.

2.4.3 ***Ficus thonningii*** Blume (Moraceae) is used as a medicinal plant in Northern and Southern Nigeria. It is found in Nigeria, Sierra Leone, Togo, Liberia and Ivory Coast. In Northern Nigeria it is called che'diya (Hausa), in Southern Nigeria it is called yor odun in yoruba. In the natural state it commences as an epiphyte and is generally propagated by stake which grows rapidly. *F. thonningii* is a sacred or emblemic tree among several tribes in northern Nigeria. The Chawai (a tribe in Zaria, Nigeria), before a hunt set the bush on fire by the ritual method of drilling two pieces of che`diya sticks (Dalziel, 1987). The bark in decoction or infusion is used for sore throat and colds. In northern Nigeria, it is used to cure pain associated with fever and for treating wounds.

2.4.4 ***Pseudocedrela kotschyi*** Harm (Meliacea) is used in Northern Nigeria for rheumatism and general body pain by traditional healers. In Northern Nigeria it is called tonas in Hausa, and in Southern Nigeria it is called emi-gbegbi in yoruba. The woody part of the plant is used in furniture and drum making (especially talking drums), mortars and bowls, barrels and canoes are also made from it (Dalziel, 1987). *P. kotschyi* root and leaves are used medicinally. In French Sudan Chevalier states the leaves are boiled and rubbed on the skin for the treatment of smallpox. The bark is bitter and exudes a dark- coloured gum. In

Togo, it is given in infusions for gastrointestinal, febrile and rheumatic conditions, and a decoction is used as a wash for ulcers. In Northern Nigeria it is used as an occasional ingredient in arrow poison.

2.4.5 ***Enantia chlorantha*** Oliv. (Annonaceae) is used in West Africa as medicine for the management of diseases in man (Keharo and Adam, 1974). It is used particularly for the management of fever, malaria and general body pains by traditional healers in Northern Nigeria (Oliver, 1960; Irvine, 1961; Dalziel, 1987.). *E. chlorantha* has been reported to contain alkaloids known as berberine and protoberberine (Virtanen., *et al.*, 1988).

The use of *E. chlorantha* for its prophylactic and healing properties on ulcers (Tan, *et al.*, 2000), effect on chloroquine resistant malaria (Kimbi and Fabgenro, 1996; Agbaje and Onabanjo, 1991), and anti-microbial (Moody, *et al.*, 1995) has been confirmed by researcher. This plant is used by traditional healers for the management of different disease states. Patients often respect any traditional healer with knowledge of "good medicinal plants". The first conviction to show that the Traditional healer is good in his field is when the drug he administers relieves pain resulting from the ailment promptly. This therefore means that most of the administered concortions may have analgesic and /or anti-inflammatory activities.

2.4.6 ***Nauclea latifolia*** Smith., (Rubiaceae) is used in West Africa as medicine for the management of diseases in both man and animals (Keharo and Adam, 1974). It is used particularly for the management of fever, pain, and malaria by traditional healers (Oliver, 1960; Irvine, 1961; Dalziel, 1987.). *N. latifolia* contains nauclefoloninine (Ngnokam, *et al.*, 2003; Shigemori, *et al.*, 2003). *E.*

chlorantha and *N. latifolia* stem bark are often mixed with the stem bark of other plants for the treatment of malaria and general body pain.

Some alkaloids from the extract of *N. latifolia* have been reported to interact *in vitro* with DNA of bacteria and mammalian cells, leading to G2-M cell cycle arrest and heritable DNA-damage, as well as inducing *in vivo* single-strand breaks in liver, kidney and blood cells (Traore, *et al.*, 2000**)**. This plant also has anti-malarial activity (Azas, *et al.*, 2002; Traore-Keita, *et al.*, 2000; Benoit-Vical, *et al.*, 1998), anti-helmintic (Onyeyili, *et al.*, 2001; Fakae, *et al.*, 2000), anti-amoebic, spasmolytic (Tona, *et al.*, 2000) and molluscicidal (Kela, *et al.*, 1989) activities.

2.4.7 ***Momordica balsamina*** Linn. (Cucurbitaceae) is a climber or trailer with stems attaining 4-5m in length (Burkill, 1985). The synonyms of this plant include Balsam apple (English), Garahuni (Hausa), Akbon-ndewe (Igbo) and Ejirin (Yoruba) (Burkill, 1985). The whole plant is used as a bitter stomachic and an infusion is used as a wash in the management of fevers and yaws. The roots are used in preparations, as an aphrodisiac and, when combined with fruits or seeds, are used as an abortificient as well as a remedy for urethral discharge. The pounded seed, when soaked in water and inserted into the neck of the womb, is used to induce abortion (Dalziel, 1987). When taken whole, the plant is used as an emetic and a vermifuge especially in children. A macerate of the whole plant is also used as a galactogogue and to massage the chest to relieve intercostals pains (Burkill, 1985).

2.4.8 ***Stachytarpheta indica*** Vahl. (Verbenaceae) is called snake weed (English) and is known by various names in different parts of Nigeria, Tsarkiyar kusu (Hausa); Írù amure (Yoruba) and other names by which it is known include Brazilian tea and devil's couch (Burkill, 1985). Indications for which the plant has been used include Abortifacient, Asthma, Headache, Alopecia, Bronchitis, Bruise, Chest Cold, Constipation, Itch, Diarrhoea, Skin Sore, Vermifuge, Dysentery, Dysmenorrhea, Erysipelas, Fever, Inflammation, Liver Disease, Poisoning, Tumor, Venereal Disease, Cataract, Sedative, Anti-Fertility, Rheumatism to mention a few (Anyensu *et al.*, 1978). In Northern Nigeria, a decoction of the leaves with natron is given for dysentery in humans and for similar conditions in horses (Burkill, 1985).

2.4.9 ***Prosopis africana*** (Guillemin & Perrottet) Taubert, (Fabaceae) is a flowering plant that has been used for a variety of diseases and conditions in most African countries such as Nigeria, Sudan, Mali, Senegal etc. In Sudan, the dried leaves of *P. africana* are used as aphrodisiac (Walters, 1994), while in Northern Nigeria, a decoction of the root is used for toothache and the bark of the root is used as a dressing for wounds (Dalziel,1987) due to its antiseptic properties. Virtually, all parts of the tree are used for some medicinal purpose.

Previous investigations show that it contains alkaloids (Piperidine type), proteins and some inorganic compounds (Khuong Huu, *et al.*, 1972 and 1982). The alkaloids, prosopine (I) and prosopinine (II) were obtained from the leaves, bark or roots of *P. Africana* (Datta, *et al.*, 2000).They were found to be active against *Staphylococci* ATCC/6538F and *E. coli*. They were observed to decrease capillary permeability, with local anaesthetic action and sedative activity on the

central nervous system (Bourrinet and Quevauviller, 1968). Scientifically, it has been reported to have anti tuberculosis activity (Odumosun *et al.*, 2006).

CHAPTER THREE
MATERIALS AND METHODS

3.1 COLLECTION AND IDENTIFICATION OF PLANT MATERIALS

Eighteen plants (*Erythrina senegalesis, Nauclea latifollia, Kizelia africana, Pseudocedrela kotchyi, Crotalaris spp., Boswellia dalzielli, Khaya senegalensis, Annona senegalensis, Xylopia aethiopica, Ficus thonningii, Cassia goratensis, Prosopis africana, Stachytapheta indica, Crinum glaucum, Holerrhena floribunda, Momordica balsamina, Enantia chlorantha* and *Sarcocephalus esculentus*) were collected between May and September 2005, based mainly on semi-structured interviews with selected knowledgeable elders (Martin, 1995; Cotton, 1996). Most of the interviews and discussions were conducted in Hausa, the official language of the people in Northern Nigeria, with the help of a translator. Interviews were held in a place where the informants were most comfortable (in their homes). Information regarding the gathering, preparation and uses of medicinal plants used for the management of pain were obtained. Additional discussions were conducted with the informants in order to ascertain the other uses of the plants. At the end of each interview, specimens of plants mentioned for the management of pain and related health problems were collected and identified (authenticated) by a taxonomist (Mr. Kareem) of the Federal College of Forestry and Prof. S.W.H Husseini of the Department of Botany, University of Jos, Jos, Plateau State, Nigeria. Voucher specimens for nine medicinal plants with analgesic activities were deposited at the School of Forestry Herbarium, Jos, Nigeria. Their herbarium numbers of nine plants investigated are SF 00105J, SF 00106J, SF 00107J, SF 00108J, SF 00109J, SF 00110J, SF 00112J,

SF 00113J, SF 00114J. In this collection, three knowledgeable elders were involved. These elders were chosen from the different sites with the assistance of a local administrator (Trad. Dr. Azija) attached to the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos. During the course of the study, each informant was visited more than once in order to verify the reliability of data obtained. If what was said during the first visit concerning the use of a particular medicinal plant by an informant did not agree with what was said during subsequent visits, the information was considered unreliable and was rejected. Repeated visits also helped to gather additional information that was not mentioned during earlier interviews. All the participants in these interviews were located in Babale, Gada biu, and Rikos of Jos North Local Government in Plateau State, Nigeria.

3.1.1 Preparation of Plant Materials

The collected plant parts were cleaned and taken to the Laboratory where they were dried in the shade. The air dried plants were separately reduced to powder using a mortar and pestle. The powders were stored in air-tight containers till use.

3.1.2 Method of Extraction

Powdered plant parts of *Erythrina senegalesis, Nauclea latifollia, Kizelia africana, Pseudocedrela kotchyi, Crotalaris spp., Boswellia dalzielli, Khaya senegalensis, Annona senegalensis, Xylopia aethiopica, Ficus thonningii, Cassia goratensis, Prosopis africana, Stachytapheta indica, Crinum glaucum,*

Holerrhena floribunda, Momordica balsamina, Enantia chlorantha and *Sarcocephalus esculentus* were Soxhlet extracted using methanol. The resultant extracts were concentrated and with the aid of a rotary evaporator. *M. balsamina* was extracted by maceration in water/ methanol (MEW) mixture (25:75) for 72 hours for large scale extraction. The resultant extracts were filtered and dried in the oven at low temperature. The resultant solid residues were stored in a refrigerator (at -4^{o}C) till use.

3.1.3 Selection of Plants for Detailed Study

Nine plants (*Sarcocephalus esculentus* Afzelius (Rubiaceae), *Cassia goratensis* Fresen (Caesalpinioideae), *Ficus thonningii* Blume (Moraceae), *Pseudocedrela kotschyi* Harm (Meliacea), *Enantia chlorantha, Nauclea latifolia, Momordica balsamina* Linn, *Stachytarpheta indica* Vahl, and *Prosopis africana*) were selected after preliminary investigation using acetic acid induced writhing reflex for study of analgesic and anti-inflammatory properties. The plant, *M. balsamina* Linn, with the highest activity and /or less toxicity was then selected for in-depth study. All the plants collected were claimed to be used for the management of pain associated with one disease condition or the other. *Stachytarpheta indica* Vahl, was also evaluated for anti-diarrhoeal properties, since it was claimed to be useful in the management of diarrhoea and constipation. *M. balsamina* Linn, was particularly claimed to be useful in the treatment of many disease conditions (stomach pain, headache, hypertension, relaxation, diarrhoea and pain associated with infection). This claim informed the study of the other pharmacological properties of *M. balsamina*.

3.2 ANIMALS USED

Albino mice of either sex (weighing 25 - 30g), albino rats of either sex (weighing 150 - 250g), guinea pigs (200 - 250g), cats (1.2 - 1.5Kg) and rabbits (0.9 - 1.3Kg) were used in the experiments. The animals were bred and housed under standard environmental conditions (26^0C) in the University of Jos Animal House, Jos, Nigeria, They were fed with standard diet (Pfizer, Nigeria Limited) and water *ad libitum.*

3.3 DRUGS AND CHEMICALS

100% acetic acid (BDH chemicals, England), *n*-Hexane, Diethylether, Ethylacetate, Butanol, Egg-albumin, Nifedipine, Propranolol, Adrenaline, Atropine, Acetylcholine, Aspirin. Also used were *M. balsamina* extract and fractions (*n*-hexane, diethylether, ethylacetate, *n*-butanol, and water fractions), sulphuric acid, ammonia solution, chloroform, Mayers' reagent and Dragendorff`s reagent, Fehling's solutions A and B, ferric chloride solution, lead acetate and distilled water.

3.4 PHYTOCHEMICAL SCREENING OF THE PLANTS STUDIED

The plants under investigation were chemically analysed for the presence of alkaloids, glycosides, saponins and tannins. The methods described in Sofowora (1982) and Trease and Evans (1983) were used for these analysis.

3.4.1 General Test for Alkaloids

About 0.1 g of the *M. balsamina* was extracted with 2 ml of sulphuric acid by warming on a water bath for 2 min. The resultant solution was centrifuged and the

supernatant alkalinized with dilute ammonia solution and extracted with 2 ml chloroform to remove the free base. The chloroform layer was filtered through a small plug of cotton wool and then evaporated to dryness. The residue was dissolved in 0.2 ml of 1% sulphuric acid to form the sulphate of any alkaloid present. The solution was divided into two portions of 0.1 ml each and Mayer's and Dragendorff`s reagents added to each respectively.

3.4.2 Test for Glycosides

Glycosides are extractable in water therefore 0.2 g of the extract was warmed with 5 ml of water on a water bath for 2 min. This was centrifuged and the supernatant pipetted off and divided into two portions. Fehling's solutions (0.1 ml each) A and B were added to each portion respectively. Each portion was then heated on a water bath for 2 min.

3.4.3 Test for Saponoins

The extract (0.1 g) was extracted with 3 ml of water by heating on a water bath for 2 min. This was centrifuged and 0.1ml of the supernatant was diluted to 1 ml of water and shaken to observe if frothing will occur.

3.4.4 Test for Tannins

The method described by Lutterodt (1988) was employed to test for tannins. About 0.5 g of the extract was boiled in 25 ml of distilled water for 5 min cooled and then treated as follows: (i) 1.0 ml of the filtered extract was made up to 10 ml

of distilled water and to this was added 5 drops of lead acetate solution to precipitate any tannins present.

(ii) 10 ml of the filtrate was treated with some drops of ferric chloride solution to test for pseudotannins.

3.4.5 Determination of Crude Protein Content

0.2g of the powdered plant material was first digested in Kjeldahl digesting apparatus according to AOAC (1975) procedure. The ammonia content of the resultant digest was determined by a micro-colorimetric method.

3.5 FRACTIONATION OF *M. BALSAMINA* EXTRACT

Approximately 45.0 g of the crude extract of *M. balsamina* (methanol / water (75:25)) was dissolved in about 200 mL of distilled water. N-hexane was added to it and after vigorous shaking of the mixture, the n-hexane layer was removed and this process was repeated twice. The three n-hexane layers were combined and evaporated on a rotary evaporator; i.e., n-hexane fraction (Mb.Hex.) and the remaining layer as the aqueous fraction (Mb.Aq). Mb.Aq was fractionated using the same procedure with dietylether, then ethylacetate and lastly water-saturated n-butanol using the same method. The fractions obtained were then tested for analgesic and anti-inflaammatory properties.

3.6 TOXICOLOGICAL STUDIES

3.6.1 Acute toxicity studies

i. LD_{50} Determination

The median lethal dose (LD50) of the methanolic extract was determined in Swiss albino mice using intraperitoneal (i.p.) route of administration (Lorke, 1983). The experiment was carried out in two phases; the first phase involved three groups of three animals per group that were administered 10, 100, and 1000 mg/kg of the methanol extract, respectively. Since no animal died in all the treated groups in the first phase after a 24-h monitoring period, the second phase comprising of three groups of one animal per group was carried out by administering 1600, 2900, and 5000 mg/kg of the extract, respectively. The animals were again monitored for 24 h. The geographic mean, which was the least dose that did not kill any of the animals and the least dose that killed one of the animals was taken as the median lethal dose (LD50) (Myrna, *et al.*, 2007).

The same procedure was repeated for the other eight plants. Intra-peritoneal and oral LD_{50} was carried out for *S. indica* because of the observed intra-peritoneal toxicity. *N. latifolia* was administered intraperitoneally at doses lower than $LD_{50.}$

ii. Histological Studies

Female rats were randomly distributed into four groups of six. Group I served as the control and received distilled water daily for seven days. Groups II, and III received the methanol/aqueous extract of *M. balsamina* at doses of 1.0 and 1.5 g /kg respectively and group IV received 500 mg/Kg aspirin (standard

control for Haematological parameters) daily for seven days (Hanefi *et al.*, 2004). Administration of the extract was done orally. They were observed daily for clinical signs of toxicity or pharmacological signs, throughout the period of study. After seven days, blood samples were collected for assay of Haematological and biochemical parameters. The animals were then sacrificed and their organs (liver, heart, testis, lungs, spleen and stomach) were removed for histological studies. The same procedure was repeated using different groups of animals with 28 days treatment periods.

iii. Histological examination

Liver, heart, testis, lungs, spleen and stomach from the treated animals were fixed in 10 percent neutral buffered-formalin prior to routine processing in paraffin-embedded blocks. Sections (4 µm thick) were cut and stained using hematoxylin-eosin stain (Hanefi *et al.*, 2004) and photo-micrographed.

The same procedure was repeated for rats treated for a period of 28 days, after which biochemical, Haematological and other investigations were carried out as stated below.

3.6.2 Haematological and Biochemical Studies

i. Haematological Parameters

Blood samples were collected from the rat's tail. The bleeding and clotting times were noted. Blood samples for packed cell volume (PCV) were collected from the tail into heparinized capillary tubes.

ii. Blood Analysis

The haematological examinations performed were according to standard methods. Haematocrit was determined by the micro-haemotacrit method described by McGown, *et al.*, (1955). Erythrocytes and total leucocytes were counted using the improved Neubauer haemacytometer. The packed cell volume of each sample was determined by using a Hawksley microhaematocrit centrifuge at 1200 g for 5 min (Dacie and Lewis, 1984).

Biochemical analysis of the serum enzymes for alanine amino-transaminase (ALAT), alkaline phosphatase (ALP) and aspartate amino-transaminase (ASAT) were assayed using Dialab kits and colorimeter. Absorbance recorded were multiplied by factors provided by Dialab and their values obtained.

iii. Biochemical Parameters

At the end of the treatment periods, 4 – 5 ml of blood was collected from the heart of each rat. They were allowed to clot and then centrifuged at 1500g for 10 min. The resultant serum was then collected into test tubes and was used for assay of the maker enzymes namely ALP, ALAT and ASAT.

i. **Alkaline Phoshatase;** Dialab laboratory reagent was used. The kit consists of two reagents. Reagent one contains, 2-Amino-2-Methyl-1-Propanol, Magnesium Acetate, and Zinc sulphate, while reagent two contains p-Nitrophenyphosphate. The reagent and the sample (serum) were mixed as stated in the table.

Testtube	Blank	Sample
Reagent 1	1000 uL	1000 uL
Sample		20 uL
Dist. Water	20 uL	

Mixed and allowed To incubate for 1 Minute. Then added

Reagent 2	250 uL	250 uL

The contents were mixed. Absorbance was read after 1, 2, and 3 min. using a colorimeter set at 420 wavelength.

Calculations

Change in absorbance= change in sample absorbance – change in blank absorbance.

Alkaline phosphatase (U/L) = change in absorbance * Factor

Factor given was 3433.

ii. **Amino-transaminase (ASAT):** Dialab laboratory reagent was used. The kit consists of two reagents. Reagent one contains L-Aspartase, while reagent two contain 2-Oxoglutarate, and NADH. The reagent and the sample (serum) were mixed as stated in the table.

Testtube	Sample
Reagent 1	1000 uL
Sample	100 uL

Mixed and allowed To incubate for 5 Minute.

Absorbance was read after 1 and 2, and 3 min. using a colorimeter set at 340 wavelength.

Calculations

Amino-transaminase (U/L) = change in absorbance * Factor

Factor given was 2143.

iii. Alanine amino-transaminase (ALAT): Dialab laboratory reagent was used. The kit consists of two reagents. Reagent one contain: L-Alanine, while reagent two contains 2-oxoglutarate, Nicotinamide adenine dinucleotide (NADH). The reagent and the sample (serum) were mixed as stated in the table.

Testtube	Sample
Reagent 1	1000 uL
Sample	100 uL

Mixed and allowed To incubate 5 minute. Then added

Reagent 2	250 uL

The contents were mixed. Absorbance was read after 1, 2, and 3 min. using a colorimeter set at 340 wavelength.

Calculations

Alanine amino-transaminase (U/L) = change in absorbance * Factor

Factor given was 2143.

3.7. TEST FOR ANALGESIA IN MICE

3.7.1 Writhing Reflex Test

Mice of either sex were divided into four groups of six each. One group received vehicle (control), another group received standard drug (aspirin) and the other groups received graded doses of plant extract i.p. (see table 7). Thirty minutes later, 0.1 ml of 1% acetic acid was injected i.p. The number of abdominal contractions (writhing movement) was observed for 15 minutes (starting from 5 minutes after injection of acetic acid), and recorded. The percentage inhibition of writhing movement was then calculated. This procedure was used for the extracts of the selected nine plants and the fractions of *M. balsamina.*

3.7.2 Hot plate Test

Rats were kept individually in a glass cylinder (open at both ends) on a hot plate, such that the rats had direct contact with the hot plate maintained at a constant temperature of 55 ± 1^0 C. Time taken for paw licking and/or jumping were recorded. Rats were divided into four groups; one group (control) received distilled water, another group received standard drug (aspirin), while the other groups received graded doses (see Table 9) of extract thirty minutes before

placement on the hot plate. This procedure was used for the extracts of the selected nine plants.

These two methods were used for the selection of the most potent drugs.

3.7.3 Tail Flick Test

Rats were kept in the restrainer for them to acclimatize to the environmental condition in the restrainer. Only rats that successfully acclimatized to the restrainer were selected for the experiment. Rats that withdrew their tail were not chosen for this experiment. The selected rats were divided into groups of six rats each; one group received distilled water, another received standard test drug (aspirin) and the remaining groups received different doses of the *M. balsamina* extract (200, 500 and 1,000 mg /Kg). Thirty minutes later, the rat's tail was inserted into a water bath containing water maintained at 55^{o} C. The time difference between the immersion time and the withdrawal time were noted and recorded. Results from the test drug group were then compared with that of the control and standard drug group. The same procedure was repeated using *M. balsamina* fractions (see Table 11).

3.7.4 Formalin Test

Rats were kept in transparent plastic containers with viewing mirror underneath (to allow for observation) for them to acclimatize to the experimental condition. The rats were then divided into three groups of six animals each. Group one received distilled water, and groups two and three received different doses of *M. balsamina* (200 and 600 mg /Kg) extract. Thirty minutes later, 2% formalin

was injected into the sub-platar surface of the right paw of the rat. The number of flicking/biting was counted for the first six minutes followed by six minute rest (with zero flicking/biting) and thereafter the number of flicks/biting were counted every six minutes for sixty minutes.

3.8 ANTI-INFLAMMATORY TEST

Acute inflammation was induced by injecting egg-albumin into the sub-platar surface of the rat's hind paw. Acute inflammation was measured by increase in the rat's right hind paw linear circumference. Animals used for this experiment were fasted for 12 h and deprived of water only during the experiment. Oedema was assessed in terms of the difference in zero time linear circumferences at the injected paw and its circumference, 1 h, 2 h, and 3 h intervals after egg-albumin injection. For routine drug testing, the increase in paw circumference 2 h after administration of the inflammation-inducing agent was adopted as a measure of effect. Animals were divided into four groups of six animals. One group received distilled water, another received standard drug (aspirin) and served as the negative and positive controls respectively while the other two groups received different doses of the extract (see Table 13). The extract was administered intra-peritoneally, 30 minutes before the inducement of inflammation. The paw circumference was measured with the aid of Vernier caliper at 1 hour, 2 hours, and 3 hours. The same procedure was repeated for the extracts of the remaing nine plant extracts and the fractions of *M. balsamina*.

3.9 ANTI-PYREXIA TEST

Fifty rats were randomly selected from a colony of rats and their body temperature was measured by inserting clinical thermometer into their anus. They were injected subcutaneously with yeast, 24 hours later, their temperature was measured, and the rats that had a temperature increase of $1^{o}C$ were selected for the experiment. The selected rats with high temperature were then divided into groups of six rats each. One group received distilled water; another standard drug (aspirin) while the remaining groups received graded doses (400 and 1,000 mg /Kg) of the *M. balsamina* extract. Their body temperature was measured at time zero, then 30 minute, 1 hour, 2 hour, 3 hour, 4 hour and 18 hours. The difference in temperature from time zero was calculated and recorded. The values obtained for the test drug were then compared statistically with that of the control and standard drug respectively.

3.10 EFFECT OF THE EXTRACT OF *M. BALSAMINA* ON GASTRIC MUCOSA

Adult Wistar rats (180–210 g) of either sex were divided into groups of five rats each. The rats were deprived of food for 18 h. *M. Balsamina* (500 mg/kg and 1,000 mg/Kg), indomethacin (40 mg/Kg), and distilled water (30 ml/Kg) were administered orally to the rats in different groups respectively. After 8 h, the animals were sacrificed and stomachs were removed and cut open along the greater curvature. The stomachs were rinsed under a slow stream of water and pinned flat on a corkboard. The stomachs were coded to eliminate observer bias and examined with a hand lens (Raji, *et al.,* 2000). Erosions formed on the glandular portion of the stomachs were counted and each one given a severity

rating on a 1–5 scale. The ulcer index was then calculated (Main and Whittle, 1975).

3.10.1 Indomethacin-induced ulcer

Adult Wistar rats (180–210 g) of either sex were divided into groups of five rats each. The rats were deprived of food for 18 h. The extract of *M. balsamina* (500 mg/kg and 1,000 mg/Kg) was administered orally 30 min prior to the oral administration of indomethacin (40 mg/Kg). Normal saline 30 ml/kg instead of the extract was administered orally to the control group. After 8 h the animals were sacrificed and stomachs were removed and cut open along the greater curvature. The stomachs were rinsed under a slow stream of water and pinned flat on a corkboard. The stomachs were coded to eliminate observer bias and examined with a hand lens (Raji, *et al.*, 2000) Erosions formed on the glandular portion of the stomachs were counted and each one given a severity rating on a 1–5 scale. The ulcer index was then calculated (Main and Whittle, 1975).

3.11 INVESTIGATION FOR THE MECHANISM OF THE ANTI-INFLAMMATORY EFFECT OF *M. balsamina*

3.11.1 Cell stability Test

Preparation of Erythrocyte Suspension

Fresh whole human blood was collected from the arm vein and transferred into four sets of ethylene-diamine-tetra-acetic acid (EDTA) centrifuge tubes. They were centrifuged for 5 minutes and washed three times with equal volume of normal saline until a clear (colourless) supernatant layer is obtained. The volume

of the blood was measured and reconstituted as a 40% suspension with isotonic buffer solution.

Effect of *M. balsamina* on Heat – Induced Haemolysis

The isotonic buffer solution (5 ml) containing 200 µg/ml of the methanol/water extract of *M. balsamina* was dispensed into ten sets of centrifuge tubes. Ten sets of tubes containing the phosphate buffer solution (5 ml) alone were used as control. Erythrocyte suspension (0.05 ml) was added to each tube and gently mixed. Five tubes containing the drug and five of the control samples were incubated at 54^0C for 20 minutes in regulated water bath, while the remaining five test samples and five control samples were incubated in a freezer (at - 4^0C). At the end of the incubation the reaction mixture was centrifuged for 3 minutes and the absorbance (OD) of the supernatant measured spectrophometrically at 540 nm using the colorimeter. The same procedure was repeated for concentrations 400 and 600 µg/ml. The percentage inhibition of haemolysis was calculated using the relation (Shinde *et al.*, 1999):

$$\text{Inhibition of Hemolysis (\%)} = 100 \times \frac{1 - (OD_2 - OD_1)}{OD_3 - OD_1}$$

Where OD_1 = Absorbance of test sample unheated

OD_2 = Absorbance of test sample heated

OD_3 = Absorbance of control sample heated

Effect of *M. balsamina* on Hypotonicity Induced Haemolysis

The hypotonic solution (distilled water; 5 ml) containing 200 µg/ml of the plant ethanolic extract was dispensed into five centrifuge tubes. Five tubes containing the distilled water alone served as control. Erythrocyte suspension (0.05 ml) was added to each tube and after gentle mixing, the mixtures were incubated for 1 hour at room temperature. After incubation the reaction mixture was centrifuged for three minutes and the absorbance (OD) of the supernatant measured spectrophotometrically at 540 nm using the colorimeter (Shinde *et. al*., 1999). The same procedure was repeated for concentrations 400, 1,000 and 2,000 µg/ml. The above procedure was repeated for concentrations 1,000 and 2,000 µg/ml under reduced hypotonicity (obtained by mixing distilled water with equal volume of normal saline). The inhibition of haemolysis was calculated using the relation. (Shinde *et. al.*, 1999)

$$\text{Inhibition of Haemolysis (\%)} = 100 \times 1 - \frac{(OD_2 - OD_1)}{OD_3 - OD_1}$$

Where:

OD_1 = Absorbance of test sample in Isotonic solution

OD_2 = Absorbance of test sample in Hypotonic solution

OD_3 = Absorbance of control sample in Hypotonic solution

3.11.2 Cell Migration Test

Effect of *M. balsamina* on Leukocyte Migration

Healthy adult albino mice of either sex (weight 25 - 31 g) were used for this experiment. The animals were grouped into four groups; the control group,

received distilled water (0.5 ml) orally. Those in groups 2, 3 and 4 received 200, 400 and 600 mg/kg of the methanol / water plant extract, one hour after drug administration. The animals received 0.25ml of dextran (ip.), four hours later, the animals were sacrificed and the peritoneal cavities washed with 1 ml of phosphate-buffered saline containing 0.1 ml of 10% EDTA. Total and differential leukocyte crusts in the peritoneal wash were taken. The inhibition (%) of neutrophil and lymphocyte migration were calculated.

Differential White Blood Cell count

A drop of the peritoneal wash was placed gently at the end of the slide and using the grounded edge of another slide, it was placed at an acute angle in front of and on top of the slide containing the peritoneal wash, until the blood runs along the edge of the slide. The cover slip was pushed forward to obtain a fine film of the wash. The films were quickly air-dried.

ii. Fixing and Staining of film

Sufficient Leishman's stain was poured on the dried film, to cover it, and allowed to stand for about 2 minutes. The stain on the slide was mixed with the buffered distilled water (7.0 = PH) and left for about 10 minutes.

iii. Counting

The film was examined systematically under the microscope by means of the movable stage at x 100 magnification. The samples were viewed with the aid of immersion oil. The number of white blood cell (WBC) in the field were viewed and counted for each of the slide.

3.12 ANTI-CONVULSIVE STUDIES

3.12.1 Chemically Induced Convulsion

Male Wistar rats were divided into four groups of six animals each. Group one received distiled water and served as the control. Groups two and three received the extract of *M. balsamina* (500 and 1,000 mg /Kg respectively), 30 minutes before the induction of convulsion. Group four received the standard drug (diazepam). Convulsion was induced with 45 mg/kg of Pentylenetetrazole intra-peritoneally. After the inducement of convulsion, the onset and duration of convulsion was noted. The number of episodes of convulsions within a period of 15 minutes was also recorded. The same proceedure was repeated using picrotoxin (6 mg /Kg) to induce convulsion and phenytoin (4 mg/Kg) as standard drug.

3.12.2 Electrically Induced Convulsion

Male Wistar rats were divided into four groups of six animals each. Group one received distiled water and served as the control. Groups two and three received the extract of *M. balsamina* (500 and 1,000 mg /Kg), 30 minutes before the inducement of convulsion. Group four received the standard drug (diazepam, 2 mg/kg). Convulsion was induced by passing diffused transcranial electrical DC curent (1 m/s pulse width and frequency 100 Hz) via two electrical electrodes cliped to the ears of the rats. The voltages required to induce convulsion was noted.

3.13 EFFECTS OF EXTRACT ON URETHANE INDUCED SLEEP

Male Wistar rats were divided into three groups of six rats each. Group one received distiled water while groups two and three received diferent doses (500 and 1,000 mg /Kg) of *M. balsamina,* 30 minutes before the inducement of sleep. Sleep was induced with 40 mg/kg of urethane. The onset and duration of sleep were noted and recorded.

3.14 ANTIMICROBIAL ACTIVITY

The antimicrobial activity of the methanolic extract of *M. Balsamina* was assayed *in-vitro* using the cup-bore method on *Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Psuedomonas aeruginosa, Proteus mirabilis*, *Staphylococci aureus* and *Salmonela typhi.* An aliquot of nutrient broth (5.0 ml) was inoculated with the test organism and incubated at 37^0 C for 24 hours. After 24 hours, using a sterile pipette, 0.2 ml of the broth culture of the test organism was added to 20 ml of nutrient agar which had been cooled to 23^0C. This was mixed well and poured into a sterile Petri dish. The agar was allowed to set and harden after which five holes were bored using a sterile cup borer on each of the agar plate. Serial dilutions of the extract and standard drug (gentamycin) were prepared and using a dropper, 0.1 ml of each dilution was introduced into five holes on each plate. The five holes in the last plate contained gentamycin, the standard drug. The plates were left for 1 hour to allow for diffusion of the extract or gentamycin before incubation at 35^0C. After 24 hours, the zones of inhibition were observed and measured. The same procedure was repeated using potatoes glucose media and ketoconazole as standard drug to assay the effectivness of *M. balsamina* in

inhibiting the growth of fungi (*Aspergilus fumigatus, Aspergilus niger, Candidas albican* and *Mucos pusillus.*).

3.15 EFFECTS ON CASTOR OIL-INDUCED DIARRHOEA IN RATS

The method described by Galvez *et al*., 1993 was employed for this study. Wistar albino rats weighing 150-220 g were fasted for 12 hours and were randomly placed into six groups each of six animals and housed in separate cages. One cage housed one animal during the experiment. Animals in group one received 1ml distilled water without castor oil, while those in groups 2, 3 and 4 were pre-treated with *M. balsamina* extract (0.1 g/kg, 0.25 g/kg, and 1.0 g/kg respectively) intra-peritoneally. The standard drug diphenoxylate (50 mg/kg, ip.) was administered to the fifth group and the last group was pre-treated with distilled water. Animals in groups 2 to 6 received castor oil orally using the orogastric canula 30 minutes after pre-treatment with the extract or standard drug.

Following treatment with castor oil, the animals were then placed in separate cages over clean white paper and were inspected for six hours (by an independent observer) for the presence of the characteristic diarrhoeal droppings; their absence was recorded as a protection from diarrhoeal (Diurno *et al.*, 1996) and the percentage protection calculated (Akah and Offiah, 1996). The same procedure was used for *S. indica* extract (1.5 g/kg and 3.0 g/kg). *S. indica* extract was administered orally. Intravenous administration of *S. indica* has been reported to be very toxic in mice with an LD_{50} less than 0.15 g/kg. (Otimenyin, *et al.,* 2006).

3.16 ANTI-DIABETIC EFFECT OF *M. balsamina*

Wister albino rats weighing 150-220g were injected with alloxan (60 mg/kg) through tail vein. Five days later, blood glucose levels of the animals were determined using a glucometer. The diabetic rats showing blood glucose levels in the range of 200–450 mg/dl were selected for the evaluation of *M. balsamina* for antidiabetic properties (Subramoniam *et al.*, 1996). Diabetic rats were divided into four groups of six each. The control group was given 0.1 ml of distilled water, ip. The test groups were given the extract of *M. balsamina* (500 mg/Kg and 1,000 mg/Kg respectively). The fourth group received chlopropramide (400 mg/kg, p.o.). Blood glucose levels were determined at 0, 1, 2, 3 and 12 hours. The animals' blood glucose concentration levels were measured using a glucometer. The rat`s blood was collected from its tail by massaging the whole length of the tail until sufficient blood accumulates at the tip. The glucometer was switched on and the glucometer code number was set to the code on the one touch glucose strip bottle. Then the strip was inserted into the glucometer as instructed by glucometer. With the aid of the surgical knife, the tip of the rat's tail was cut off and the blood dropped on the appropriate portion of the glucose strip inserted in the glucometer. After some few seconds the glucometer starts counting down from 45 seconds to 1 second and then displays on its screen the glucose concentration in mg/dl. This procedure was repeated for all the rats used in the experiment and their blood glucose level noted.

3.17 EFFECTS ON ISOLATED RABBIT JEJUNUM

Four rabbits were sacrificed by a blow on the head, dislocating the neck, and exsanguinated. Segments of the jejunum, about 2.0 cm long, were removed

and dissected free of adhering mesentery. The intestinal contents were removed by flushing with Tyrode's solution of the following composition in millimoles (mM): NaCl, 136.8; KCl, 2.7; CaCl, 1.3; $NaHCO_3$, 12.0; MgCl, 0.5; $NaPO_4$, 0.14; and glucose, 5.5. The tissue was mounted in a 50 ml organ bath containing Tyrode's solution maintained at 36^0 C and aerated with air. A load of 0.5 g was applied. Equilibration period of 60 minute was allowed during which the physiological solution was changed every 15 min. At the end of the equilibration period, the effects of Acetylcholine, was determined. The effects of graded doses of the extracts of *M. balsamina* were recorded. Also the effects of the various doses of the extract in the presence of antagonists: Atropine, and Nifedipine, which were incubated for 1 minute after obtaining control responces of the extract, was recorded. The contact time for each concentration was 1 min, which was followed by washing. The tissue was allowed a resting period of 15 min before the fresh challenge. Responses were recorded iso-metrically using Ugo Basile Unirecorder 7050.

3.18 DIRECT BLOOD PRESSURE

Measurement in Cats

Normal cats (five) were used in this study. These animals were anaesthetized using sodium pentobarbital (40 mg/kg i.p.). At the stage of surgical anaesthesia, the trachea was intubated to facilitate spontaneous respiration. The femoral vein was cannulated with heparinized polyethylene tubing (PE-50) for intravenous injection of *M. balsamina*, and drugs (Adrenaline, Acetylcholine, propranolol, etc.), while the carotid artery was cannulated and connected to a

Bentley Trantec Pressure Transducer for blood pressure recording on Ugo Basile Microdynamometer 7050. The Cat's body temperature was maintained at 37 °C by means of thermostatically controlled dissecting table. Animals whose blood pressure fluctuated by more than 10% within the first 30 min of recording were discarded. After a 30 min equilibration, intravenous administration of graded doses of *M. balsamina* and other drugs (Adrenaline, Acetylcholine and propranolol,) were carried out slowly for about 30 s. The maximum volume of extract or drugs injected was not greater than 0.4 ml. This is to help check medullary influences on the blood pressure.

Studies on Isolated Rat Atria

Five adult Wistar rats of either sex were killed and exsanguinated. The thoracic region was opened, and the heart was rapidly removed and placed in Locke's solution of the following composition (mM): NaCl, 153.8, KCl, 5.6, $CaCl_2$, 2.1, $NaHCO_3$, 5.9 and glucose, 5.5. This was kept at a temperature of 37 °C and aerated with 100% oxygen. The atria both right and left were carefully dissected out and mounted in an organ bath containing 25 ml Locke's solution. A resting load of 0.5 g was applied and the tissue was allowed to equilibrate for a period of 60 min during which the physiological solution was changed every 15 min. The effect of graded doses of *M. balsamina* and standard drugs was recorded on the Ugo Basile Unirecorder 7050 via an isometric transducer 7004.

3.19 STATISTICAL ANALYSIS

Differences between control and treatment groups were analyzed by ANOVA and student t- test (Snedecor and Cochran, 1967).

CHAPTER FOUR
RESULTS

4.1 COLLECTION AND SELECTION OF PLANTS

The eighteen plants (*Erythrina senegalesis, Nauclea latifollia, Kizelia Africana, Pseudocedrela kotchyi, Crotalaris spp., Boswellia dalzielli, Khaya senegalensis, Annona senegalensis, Xylopia aethiopica, Ficus thonningii, Cassia goratensis, Prosopis africana, Stachytapheta indica, Crinum glaucum, Holerrhena floribunda, Momordica balsamina, Enantia chlorantha* and *Sarcocephalus esculentus*) collected are shown in Table 1. All the plants collected were claimed to be effective in the management of pain and related diseases. After preliminary investigations using acetic acid induced writhing reflex, nine (*S. esculentus, C. goratensis, F. thonningii, P. kotschyi, E. chlorantha, N. latifolia, M. balsamina, S. indica, P. africana*) plants were selected for screening (Table 2). They were screened for analgesic and anti-inflammatory activities. *M. balsamina* (most potent) was then chosen for in-dept study.

4.2 PHYTOCHEMICAL SCREENING OF THE PLANTS STUDIED

Phytochemical studies revealed that all the plants contain flavoniods and other chemical constituents as shown in the table 2 below.

4.3 TOXICOLOGICAL STUDIES

4.3.1 *LD_{50}* and Percentage Yield

The intra-peritonial LD_{50} values and percentage yield of the extracts are shown in Table 3. The result shows that *S. indica* is the most toxic intra-peritoneally, (this made it impossible to use this route for pharmacological investigations), while *F. thonningii* is the safest intra-peritoneally. *N. latifolia* `s intra-peritonial LD_{50} value revealed that it is toxic when high dose (>0.80 g/Kg) is administered via this route. The result also shows that *S. indica,* though toxic intra-peritoneally, was very safe orally with oral LD_{50} values greater than 7.00 g/ Kg. (Table 3).

C. goratensis and *P. kotschyi* intra-peritonial LD_{50} were 1.37 and 1.50 g/ Kg respectively. Their intra-peritonial LD_{50} show that these plants have low safty margins. *P. africana, S. esculentus E. chlorantha, F. thonningii* and *M. balsamina* were better tolerated when administered intra-peritoneally.

Table 3; Percentage Yield and LD_{50} of Methanolic Extracts of Plants Studied

Plant	Intra-peritoneal LD_{50} (g/kg)	Oral LD_{50} (g/kg)	% yield
S. indica	<0.15	>7.00	23.52
N. latifolia	0.80	3.72	14.70
C. goratensis	1.37		19.99
P. kotschyi	1.50		38.79
E. chlorantha	2.59		12.30
P. africana.	3.23		20.31
S. esculentus	3.50		11.70
M. balsamina	4.15		23.07
F. thonningii	7.00		12.31

4.3.2 Histological Examination

Histological examination showed that *M. balsamina* extract did not have any significant effect on liver, stomach, spleen, testis and heart cells (Apendixes 1-18). There was no sign of inflammation or cell abnormality. The blood vessels in the organs were intact without any lesion.

4.3.3 Haematological Parameters

The mean ± SEM values of the bleeding time, clotting time and packed cell volume of rats treated with the extract of *M. balsamina* are shown in table 4 and 5. The mean PCV values of treated animals and animals that received aspirin were not significantly different from that of the control. This shows that the extract of *M. balsamina* has no effect on packed cell volume and thus erythropoiesis (Table 5). Bleeding time and clotting time were also not affected by the extract ($P>0.05$) when compared to control valus. Aspirin significantly ($P<0.05$) increased the bleeding and clotting times when compared to the control (Table 4).

The mean RBC, WBC and Platelet count were not significantly ($P>0.05$) affected by the doses of the extract used when compared with the control, (table 6). These results show that the extract has no significant effect on haematological parameters. It was also observed that Aspirin did not have ($P>0.05$) any effect on the red blood cells, white blood cells and platelet counts.

Table 4; Effect of Methanolic Extract of *M. balsamina* on Bleeding time, and Clotting Time

Grouping	Bleeding time (sec)	Clotting time (sec)
Control		
Vehicle (dist. Water)	99.00±9.10	84.00±3.89
M. balsamina		
1.0 g/kg	99.00±6.80	72.00±2.68
1.5 g/kg	112.50±5.30	82.50±15.91
Aspirin		
0.5 g/kg	341.25±28.25*	161.25±14.40*

*P < 0.05, when compared with the control values, using student's t test.

N = 5 animals per group

Table 5; Effect of Methanolic Extract of *M. balsamina* on Heamoglobin and Packed cell volume in Rats

Grouping	**Packed cell volume**	**Heamoglobin gHb/DL**
Control		
Vehicle (dist. Water)	51.40±1.57	12.84±1.56
M. balsamina		
1.0 g/kg	51.80±3.67	11.05 ± 0.43
1.5 g/kg	45.00±0.71	9.02 ± 0.21
Aspirin		
0.5 g/kg	49.75±0.65	14.71 ± 0.18

*P < 0.05, when compared with the control values, using student,s t test.

n = 5 per group

Table 6; Effect of Methanolic Extract of *M. balsamina* on Haematological Parameters in Rats

Grouping	**Red Blood Cell Count x10^{6}cell/cm^3**	**White Blood Cell Count x10^4 cell/cm**	**Platelet Count x10^5 cell/cm^3**
Control			
Vehicle (dist. Water)	7.27±0.14	1.25±0.14	1.04±0.04
M. balsamina			
1.0 g/kg	7.94 ± 0.39	0.99 ± 0.15	1.46 ± 0.17
1.5 g/kg	6.90 ± 0.96	1.20 ± 0.16	0.85 ± 0.01
Aspirin			
0.5 g/kg	6.65 ± 0.15	1.41 ± 0.23	0.51 ± 0.04

*P < 0.05, when compared with the control values, using student t test.

n = 5 animals per group

4.3.4 Biochemical Parameters

The blood concentrations of the three most prominent liver enzymes, [alkaline phosphatase (ALP), alanine aminotransaminase, (ALAT) and aspartate aminotransaminase, (ASAT)] were not affected (P>0.05) by the extract of *M. balsamia* (1.0 and 1.5 g/Kg).

The blood concentration of alkaline phosphatase insignificantly (P>0.05) decreased, at a dose of 1.0 g/Kg to 397.26 ± 0.59 u/l when compared with control value, 403.35 ± 1.02 u/L. As the dose was increased to 1.5 g/Kg, the blood concentration further decreased (395.19 ± 1.04 u/L). The values obtained were not significantly different from that of the control animals. (Table 7).

Alanine transaminase blood level decreased in the rats pretreated with the two doses of the extract. At a dose of 1.0 g/Kg the blood level was insignificantly (P>0.05) reduced to 26.24 ± 1.11 u/L from the control value of 28.01 ±0.94 u/L. The alanine transaminase value further decreased to 25.14 ± 0.99 u/L (P>0.05) when the dose of the extract was increased to 1.5 g/Kg. The decreases observed were not significantly (P>0.05) different from the values of the control (Table 7). There was no significant (P>0.05) decrease in the blood concentration values of aspartate amino transaminase in the rats pretreated with various doses of *M. balsamina* (1.0 and 1.5 g/Kg) of the extract when compared with control values. The overall results show that the extract has no effect on the liver cells and enzymes, (Table 7, Apendixes 1, 2, 3, and 4).

Table 7; Effect of Methanolic Extract of *M. balsamina* on Some Biochemical Parameters in Rats

Grouping	Parameters Assayed.		
	ALP(U/L)	ALAT (U/L)	ASAT(U/L)
Control	403.35 ± 1.02	28.01 ± 0.94	56.14 ± 0.43
1.0 g/kg	397.26 ± 0.59	26.24 ± 1.11	54.23 ± 0.73
1.5 g/kg	395.19 ± 1.04	25.14 ± 0.99	53.96 ± 0.95

Values are mean ± S.E.M.,

n = 5 animals per group

4.4 ANALGESIC ACTIVITY

The nine plants under investigation were evaluated for peripheral (writhing reflex test) and central (hot plate test) analgesic activities and anti-inflammatory effects. The less toxic and most effective plant was then selected for indept study of analgesic activities. In this case *M. balsamina* was selected for indept study of analgesic activities.

4.4.1 Writhing Reflex

All the nine plants (*S. esculentus, C. goratensis, F. thonningii, P. kotschyi, E. chlorantha, N. latifolia, M. balsamina, S. indica, P. africana*) investigated had varying degrees of analgesic activity (Table 8). *M. balsamina* was found to be the most effective while *N. latifolia* was the least effective (Table 8) in writhing reflex test.

S. indica, *S. esculentus*, and *E. chlorantha* were observed to have weak analgesic effects compared to *P. Africana, C. goratensis, F. thonningii, P. kotschyi, and M. balsamina*.

It was also observed that the fractions of *M. balsamina* had varying degrees of analgesic activities (Table 9).

TABLE 8; Effect of Methanolic Extract on Acetic Acid-induced Writhing Reflex

Treatment **Plant**	**Dose (mg/kg, i.p)**	**Number of writhes**	**Inhibition (%)**
Control		51.17 ± 4.12	----
Aspirin	100	10.03 ±3.01**	80.03
	200	07.51 ±2.53**	85.32
M. balsamina	400	15.00 ± 2.60**	79.69
	1,000	08.16 ±2.30**	84.05
F. thonningii	500	15.00 ± 1.29**	79.69
	1,000	08.50 ±1.55**	83.39
C. goratensis	500	15.50 ± 2.10 **	69.71
	1,000	8.17 ± 2.70**	84.03
P. kotschyi	300	23.33 ± 0.42 *	54.41
	600	14.00 ± 0.58**	72.64
P africana	100	24.17 ± 2.99*	52.77
	200	23.00 ±2.53*	55.05
E. chlorantha	400	25.70 ± 3.20*	49.78
	1,100	14.12 ±3.70**	72.41
S. esculentus	500	27.00 ± 4.4*	47.23
	1,500	15.33 ±4.90**	70.04
S. indica	200	30.04 ± 5.01*	41.29
	400	17.00 ± 3.21**	66.78
N. latifolia	150	39.98 ± 6.90*	21.87
	500	30.71 ± 3.70*	39.98

*P<0.05, **P<0.005 when compared with the control using student`s t test.

n = number of mice per group = 6

TABLE 9; Effect of various fractions of *M. balsamina* Extracts on Acetic Acid-induced Writhing Reflex

Treatment Plant	Dose (mg/kg, i.p)	Number of writhing	Inhibition (%)
Control		49.32 ± 3.23	----
N- Hexane	500	43.62 ± 1.52	11.56
	1,000	40.18 ±1.95	18.53
Chloroform	500	30.20 ± 2.32*	38.77
	1,000	25.25 ± 1.46*	48.80
DEE	500	20.15 ± 3.31*	59.14
	1000	14.36 ± 2.32**	70.88
EA	500	17.20 ± 2.41**	65.13
	1,000	12.42 ±1.99**	74.82
Butanol	500	15.91 ± 1.15**	67.74
	1,000	09.31 ±1.37**	81.23
Water	500	10.32 ± 1.01**	79.08
	1,000	07.29 ±1.00**	85.22
M. balsamina	400	15.00 ± 2.60**	69.90
(crude)	1,000	08.16 ±2.30**	83.45
Aspirin	100	10.03 ±3.01**	79.66
	200	07.51 ±2.53**	84.77

*P<0.05, **P<0.005 when test is compared with the control

n = number of mice per group = 6

4.4.2 Hot Plate Test

P. africana, S. *indica, N. latifolia and S. esculentus did* not have inhibiting or potentiating effects in hot plate test (Table 10). *M. balsamina* was the most potent of all the plants (*S. esculentus*, *C. goratensis*, *F. thonningii*, *P. kotschyi*, *E. chlorantha, N. latifolia*, *M. balsamina*, *S. indica*, *and P. africana*) evaluated using hot plate method. The observed effect with *M. balsamina* was comparable to that of aspirin at the doses used for this experiment.

E. chlorantha was observed to have weak analgesic activity in hot plate model.

Table 10; Antinociceptive Effect of Methanolic Extracts on the Hot-plate Test

Treatment	**Dose (mg/kg)**	**Latency (S)**
Control	----	1.79 ± 0.67
S. indica	200	1.52 ± 0.32
	400	1.01 ± 0.43
P. Africana	100	1.78 ± 0.55
	200	1.99 ± 0.41
N. latifolia	150	1.99± 0.54
	500	1.78 ± 0.48
S esculentus	1,500	1.83 ± 0.75
	1,500	2.17 ± 0.41*
E. chlorantha	400	3.00± 1.46*
	1,100	3.83 ± 1.93*
P. kotschyi	300	3.52± 0.41*
	600	9.33 ± 1.20**
F. thonningii	500	6.50 ± 0.65**
	1,000	6.75± 0.48**
C goratensis	500	6.00± 1.55**
	1,000	10.83 ± 2.93**
M. balsamina	400	8.55 ± 0.57**
	1,000	11.16 ± 0.51**
Aspirin	100	9.01 ± 1.52**
	200	15.59 ±2-58**

*P<0.05, **P<0.005 when compared with the control (student T test)

n = number of rats per group = 6

4.4.3 Tail Flick Test

M. balsamina was chosen for further studies because it has the most potent analgesic activity in both writhing reflex and hot plate test and is well tolerated. *M. balsamina* prolonged the stay time of rats tail in hot water regulated at a temperature of 55^{o}C (Table 11) at higher doses (500 and 1,000 mg /Kg) but not at the low dose (200 mg /Kg). The result showed that *M. balsamina* is effective in hot water tail flick test. The values obtained were comparable to that of aspirin. Aspirin significantly prolonged the stay time of the rat`s tail in hot water regulated at 55^{o}C.

Fractions of *M. balsamina* showed varying degrees of inhibition of tail flick at the same temperature (55^{o}C) as shown in Table 12.

Table 11; Effect of *M. balsamina* on Hot Water Tail Flick Test

Treatment Mg /Kg	**Latency ± SEM**	**% inhibition**
Control	0.19 ± 0.07	-----
M. balsamina		
200	0.17 ± 0.11	0
500	0.82 ± 0.31*	431
1000	2.38 ± 0.72*	1152
Aspirin	1.41 ± 0.93*	742

SEM = Standard error mean

*P<0.05, when test is compared with the control

n = 6 animals per group

Table 12; Effect of *M. balsamina* fractions on Hot Water Tail Flick Test

Treatment	**mg /Kg**	**N**	**Latency (sec) ± SEM**	**% Inhibition**
Control		5	0.20 ± 0.12	---
DEE				
	500	5	0.18 ± 0.09	10
	1,000	5	0.22 ± 0.14	10
EA				
	500	5	0.17 ± 0.09	15
	1,000	5	0.28 ± 0.07	40
Chloroform				
	500	5	0.19 ± 0.12	5
	1,000	5	0.25 ± 0.13	25
Butanol				
	500	5	0.49 ± 0.11*	145
	1,000	6	0.82 ± 0.31*	310
Water				
	200	6	1.17 ± 0.11*	485
	500	6	2.82 ± 0.07*	1310
M.balsamina				
	200	6	0.17 ± 0.11	15
	500	6	0.82 ± 0.31	409
	1,000	6	2.38 ± 0.72	1090

SEM = Standard error mean

*P<0.05, when test were compared with the control.

4.4.4 Formalin Test

M. balsamina effectively inhibited peripherally and centrally induced paw flick and paw lick in formalin test (Table 13 and 14). This futher shows that *M. balsamina* possesses both central and peripheral analgesic properties. *M. balsamina* at the doses used (200 and 600 mg/Kg) significantly reduced the number of paw flick and paw licks in the first 6 seconds. No noticable movement of the rats paw was observed for the following 6 seconds in all the groups. *M. Balsamina* also significantly reduced the number of paw flick and paw lick in counts for subsequent six seconds and counts for every six seconds up to sixty seconds (Tables 13 and 14).

4.5 ANTI-INFLAMMATORY ACTIVITY

Fresh egg-albumin caused a progressive increase in the rat paw circumference in the control rats. The extracts under investigation showed anti-inflammatory activity against acute inflammation (Tables 15) induced by egg albumin. On average, *S. indica, N. latifolia* and *E. chlorantha* extract exhibited the least potency. All the plant extracts (*S. esculentus, C. goratensis, F. thonningii, P. kotschyi, E. chlorantha, N. latifolia, M. balsamina, S. indica, P. africana*) under investigation suppressed the increase in the rat paw edema in a dose dependent manner. The inhibition was significant ($p<0.05$) two to three hours after the inducement of inflammation. *M. balsamina* was found to be the most potent of all the herbs investigated. Its effect was comparable to that of Aspirin at the dose used.

S. indica, N. latifolia and *E. chlorantha* were observed to exhibit weak anti-inflammatory activity when compared to other plants (*S. esculentus, C. goratensis, F. thonningii, P. kotschyi, M. balsamina, P. africana*) studied.

Fractions of *M. balsamina* had inhibitory effects on egg white induced inflammation in rats (Table 16).

4.6 TEMPERATURE LOWERING EFFECT

The results in Table 17 show that *M. balsamina* reduced yeast induced hyperthermia in albino rats. The effects observed at higher dose was the same as the effects observed at lower dose. This may suggest that the anti-pyretic effect of *M. balsamina* is independent of the dose administered and it lasted up to 18 hours. Aspirin was also observed to significantly reduce the body temperature of experimental animals. The values obtained for aspirin were not significantly different from the value obtained with *M. balsamina* at time 18 hours, but was significantly different from that of control.

4.7 ULCEROGENIC AND ANTI-ULCEROGENIC EFFECT OF *M. balsamina*

M. balsamina at low and high doses did not have any significant ($P>0.05$) ulcerogenic effect (Table 18), but indomethacin significantly ($P<0.05$) produced ulceration of the gastrointestinal wall of test rats when compared with control rats.

It was also observed that this plant had slight protective effect on the gastro-intestinal wall. It insignificantly ($P>0.05$) protected the rats against indometacin induced gastric ulcer (Table 19).

Key.

Ulcer Scale

0 = Absence of damage

1 = Redness of mucosa

2 = Erosion of the gastric mucosa

3 = Ulcer

$$\text{Ulcer index} = \frac{\text{Mean degree of ulceration x \% of group ulceration}}{100}$$

% inhibition of ulceration

$$= \frac{\text{Ulcer index in control} - \text{Ulcer index in test}}{\text{Ulcer index in control}} \times 100$$

Table 18; Effect of *M. balsamina* on the gastric Ulceration

Treatment	Ulcer score	Ulcer index	% ulcerogenicity
Distilled water	0.00 ± 0.00	0.00 ± 0.00	0.00
M. balsamina			
1,000	0.00 ± 0.00	0.00 ± 0.00	0.00
2,000	0.00 ± 0.00	0.00 ± 0.00	0.00
Indomethacin			
60 mg/Kg	1.60 ± 0.51*	0.85 ± 0.27*	45.00

*P<0.05., when tests are compared with the control, using Student's t test.

n = 5 animals per group

Table 19; Effect of *M. balsamina* on indomethacin (60 mg/Kg) **induced ulcer**

Treatment	**Ulcer score**	**Ulcer index**	**% ulcerogenicity**
Distilled water	1.60 ± 0.51	0.85 ± 0.27	
M. balsamina			
1,000 mg/Kg	1.20 ± 0.00*	0.48 ± 0.20*	43.52

*P<0.05., when tests are compared with the control, using Student's t test.

n = 5 animals per group

4.8 INVESTIGATION OF THE MECHANISM OF ANTI-INFLAMMATORY EFFECT OF *M. balsamina*

Results obtained showed that *M. balsamina* extract significantly ($P<0.05$) inhibited heat-induced and hypotonicity induced heamolysis (Tables 20 and 21). The membrane stabilizing effects of the plant could to explain its claimed anti-inflammatory and analgesic activities.

M. balsamina (400 and 600 mg/Kg) also inhibited white blood cell migration. It significantly ($P<0.05$) reduced the mean number of neutrophils and lymphocytes at the inflammatory site (Table 22). The inhibition of neutrophils and lymphocytes migration to the inflammatory site (thereby inhibiting inflammatory processes) can account for the claimed uses of *M. balsamina* in folk medicine as anti-inflammatory and analgesic herb.

Table 20; effects of *M. balsamina* extract on heat – induced haemolysis

Dose (mg/ml)	Mean Inhibition of Haemolysis (%)
Control	9.0 ± 2.0
200	12.99±5.77
400	23.32 ± 5.34*
600	68.18 ± 6.26**

*P<0.05, **P<0.005., when tests are compared with the control, using Student,s t test.

n = 5 animals per group

Table 21; Effects of *M. balsamina* Extract on Hypotonicity – Induced Haemolysis

Dose (µg/ml),	**Mean Inhibition haemolysis (%)**
Control	3.4 ± 1.21
200	3.5 ± 1.91
400	3.5 ± 1.29
1000	6.5 ± 3.32
2000	7.5 ± 1.73*

*P<0.05, when tests are compared with the control, using Student,s t test.

n = 5 animals per group

Table 22; Effects of *M balsamina* Linn extracts on white blood cells migration

Dose (mg/kg)	Mean No of Neutrophil	Mean No of Lymphocyte	Mean % Inhibition of Neutrophil Migration	Mean % Inhibition of Lymphocyte Migration
Control	2.33 ± 0.58	2.67 ± 0.58	-----	
M. balsamina				
200	2.00 ± 0.00	2.33 ± 0.58	14.16	12.73
400	2.00 ± 0.00	2.33* ± 0.58	28.33*	12.73
600	1.67*± 0.58	2.00* ± 0.00	28.33*	25.81*

*P<0.05, when compared with the control using student t test

n = 5 animals per group

4.9 ANTI-CONVULSANT EFFECT

M. balsamina did not inhibit pentylenetetrazole and picrotoxin induced convulsion in rats. This shows that *M. balsamina* has no significant anti-convulsant effect, on pentylenetrazole and picrotoxin induced convulsion (Tables 23 and 24). Diazepam significantly inhibited pentylenetetrazole induced convulsion in rats (Table 24). Phenytoin (4mg/kg) significantly inhibited Picrotoxin (6 mg/Kg) induced convulsion in rats (Table 24). Similarly, *M. balsamina* had no effect on electrically induced convulsion while Phenytoin, the positive control, significantly inhibited electrically induced convulsion (Table 25).

Table 23; Effect of Methanolic Extract of *M. balsamina* on Pentylenetetrazole Induced Convulsion

Dose mg/Kg	Onset of Convulsion (sec) X± SEM	%	Number of animals that convulsed	Number of attacks during 15 min X± SEM	%
Control	70 ±12.30	100	6/6	4.32 ± 0.38	100
M. balsamina					
500	74 ± 23.10	100	6/6	3.99 ± 0.59	98
1000	65± 20.30	97	6/6	4.32 ± 0.38	99
Diazepam					
2	145±32.4^{+}	50	2/6	1.05± 0.02^{+}	24

Values are mean ± SEM, $^{+}P<0.05$ when test iscompared with control, student`s t- test

n = 6 animals per group

Table 24; Effect of Methanolic Extract of *M. balsamina* on Picrotoxin Induced Convulsion

Dose mg/Kg	**Onset of Convulsions (min) X± SEM**	**Number of episodes (±SEM min per 15 min)**	**Number of death**
Control	21.5 ± 4.73	7±1.0	4
M. balsamina			
500	18.0 ± 4.24	6 ± 2.8	2
1000	17.5 ± 3.53	6 ± 2.8	1
Phenytoin			
(4mg/kg)	26.0± 4.24^{+}	3 ± 1.4^{+}	1

$^{+}P < 0.05$., when each group is compared with the control

n = 5 = animals per group

Table 25: Effect of Methanolic Extract of *M. balsamina* on Electrically Induced Convulsion

Doses (mg/kg)	**Convulsion Voltage (±SEM) Min**
Control (dist water)	13.9 ± 4.59
M. balsamina	
500	14.7 ± 3.56
1000	19.0 ± 6.52
Phenytoin 4mg/kg	22.0± 2.14*

*P<0.05, when each group is compared with the control using Student t test.

n = 5 animals per group

4.10 URETHANE INDUCED SLEEP

Methanolic extract of *M. balsamina* significantly ($P<0.05$) reduced the onset of urethane induced sleep (not at lower dose) and significantly prolonged the duration of sleep at doses used for this experiment (Table 26). *M. balsamina* when given alone, did not induce sleep in experimental rats.

Table 26; Effect of Methanolic Extract of *M. balsamina* on Urethane induced Sleep

Dose mg/Kg	Onset of sleep(Min.)	Duration of sleep (Min.)
Control	41±5.93	64±3.56
M. balsamina		
500	30±3.71	92±5.31*
1,000	21±4.20*	107±6.22*

Values are mean ± S. E. M,

* P<0.05 vs. control, student's t-test.

n = 6 animals per group.

4.11 ANTI-MICROBIAL ACTIVIIES

The crude extract of *M. balsamina* was able to inhibit (P<0.05) some of the tested bacterial strains. The antibacterial activity of the extract of *M. balsamina* against the tested strains is shown in Table 27. The crude extract showed some activity against *B. subtilis, E. coli, P. aeruginosa, and P. mirabilis*, but showed marginal activity against *S. aureus K. pneumonia, and S. typhi.* The activity of the extract against *B. subtilis and E. coli* was comparable to that of gentamycin.

The crude extract of *M. balsamina* was unable to inhibit (P>0.05) the tested fungi strains. The antifungal activity of the extract of *M. balsamina* against the tested strains is shown in Table 28. The crude extract showed no activity against *A fumigatus A. niger C. albicans and M. pusillus.* Ketoconazole was very effective in inhibiting all the fungi used for this study.

M. balsamina crude extract has great potential as antimicrobial agent against bacteria but not against fungi and it can be used in the treatment of infectious diseases caused by resistant bacteria. *E. coli* showed maximum suseptibility.

4.12 ANTI-DIARRHOEAL EFFECT

4.12.1 Effects on Castor Oil-induced Diarrhoea

The extracts of *M. balsamina and S. indica* and loperamide (50 mg/kg) significantly ($P< 0.05$) protected rats against castor oil-induced diarrhoea when compared with the control. *M. balsamina and S. indica* gave 87.5% and 64.5% protection respectively, (Table 29 and 30) while loperamide gave 100% protection. *M. balsamina* was more effective than *S. indica.*

The control that received distilled water only without castor oil did not show any diarrhoea while the control group that was administered distilled water with castor oil showed 100 percent diarrhoea when compared with the first control. This showed that the animals used for the experiments were healthy and were not having diarrhoeal stool.

Table 29; Effect of *M. balsamina* Linn on Castor Oil Induced Diarrhoea in Albino Rats

Dose mg/kg	Mean Freq of Diarrhoeal stool	% Inhibition of Diarrhoea
CONTROL	4.0 ± 1.274	
M. balsamina		
100 mg/kg (IP)	0.5 ± 0.45*	87.5
250 mg/kg (IP)	0.5 ± 0.43*	87.5
1000 mg/kg (PO)	0.00 ± 0.00	--
Diphenoxylate		
50mg/kg	0.00 ± 0.00	100
Distiled water	0.00 ± 0.00	

* $p<0.05$, when compared with castor oil control and diphenoxylate (50mg/kg) using the student's t-test

n= 6 animals per group

Table 30; Effect of *S. indica* on Castor Oil induced Diarrhoea in Albino Rats

Dose mg/kg	Mean Freq of Diarrhoeal stool	% Inhibition of Diarrhoea
CONTROL	7.75 ± 0.74	
S. indica		
1500 mg/kg	2.75 ± 0.22*	60
3000 mg/kg	3.25 ± 0.65*	64.52
Distilled water	0.00 ± 0.00	-
Diphenoxylate		
50mg/kg	0.00 ± 0.00	100

* $p<0.05$, when compared with castor oil control and diphenoxylate (50mg/kg) using the student's t-test.

n= 6 animals per group

4.12.2 Effects on Isolated Rabbit Jejunum

The effects of plant extracts on the jejunum revealed that *M. balsamina* produced an initial contraction followed by relaxation while *S. indica* produced sustained contraction which was inhibited by atropine and nifedipine. (Figures 1, 2, and 3). The contraction observed with *M. balsamina* was antagonized by atropine and to a greater extent by nifedipine.

4.13 ANTI-DIABETIC EFFECT

M. balsamina crude extract significantly ($P<0.05$) reduced blood glucose level in aloxan induced hyperglycemic rats. The effect of *M. balsamina* at 1,000 mg/Kg was comparable to that of chlopropamide (Table 31). The reductive effect of *M. balsamina* was not evident until the twelveth hour of administration This implies that it can be administered dailly as single or in two doses.

4.14 EFFECT OF THE EXTRACT OF *M. balsamina* ON BLOOD PRESSURE

4.14.1 Effect of Extract on Cat`s Blood Pressure

This aspect of the work was carried out in Ahmadu Bello University, Zaria, Nigeria. It was observed that the methanolic extract of *M. balsamina* lowered cat's blood pressure in a dose dependent maner (Figures 7 and 8). The effects of the extract were comparable to that of propranolol (Figure 5).

Effect of Adrenaline

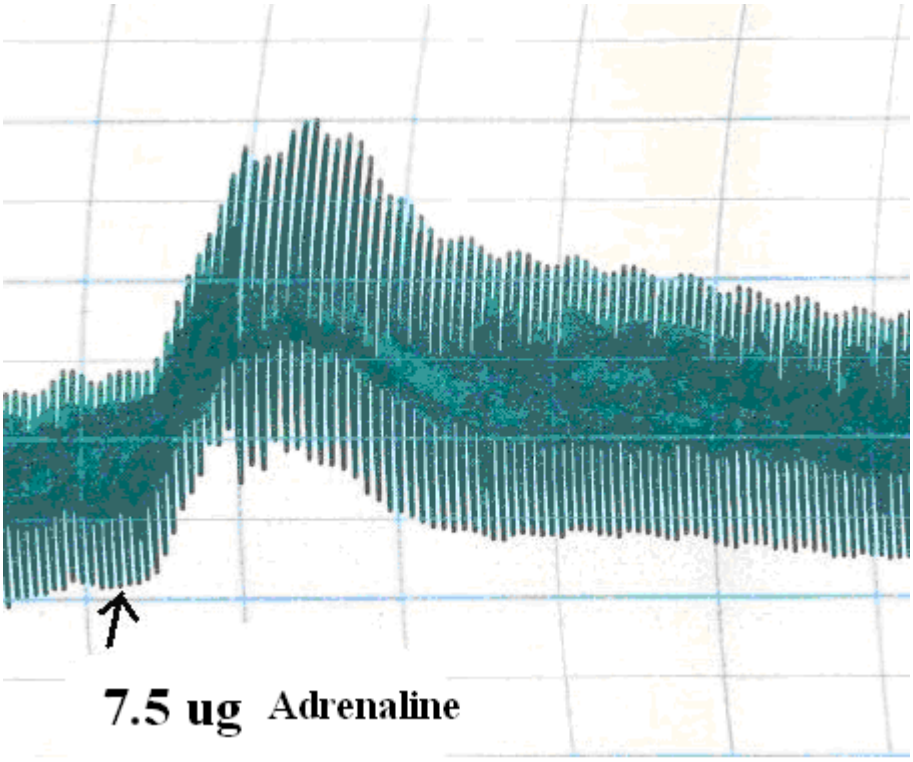
Adrenalin dose dependently increased cat's blood pressure (Figure 4). *M. balsamina* significantly ($P<0.05$) reduced adrenaline induced increase in cat's blood pressure (Figure 9, 10, 11, 12 and 13). Figure 14, showed that repeated dose of the extract produced an effect that was higher than the one produced by the same dose (earlier) before adrenaline.

Effect of Atropine

It was also observed that the blood pressure lowering effect of the extract of *M. balsamina* was inhibited by atropine (Figure 15). This suggests that the extract may have exhibited its action via the cholinergic pathway. This result supports the results obtained from rabit the jejunum (Figure 1). The extract potentiated the effect of acetylcholine on cat's blood pressure (Figure 16).

Effect of Nifedipine

Nifedipine was also observed to reduce the cat's blood pressure in a gradual fashion. The reduction in blood pressure was not as pronounced as that of the extract, acetycholine, and propranolol (Figure 6).



Figur 4; Effect of adrenaline (7.5ug) on cat`s blood pressure

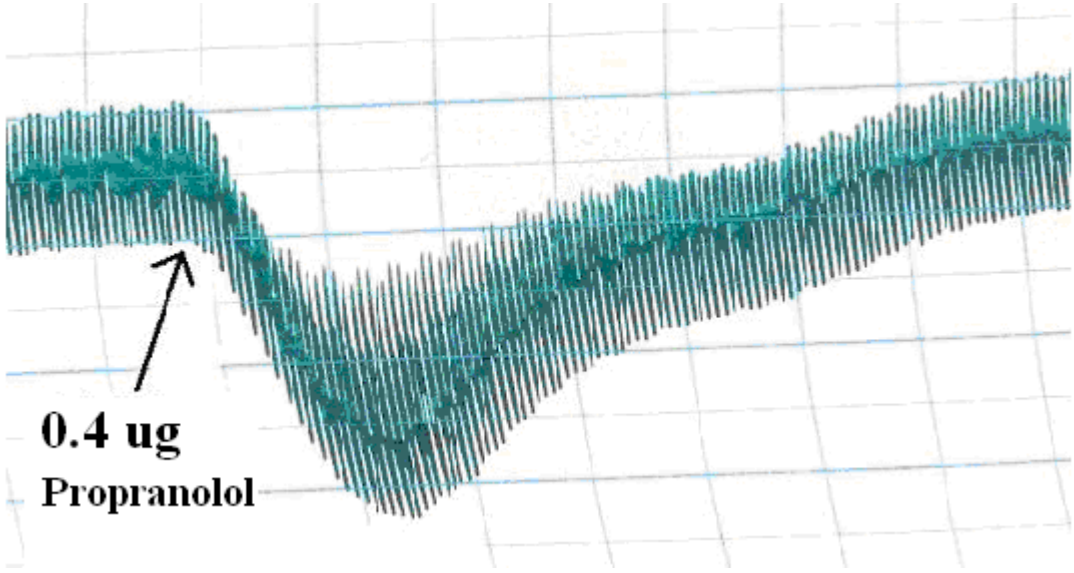


Figure 5; Effect of Propranolol (0.4 ug) on cat`s blood pressure

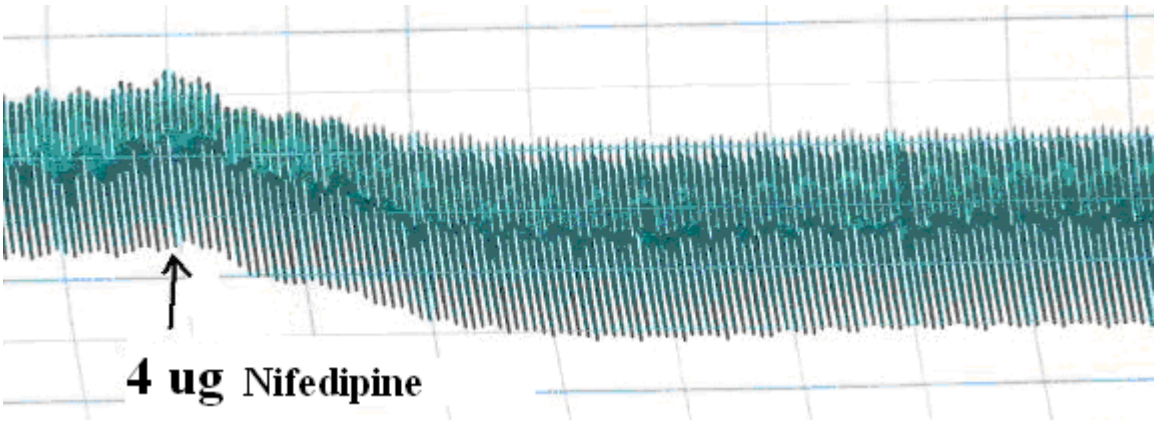


Figure 6; Effect of Nifedipine (4 ug) on cat`s blood pressure

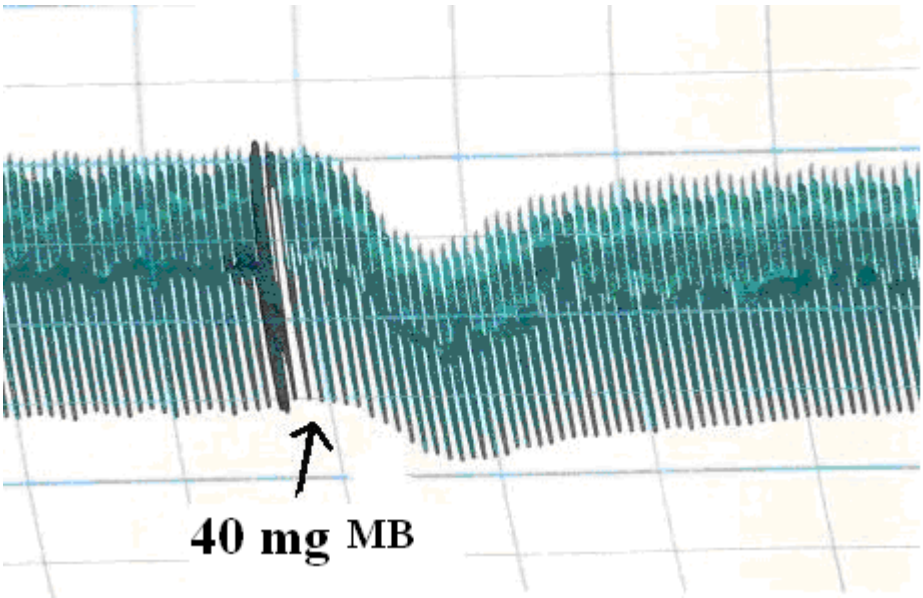


Figure 7; Effect of *M. balsamina* (MB) extract (40 mg) on cat`s blood pressure

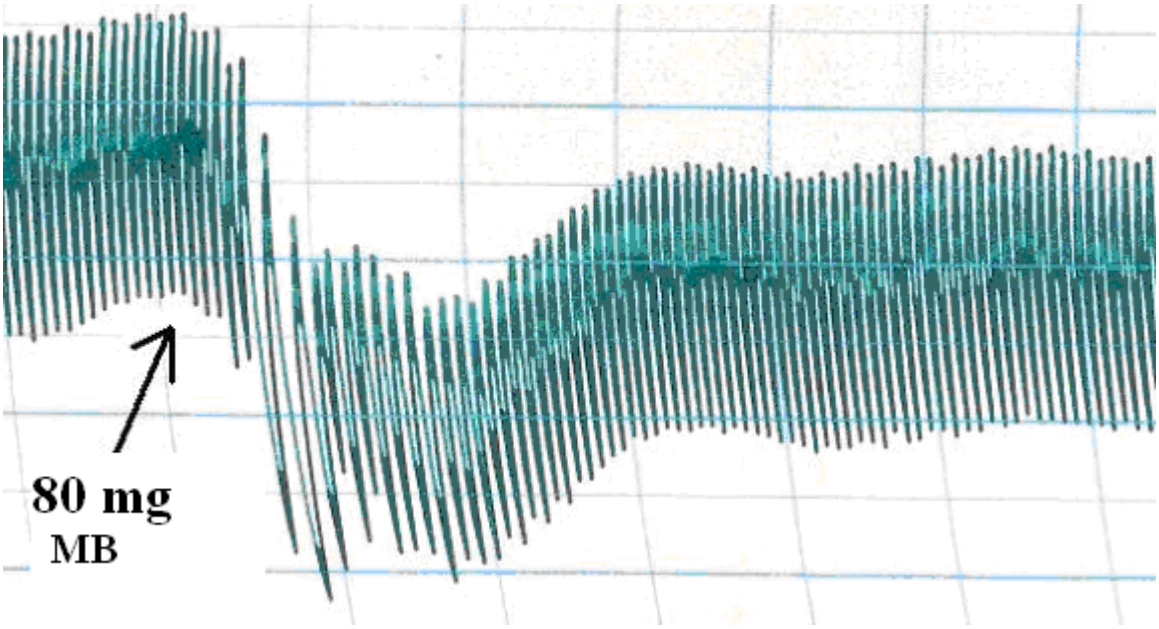


Figure 8; Effect of *M. balsamina* extract (80mg) on cat`s blood pressure

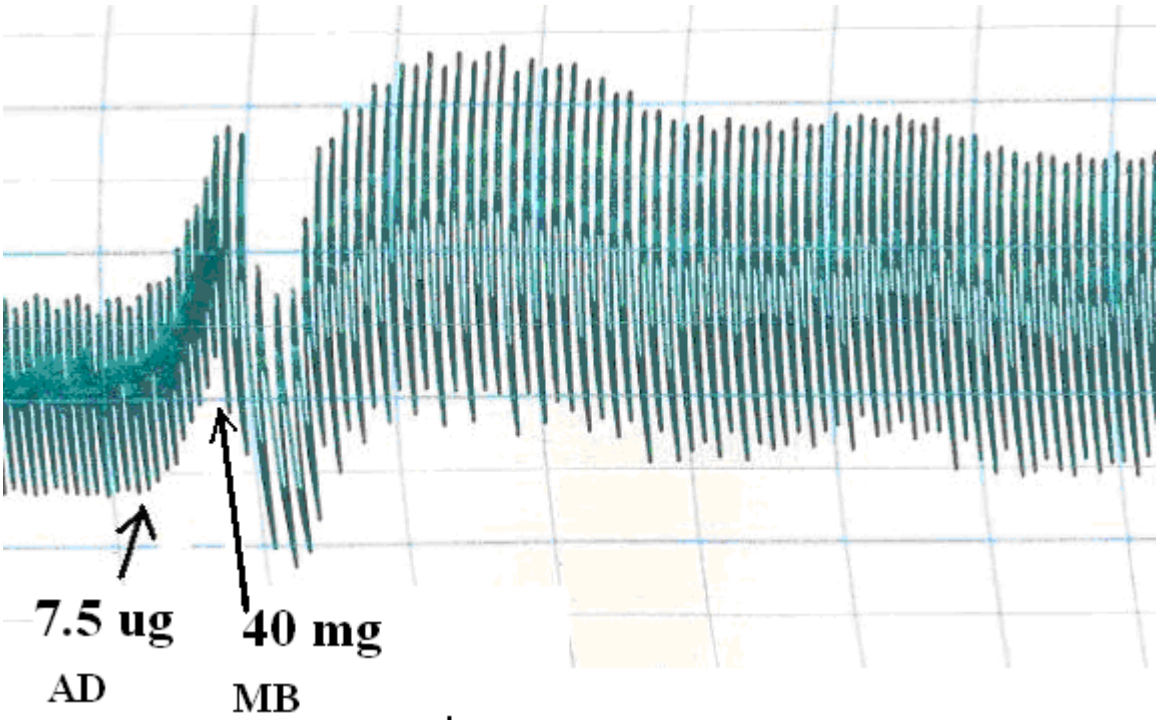


Figure 9; Effect of *M. balsamina* extract (40mg) on cat`s blood pressure in the presence of adrenaline (7.5 ug)

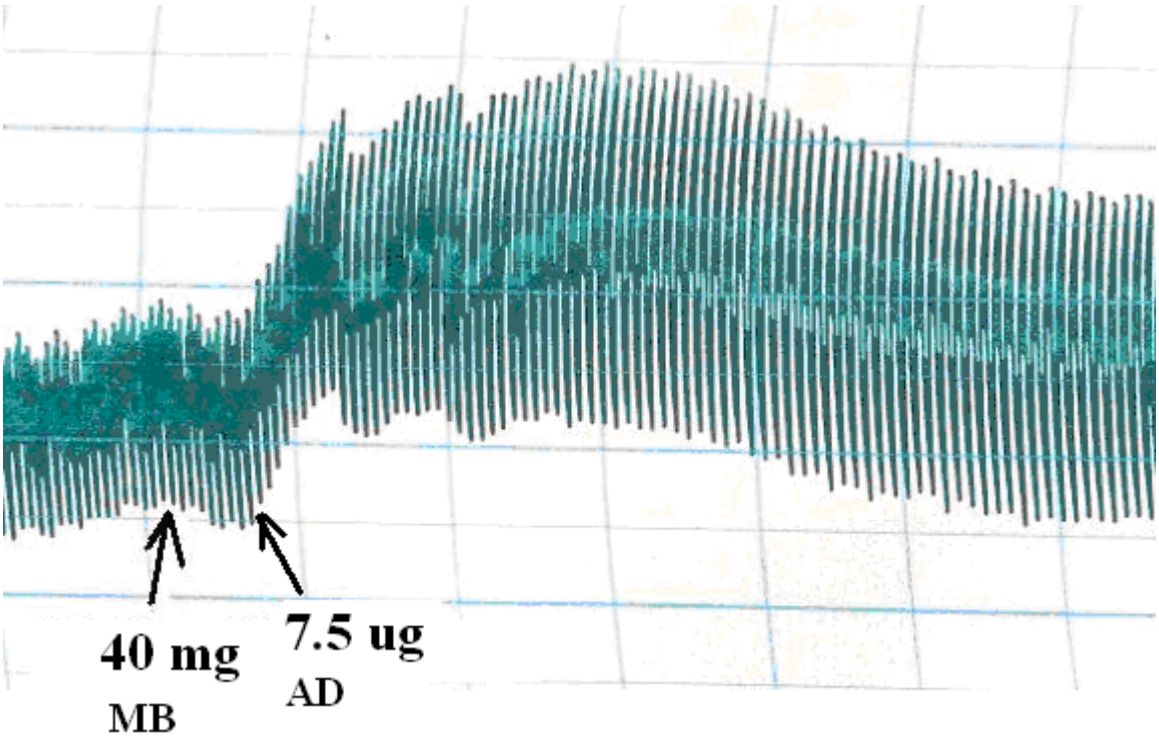


Figure 10; Effect of Adrenaline (7.5 ug) on cat's blood pressure in the presence of *M. balsamina* extract (40 mg)

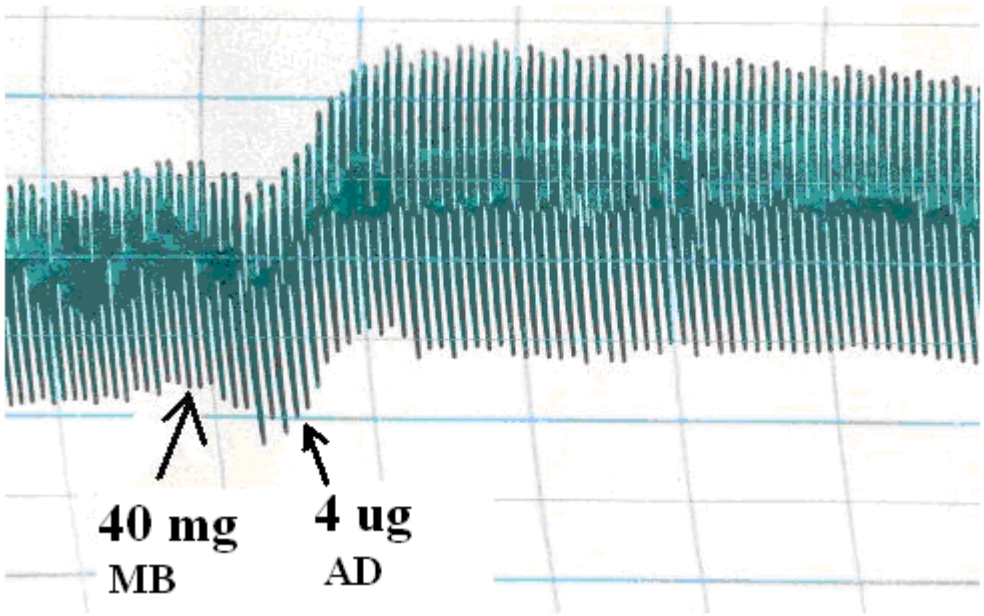


Figure 11; Effect of Adrenaline (4 ug) on cat's blood pressure in the presence of *M. balsamina* extract (40 mg)

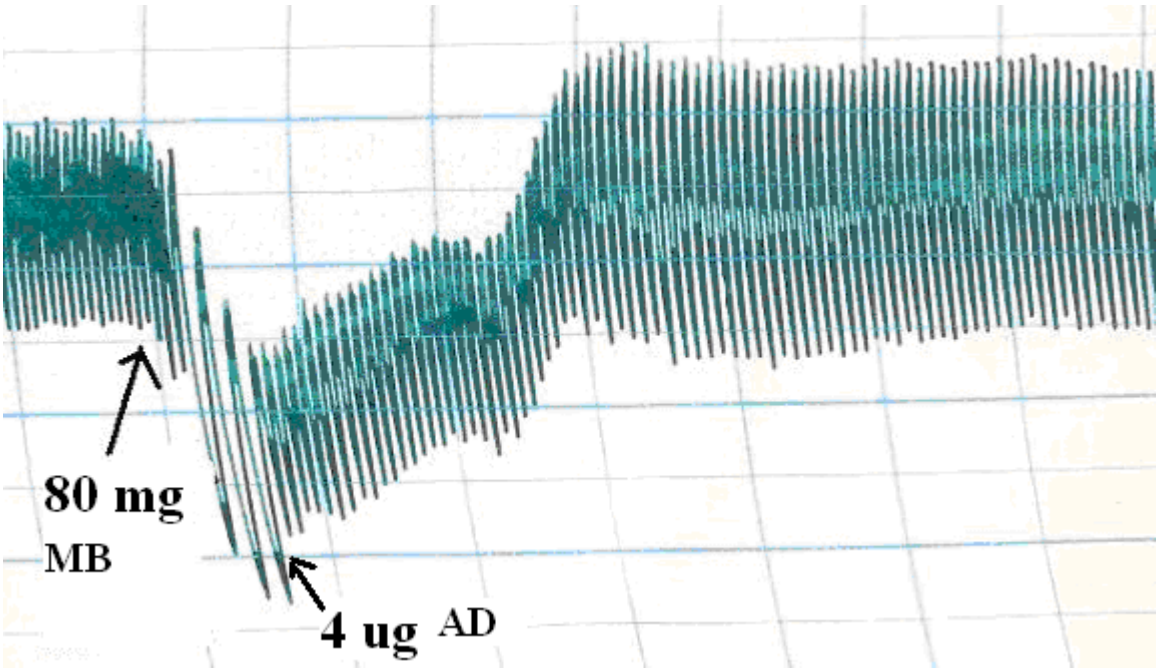


Figure 12; Effect of Adrenaline (4 ug) on cat's blood pressure in the presence of *M. balsamina* extract (80 mg)

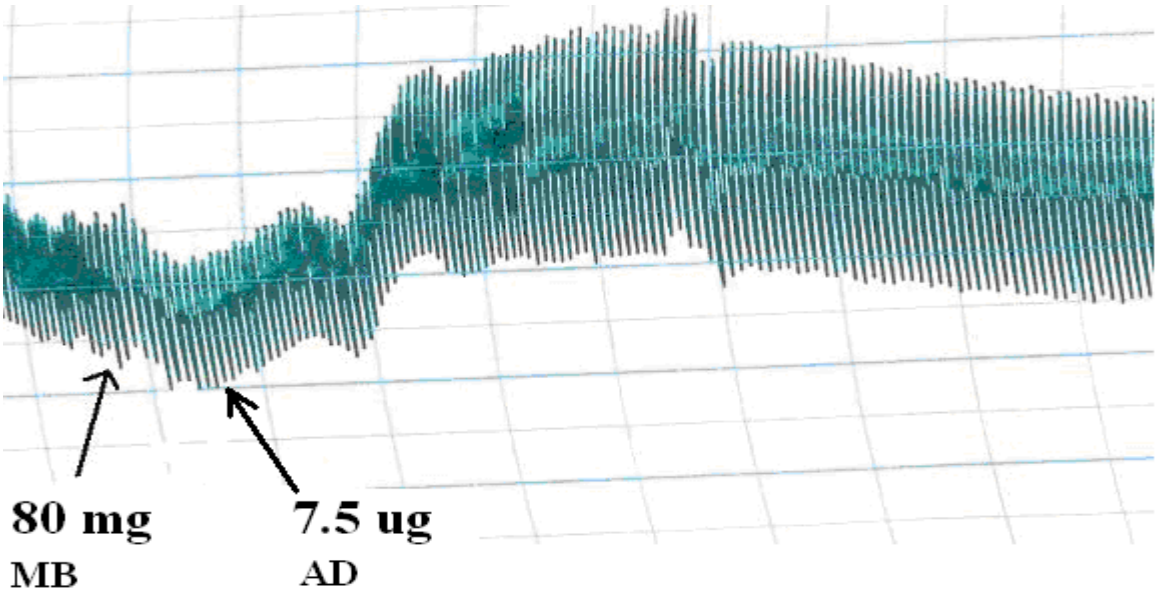


Figure 13; Effect of Adrenaline (7.5 ug) on cat's blood pressure in the presence of *M. balsamina* extract (80 mg)

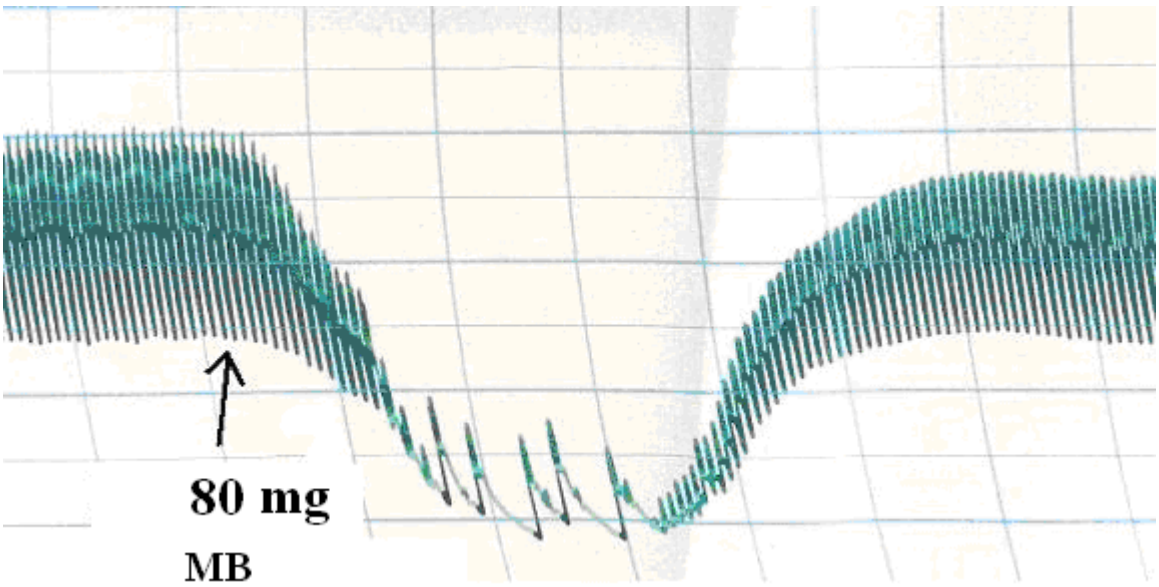


Figure 14; Effect of repeated dose of *M. balsamina* extract (80mg) on cat`s blood pressure

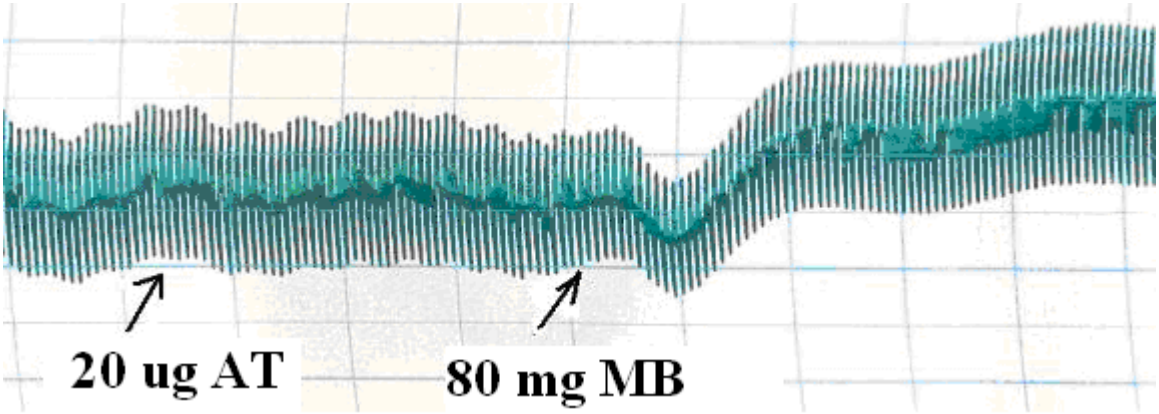


Figure 15; Effect of *M. balsamina* extract (80 mg) on cat`s blood pressure in the presence of Atropine (20 ug)

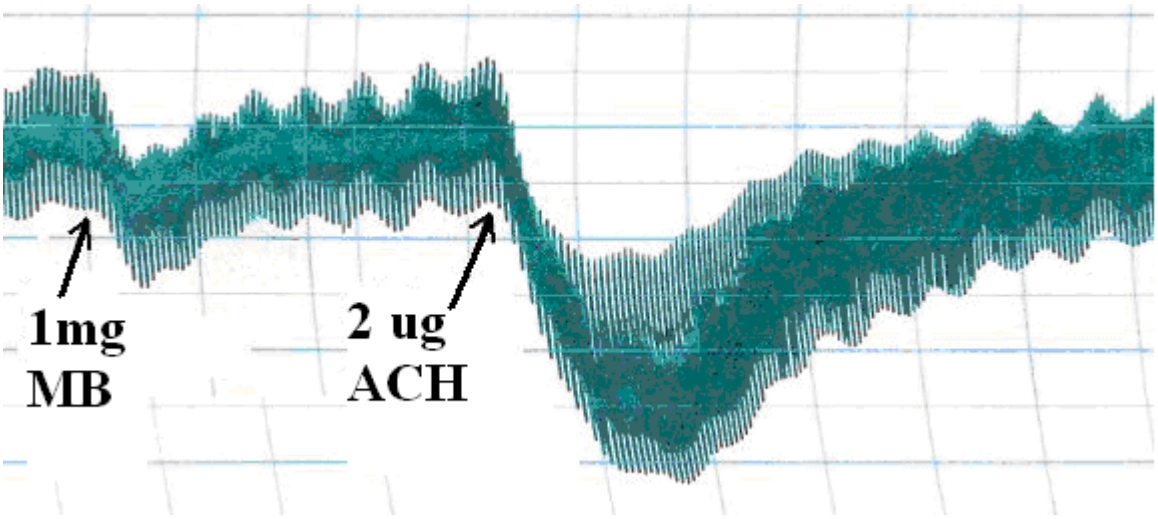


Figure 16; Effect of Acethylcholine (2 ug) on cat's blood pressure in the presence of *M. balsamina* extract (1mg)

4.14.2 EFFECT ON RAT'S ATRIA

This study revealed that *M. balsamina* had no significant effect on rat's atria at low doses, but at higher doses, it gradually reduced the rat's atria contraction (Figure 20 and 21). Adrenaline and calcium ion had contractile action on rat's atria muscle (Figure 17, 18 and 19). The extract did not have any significant effect on the effects of adrenaline and Calcium ion on rat's atria (Figure 22 and 23 respectively).

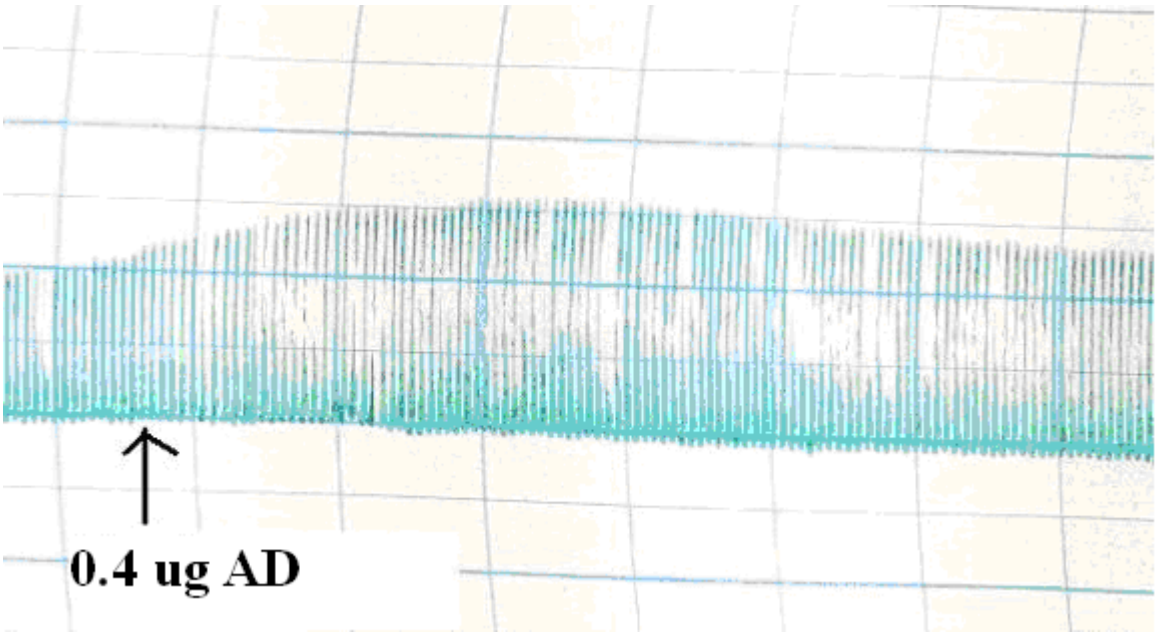


Figure 17; Effect of Adrenaline (0.4 ug) on rat's Atria

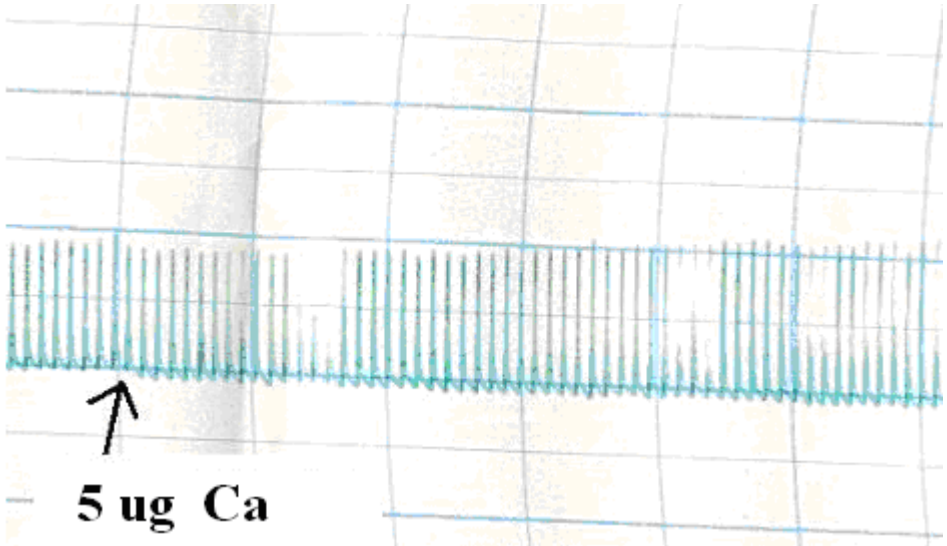


Figure 18; Effect of Calcium (5 ug) on rat's Atria

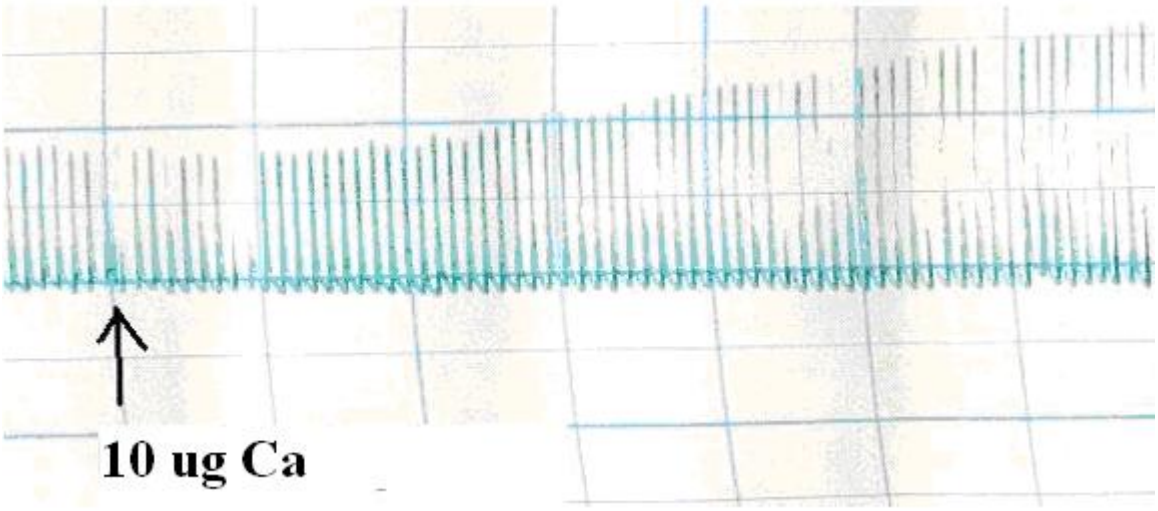


Figure 19; Effect of Calcium (10 ug) on rat's Atria

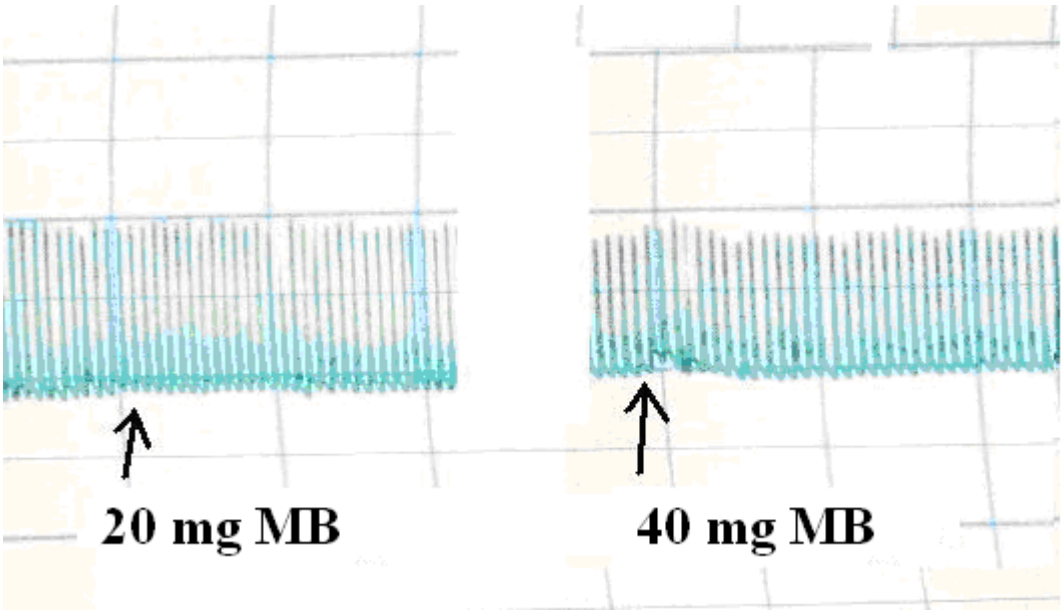


Figure 20; Effect of graded doses (20mg and 40mg) of *M. balsamina* extract on rat's Atria

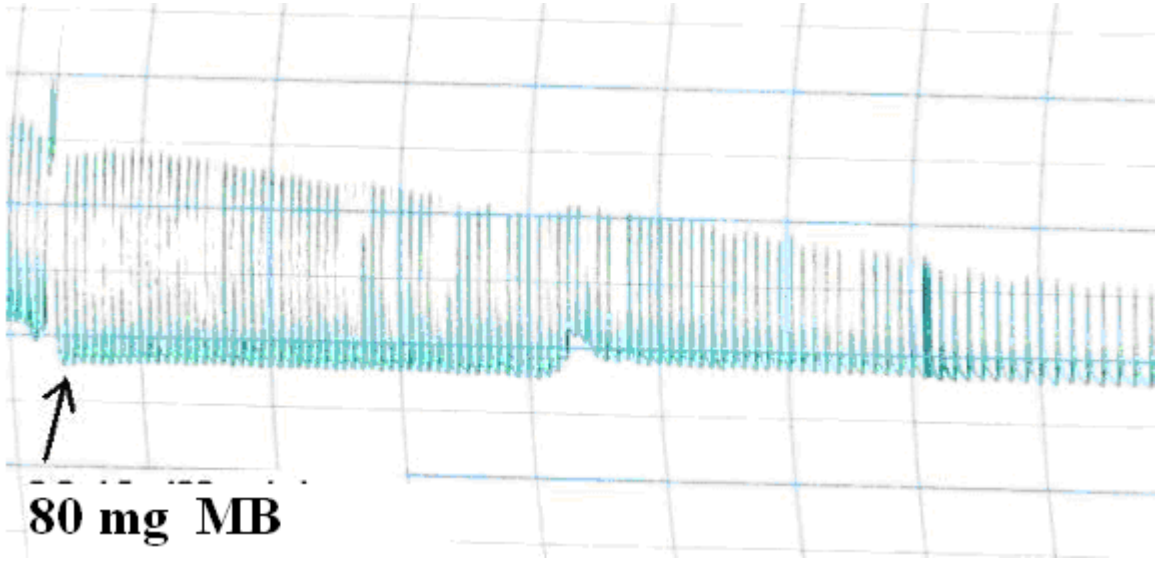


Figure 21; Effect of *M. balsamina* extract (80mg) on rat's Atria

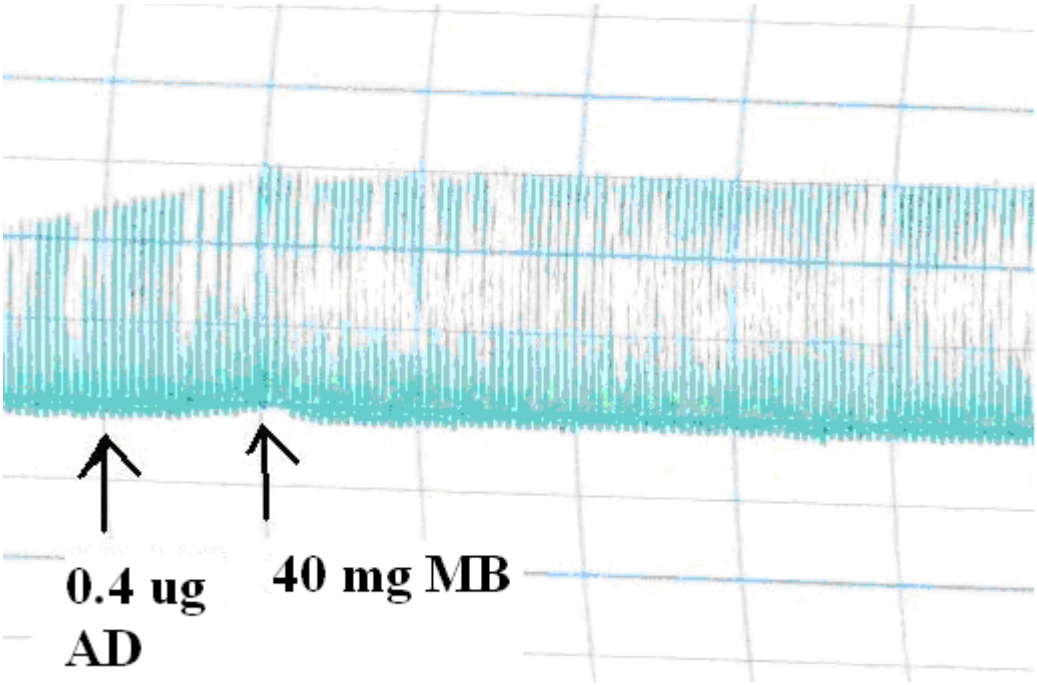


Figure 22; Effect of *M. balsamina* extract (40 mg) on rat's Atria in the presence of Adrenaline (0.4 ug)

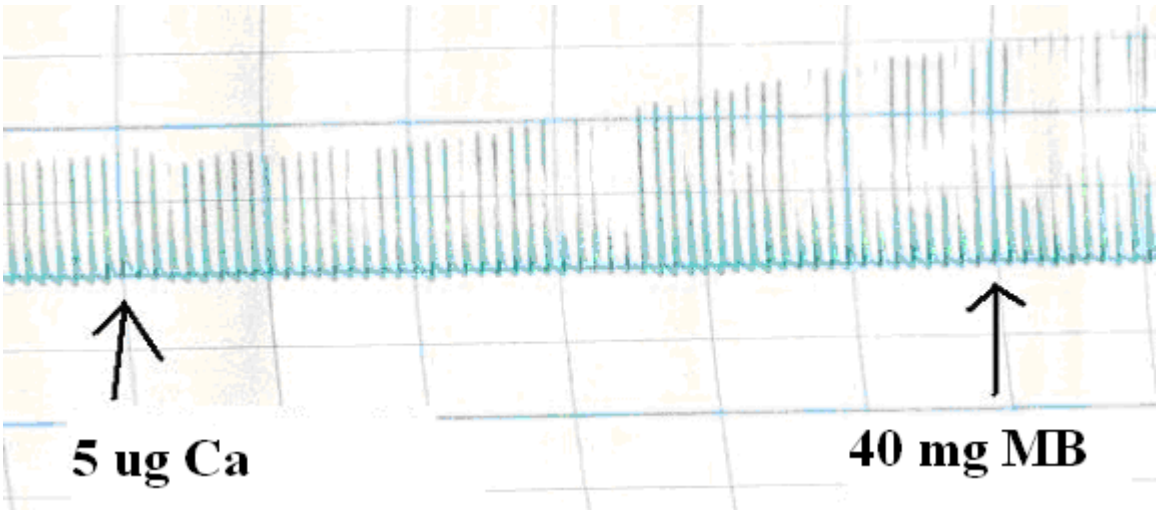


Figure 23; Effect of *M. balsamina* (40 mg) on rat's Atria in the presence of Calcium (5 ug)

CHAPTER FIVE
DISCUSSION

5.1 PHYTOCHEMICAL STUDIES

Phytochemical studies of the plants revealed that all the plants investigated contain flavoniods, tannins, and carbohydrates. Other chemical constituents present in some of the plants include alkaloids, saponins, anthroquinones, steroids, proteins and cardaic glycosides (Table 2). Flavonoids and protein were found at a higher concentration than other constituents. The presence of flavonoids may explain their biological activity. Previous studies have revealed that flavonoids in plants are responsible for the analgesic and anti-inflammatory activities of most medicinal plants (Moroney, *et al.,* 1988). Other constituents present e.g. alkaloids, may be responsible for the other pharmacological activities associated with *M. balsamina*.

5.2 TOXICOLOGICAL STUDIES

5.2.1 LD_{50} and Acute Toxicity Assay

Scientifically, plants are evaluated for LD_{50} values to establish their safety. Acute toxicity test gives an idea of the effect of short term use of the drug on body organs and systems. The results from this study show that the intra-peritonial LD_{50} values (Table 3) for most of the plants investigated are within safety margins. They are therefore safe for consumption, when given at safe doses. *S. indica* and *N. latifolia* (with LD_{50} 0.15 and 0.80 g /Kg respetively) were found to be the most toxic intra-peritoneally. This may explain the traditional use of *S. indica* as arrow

poison by hunters. When *S. indica* was first administered intra-peritoneally, the mice in the treated groups died few minutes after the administration of the extract (*S. indica).* At reduced doses, mice showed signs of restlessness, piloerection and were whistling before death. It was only at doses below 0.15 g/Kg that the animals survived, but were restless and whistling. The restlessness and whistling observed may suggest that *S. indica* has central nervous system activity. *S. indica* was not toxic when administered orally; the oral LD_{50} values (Table 3) was greater than 7.00 g/ Kg. Phyto-chemical analysis revealed that the plant (*S. indica)* is very rich in saponins. Saponins are known to haemolyse red blood cells. This may possibly be the cause of death and intra-peritoneal toxicity. Saponins, when taken orally, are broken down to harmless substances and thus not absorbed into the system. This may explain why this plant extract was not toxic orally.

S. indica is used in Brazil for the adulteration of tea. Since there has been no record of toxicity resulting from the frequent ingestion of this plant along side tea, this may be another evidence to support the safety of this plant by oral route of administration.

The LD_{50} for methanolic extract of *N. latifolia* was 0.80 g/kg. This shows that this plant is very toxic if administered intra-peritoneally. This result supports the report by Traore and his team 2000, that toxicity resulting from *N. latifolia* occur as a result of the interaction *in-vitro* with DNA of mammalian cells, leading to G_2-M cell cycle arrest and heritable DNA-damage, as well as inducing *in-vivo* single-strand breaks in liver, kidney and blood cells. The plant extract should be taken with care, and traditional healers educated on the potential danger of administering the ethanolic preparations of *N. latifolia.* Plants with toxic effect

have been reported to be of pharmacologic importance. For example, eserine from Calabar bean, which was once used as an ordeal poison to assess the guilt or innocence of suspected criminals and heretics, has been found to be of pharmacological importance. The substance physostigmine, which was discovered to be responsible for its toxicity, is now an effective drug for the management of glaucoma (Rang and Dale, 1998). It has however been reported that the aqueous extract of *N. latifolia* is safe for consumption and effective as an analgesic (Ngnokam *et al.*, 2003). Methanol may have extracted more of the toxic principles than water. Traditional healers should therefore be advised to use aqueous preparations rather than the ethanolic preparations of *N. latifolia.*

Most of the plants investigated were safe for consumption, *F. thonningii,* from the result, was better tolerated intra-peritoneally (Table 3) than the other plants investigated.

5.1.2 Histological Examination

Histological examination showed that *M. balsamina* extract did not have any significant effect on liver, spleen, lungs, stomach, and heart cells of treated animals (Apendix 1-18). The data for liver supports the results obtained from blood assay of liver enzymes (Table 7).

5.1.3 Haematological Parameters

The mean ± SEM values of the bleeding time and clotting time of rats treated with the extract of *M. balsamina* (1,000 and 1,500 mg /Kg) are as shown in Table 4. The mean PCV values of treated animals and animals that received aspirin were not significantly different from that of the control. This shows that

the extract of *M. balsamina* has no effect on packed cell volume and thus on erythropoiesis (Table 5). Bleeding time and clotting time were also not affected by the extract ($P>0.05$) when compared to control values. Aspirin significantly ($P<0.05$) increased the bleeding and clotting time when compared to the control and the value for *M. balsamina* (Table 4).

The mean RBC, WBC and platelet count were not significantly ($P>0.05$) affected by the doses of the extract used, (Table 6). These results show that the extract has no significant effect on haematological parameters. It was also observed that aspirin did not have ($P>0.05$) any effect on the red blood cells, white blood cells and platelet counts. The results obtained for WBC is not in consonance with the result obtained by (Matawalli *et al.*, 2004). These authors claimed that *M. balsamina* increases WBC count. The disparity in this result may be due to the part of the plants used. They used the leaves only while in this work the whole plant was used.

5.1.4 Biochemical Parameters

The blood concentrations of the three most prominent liver enzymes, [alkaline phosphatase (ALP), alanine aminotransaminase, (ALAT) and aspartate aminotransaminase, (ASAT)] were not affected ($P>0.05$) by *M. balsamina* (1.0 and 1.5 g/Kg) extract.

The blood concentration of alkaline phosphatase insignificantly ($P>0.05$) decreased, at a dose of 1.0 g/kg to 397.26 ± 0.59 u/L when compared with control value, 403.35 ± 1.02 u/L. As the dose was increased to 1.5 g/Kg, the blood

concentration further decreased (395.19 ± 1.04 u/L). The values obtained were not significantly different from that of the control animals (Table 7).

Alanine transaminase blood level decreased in the rats pretreated with the two doses of the extract. At a dose of 1.0 g/Kg the blood level was insignificantly ($P>0.05$) reduced to 26.24 ± 1.11 u/L from the control value of 28.01 ±0.94 u/L. The concentration further reduced to 25.14 ± 0.99 u/L ($P>0.05$) when the dose of the extract was increased to 1.5 g/Kg. The decreases observed were not significantly ($P>0.05$) different from the values of the control, (Table 7). There was no significant ($P>0.05$) decrease in the blood concentration values of aspartate amino transaminase in the rats pretreated with various doses (1.0 and 1.5 g/Kg) of the extract when compared with control value. The overall results show that the extract has no effect on the liver cells and enzymes, (Table 7, Apendixes 1, 2, 3 and 4). The observed effects on liver cells are an advantage to *M. balsamina.* This gives it an advantage over analgesics (like paracetamol), which have distructive effect on the liver cells at high doses.

5.3 ANALGESIC ACTIVITY

5.3.1 Writhing Reflex

This test was used for preliminary screening of the plants collected. The plants that were not effective in this test were not selected. This may explain why all the nine plants showed activity in writhing reflex test (Table 8). According to Turner (1965), plants that are effective in this test have peripheral analgesic activity. The results show that these plants can be useful for the management of peripherally induced pain. *M. balsamina* was the most effective of the nine plants

studied when compared with other plants investigated with respect to their potency and safety. It was then selected for in-depth study of analgesic activities**.** *N. latifolia* was the least effective (Table 8) at the dose utilized. *P. africana* and *S. indica* were observed to have weak analgesic effects in this test.

M. balsamina fractions were equally effective in inhibiting acetic acid induced writhing reflex (Table 9). *N*-hexane fraction had no effect on acetic acid induced writhing reflex, but diethylether, ethylacetate, *n*-butanol and water factions had varing degrees of inhibition (Table 9).

5.3.2 Hot Plate Test

P. africana, S. indica and *N. latifolia* were not effective in hot plate test (Table 10). *S. esculentus* was only effective at high dose and not at the low dose. *M. balsamina* was the most potent of all the plants assayed in hot plate test. The observed analgesic effect with *M. balsamina* was comparable to that of aspirin at the doses used for this experiment. Hot plate test is a model for assaying effect of drugs on central pain. Drugs that are effective in this model have central analgesic effect (Turner, 1965). *P. africana, S. indica* and *N. latifolia* can be said to have only peripheral analgesic effect, while the other plants evaluated have both central and peripheral analgesic activity.

5.3.3 Tail Flick Test

Tail flick test is a model for evaluating analgesics with central analgesic effects. Drugs effective in this model exhibit their analgesic effect centrally. *M. balsamina* was observed to be effective in prolonging the stay time of rat`s tail in

hot water regulated at a temperature of 55^{o}C (Table 11). This activity was only evident at higher doses but not at the low doses. The result showed that *M. balsamina* exhibits its analgesic effect centrally and supports the earlier result obtained from the hot plate test.

The fractions of *M. balsamina* prolonged the stay time of the rat's tail in hot water to varing degrees, except the *n*-hexane fraction (Table 12) which had no effect. Water-soluble fraction was more effective followed by the *n*-butanol fraction.

This result suggests that water decoction will produce maximal effect.

5.3.4 Formalin Test

M. balsamina effectively inhibited peripherally and centrally induced paw flick and paw licks after intra-dermal injection of formalin into the rat`s paw (Table 13, 14). This further shows that *M. balsamina* possesses both central and peripheral analgesic properties and supports the results obtained earlier. The number of paw flick or licks within the first six minutes of administration was significantly lower in the test group than the control group (Table 13, 14). The first phase, which occurs between zero to six minutes, is elicited by the stimulation of peripheral nociceptors (Padi *et al.*, 2006) and reveal peripheral activity. It was also observed that the later phase that started twelve minutes after administration of formalin (elicited centrally) was significantly inhibited by the extract of *M. balsamina*. This phase occurs as a result of the stimulation of central nociceptive receptors (Padi *et. al.*, 2006). This result further reveal that *M.*

balsamina is effective for the management of peripherally and centrally induced pain.

5.4 ANTI-INFLAMMATORY ACTIVITY

Fresh egg-albumin caused a progressive increase in the rat paw circumference in the control rats. The extracts of the plants under investigation showed anti-inflammatory activity against acute inflammation induced by egg-albumin (Tables 15). On average, *S. indica, E.chlorantha and N. latifolia* extracts exhibited least potency. All the extracts under investigation suppressed the increases in rat paw edema in a dose dependent manner. The inhibition was significant ($p<0.05$) two to three hours after the injection of inflammatory agent. *M. balsamina* was found to be the most potent of the herbs investigated. The results show that these herbs exhibit both analgesic and anti-inflammatory activities. This implies that they will be useful in the management of inflammatory pain, a condition which occurs together in disease states. Pain and inflammation are complementary and always occur together. The plants were reported by traditional healers to be effective in the management of specific pain conditions (like stomach pain). Inflammation has been linked to the aetiology of most disease conditions (Giri, 2003; Tillie-Leblond *et. al.*, 2005; Fireman, 2003; Madamanchi *et. al.*, 2005; Kanai and Watanabe, 2004; Hotamisligil, 2003.). Its control can improve the effects of specific drugs used in the management of these specific disease conditions. Pain receptors can be stimulated by substances (e.g. histamine) released during inflammation. Control of inflammation can therefore contribute to the reduction of pain sensation.

It was also observed that all the fractions of *M. balsamina,* except *n*-hexane fraction, inhibited egg white induced rat paw inflammation, with water-soluble fractions having the highest activity followed by *n*-butanol fraction. These results were comparable to that of aspirin (Table 16).

5.5 OTHER PHARMACOLOGICAL EFFECTS OF *M. balsamina*

Pain occurs as a pathological condition in rare cases. In most cases pain is associated with disease conditions. This may explain why most of the herbs collected were described to be useful in the management of pain associated with some disease conditions. This study covered some of the claimed pharmacological effects of the plants studied, especially *M. balsamina.* In practice, analgesic agents are prescribed together with drugs that cure or modify the underlining disease conditions.

Analgesics with additional pharmacological effects will be of great advantage. It will reduce the number of medications prescribed to a patient. This will improve compliance and provide effective management of the disease conditions. *M. balsamina* has shown great potentials in this respect. It was found to have wide applications.

5.5.1 Temperature Lowering Effect

The results above show that *M. balsamina* reduced yeast-induced hyperthermia in albino rats (Table 17). The effects observed at higher dose were the same as the effects observed at lower dose. This may suggest that the anti-pyrexia effect of *M. balsamina* is independent of the dose administered. *M.*

balsamina protected the animals for up to 18 hours. This gave it an advantage over aspirin and may also explain why it is prescribed for use twice daily by traditional healers. Increases in body temperature are signs that there are some pathological conditions in the body. It is often associated with the release of inflammatory mediators, which affect the temperature regulating centres in the hypothalamus. Pain and increase in body temperature are the body`s alarm system, that inform an organism that there are some pathological conditions going on in the body. Any animal that has a defect in this alarm system will not know when to seek medical attention or take drugs or herbs. They are the signals that drive most patients to the hospital or to seek the advice of a traditional healer.

Control of hyperthermia and pain is normally taken as relief by patients and even the traditional healers. Continuous use of medications or herbs that have only these effects without drugs or herbs that modify the pathological conditions is normally very dangerous. *M. balsamina* with multiple actions will be an advantage because such scenario will be prevented in man.

5.5.2 Ulcerogenic and Anti-ulcerogenic Effect of *M.balsamina*

M. balsamina was shown to have no ulcerogenic properties (Table 18). This property makes it a better analgesic / anti-inflammatory drug than most of the conventional analgesics / anti-inflammatory. It also had slight insignificant protective effect against indomethacin induced ulcer (Table 19).

5.5.3 Mechanism of Anti-inflammatory Effect of *M. balsamina.*

Results obtained from heat-induced haemolysis and hypotonicity-induced haemolysis (Tables 20 and 21), showed a significant difference between the means of inhibition of haemolysis (%) at $P<0.05$, implying that *M balsamina* Linn extract stabilizes the red blood cell membranes to the destructive effects of heat (54^0C) and hypo-tonicity. The membrane stabilizing effects of the plant could account for its claimed anti-inflammatory and analgesic activities,

Mast cell membrane has receptors both for a special class of antibody, immunoglobin E (1GE) and for complement components C3a and C5a. The cell can be activated to secrete mediators through these receptors. Physical damage of mast cell can also lead to the release of the content of mast calls. One of the main substances released by the mast cells is histamine; others include heparin, leukotrienes, PGD_2 (prostanoid), nerve growth factors and some interleukins, (Rang and Dale, 1998).

Histamine released is capable of producing many of the effects of inflammation and hypersensitivity: vasodilation, increased vascular permeability and the spasm of smooth muscle. Histamine has a significant role only in some sorts of Type 1 hypersensitivity reaction, such as allergic reactions and urticaria, (Rang and Dale, 1998).

Prostanoids are powerful vasodilators in their own right and synergize with other inflammatory vasodilators such as histamine and bradykinin. It is this combined dilator action on precapillary arterioles that contributes to the redness and increased blood flow in areas of acute inflammation. The prostanoids do not directly increase the permeability of the post capillary venules, but they potentiate

this effect of histamine and bradykinin. Similarly, they do not themselves produce pain but potentiate the effect of bradykinin by sensitizing afferent fibres (Rang and Dale, 1998). Leukotrienes can be found in inflammatory exudates and is present in inflamed tissues in many inflammatory conditions, including rheumatoid arthritis, psoriasis and ulcerative colitis. The cysteinyl-leukotrienes are present in the sputum of chronic bronchitis in amounts that are biologically active (Rang and Dale, 1998).

Mast cell membrane rupture therefore results in the release of mediators that play varieties of vital roles in inflammatory responses. *M balsamina* Linn extract, by stabilizing the mast cell membrane, prevents the release of these mediators, thus accounting for the claimed anti-inflammatory and analgesic effects. This may account for the mechanism of analgesic effects, because stimulation of nociceptors will not occur, since the cell content that acts on pain receptors are not released.

M. balsamina also significantly ($P<0.05$) reduced the mean number of neutrophils and lymphocytes at the inflammatory site (Table 22). During inflammation, neutrophils and lymphocytes migrate to the site of inflammation where they engulf, kill and digest the microorganisms, (Rang and Dale, 1998). The inhibition of neutrophils and lymphocytes migration to the inflammatory site (thereby inhibiting inflammatory processes) can account for the claimed uses of *M balsamina* in folk medicine as anti-inflammatory and analgesic medicinal herb. This action, though advantageous, may predispose patients to septicemia and complications of microorganism infection. This is not usually the case since from

these research findings, *M. balsamina* has anti-microboial activities. *M. balsamina* is therefore a promising drug for the management of infectious diseases.

5.5.4 Anti-convulsive Effect

M. balsamina did not (P>0.05) inhibit pentylenetetrazole and picrotoxin induced convulsion in rats (Table 23 and 24). It was also not effective in reducing convulsive episodes or protecting the animals against electrically induced convulsion (Table 25). This shows that *M. balsamina* may not be effective in the management or control of convulsion as claimed by traditional healers.

5.5.5 Urethane Induced Sleep

Methanolic extract of *M. balsamina* significantly (P<0.05) reduced the onset of urethane-induced sleep and significantly (P<0.05) prolonged the duration of sleep at the doses used for this experiment (Table 26). *M. balsamina*, when given alone, did not induce sleep in experimental rats. This was evident in all the experiments carried out in this study. The effect of this extract on sleep may augument its analgesic effects or may be the basis for its analgesic activity. Most sedatives (e.g. diazepam) have analgesic activity. They reduce the resting action potential of the nociceptive nerves and make it difficult for the nerves to be stimulated.

5.5.6 Anti-microbial effect of *M. balsamina*

The crude extract of *M. balsamina* inhibited (P<0.05) some of the tested bacterial strains. The antibacterial activity of the extract of *M. balsamina* against the tested strains is shown in Table 27. The crude extract showed some activity

against *B. subtilis, E. coli, P. aeruginosa, and P. mirabilis*, but showed marginal activity against *S. aureu,s K. pneumonia, and S. typhi.* The activity of the extract against *B. subtilis and E. coli* was comparable to that of gentamycin.

The crude extract of *M. balsamina* was unable to inhibit (P>0.05) the tested fungi strains (Table 28). It showed no activity against *A. fumigatus, A. niger, C. albicans and M. pusillus.* Ketoconazole was very effective in inhibiting all the fungi used for this study (Table 28). The antibacterial properties observed may augument the analgesic and anti-inflammatory properties of the herb. This implies that *M. balsamina* alone will effectively combat the infection and pain associated with infectious diseases thus reducing the number of drugs used and preventing the complications of poly pharmacy.

M. balsamina crude extracts have great potential as antimicrobial agent against bacteria but not against fungi and they can be used in the treatment of infectious diseases caused by resistant bacteria strains. *E. coli* showed maximum susceptibility.

5.5.7 Anti-diarrhoea Effect of *M. balsamina*

A. Effects on Castor Oil-induced Diarrhoea

This study was conducted for *M. balsamina* and *S. indica.* The study on *S. indica* was conducted alongside with that of *M. balsamina* because most of the traditional healers reported that *S. indica* is used for the management of pain associated with diarrhoea and colic pain. The extracts of *M. balsamina, S. indica* and diphenoxylate (50 mg/kg) significantly (P<0.05) protected rats against castor

oil-induced diarrhoea. The percentage protections were 87.5% and 64.5% for *M. balsamina and S. indica* respectively, (Table 29 and 30) while diphenoxylate gave 100% protection. Diarrhoea, induced by castor oil, occurs when there is hydrolysis of the oil by intestinal lipases resulting in the release of ricinoleic acid. The ricinoleic acid released produces an irritating reaction on the wall of the intestine thus enhancing the peristaltic activity of the small intestine. Also, ricinoleic acids, like other anionic surfactants, reduce the net absorption of water and electrolytes (Almieda *et al.,* 1995) causing diarrhoea. This effect, coupled with the latter is responsible for the diarrhoea observed when castor oil is administered. Diphenoxylate, a drug widely used in the management of diarrhoea disorders, has been reported to be effective in the prevention of diarrhoea induced by castor oil, prostaglandins, and cholera toxin (Farack *et al*, 1981). The pharmacological effect of Diphenoxylate is due to its anti-motility and anti-secretory properties (Karim, and Adeikan, 1977). From this investigation, it is likely that the extracts mediate their effects through similar mechanisms. Prostaglandins are implicated in the patho-physiology of diarrhoea, (Haruna *et al*, 1997). The phyto-chemical analysis of the extracts of *M. balsamina* (Otimenyin, and Uguru 2005) *and S. indica* (Otimenyin *et al*, 2006) revealed that they contain flavonoids. Flavonoids are known to modify the production of cyclo-oxygenase 1 and 2 (COX-1, COX-2) and lipo-oxygenase (LOX) (Moroney, *et al.,* 1988) thereby inhibiting prostaglandin production. The activation of LOX is induced by fatty meals while COX-1 and COX-2 is by diarrhoea-genic agents. Though several constituents are present in the extracts, it is most likely that flavonoids,

singly or in combination with tannins, and possibly other constituents, are responsible for the observed anti-diarrhoea effects of *M. balsamina and S. indica*.

B. *In-vitro* Anti-diarhoeal Effect of *M. balsamina*

Drugs that reduce gastro-intestinal motility are potent anti-diarrhoeal agents. The result of this study showed that *M. balsamina* but not *S. indica* effectively reduced spontanuous contractions of the rabbit jejunum. This result supports the use of *M. balsamina,* but not *S. indica* in the management of colic pain and diarhoea. The extract of *M. balsamina* relaxed the rabbit jejunum after transient contraction. It produced contraction at low concentrations while at a higher concentration the contraction produced was followed by relaxation. Higher concentrations produced marked relaxation, (Figure 1). The spasms observed were short lived not lasting more than few seconds.

The effect of S. *indica* on the rabbit jejunum contradicts its use in the management of diarrhoea as supported by the inhibitory effect of the extract on Castor oil induced diarrhoea. Almeida *et al.*, (1995) reported that *S. cayenesis*, (a plant that belongs to the same family) extract exhibited anti-diarrhoea activity through its positive action on intestinal transport of water, and gastrointestinal propulsion. Their results agree with the results obtained from *in-vivo* study of the effect of *S. indica* on castor oil induced diarrhoea, but not the *in-vitro* results, (Figure 2). They stated that *S. cayenesis* reduced gastrointestinal propulsion rate (Almeida *et al*, 1995), but from these results the extract of *S. indica* caused sustained contraction of isolated rabbit jejunum.

Anyensu (1978) reported that *S. indica* is used by traditional healers in the management of constipation as well as diarrhoea. He noted that it is often mixed with natron (NaCl) for the management of diarrhoea. NaCl used in the preparation (alone or in combination with the plant constituents) may be responsible for the anti-diarrhoea effect of the plant. NaCl is one of the components of "Oral Rehydration Therapy" (ORT). It is known to assist in the absorption of glucose from the gastrointestinal tract, which is impaired during diarrhoea. The principle behind this therapy is the replacement of lost body fluids. The arguments for the use of this therapy are based on the belief that death resulting from diarrhoea is mainly due to body fluid depletion. This therapy is particularly useful in children, because severe alteration in body fluid volume can be fatal.

From the results obtained from the *in-vitro* experiments, it could therefore be said that the effect produced by *S. indica* at the doses employed would be rather more useful in the management of constipation than diarrhoea.

Some anti-diarrhoea agents like morphine increase the tone and rhythmic contractions of the intestine but diminish propulsive activity; the overall effect being constipation. This activity is mediated through μ and δ receptors through both peripheral and central sites of action (Rang *et al.*, 2001). It is possible that the contraction produced by *S. indica* may be the same as the one produced by morphine, in which case this result will support the findings of Almeida *et al.*, (1995) that the extract of *S. cayenesis* reduced gastrointestinal propulsion rate. If this is the case, the results obtained support the use of *S. indica* for the management of diarrhoea. The effects of these extracts on smooth muscle and

castor oil-induced diarrhoea justify their usage in the treatment of diarrhoea and colic pain.

5.5.8 Effects on Isolated Rabbit Jejunum

As earlier discussed, *M. balsamina* produced a transient contraction of the rabbit jejunum followed by relaxation while *S. indica* produced sustained contraction, which was inhibited by atropine and nifedipine, (Figure 1, 2, and 3). The contraction observed with *M. balsamina* was antagonized by atropine and to a greater extent by nifedipine.

In the presence of atropine, there was a reduction in the height of contraction compared with the preceding control; however pronounced relaxation followed each contraction. Total relaxation followed contraction at very high concentration. It was also observed that the relaxation produced by *M. balsamina* was potentiated by Ca^{+} channel blocker (Nifedipine) while the transient contraction was almost abolished. This may suggest that *M. balsamina* contains substances with Ca^{+} channel blocking properties. According to Gilani *et al*., (2005), "the spasmolytic constituents of various plants are mediated through blockage of Ca^{+} channels". The total relaxation produced by the extract at higher concentration (figure 1 and 2), supports this assertion. The total relaxation may be due to a synergistic blockade of Ca^{+} channels thereby producing a relaxation that could not be produced by the extract alone. The blockage of the Ca^{+} channels would result in reduced influx of Ca^{+} ions into the sarcoplasmic reticulum, thus causing a reduction in cytosolic Ca^{+} ion which in turn causes a reduced binding of calcium to the protein calmodulin. The calcium-calmodulin complex activates

myosin light chain kinase with the resultant phosphorylation of the light chains and interaction between actin and myosin. This interaction results in smooth muscle contraction (Gilani, *et al,* 2005). Inhibition of Ca^{+} would result in a break in this cascade, producing relaxation. Potientation of the relaxation effect of rabbit jejunum suggests that smooth relaxing effect of *M. balsamina* may be mediated through Ca^{+} channel blockade.

On the rabbit jejunum, *S. indica*, at concentrations used, produced maximum contractions (Fig 2). However, in atropinized preparation, the spasmogenic activity observed was reduced and in the presence of Nifedipine, no blockade was observed even though there was a reduction in the duration of contraction. It can be inferred from this that the contractions observed were mediated through muscarinic cholinoceptors present in the gastrointestinal tract.

5.5.9 Blood Pressure Lowering Effects of *M. balsamina*

A. Effect *M. balsamina* on Cats Blood Pressure

Standard blood pressure drugs showed dose dependent effect on cat's blood pressure. It was observed that the methanolic extract of *M. balsamina* lowered cat's blood pressure in a dose dependent maner (Figures 7, and 8). The effects of the extract were comparable to that of propranolol (Figure 5).

Adrenalin dose-dependently increased cat's blood pressure (Figure 4). *M. balsamina* significantly ($P<0.05$) reduced adrenaline induced increase in cat's blood pressure (Figure 9, 10, 11, 12 and 13). Figure 14, showed that repeated doses of the extract produced an effect that was higher than the one produced earlier by the same dose.

It was also observed that the blood pressure lowering effect of the extract of *M. balsamina* was inhibited by atropine (Figure 15). This suggests that the extract may have exhibited its action via the cholinergic pathway. This result supports the results obtained from rabbit jejunum (Figure 1). The extract potentiated the effect of acetylcholine on cat's blood pressure (Figure 16).

Nifedipine was also observed to reduce the cat's blood pressure in a gradual fashion. The reduction in blood pressure was not as drastic as that of the extract, acetycholine, and propranolol (Figure 6).

Drugs that interact with blood pressure affect the perception of pain. This interaction was first reported by Lovick (1997). Increase in blood pressure (hypertension) is often associated with pain, which results from pressure on the vascular wall. Drugs that lower the blood pressure will reduce the pressure on the vascular wall and thereby reduce pain (especially headache and migrain pain). Drugs with hypotensive, analgesic and anti-inflammatory effects will be of great benefit. Their anti-inflammatory effects will prevent the complications of hypertension. *M. balsamina,* from these results, have these effects. It can be of benefit in the management of pain associated with hypertension. It is in fact used by traditional healers for this purpose and for the management of hypertension.

B. Effect of *M. balsamina* on Rat's Atria

This study revealed that *M. balsamina* had no significant effect on rat's atria at low doses (Figure 20), but at higher doses, it gradually reduced the rat's atria contraction (Figure 21). Adrenaline and calcium ion had contractile effects on rat's atria muscle (Figure 17, 18, and 19). The extract did not have any

significant effect on the effects of adrenaline and Calcium ion on rat's atria (Figure 22 and 23 respectively).

5.6 SUMMARY

i. Eighteen plants (*Erythrina senegalesis, Nauclea latifollia, Kizelia africana, Pseudocedrela kotchyi, Crotalaris spp., Boswellia dalzielli, Khaya senegalensis, Annona senegalensis, Xylopia aethiopica, Ficus thonningii, Cassia goratensis, Prosopis africana, Stachytapheta indica, Crinum glaucum, Holerrhena floribunda, Momordica balsamina, Enantia chlorantha and Sarcocephalus esculentus*) were collected and identified out of which nine plants (*S. esculentus, C. goratensis, F. thonningii, P. kotschyi, E. chlorantha, N. latifolia, M. balsamina, S. indica, P. africana*) were selected for study for possible analgesic and anti-inflammatory activities after preliminary investigations.

ii. The nine plants (*S. esculentus, C. goratensis, F. thonningii, P. kotschyi, E. chlorantha, N. latifolia, M. balsamina, S. indica, P. africana*) selected were confirmed to have varing degrees of activities.

iii. *S. indica* was found to be toxic when administered intraperitoneaally, but safe when administered orally. This supports its use for the adultration of tea in Brazil and as an arrow poison. *S. indica* exhibited anti-diarrhoeal activity and produced sustained contraction which was inhibited by atropine and nifedipine.

iv. *N. latifolia* was also not safe for intra-peritoneal administration. Traditional healers should use aqueous preparations rather than the ethanolic preparations of *N. latifolia.*

v. *F. thonningii* from the result was found to be better tolerated intra-peritoneally.

vi. *M. balsamina* extract was well tolerated and had no effect on the stomach, testis, lungs, heart and liver cells. Liver enzymes, PCV, RBC and WBC counts were not also affected. It may not be harmful when used for up to 28 days. It has central and peripheral analgesic, anti-inflammatory, hypothermic, anti-diarrhoea, hypotensive, but not anti-convulsant effects *M. balsamina* produced a transient contraction of the rabbit jejunum followed by relaxation. The contraction observed was antagonized by atropine and to a greater extent by nifedipine. This suggests that the extract may have exhibited its action via the cholinergic pathway and/or through the blockade of Calcium channels.

vii. *S. indica* had contradictory effects in the management of diarrhoea. It was effective *in-vivo* but not *in-vitro* models of diarrhoea.

viii. *P. africana* and *S. indica* were observed to have weak analgesic effects. *P. africana, S. indica* and *N. latifolia* were not effective in hot plate test. *S. esculentus* was only effective at high dose and not at the low dose used for the experiment. *P. africana, S. indica* and *N. latifolia* can be said to have only peripheral analgesic effect.

ix. *M. balsamina* fractions (exception of *n*-hexane fraction) were observed to have analgesic and anti-inflammatory activities. Water fraction contained more potent agent.

x. *M. balsamina* extract inhibited the growth of the test bacteria against *B. subtilis, E. coli, P. aeruginosa, and P. mirabilis*, but showed marginal activity against *S. aureus, K. pneumonia, and S. typhi.* The activity of the extract against *B. subtilis and E. coli* was comparable to that of gentamycin. The extract did not inhibit the growth of fungi strains.

xi. The extracts of *M. balsamina* had slight insignificant gastric protective effect. Unlike most non-steroidal anti-inflammatory drugs, it had no ulcerogenic effect.

xii. The extracts of *M. balsamina* stabilized red blood cells and reduced neutrophil and lymphocyte cell migration to the site of inflammation. This may explain the mechanism of it anti-inflammatory and possibly its analgesic properties since mast cell stabilization will prevent the release of chemical mediators of pain.

This study has confirmed that *M. balsamina* has most of the acclaimed uses in folk medicine.

5.7 CONCLUSION

5.7.1 Highlights of the major findings of this research and contribution to knowledge.

The practice of traditional medicine in Plateau State is as old as the existence of man. This study revealed that some of the herbs used for the management of pain and inflammation are effective for the management of these disease conditions. Nine plants studied (*S. esculentus*, *C. goratensis*, *F. thonningii*, *P. kotschyi*, *E. chlorantha*, *N. latifolia*, *M. balsamina*, *S. indica*, *P. africana*) for analgesic and anti-inflammatory effects were effective with *M. balsamina* having the highest activity. *M. balsamina* was also effective in tail flick and formalin models of pain. It has no anti-convulsant activity but had hypotensive, reduction of sleep onset, prolongation of duration of sleep, anti-pyretic and anti-diarrhoea activity. Sub acute toxicity studies revealed that *M. balsamina* had no significant effect on body organs, biochemical and Haematological parameters.

5.8 CONTRIBUTIONS TO KNOWLEDGE

- Nine plants (*S. esculentus*, *C. goratensis*, *F. thonningii*, *P. kotschyi*, *E. chlorantha*, *N. latifolia*, *M. balsamina*, *S. indica*, *P. africana*) studied have analgesic and anti-inflammatory activities.
- *M. balsamina* has analgesic, anti-inflammatory, hypothermic, hypotensive effects, but is devoid of anti-convulsive effect. The multiple actions of *M. balsamina* make it a promising herb for the management of pain associated with these disease conditions and at the same time treat the underlying pathology.
- Methanolic preparations of *N. latifolia* should be avoided.

- Extract of *S. indica* is safe if given orally, but toxic when administrered intraperitoneally.
- *S. indica* has anti diarrhoea effect and may also be useful in the management of constipation.

REFERENCES

Agbaje, E.O., and Onabanjo, A.O., (1991). The effects of extracts of *Enantia chlorantha* in malaria. *Annal Tropical Medical Parasitology*; 85(6):585-90.

Ahlquist D.A., (2001). Constipation and Diarrhoea. In: Principles of Internal Medicine. (Edited by Hauser S, Longo D, Jameson L. Braunwald E. Fauci As, Kasper D.) Vol. 1 Mcgraw Hill Medical Publishing Division, New York, p 241 – 247.

Akah, P.A., Offiah, V.N., 1996. Gastrointestinal effects of *Allamanda cathartica* leaf extracts. *International Journal of Pharmacognosy*; 30: 213–217.

Alade, P. I., Irobi, O.N., (1993): Antimicrobial activities of Crude Leaf extract of *Acalypha wikensiana*. *Journal Ethnopharmacology;* 39:71-174.

Almeida CE, Karnikowski MG, Foleto R, Baldisserotto B., (1995). Analysis of antidiarrhoeaic effect of plants used in popular medicine. *Rev Saude Publica*; 29(6):428-433.

Anyensu E.S. (1978). Medicinal Plants of West Africa; p. 110.

AOAC, (1975). Official methods of analysis. 12th edition. Association of Official Agricultural Chemists. Washington DC, USA

Audu, R., Umilabug, S.A., Renner, J.K., Awodiji, J.A., (2000). Diarrhoea management. *Journal Nigerian Infection Control association;* 3:15-17.

Azas, N., Laurencin, N., Delmas, F. Di G.C., Gasquet, M., Laget, M., and Timon-David, P., (2002). Synergistic in vitro antimalarial activity of plant extracts used as traditional herbal remedies in Mali. *Parasitology Research;* 88(2):165-71.

Bax, R., Mullan, V.,(2000): The millennium bugs-the need for and development of new antibacterials. *International Journal of Antimicrobial Agent;* 16 : 51-59.

Beckonert, O., Bollard, M.E., Ebbels, T.M.D., Keun, H.C., Antti, H., Holmes, E., Lindon, J.C., and Nicholson, J.K., (2003). NMR-based metabonomic toxicity classification: hierarchical cluster analysis and k-nearest-neighbour approaches. *Analytica Chimica Acta*; 490: 3– 8.

Benoit-Vical, F., Valentin, A., Cournac, V., Pelissier, Y., Mallie, M., and Bastide, J.M., (1998). *In vitro* antiplasmodial activity of stem and root extracts of *Nauclea latifolia* S.M. (Rubiaceae). *Journal of Ethnopharmacology*; 61(3):173-178.

Blumenthal, M., (1999). Herb market levels after five years of boom. *HerbalGram;* 47: 64–65.

Bourrinet P., and Quevauviller A., (1968), Prosopinine, an alkaloid from *Prosopis africana.* Effect on the central and autonomic nervous systems. *Comptes Rendus des Seances de la Societe de Biologie et de Ses Filiales;* 162(5-6): 1138-1140.

Brevoort, P., (1998). The booming US botanical market. *HerbalGram;* 44: 33–48.

Carlson, N. R., (2004). Neurotransmitters and Neuromodulators. In *Physiology of Behavior*; 8: 112-130.

Carroll, M.N. and Lim, R.K., (1960). Observations of the neuropharmacology of morphine-like analgesia. *Archieve International Pharmacodynamics and Therapeutics;* 125:383–403.

Chan, K., (2005). Chinese medicinal materials and their interface with Western medical concepts. *Journal of Ethnopharmacology*; 96: 1– 18.

Charlton, K. H., (2005)A Chiropracticness Test.*Chiropr Osteopat.*; 24:13:24.

Chopra, I., Hawkey, P.M., Hilton, M.,(1992): Tetracyclines, molecular and clinical aspects. *Journal of Antimicrobial Chemotherapy; 29: 245-277.*

Cragg, G.M., and Newman, D.J., (2001). Natural product drug discovery in the next millennium. *Pharmaceutical Biology;* 39: 8–17.

Cohen, M.L., (1992): Epidemiology of drug resistance implications for a post antimicrobial era. *Science;* 257:1050-1055.

Corda MG, Giorgi O, Logoni B, Orlandi M, Biggi G, (1990); Decrease in the function of the gamma-aminobutyric acid-coupled chloride channel produced by the repeated administration of pentylenetetrazol to rats. *Journal Neurochemistry;* 55(4): 1216-1221.

Cotton, C.M., (1996). Ethnobotany: P*rinciples and Applications*; p 97.

Cowan, M.M., (1999): Plant products as antimicrobial agents. *Clinical Microbiology Reviews;* 12: 564-582

Dacie, J.V., and Lewis, S.M., (1984). Practical haematology. 6th Ed. Churchill Livingston, Edinburgh; p. 24 – 36.

Dalziel J. M., (1987). The Useful Plants of West Africa. The Crown Agents for Overseas Colomes, London; p. 401.

Datta, A., Ravi, K., and Jalluri, S., (2000). Book of Abstracts, 219th ACS National meeting, San Francisco, *American Chemical Society*, Washington, D.C. CA ORGN-762.

Davies, S., (2001). Pain benefits and control *http://www.sycd.co.uk/only_connect/pdf/explore/other resources/catalyst_pain.pdf*. (12/5/2006)

Dennis, S.G., and Melzack, R., (1983). Effects of cholinergic and dopaminergic agents on pain and morphine analgesia measured by three pain tests. *Experimental Neurology*; 81(1):167-76.

Diurno, M.V., Izzo, A.A., Mazzoni, B., Bologgnese, A., and Capasso, F., (1996). Antidiarrhoeal activity of new thiazolidinones related to loperamide. *Journal of Pharmacy and Pharmacology*; 8: 760–762.

Eschalier, A., Pelissier, T., Alloui, A., Caussade, F., Dubray, C., Cloarec, A., and Lavarenne, J., (1988). Paracetamol exerts a spinal antinociceptive effect involving an indirect interaction with 5-hydroxytryptamine3 receptors: *in-vivo* and *in-vitro* evidence. *Journal of Pharmacology and Experimental Therapeutics;* 278 (1): 8–14.

Evans, W.O., (1961). A new technique for the investigation of some drugs on a reflexive behavior in the rat. *Psychopharmacology* (Berl); 2:318–325.

Fakae, B.B., Campbell, A.M., Barrett, J., Scott, I.M., Teesdale-Spittle, P,H., Liebau, E., and Brophy, P.M., (2000). Inhibition of glutathione S-transferases (GSTs) from parasitic nematodes by extracts from traditional Nigerian medicinal plants.*Phytotherapy Research;* 14(8):630-4.

Farack, U.M., Kantz, U., Loeseke, K., (1981). Loperamide reduces the intestinal secretion but not the mucosa cAMP accumulation induced by cholera toxin. *Naungn Schmiedebergs Archive of Pharmacology*; 317: 178–179.

Farnsworth, N.R., Kinghorn, A.D., Soejarto, D.D., and Waller, D.P., (1985). "Siberian Ginseng *(Eleutherococcus senticosus)*: Current Status as an Adaptogen" In H. Wagner, H.Hikino and N.R. Farnsworth (eds.). *Economic and Medicinal Plant Research.* Vol. 1. Orlando, FL: Academic Press. p. 155-215.

Fennell, C.W., Light, M.E., Sparg, S.G., Stafford, G.I., and van Staden, J., (2004a). Assessing African medicinal plants for efficacy and safety: agricultural and storage practices. *Journal of Ethnopharmacology;* 95: 113–121.

Fennell, C.W., Lindsey, K.L., McGaw, L.J., Sparg, S.G., Stafford, G.I., Elgorashi, E.E., Grace, O.M., and van Staden, J., (2004b). Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. *Journal of Ethnopharmacology;* 94: 205–217.

Fireman, P., (2003). "Understanding asthma pathophysiology" *Allergy Asthma Proceeding*; 24(2):79-83.

Galvez, J., Crespo, M.E., Jimnenez, J., Suarez, A. and Zarzuelo, A., (1993) Antidiarrhoeic activities of quercitrin in mice and rats. *Journal of Pharmacology*; 47: 157-159.

Gilani, A.H., Molla, N., Atta-ur-Rahman, and Shah, B.H., (1992). Role of natural products in modern medicine. *Journal of Pharmaceutical Medicine;* 2: 111–118.

Gilani, A.H., (1998). Novel developments from natural products in cardiovascular research. *Phytotherapy Research;* 12 (Suppl. 1): S66–S69.

Gilani, A.H., Bashir, S., Janbaz, K.H., and Khan, A., (2005). Pharmacological basis for the use of *Fumaria indica* in constipation and diarrhoea. *Journal of Ethnopharmacology;* 96: 585–589.

Giri, R.K., (2003). "Amyloid peptide-induced cytokine and chemokine expression in THP-1 monocytes is blocked by small inhibitory RNA duplexes for early growth response-1 messenger RNA" *Journal of Immunology*;170(10):5281-5284.

Green, A.F., Young, P.A., and Godfrey, E.I., (1951). A comparison of heat and pressure analgesimetric methods in rats. *British Journal of Pharmacology;* 6:572–585.

Hanefi O., Gulcin S. C., Haluk D., Serdar U. and Betul S., (2004). Hepatoprotective and anti-inflammatory activities of *Bollota glandulosissima*. *Journal of Ethnopharmacology;* 95: 143-149.

Haruna, A.K., Ilyas, M., and Ilyas, N., (1997). Antidiarrhoeal action of the aqueous extract of *Macrophylla parinari* (Rosaceae). *Phytotherapy Research*; 11: 307–309.

Holmes, E., Nicholls, A.W., Lindon, J.C., Connor, S.C., Connelly, J.C., Haselden, J.N., Damment, S.J.P., Spraul, M., Neidig, P., and Nicholson, J.K., (2000). Chemometric models for toxicity classification based on NMR spectra of biofluids. *Chemical Research in Toxicology* ;13: 471–478.

Hotamisligil, G.S., (2003). "Inflammatory pathways and insulin action" *International Journal of Obesity Related Metabolic Disorder;* 27: Suppl 3:S53-S55.

Irvine, F.R., (1961). Woody plants of Ghana with special references to their uses. Oxford University Press, London. p. 868.

Jurna, I., (2003). [Serturner and morphine--a historical vignette] *Schmerz*;17(4):280-283.

Kanai, T., and Watanabe, M., (2004). "Regulatory T cells and inflammatory bowel diseases"(Article in Japanese) *Nihon Rinsho Meneki Gakkai Kaishi*; 27(5):302-308.

Karim, S.M.M., and Adeikan, P.G. (1977). The effects of loperamide on prostaglandin-induced diarrhoeal in rats and man. *Prostaglandins*; 13: 321–331.

Keharo, J., and Adam, J. C., (1974). La Pharmacopee senegalaise traditionnelle: Plantes medicinales et toxiques. Ed. Vigot & Freres. Paris; p.1011.

Khuong H. Q., Ratle G., Monseur X., and Goutarel R., (1972). Piperidine alkaloids II. Structures of prosopine and prosopinine, alkaloids from *Prosopis africana., Bulletin des societies Chimiques Belges;* 81(7-8): 425-431.

Khuong H. Q., Monseur X., Gasic M., Wovkulich P., and Wenkert E., (1982) ,*A carbon-13 NMR spectral analysis of Prosopis africana alkaloids. Journal of the Chemical Society of Pakistan*; 4(4): 267-269.

Kimbi, H.K., and Fagbenro-Beyioku, A.F., (1996). Efficacy of *Cymbopogon giganteus* and *Enantia chlorantha* against chloroquine resistant *Plasmodium yoelii nigeriensis*. *East African Medical Journal*; 73(10):636-637.

Kumar, P. and Clark, M. (2005). Clinical Medicine, sixth edition, WB Saunders Company, London. p 592.

Le Bars D., Manuela G., and Samuel W. C., (2001) Animal models of Nociception. *Pharmacology Reveiw;* 53: 597-652

Lindon, J.C., Holmes, E., and Nicholson, J.K., (2004). Metabonomics: Systems biology in pharmaceutical research and development. *Current Opinion in Molecular Therapeutics;* 6: 265–272.

Lorke, D., 1983. A new approach to partial acute toxicity testing. *Archives of Toxicology*; 54, 275–287.

Loscher, W., 1998. New visions in the pharmacology of anticonvulsion. *European Journal of Pharmacology*; 342, 1–13.

Lovick, T.A., (1997). The medulary raphe nuclei: a system for intergration and gain control in autonomic and somatomotor responsiveness. *Experimental Physiology;* 82: 31-41.

Lutterodt, G. D. (1988). Abortificient properties of an extract of *Sida veronicifolia. Journal of Ethnopharmacology*; 23, (1), p. 27-37

Macdonald RL, Kelly KM. (1993) Antiepileptic drug mechanisms of action. *Epilepsia*; 34(Suppl 5): S1-S8.

Macdonald RL, Barker JL. (1977) Phenobarbital enhances GABA-mediated postsynaptic inhibition in cultured mammalian neurons. *Trans American Neurology Association;*102:139-40.

McGowen, J.J., Jones, A.A.R. and Steinerg, A.G. (1955). The haematocrit of carpillary blood. *New England Journal Medicine;* 253:308.

Madamanchi, N.R., Vendrov, A., and Runge, M.S., (2005). Oxidative stress and vascular disease. *Arterioscler Thrombosis and Vascular Biology;* 25(1):29-38.

Malone, M.H., and Robichaud, R.C., (1962). A Hippocratic screen for pure and crude drug materials. *Lloydia;* 25: 320–332.

Mander, J., Quinn, N.W., and Mander, M., (1997). Trade in Wildlife Medicinals in South Africa. Investigational Report Number 157. Institute of Natural Resources, Pietermaritzburg, South Africa; p. 7.

Martin, G.J., (1995). Ethnobotany: A Conservation Manual. – Chapman & Hall, London. p 13.

Mashelkar, R.A., (2005). Global voices of science: India's R&D: reaching for the top. *Science*; 307: 1415–1417.

Matawalli, A, G., Issa P., and Hajjagna L., (2004). Effects of aqueous stem bark extract of *Momordica balsamina* Linn on Serum Electrolytes and Some Haematological Parameters in Normal and Alcohol Fed rats. *Pakistan Journal of Biological Sciences;* 7 (8): 1430-1432.

Mitchell, R.N., and Cotran, R.S., (2000) Cell injury, adaptation, and death. In: KumarV, Cotran RS, Robbins SL, editors. New York: McGraw-Hill. p. 642-646.

Moody, J.O., Bloomfield, S.F., and Hylands, P.J., (1995). *In-vitro* evaluation of the antimicrobial activities of *Enantia chlorantha* Oliv. extractives. *African Journal Medical Sciences*; 24(3):269-273.

Moroney, M.A., Alcaraz, M.J., Folder, R.A., Carey, F., Hoult, S.R.S., (1988). Selectivity of neutrophil 5-lipoxygenase and cycloxygenase inhibition by anti-inflammatory flavonoidglycoside and related aglycone flavonoids. *Journal of Pharmacy and Pharmacology;* 40: 787-792.

Myrna, D., Isabel R., Myriam A., Gabriela C., Guadalupe E. A., Andrés Navarrete and Rachel M., (2007). Acute toxicity and mutagenic activity of Mexican plants used in traditional medicine. *Journal of Ethnopharmacology*; 110, (2), P. 334-342.

Nascimento, G.F., Juliana, L., Paulo, C.F., Giuliana, L.S., (2000): Antibacterial activity of plant extracts and Phytochemcicals on antibiotic resistant bacteria. *Brazilian Journal Microbiology;* 31:247-256.

National Foundation of Alternative Medicine (NFAM), Neveu, M., (2004). Alternative Versus Conventional Medicine; p. 1–5, *http://www.nfam.org/neveualtverusconv.html*. (12/06/06)

Ngnokam, D., Ayafor, J.F., Connolly, J.D.; and Nuzillard, J.M., (2003) Nauclefolinine: A new alkaloid from the roots of *Nauclea latifolia* . *Bulletin of the Chemical Society of Ethiopia;* 4: 173-176.

National Institute of Health (NIH), (1992). Report, Alternative Medicine: Expanding Medical Horizons, p. 1–44, at *http://www.elixir.net/RR-NIH-hpage*. 12/05/06

Odumosun, O.P., Otimenyin, O. S., and Ngwai Y., (2006). The possible Value of *Prosopis africana* (Leguminaceae) in the treatment of Tuberculosis. *Nigerian Journal of Pharmaceutical Research;* 5 (1): 91-96.

Onyeyili, P.A., Nwosu, C.O., Amin, J.D. and Jibike, J.I., (2001). Anthelmintic activity of crude aqueous extract of *Nauclea latifolia* stem bark against ovine nematodes. *Fitoterapia;* 72(1):12-21

Oliver, B., (1960). Medicinal plants in Nigeria. Ibadan College of Arts and Sciences and Technology; p. 358.

Otimenyin, O. S. Uguru, O.M and Ojeka. K. (2006). Acute toxicity studies and some pharmacological properties of *Stachytarpheta indica*. Presented at International Society for Developmental Neuroscience (ISDN) conference, Canada, p. 19.

Padi, V.S.S., Naldu, S.P., and Kulkarni, K. S., (2006). Involvement of peripheral prostaglandins in formalin-induced nociceptive behaviours in the orofacial area of rats. *Inflammopharmacology;* 14: 57-61.

Patwardhan, B., Vaidya, A.B.D., Chorghade, M., (2004). Ayurveda and natural products drug discovery. *Current Science;* 86: 789–799.

Patwardhan, B., (2005a). Botanical immunodrugs: scope and opportunities. *Drug Discovery Today* ;10: 495–502.

Patwardhan, B., (2005b). Classification of human population based on HLA gene polymorphism and concept of Prakriti in Ayurveda. *Journal of Alternative and Complementary Medicine;* 11: 349–353.

Patwardhan, B., (2005c). Traditional medicine: modern approach for affordable global health. Commission on Intellectual Property Innovation and Public Health. WHO, Geneva. *http://www.who.int/ intellectualproperty /studies/traditional medicine/en/.* (12/06/06)

Patwardhan, B. (2005d) Ethno-pharmacology and drug discovery *Journal of Ethno-pharmacology;* 100: 50–52.

Pellock, J.M., 1995. Antiepileptic drug-therapy in the United-States—a review of clinical-studies and unmet needs. *Neurology;* 45, S17–S24.

Prabhu, V., Karanth, K.S., Rao, A., 1994. Effects of *Nardostachys jatamansi* on biogenic-amines and inhibitory amino-acids in the rat-brain. *Planta Medica* 60, 114–117.

Rang, P. H., and Dale, M.M., (1998). Pharmacology; Second edition. Churchill Livingston, Edinburgh; p. 396,319.

Rang, H., Dale, M., and Ritter, J.M., (2001). Pharmacology, London, Harcourt Publishers fourth Edition; p. 318.

Rome, J., (2002). Mayo Clinic on Chronic Pain. *Mayo Clinic Health Information;* Rochester Mn.; p. 4.

Rec. Gscc (DGKC). (1972). Optimised standard colorimetric methods. *Journal of Clinical Chemistry and Clinical Biochemistry*; 10:182.

Reitman, S., and Frankel, A.S., (1957). A colorimetric method of determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*; 28:53-63.

Rietbrock, N., and Woodcock, B.G., (1985). Two hundred years of foxglove therapy: *Digitalis purpurea* (1785–1985). *Trends in Pharmacological Sciences;* 6: 267–269.

Sabers, A., Gram, L., (1996). Drug treatment of epilepsy in the 1990s—achievements and new developments. *Drugs*; 52, 483–493.

Sandberg, F., Verpoorte, R., and Cronlund, A., (1971). Screening of African Strychnos species for convulsant and muscle-relaxant effects. *Acta Pharmaceutica Suecica;* 8: 341–350.

Sherrington, C.S., (1910). Flexion-reflex of the limb, crossed extension-reflex and reflex stepping and standing. *Jorunal of Physiology* (London); 40:28–121.

Shigemori, H., Kagata, T., Ishiyama, H., Morah, F., Ohsaki, A., and Kobayashi, J., (2003). Naucleamides A-E, new monoterpene indole alkaloids from *Nauclea latifolia*. *Chemical and Pharmaceutical Bulletin* (Tokyo); 51(1):58-61.

Shinde, U. A., Phadke A. S., Nair A. M., Mungantiwar A. A., Dikshit V. J. and Saraf M. N. (1999). Studies on the anti-inflammatory and analgesic activity of *Cedrus deodara* (Roxb.) Loud. wood oil. *Journal of Ethnopharmacology*; 65: (1): 21-27

Snedecor, G.W., and Cochran, W.C., (1967). Statistical Methods. Sixth Edition Ames, Iowa: The Iowa State University Press; p 423.

Sofowora, A. (1982). Medicinal Plants and Traditional Medicine in Africa. John Wiley and Sons Ltd., New York, p. 6.

Stucky, C.L., Gold, S.M., and Zang, X., (2001). Mechanisms of Pain. *Proceedings of the National Academy of Science;* 98: 11845-11846.

Sun, W.J., (1986). Determination of syringin in Acanthopanax senticosus by HPLC. *Zhong Yao Tong Bao*; 11(4):42-3.

Syder, J.H., Merson, M.H., (1982). The magnitude of the global problem of acute diarrhoeal disease. A review of active surveillance data. *Bulletin of World Health Organization*; 60: 605.

Tan, P.V., Nyasse, B., Enow-Orock, G.E., Wafo, P., and Forcha, E.A., (2000). Prophylactic and healing properties of a new anti-ulcer compound from *Enantia chlorantha* in rats. *Phytomedicine*; 7(4): 291-296.

Tillie-Leblond, I., Gosset, P., and Tonnel, A.B., (2005). "Inflammatory events in severe acute asthma" *Allergy ;* 60(1):23-29.

Traore-Keita, F., Gasquet, M., Di Giorgio, C., Ollivier, E., Delmas, F., Keita, A., Doumbo, O., Balansard, G., and Timon-David, P., (2000). Antimalarial activity of four plants used in traditional medicine in Mali. *Phytotherapy Research*; 14(1):45-7.

Traore, F., Gasquet, M., Laget, M., Guiraud, H., Di Giorgio, C., Azas, N., Doumbo, O., and Timon-David, P., (2000). Toxicity and genotoxicity of antimalarial alkaloid rich extracts derived from Mitragyna inermis O. Kuntze and *Nauclea latifolia*. *Phytotherapy Research*.; 14(8):608-611.

Trease, G. E. and Evans, W. C. (1983). A Textbook of Pharmacognosy, twelveth Edition. Publ. Baillere Tindall, London, p. 241

Tona, L., Kambu, K., Ngimbi, N., Mesia, K., Penge, O., Lusakibanza, M., Cimanga, K., De Bruyne, T., Apers, S., Totte, J., Pieters, L., and Vlietinck, A.J., (2000). Antiamoebic and spasmolytic activities of extracts from some antidiarrhoeal traditional preparations used in Kinshasa, Congo. *Phytomedicine*; 7(1):31380.

Turner, R.A., (1965). Screening methods in pharmacology. Academic Press, New York p. 106.

Vaidya, A.D.B., (2005). Asian medicine—a global blessing. In: Indian Association of Studies in Traditional Asian Medicine (IASTAM) Silver Jubilee Convention Commemorative Volume, Pune, India; p. 17.

Van der Geest, S., (1997). Is there a role for traditional medicine in basic health services in Africa? A plea for a community perspective. *Tropical Medicine and International Health;* 2: 903–911.

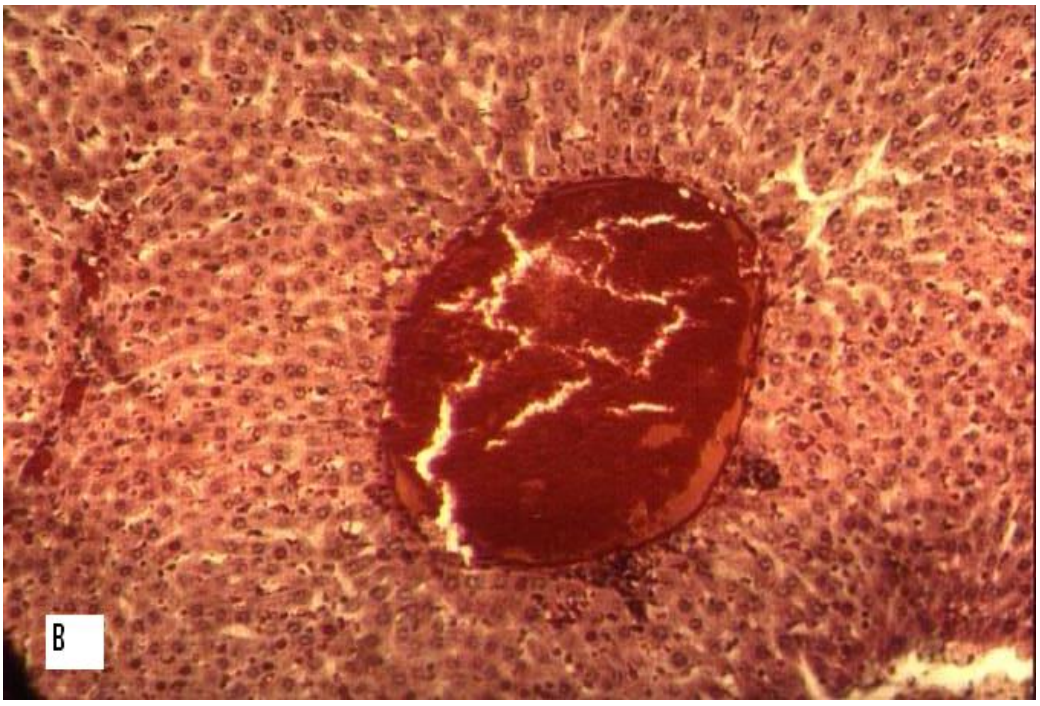
Verpoorte, R., and Bohlin, L., (1976). Screening of African Strychnos species for convulsant and muscle-relaxant effects. *Acta Pharmaceutica Suecica;* 13: 245–250.

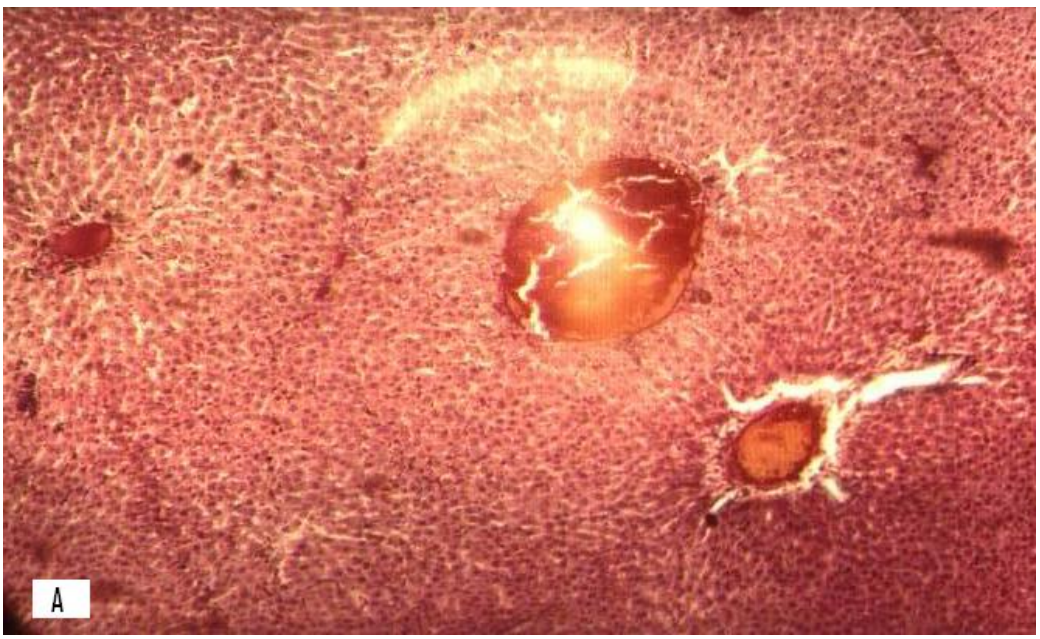
Vasquez, M .J. Implications of the 1992 ethics code for the practice of individual psychotherapy. *Prof Psychol Res Pr.* 1994 ;25(4):321-8.

Vidya S. Raoa, Anjali Raob, K. Sudhakar Karanth c (2005) Anticonvulsant and neurotoxicity profile of *Nardostachys jatamansi* in rats; *Journal of Ethnopharmacology;* 102: 351–356

Walters, A.J., (1994). The comforting role in critical care nursing practice: a phenomenological interpretation. *Intenational Journal of Nursing Studies*; 31(6):607-616.

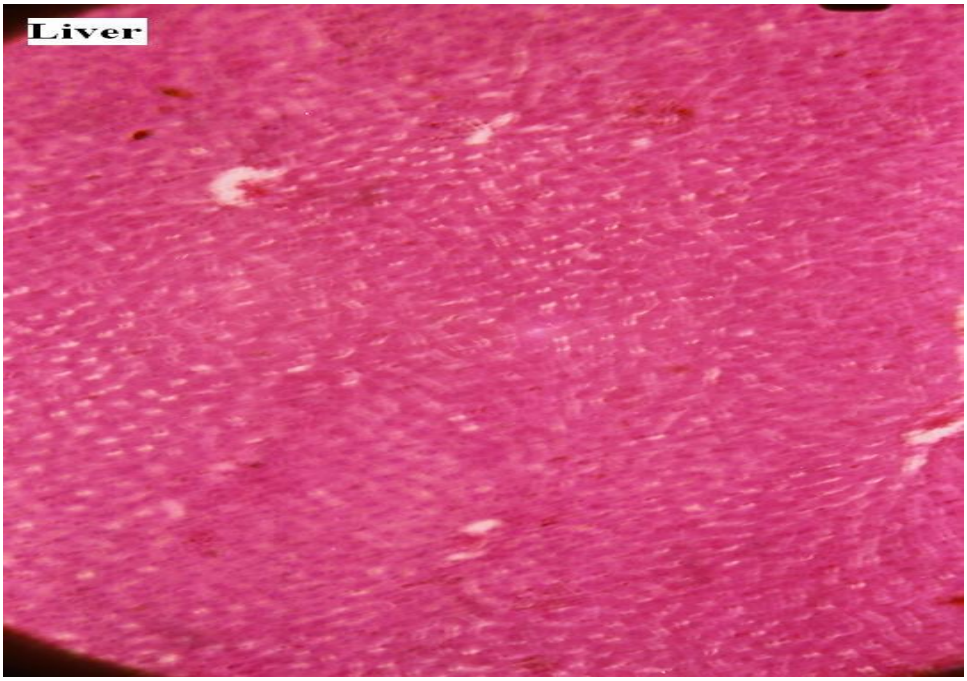
APPENDICES



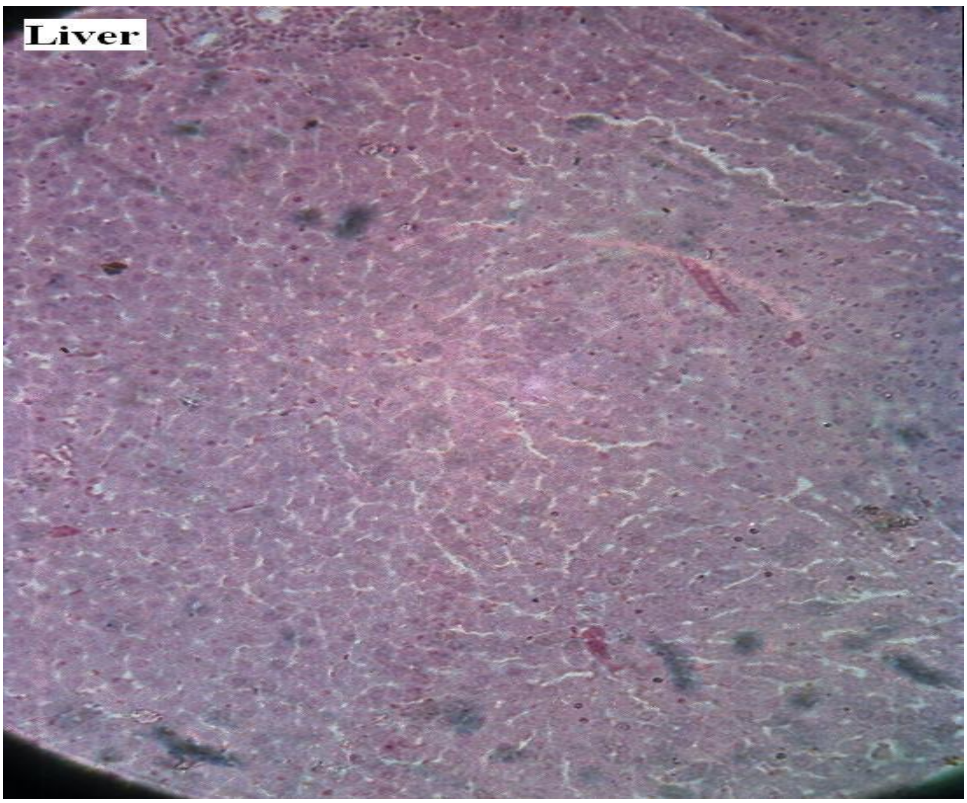


A. Test Liver showing normal cells (3.52). B. Control Liver showing normal cells (3.51)

APPENDIX 1; Effect of Methanol/Water Extract of *M. balsamina* (1000 mg/Kg) on Liver after Seven Days Treatment

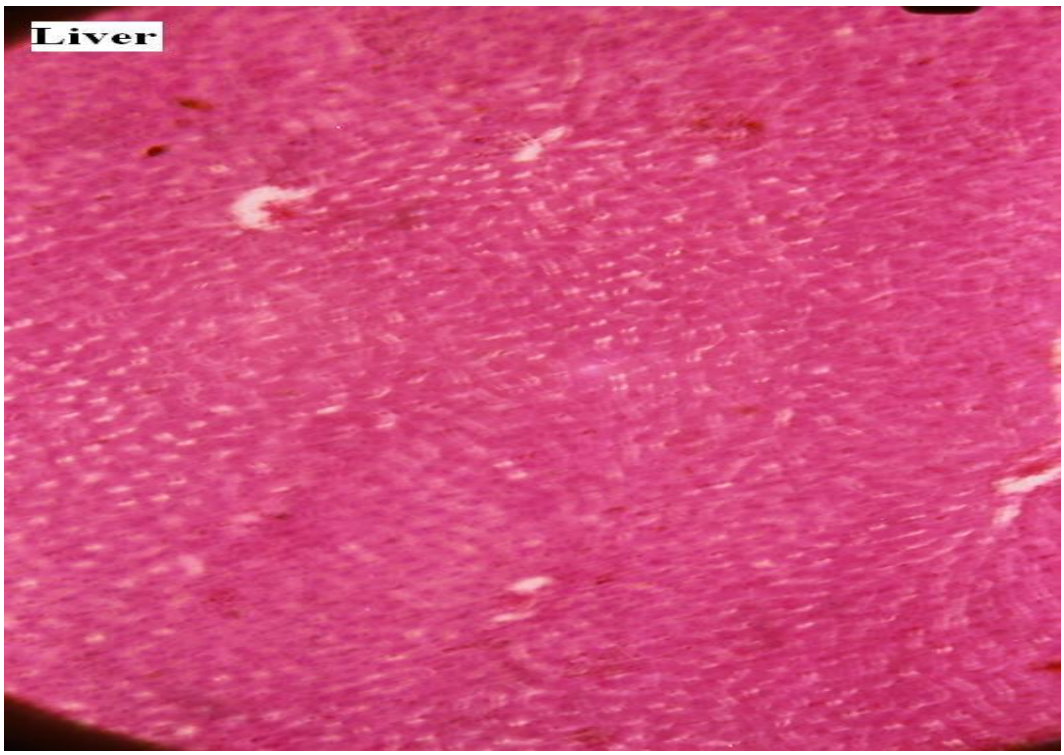


Control (distilled water) Liver showing normal cells (3.52)

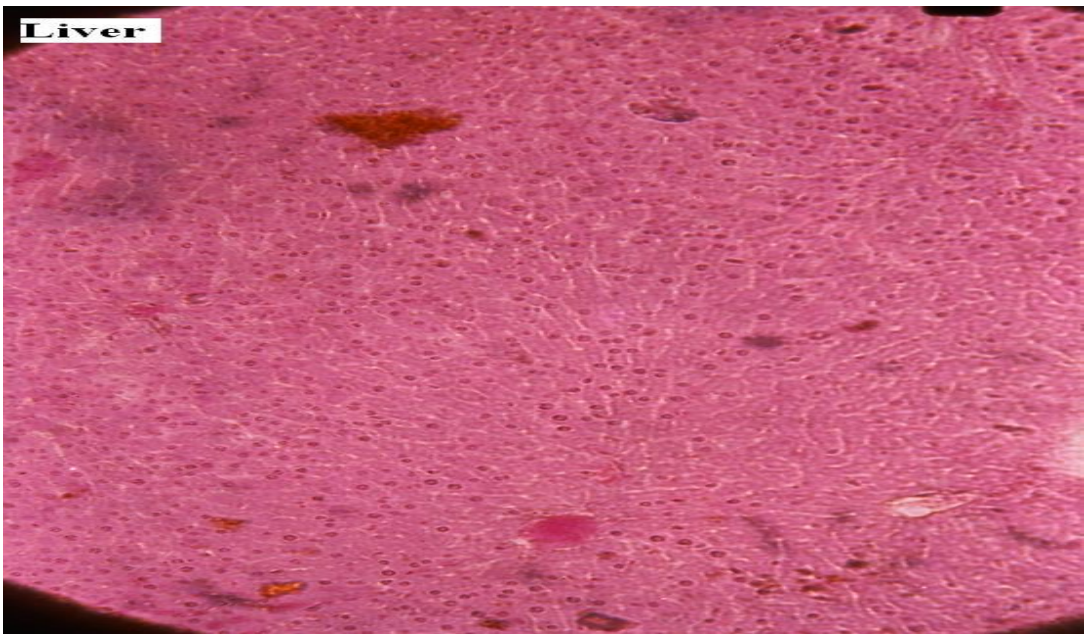


Test (*M. balsamina*, 500 mg /Kg) Liver showing normal cells (3.51)

APPENDIX 2; Effect of Methanol/Water Extract of *M. balsamina* (500 mg/Kg) on Liver after Twenty-eight Days Treatment

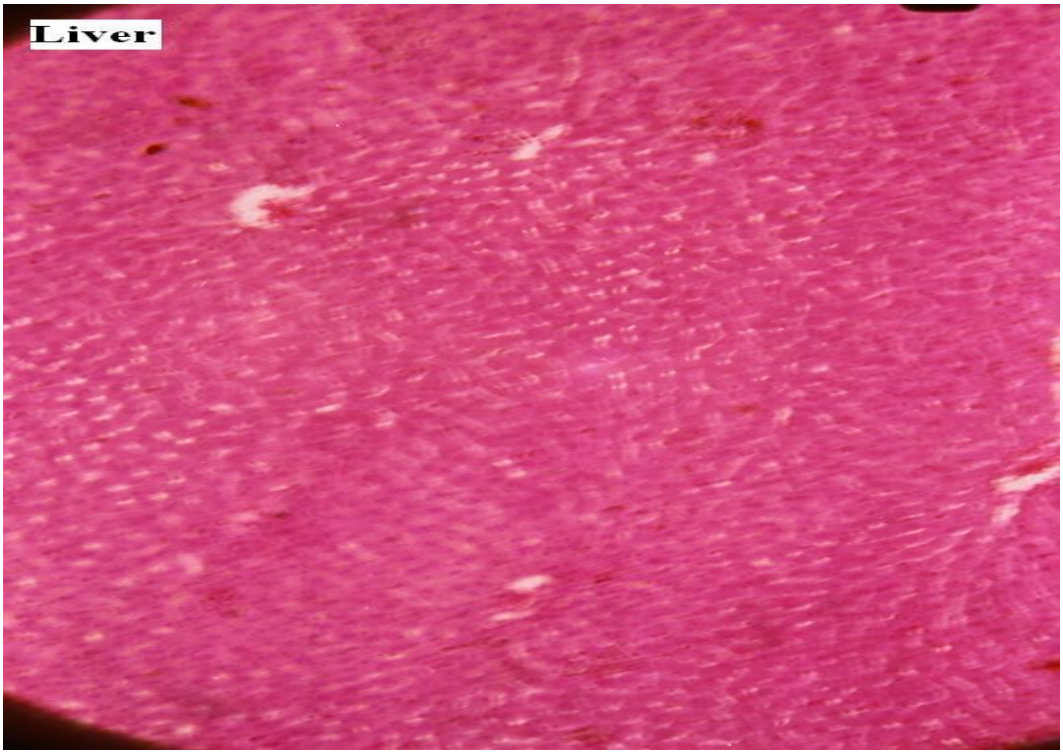


Control (distilled water) liver showing normal cells (3.52)

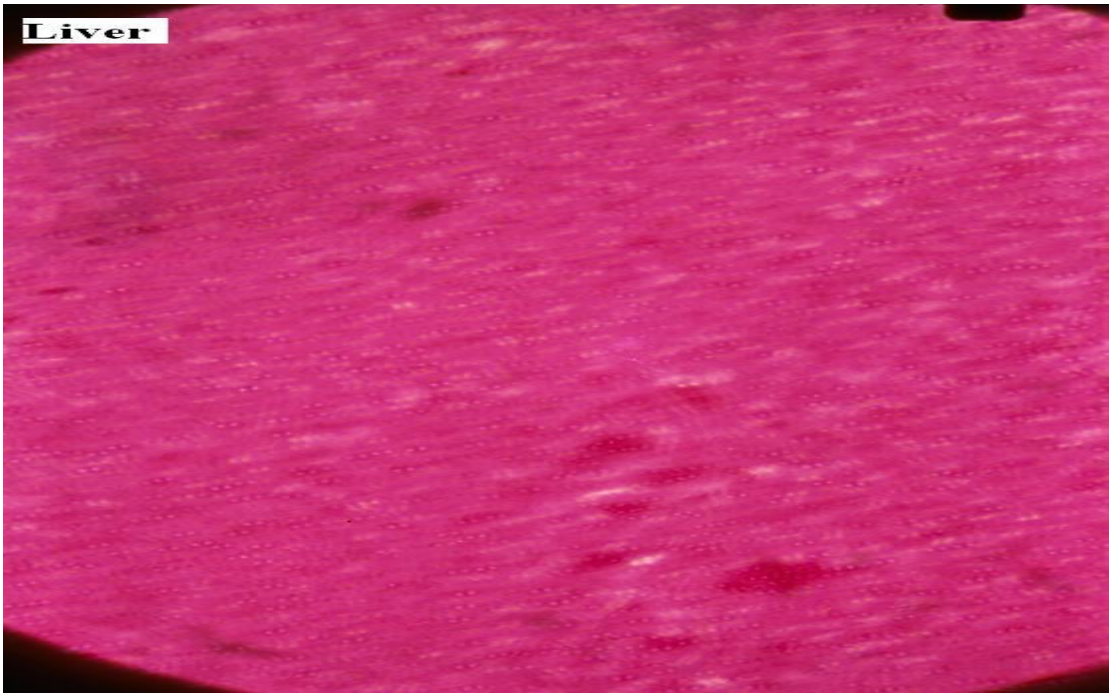


Test (*M. balsamina*, 1000 mg /Kg) liver showing normal cells (3.52)

APPENDIX 3; Effect of Methanol/Water Extract of *M. balsamina* (1000 mg/Kg) on Liver after Twenty-eight Days Treatment

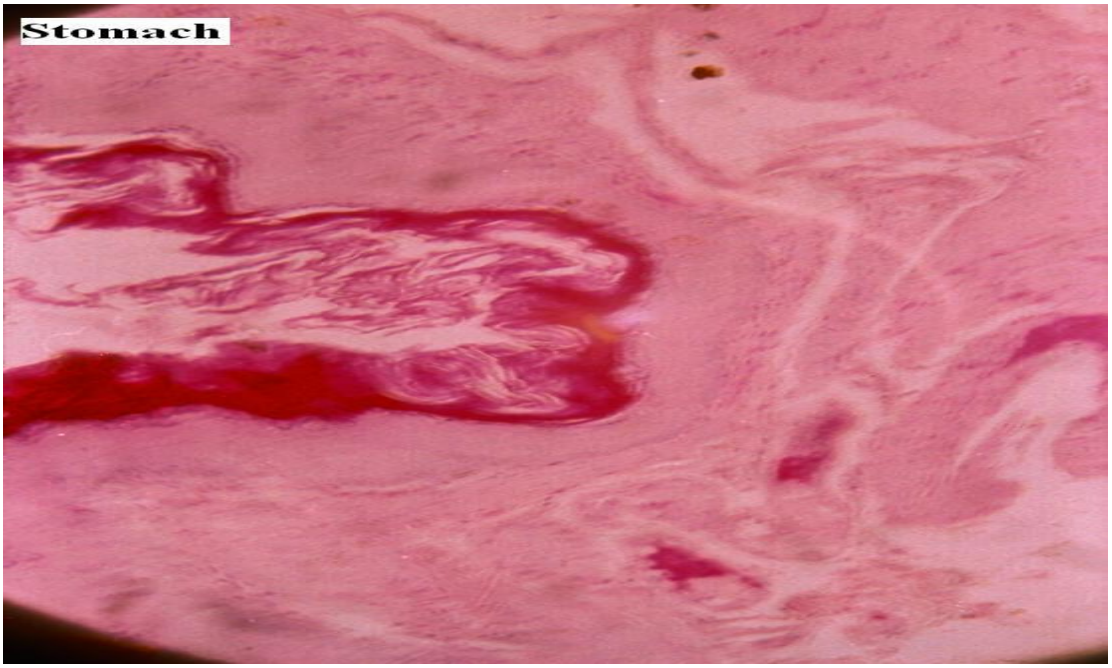


Control (distilled water) liver showing normal cells (3.52)

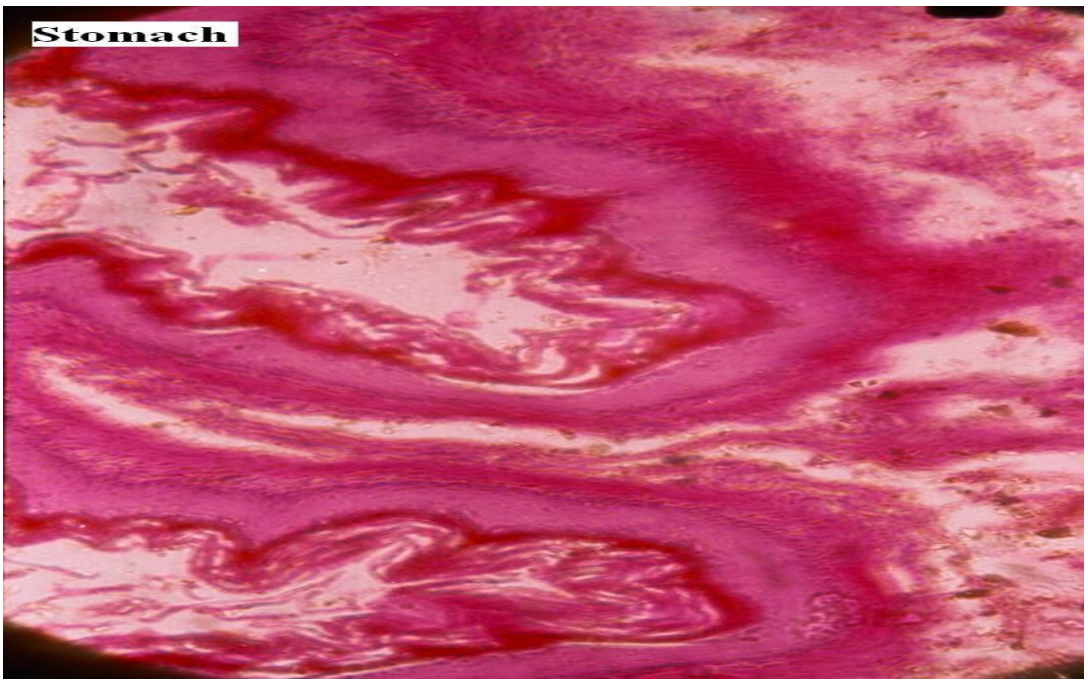


Test (*M. balsamina*, 1500 mg /Kg) lung showing normal cells (3.52)

APPENDIX 4; Effect of Methanol/Water Extract of *M. balsamina* (1500 mg/Kg) on Liver after Twenty-eight Days Treatment

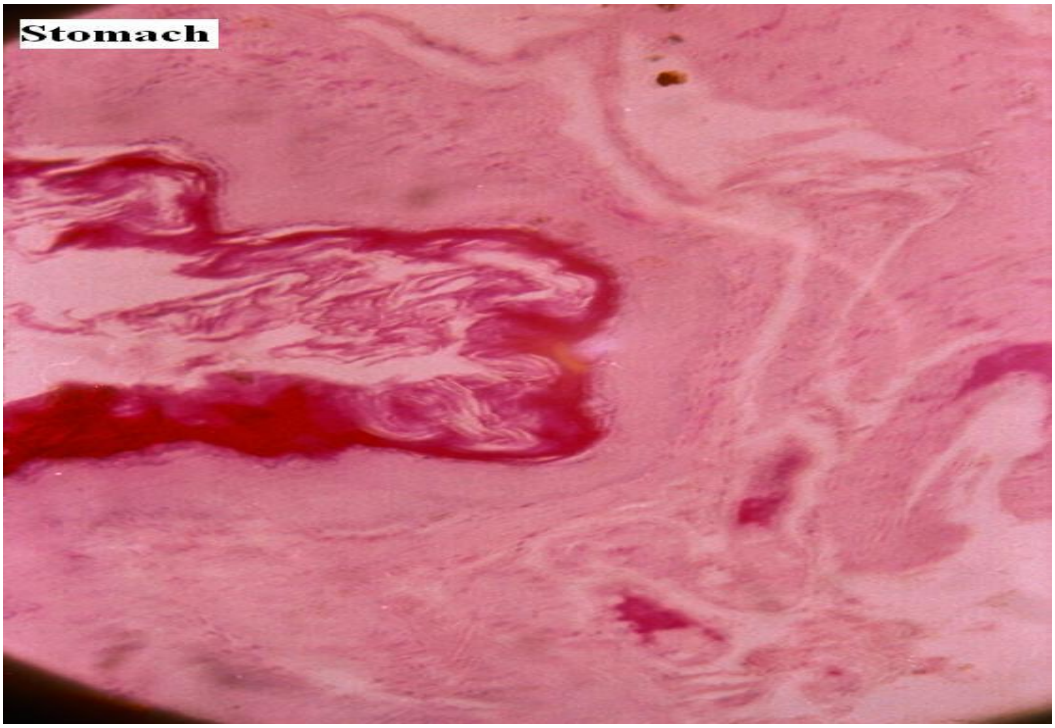


Control stomach showing normal cells (3.51)

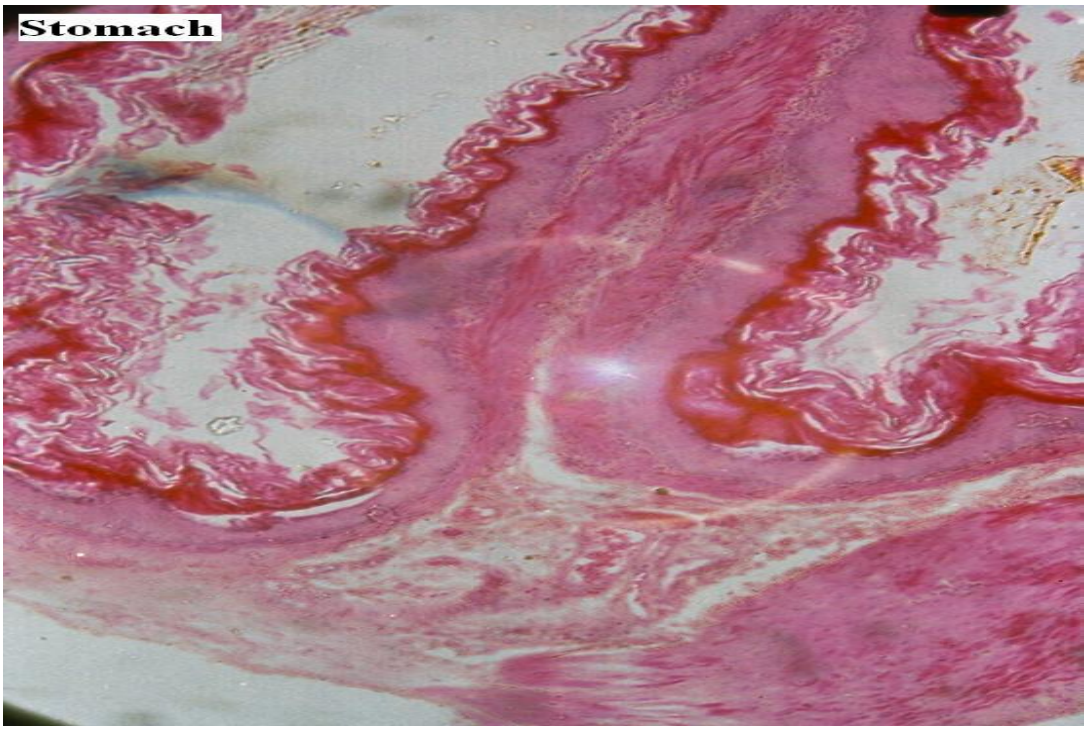


Test (*M. balsamina* 500 mg /Kg) stomach showing normal cells (3.52).

APPENDIX 5; Effect of Methanol/Water Extract of *M. balsamina* (500 mg/Kg) on Stomach after Twenty-eight Days Treatment

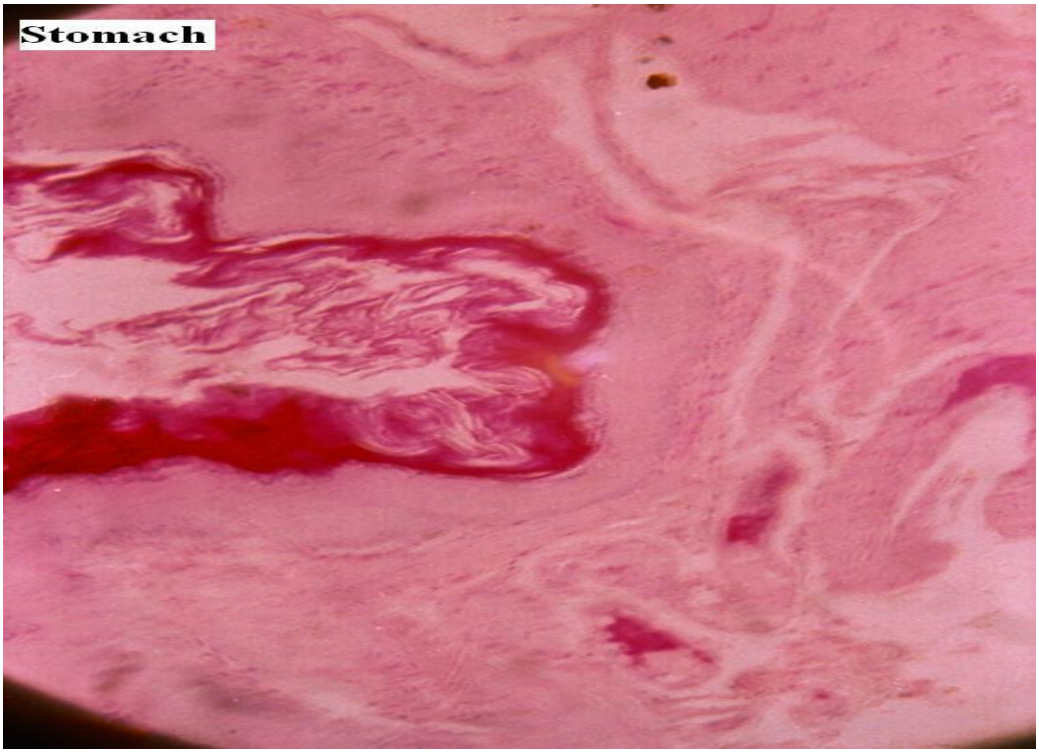


Control (distilled water) stomach showing normal cells (3.51)

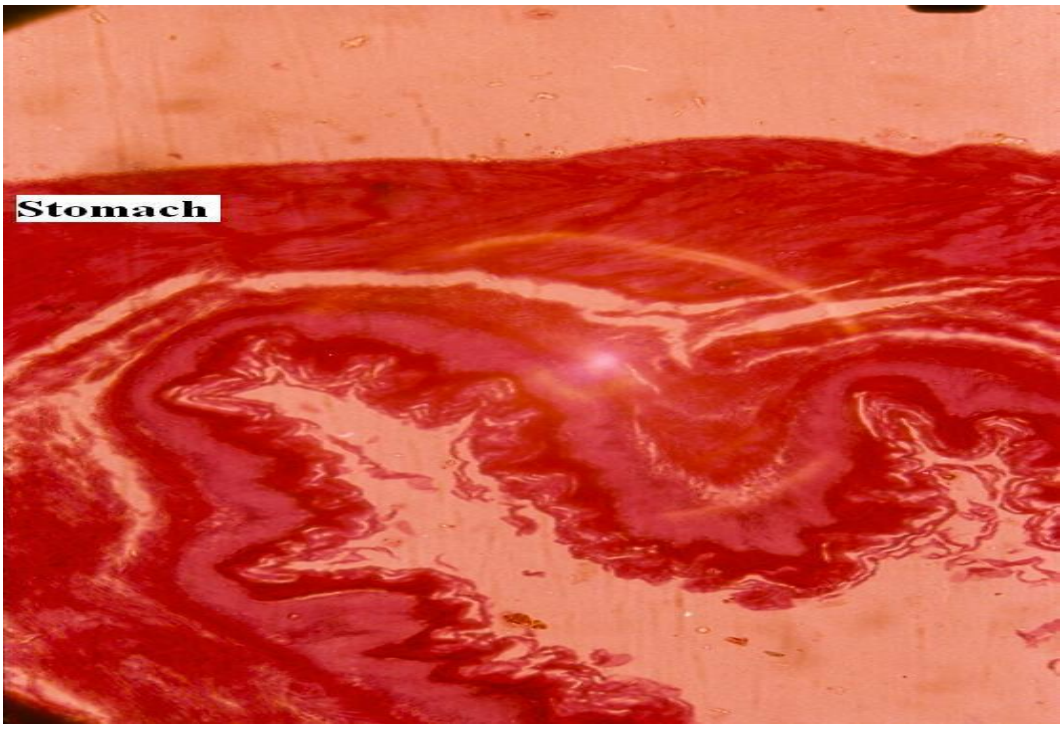


Test (*M. balsamina*, 1000 mg /Kg) stomach showing normal cells (3.51)

APPENDIX 6; Effect of Methanol/Water Extract of *M. balsamina* (1000 mg/Kg) on Stomach after Twenty-eight Days Treatment

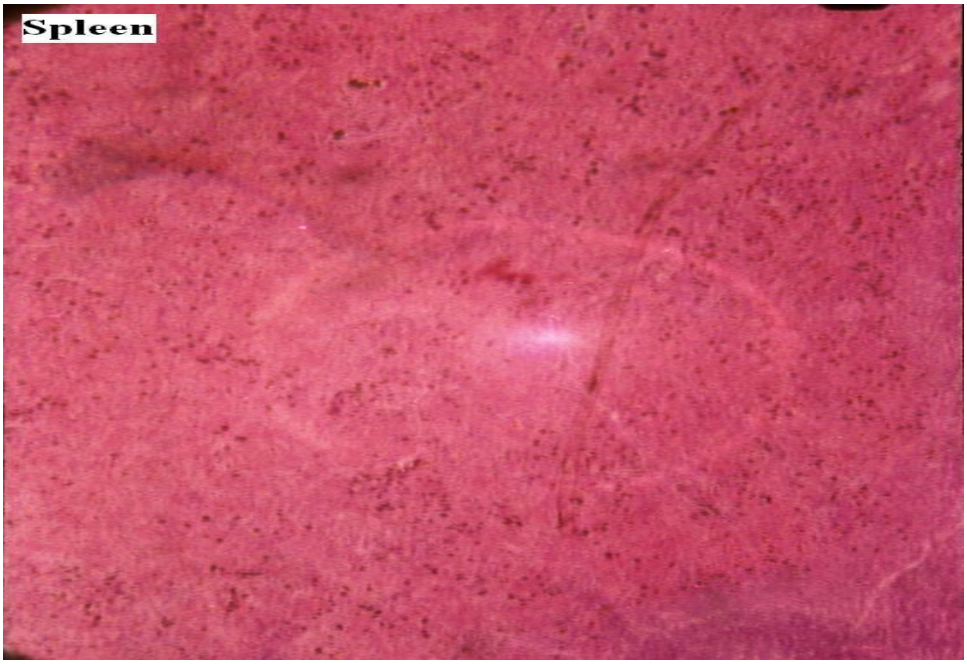


Control (distilled water) stomach showing normal cells (3.51)

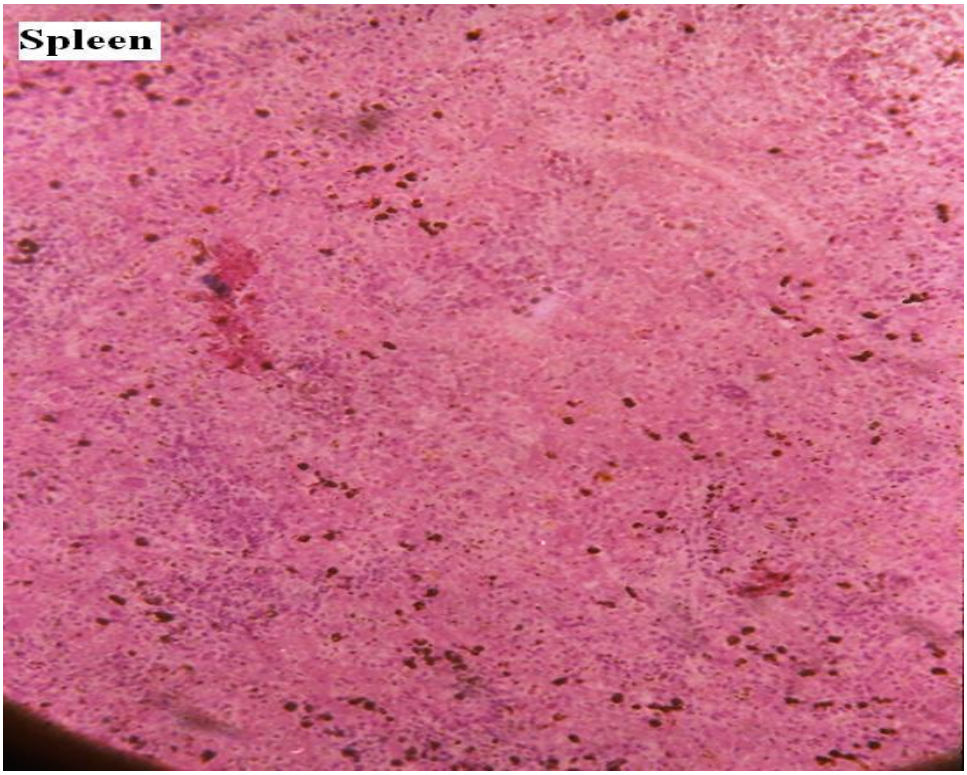


Test (*M. balsamina*, 1500 mg /Kg) stomach showing normal cells (3.52)

APPENDIX 7; Effect of Methanol/Water Extract of *Momordica balsamina* (1500 mg/Kg) on Stomach after Twenty-eight Days Treatment

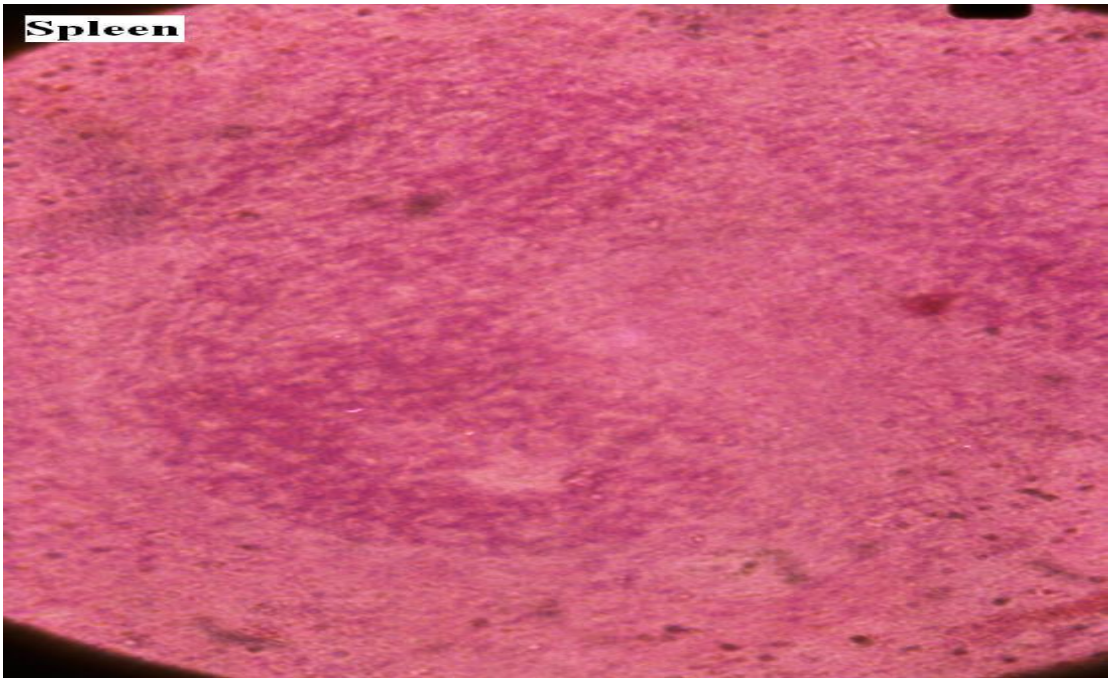


Control spleen showing normal cells (3.52)

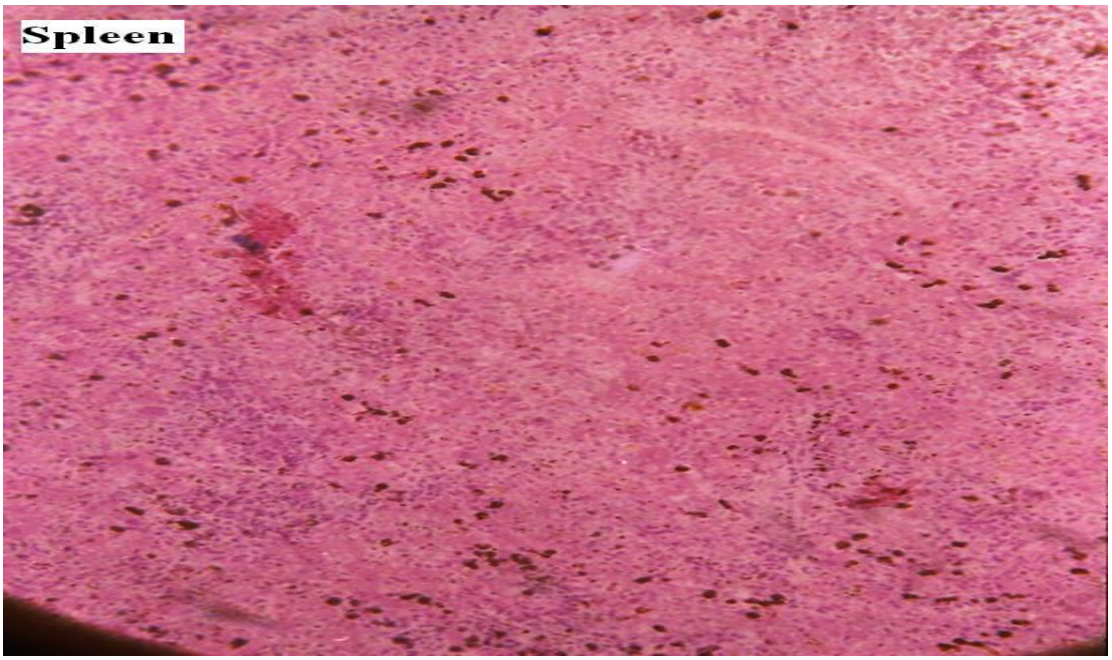


Test (*M. balsamina*, 500 mg /Kg) spleen showing normal cells (3.51)

APPENDIX 8; Effect of Methanol/Water Extract of *M. balsamina* (500 mg/Kg) on Spleen after Twenty-eight Days Treatment

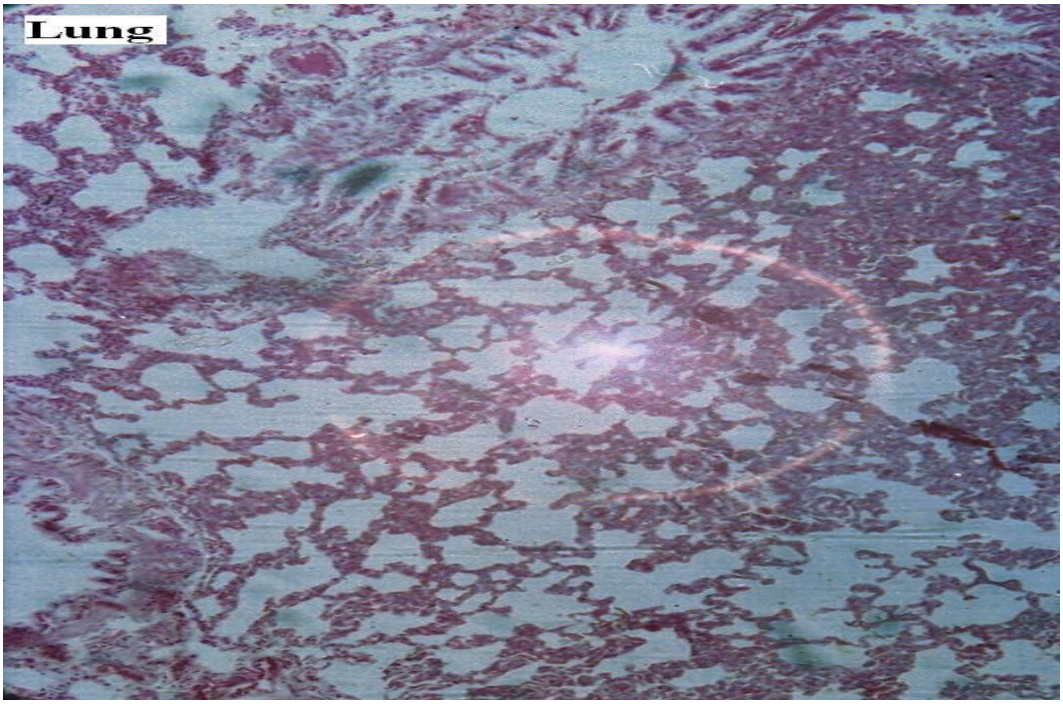


Control (distilled water) spleen showing normal cells (3.52)

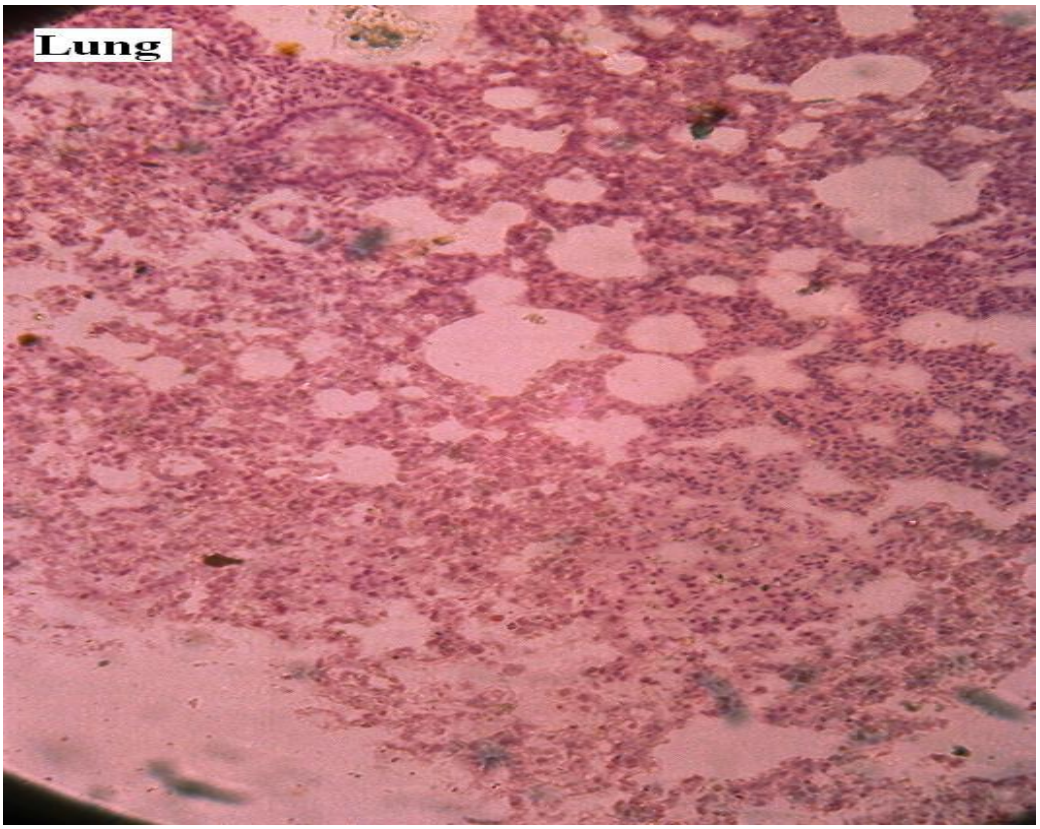


Test (*M. balsamina*, 1500 mg /Kg) spleen showing normal cells (3.51)

APPENDIX 9; Effect of Methanol/Water Extract of *M. balsamina* (1500 mg/Kg) on Spleen after Twenty-eight Days Treatment

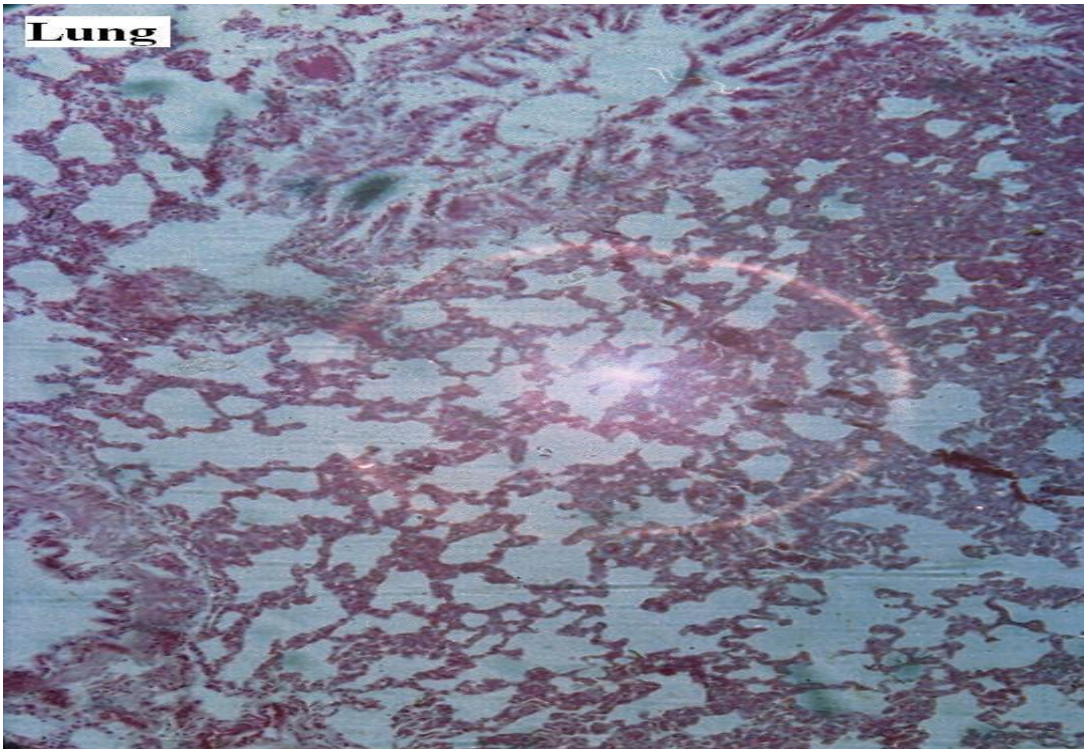


Control (distilled water) Lung showing normal cells (3.51)

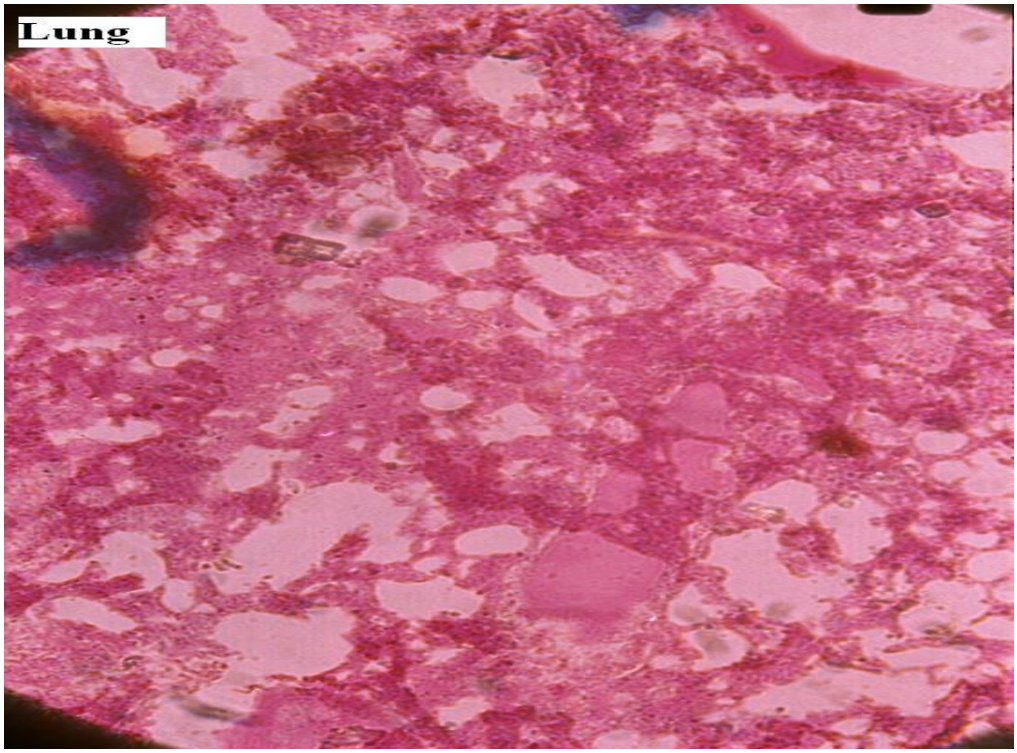


Test (*M. balsamina*, 500 mg /Kg) Lung showing normal cells (3.51)

APPENDIX 10; Effect of Methanol/Water Extract of *M. balsamina* (500 mg/Kg) on Lungs after Twenty-eight Days Treatment

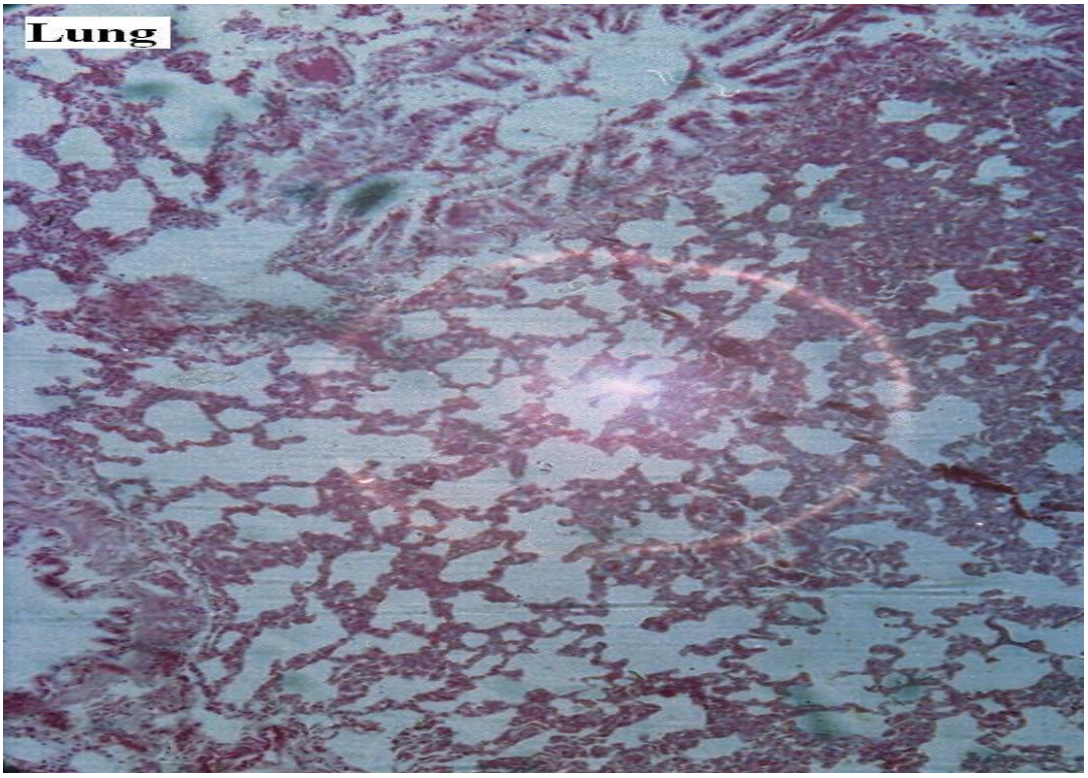


Control (distilled water) lung showing normal cells (3.51)

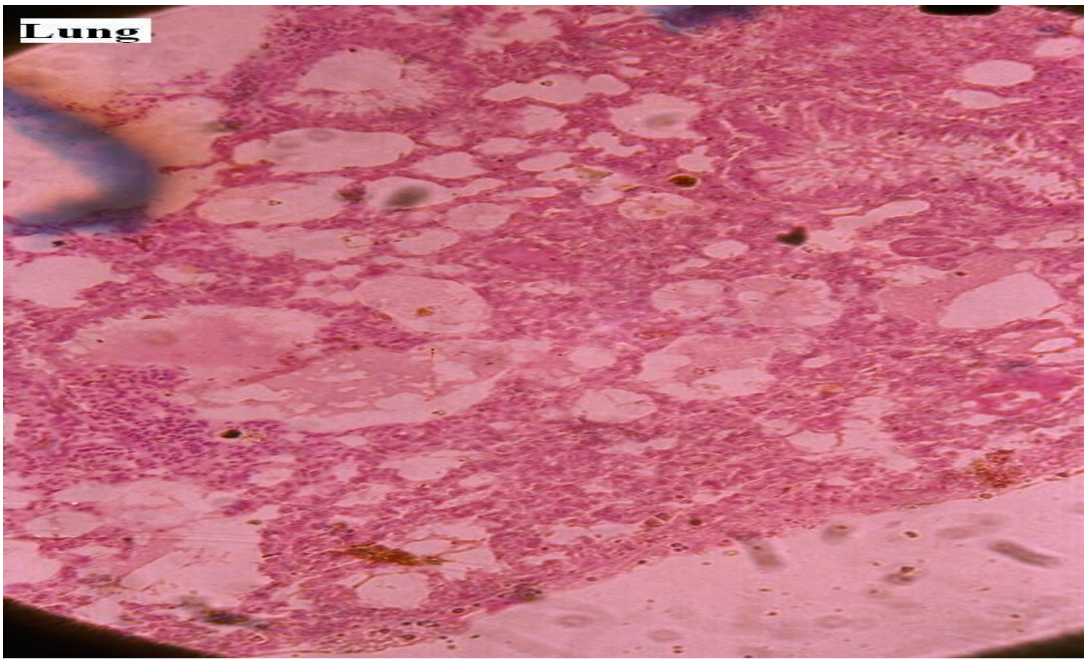


Test (*M. balsamina*, 1000 mg /Kg) lung showing normal cells (3.51)

APPENDIX 11; Effect of Methanol/Water Extract of *M. balsamina* (1000 mg/Kg) on Lung after Twenty-eight Days Treatment

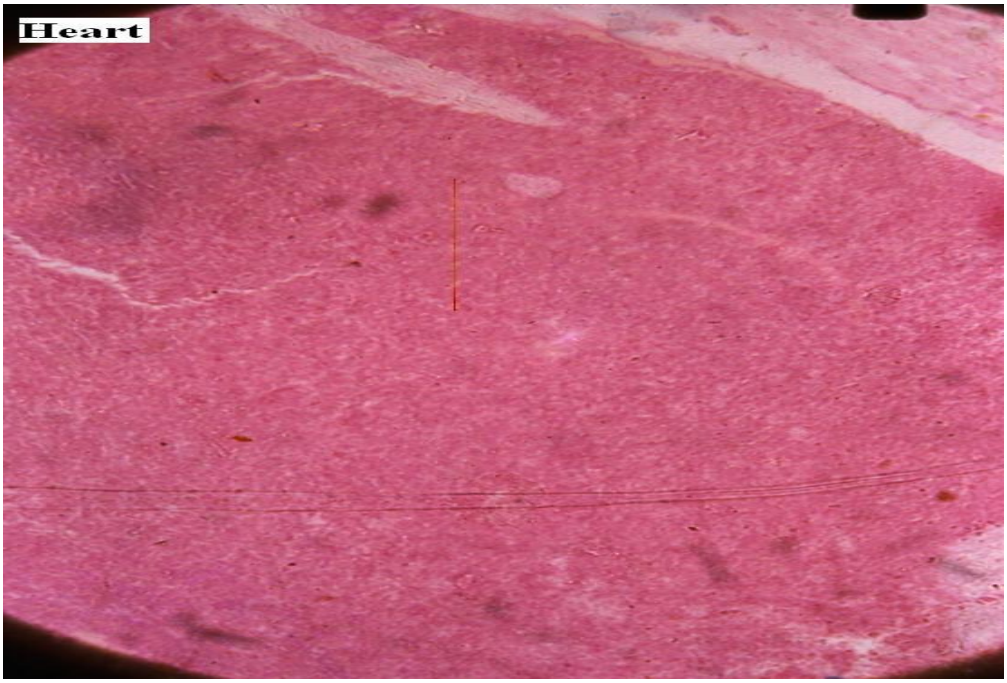


Control (distilled water) lung showing normal cells (3.51)

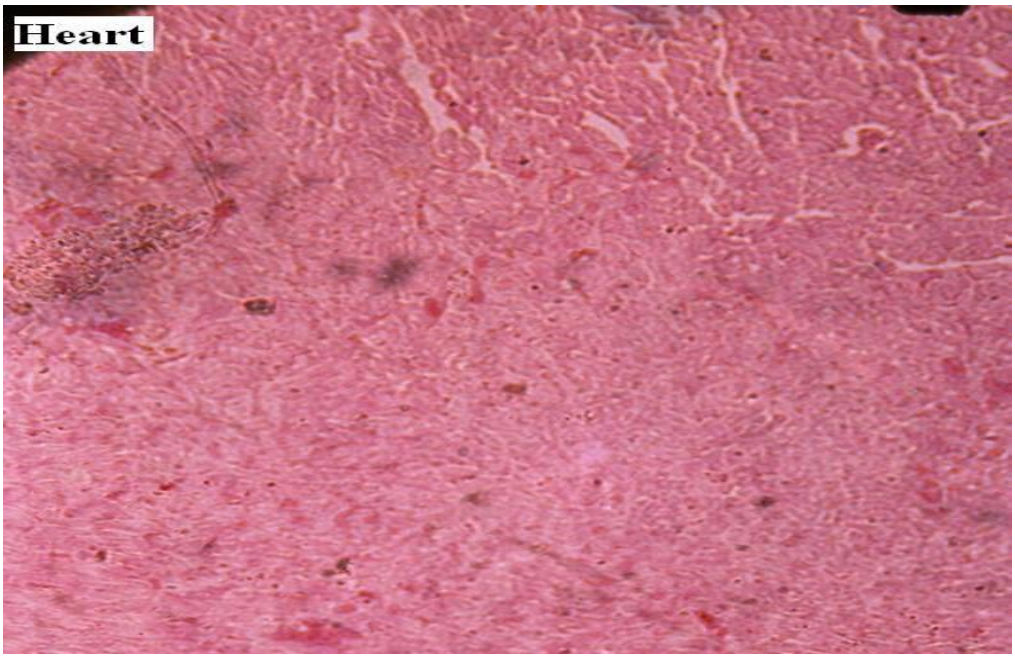


Test (*M. balsamina*, 1500 mg /Kg) lung showing normal cells (3.51)

APPENDIX 12; Effect of Methanol/Water Extract of *M. balsamina* (1500 mg/Kg) on Lungs after Twenty-eight Days Treatment

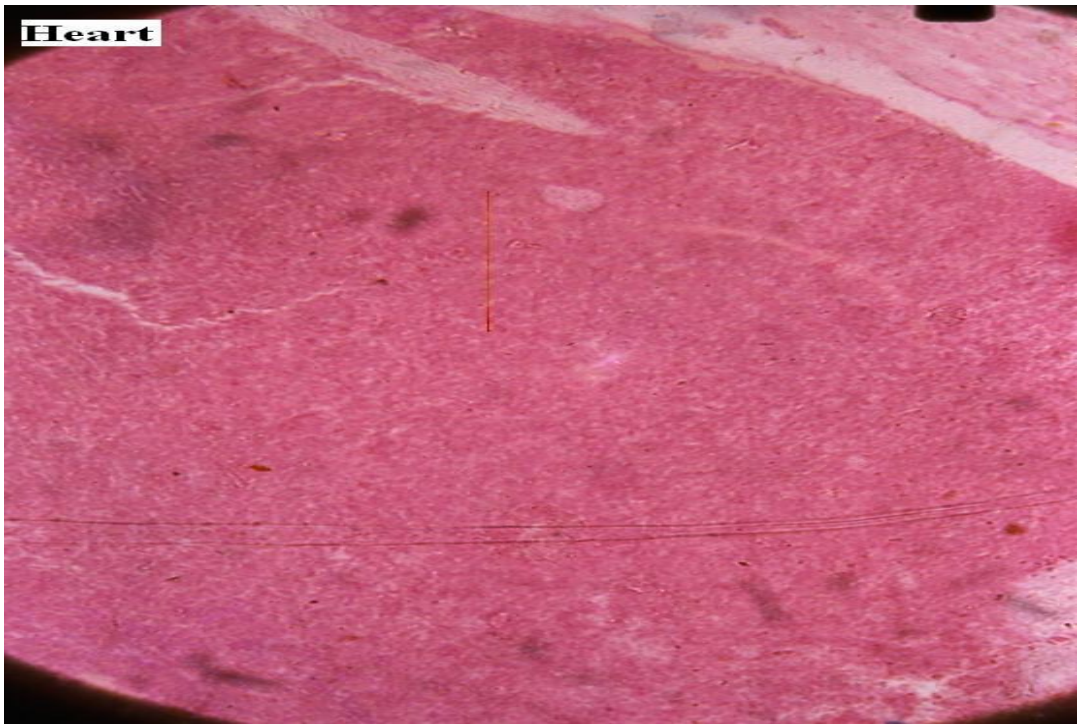


Control (distilled water) Heart showing normal cells (3.52)

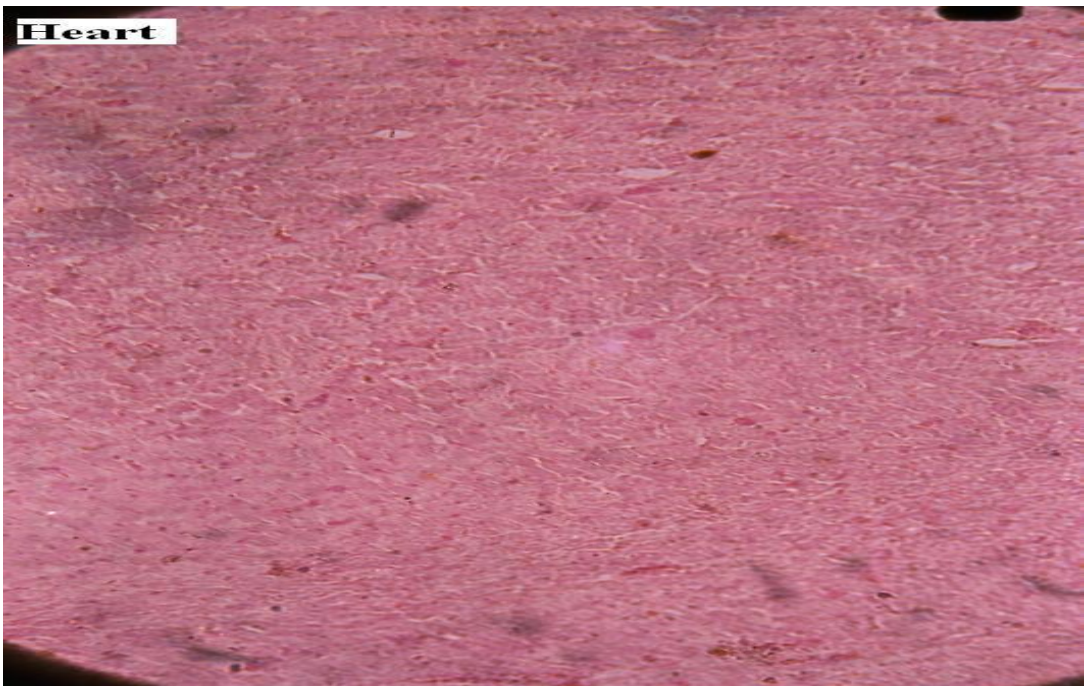


Test (*M. balsamina*, 500 mg /Kg) heart showing normal cells (3.51)

APPENDIX 13; Effect of Methanol/Water Extract of *M. balsamina* (500 mg/Kg) on Heart after Twenty-eight Days Treatment

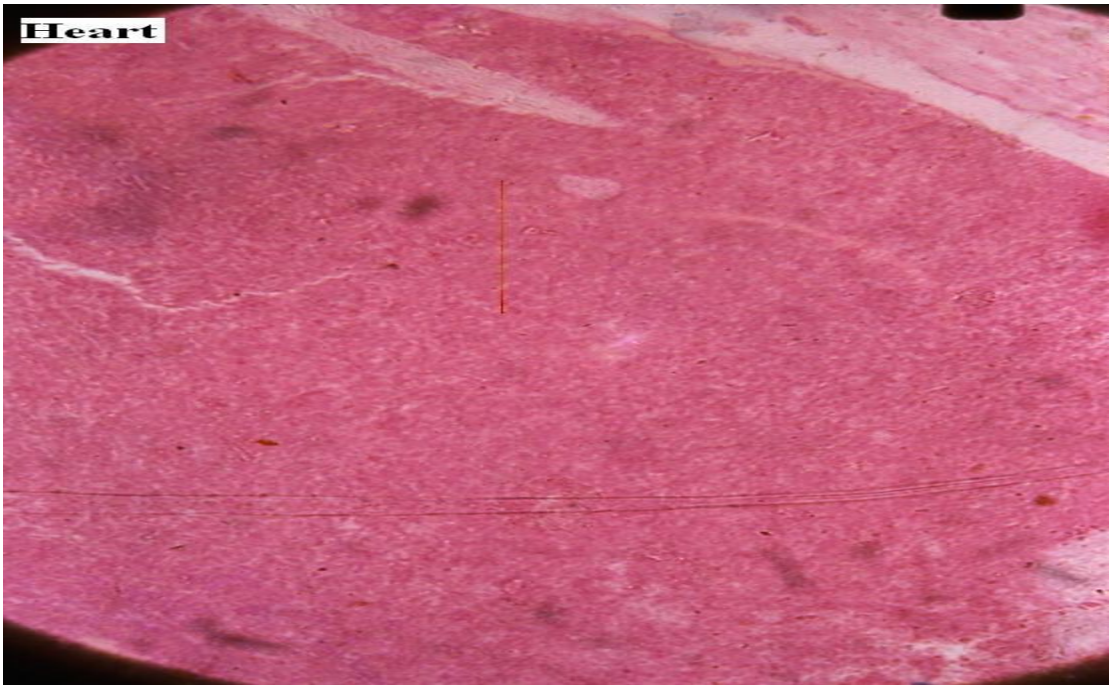


Control (distiller water) heart showing normal cells (3.52)

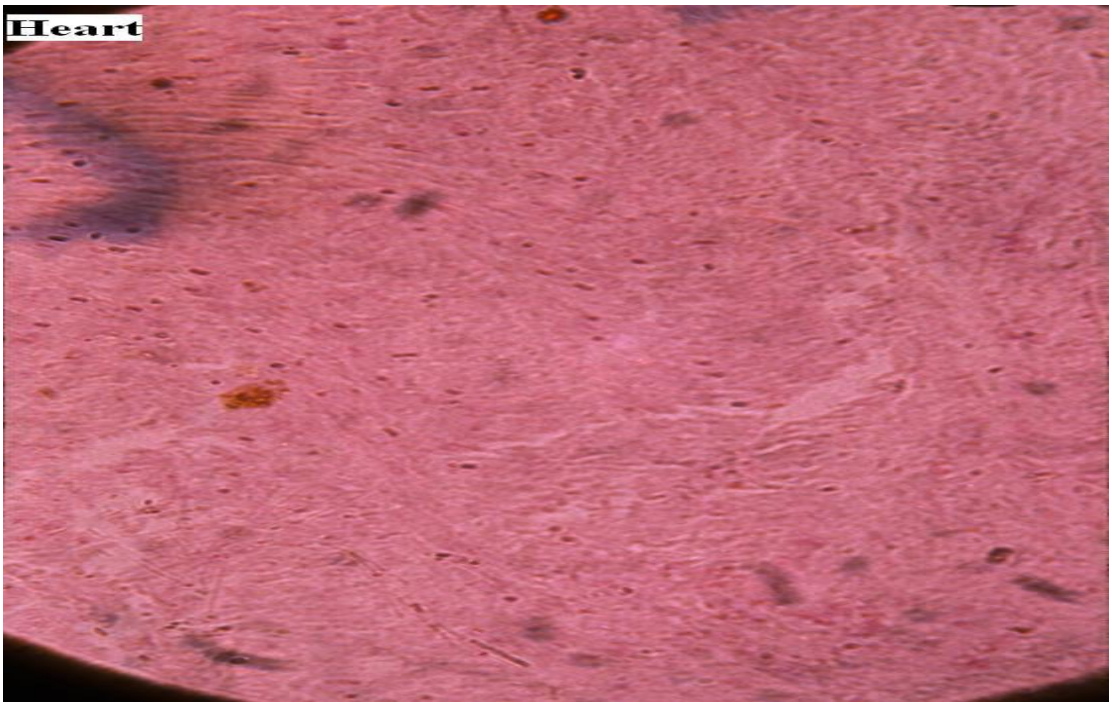


Test (*M. balsamina*, 1000 mg /Kg) heart showing normal cells (3.51)

APPENDIX 14; Effect of Methanol/Water Extract of *M. balsamina* (1000 mg/Kg) on Heart after Twenty-eight Days Treatment

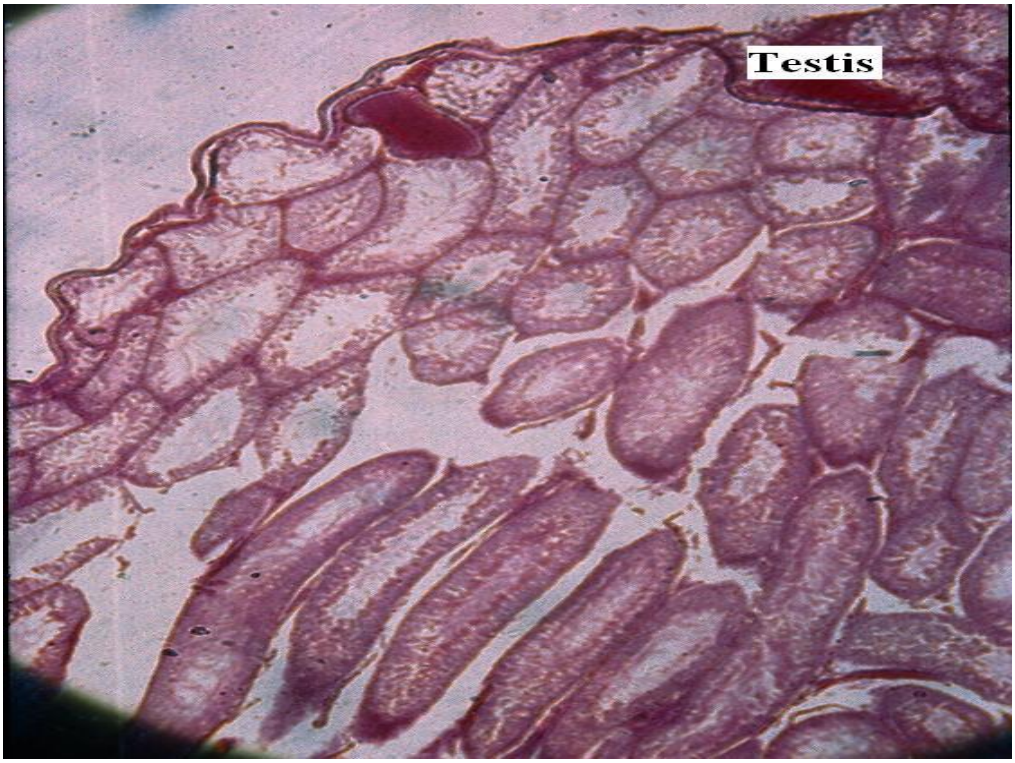


Control (distilled water) heart showing normal cells (3.52)

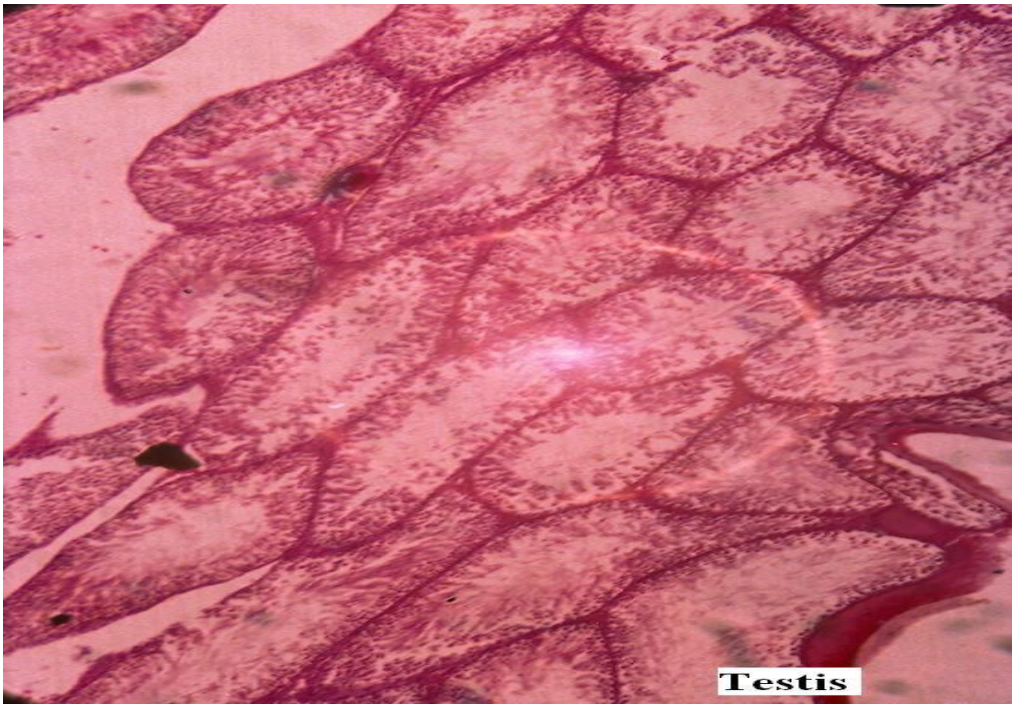


Test (*M. balsamina*, 1500 mg /Kg) heart showing normal cells (3.51)

APPENDIX 15; Effect of Methanol/Water Extract of *M. balsamina* (1500 mg/Kg) on Heart after Twenty-eight Days Treatment

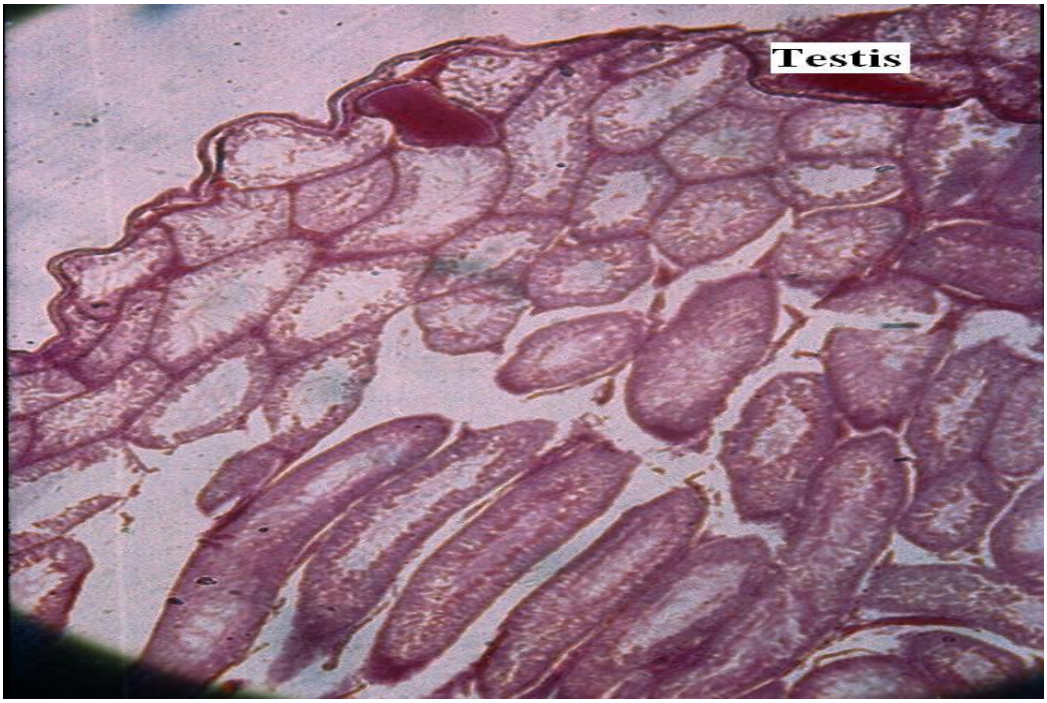


Control (distilled water) testis showing normal cells (3.52)

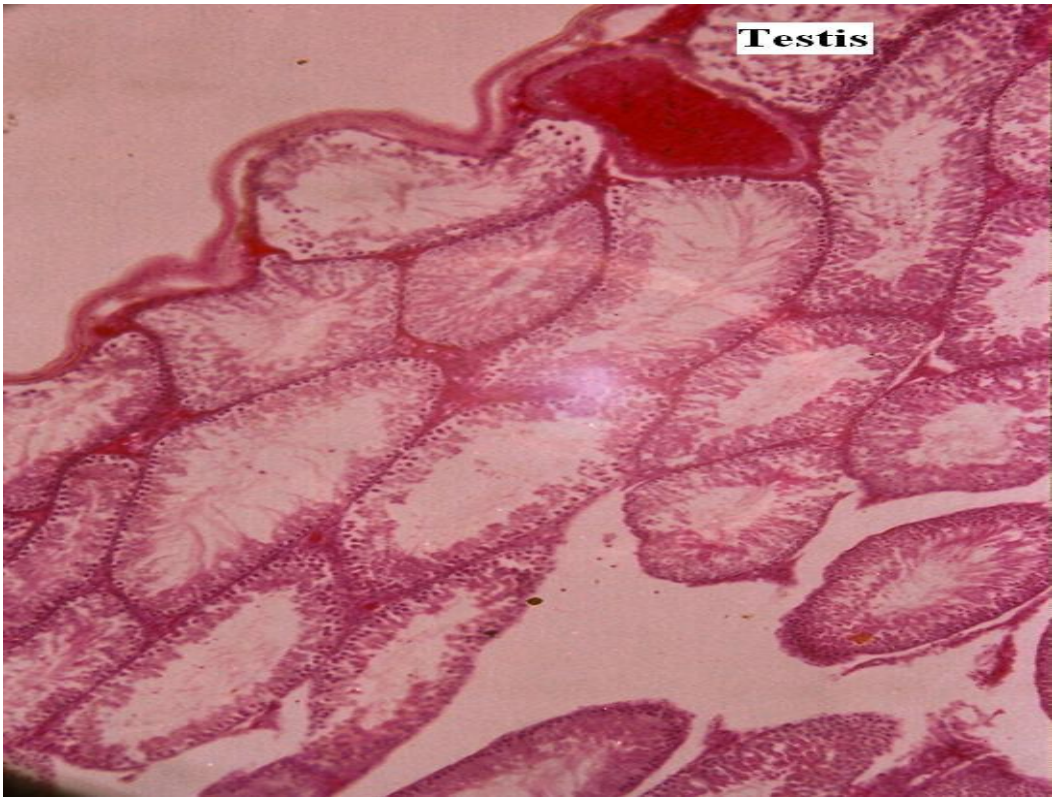


Test (*M. balsamina*, 500 mg /Kg) testis showing normal cells (3.51)

APPENDIX 16; Effect of Methanol/Water Extract of *M. balsamina* (500 mg/Kg) on Testis after Twenty-eight Days Treatment

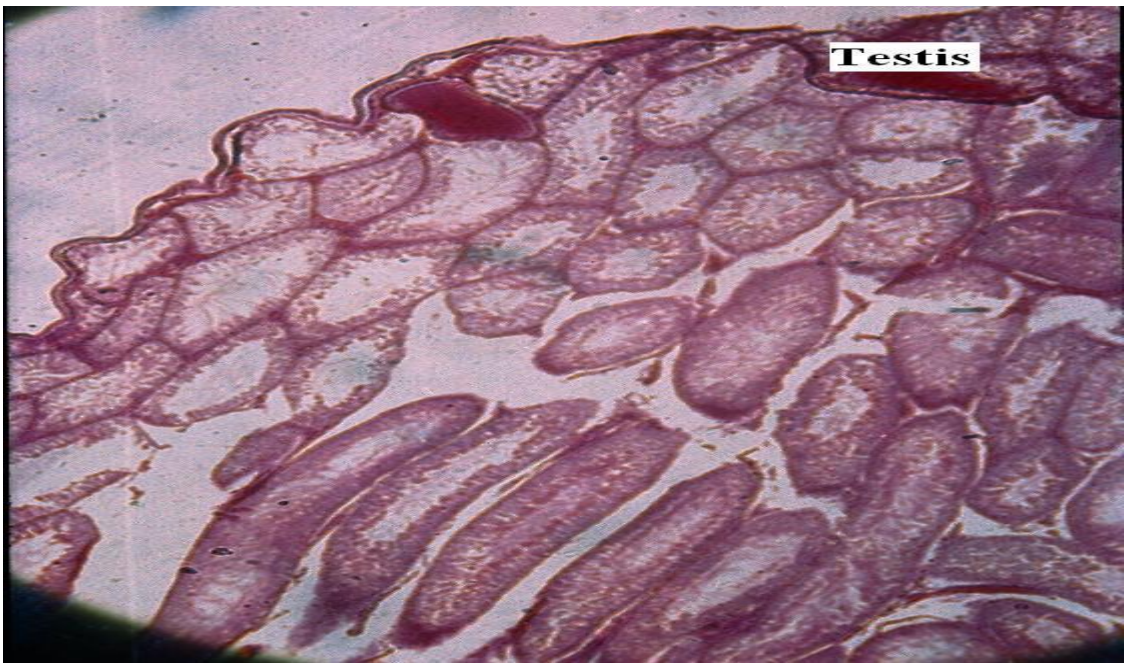


Control (distilled water) testis showing normal cells (3.52)

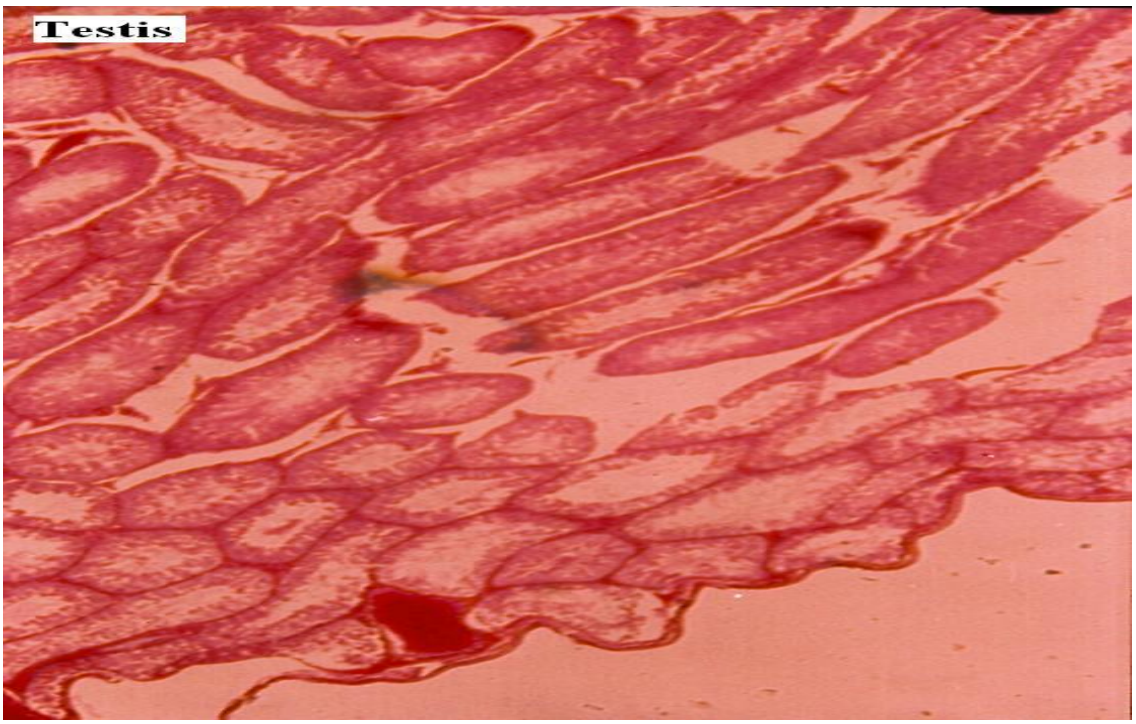


Test (*M. balsamina*, 1000 mg /Kg) testis showing normal cells (3.51)

APPENDIX 17; Effect of Methanol/Water Extract of *M. balsamina* (1000 mg/Kg) on Testis after Twenty-eight Days Treatment

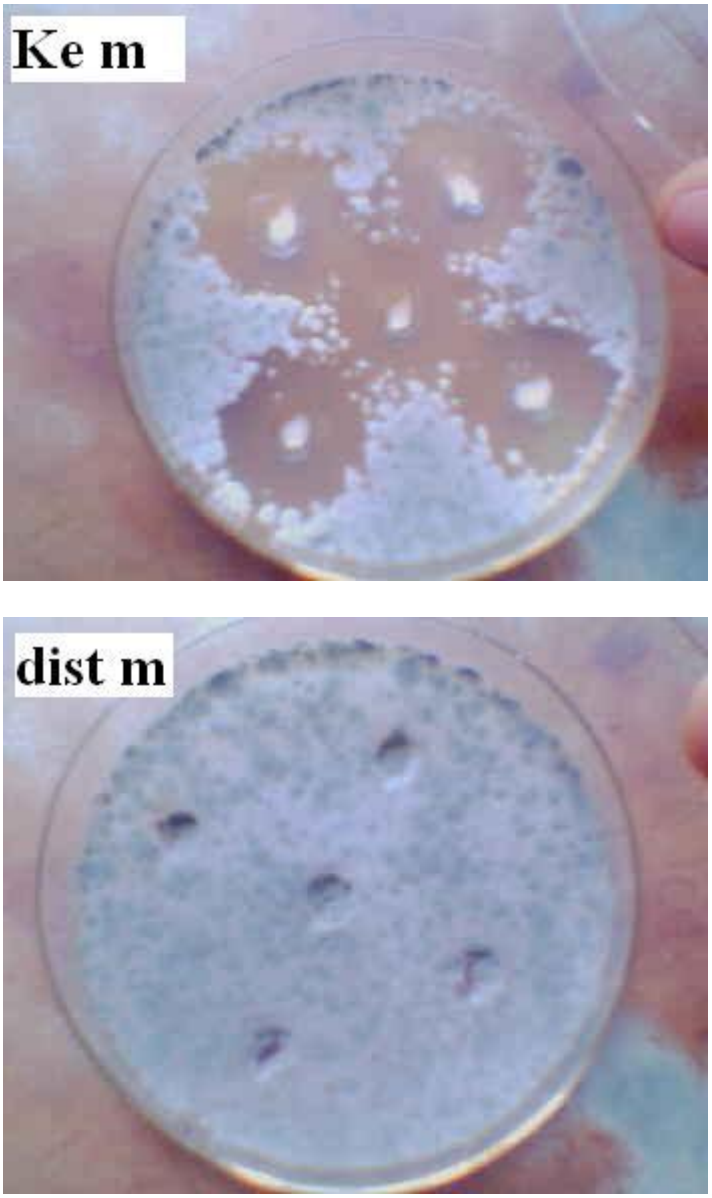


Control (distilled water) testis showing normal cells (3.52)

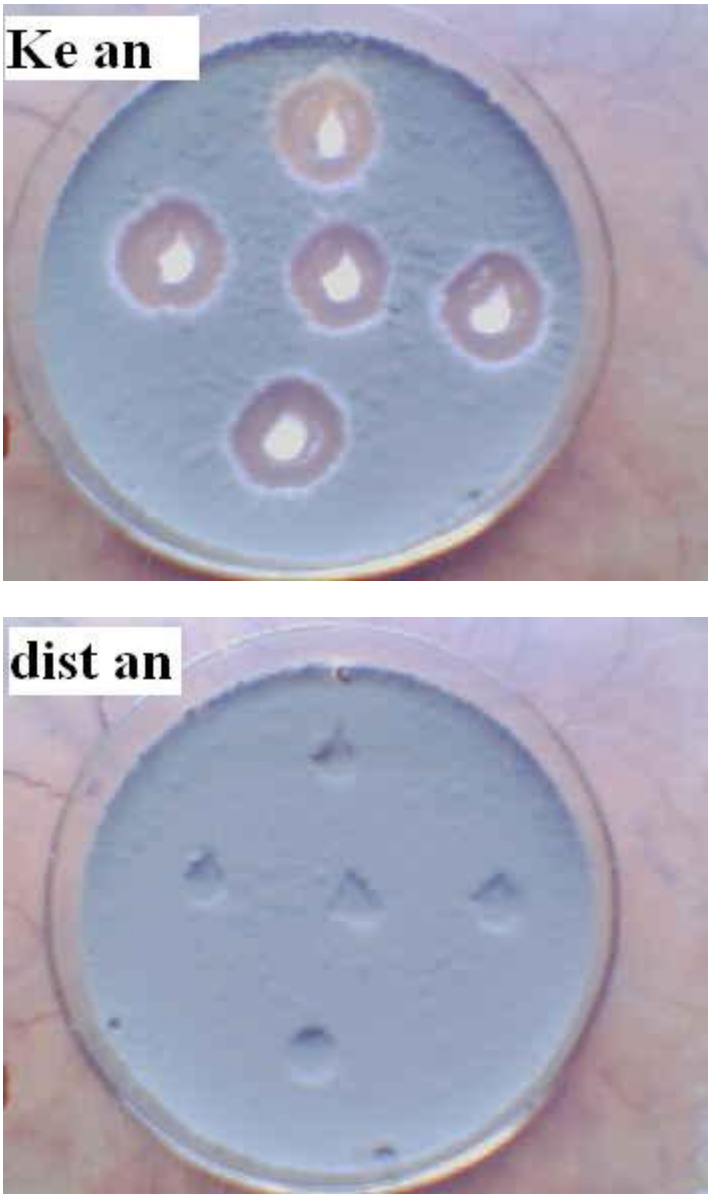


Test (*M. balsamina,* 1500 mg /Kg) testis showing normal cells (3.52)

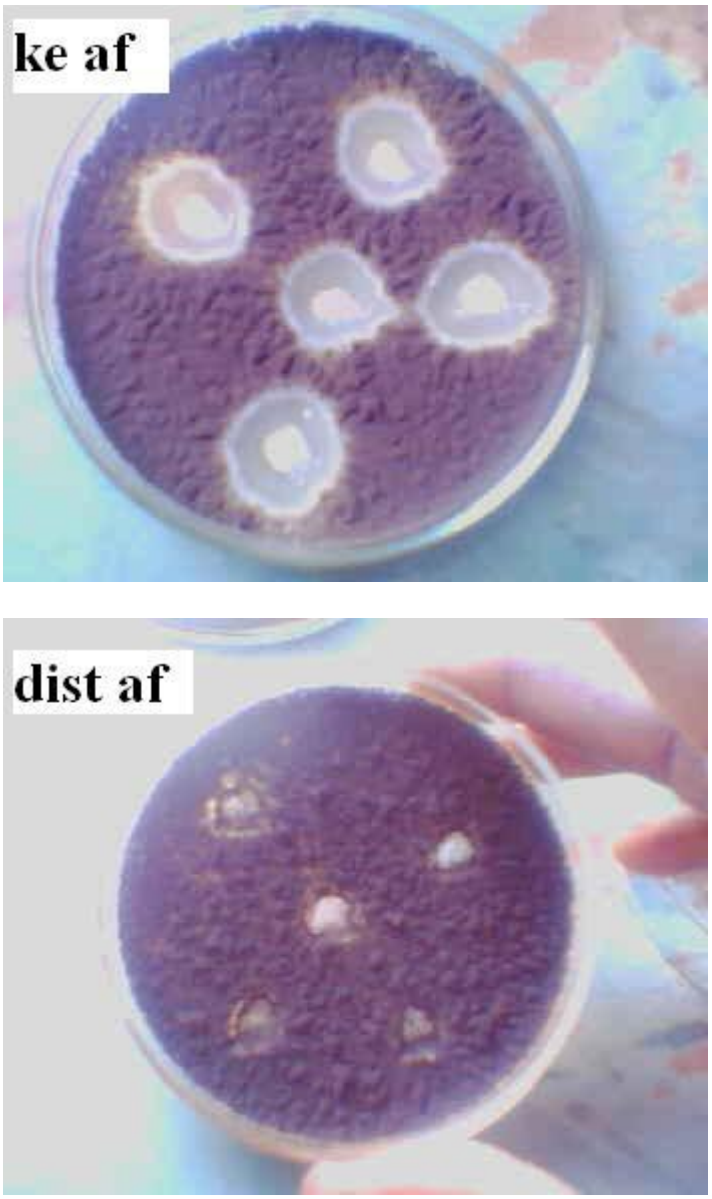
APPENDIX 18; Effect of Methanol/Water Extract of *M. balsamina* (1500 mg/Kg) on Testis after Twenty-eight Days Treatment



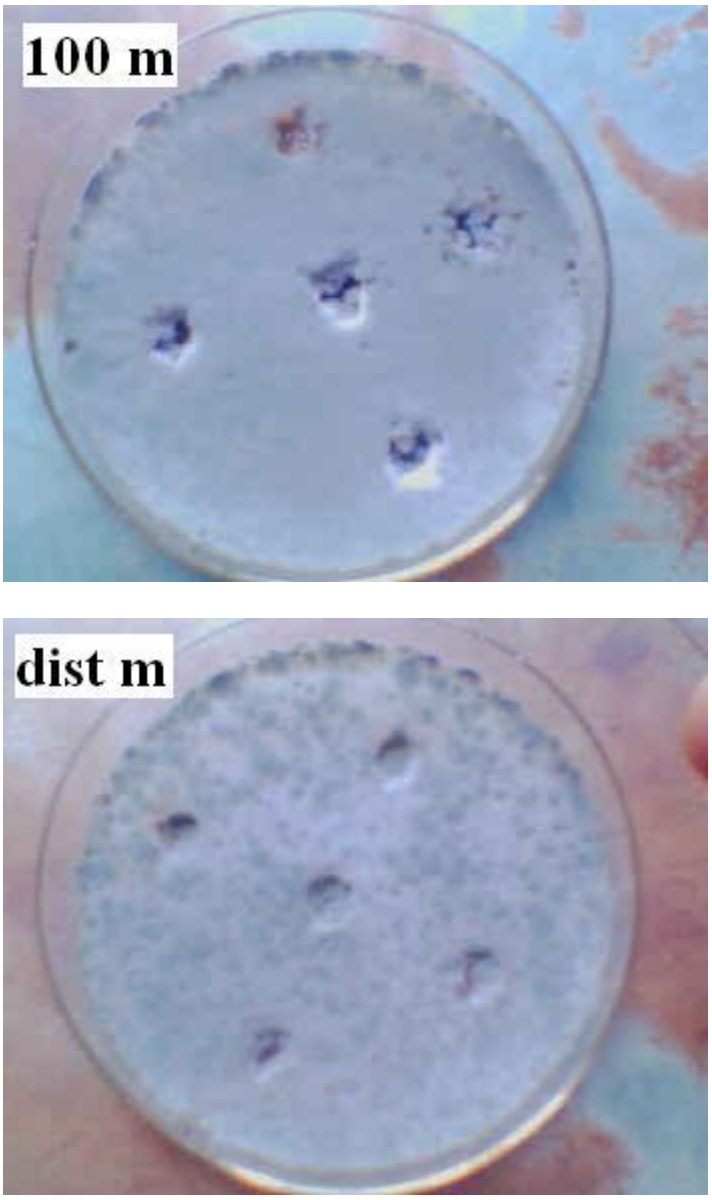
Appendix 19; Effect of Ketoconazole (1 mg) on *Mucus pusillus*



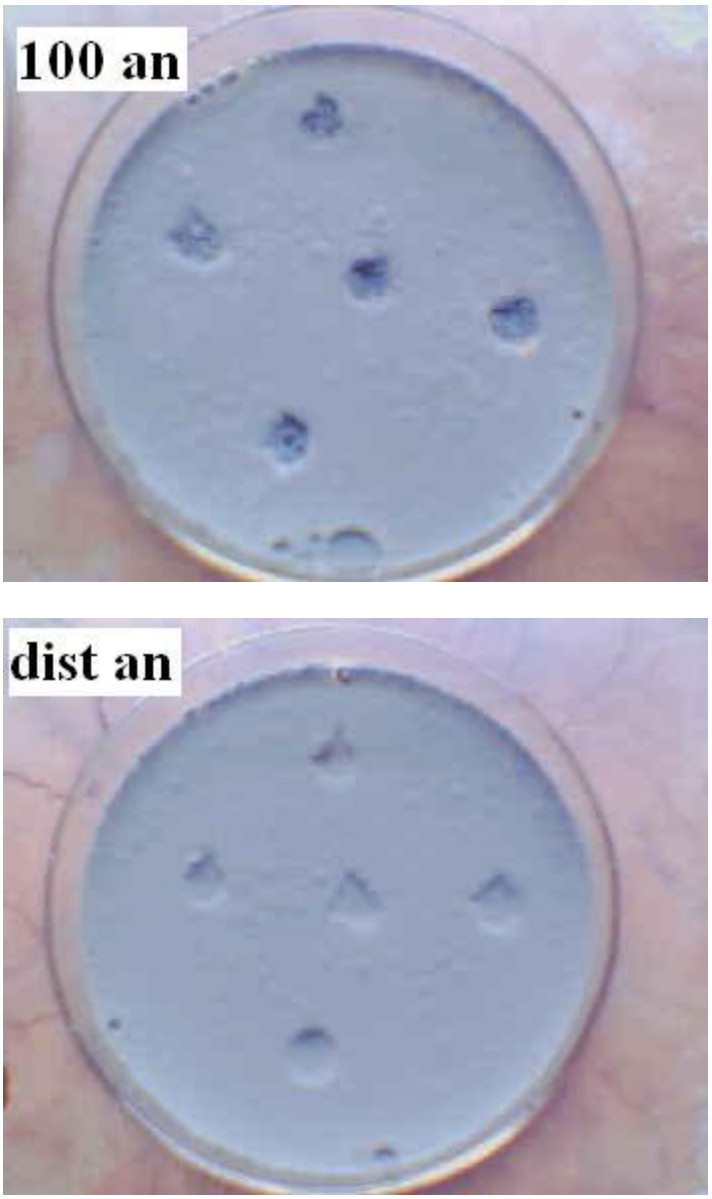
Appendix 20; Effect of Ketoconazole on *Aspargilus niger.* (ke = ketoconazole; dist. = distilled water)



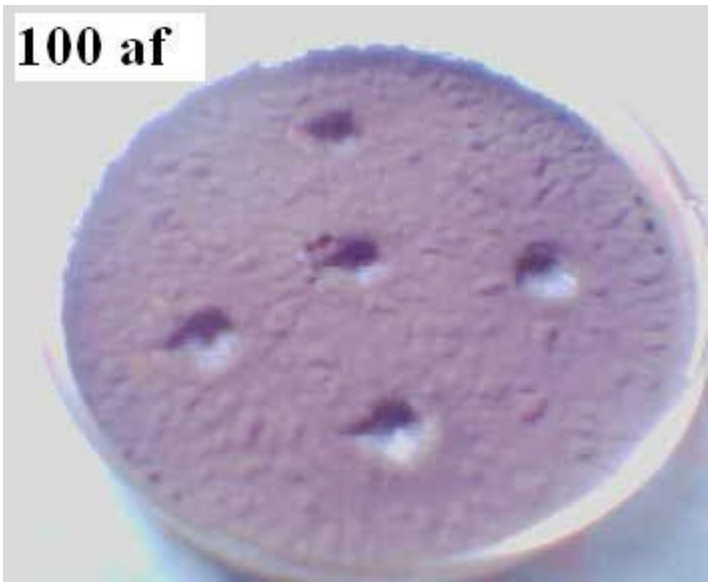
Appendix 21; Effect of Ketoconazole on *Aspergillus fumigatus*.. (ke = ketoconazole; dist. = distilled water)



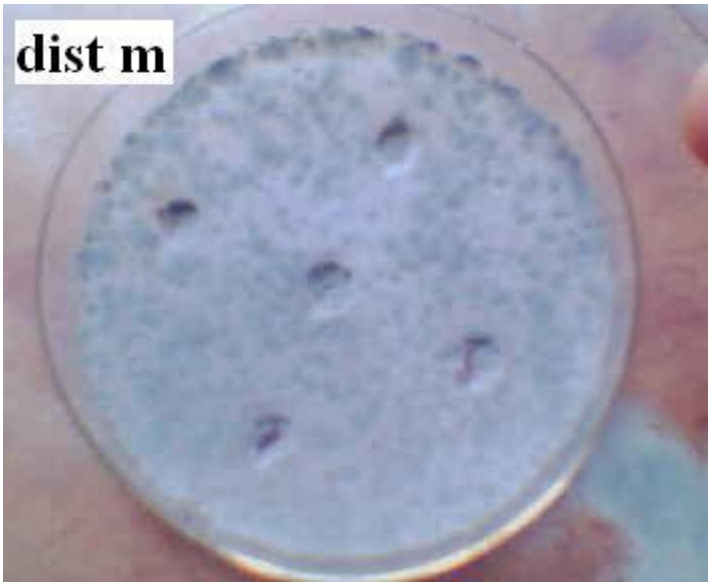
Appendix 22; Effect of *M. balsamina* (100 mg) on *Mucus. pusillus.* (m = *Mucus. pusillus* ; dist. = distilled water)



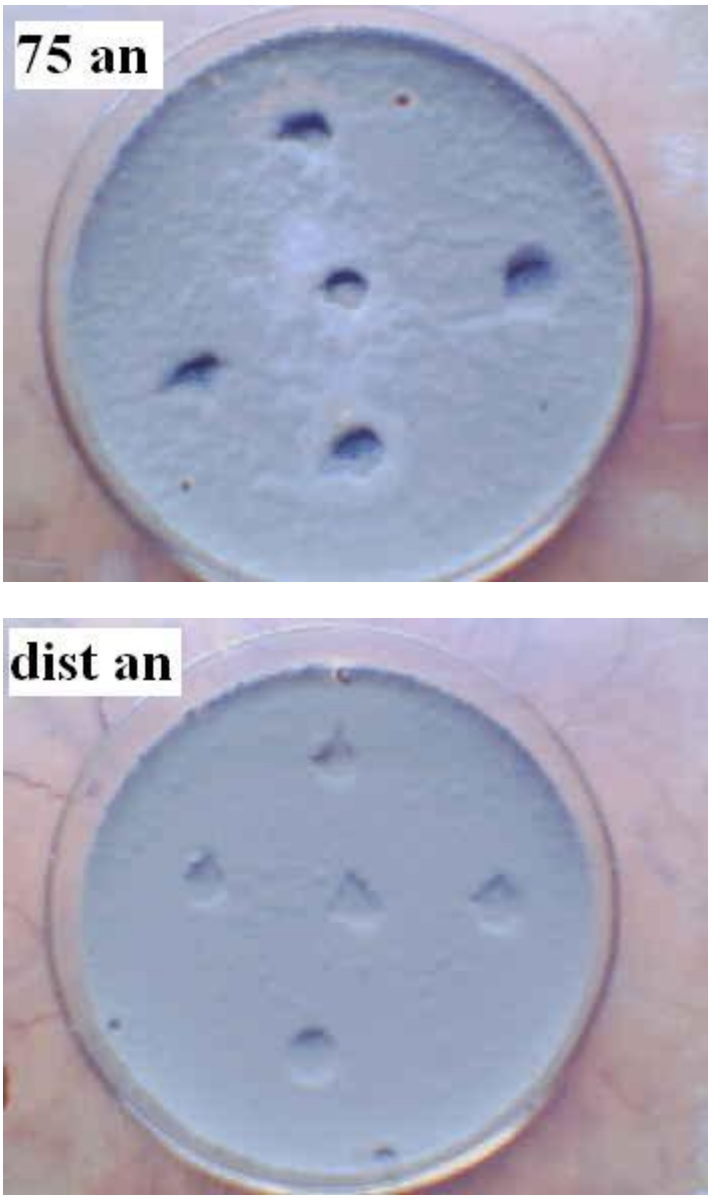
Appendix 23; Effect of *M. balsamina* (100 mg) on *Aspergillus niger.* (an = *A. niger*; dist. = distilled water)



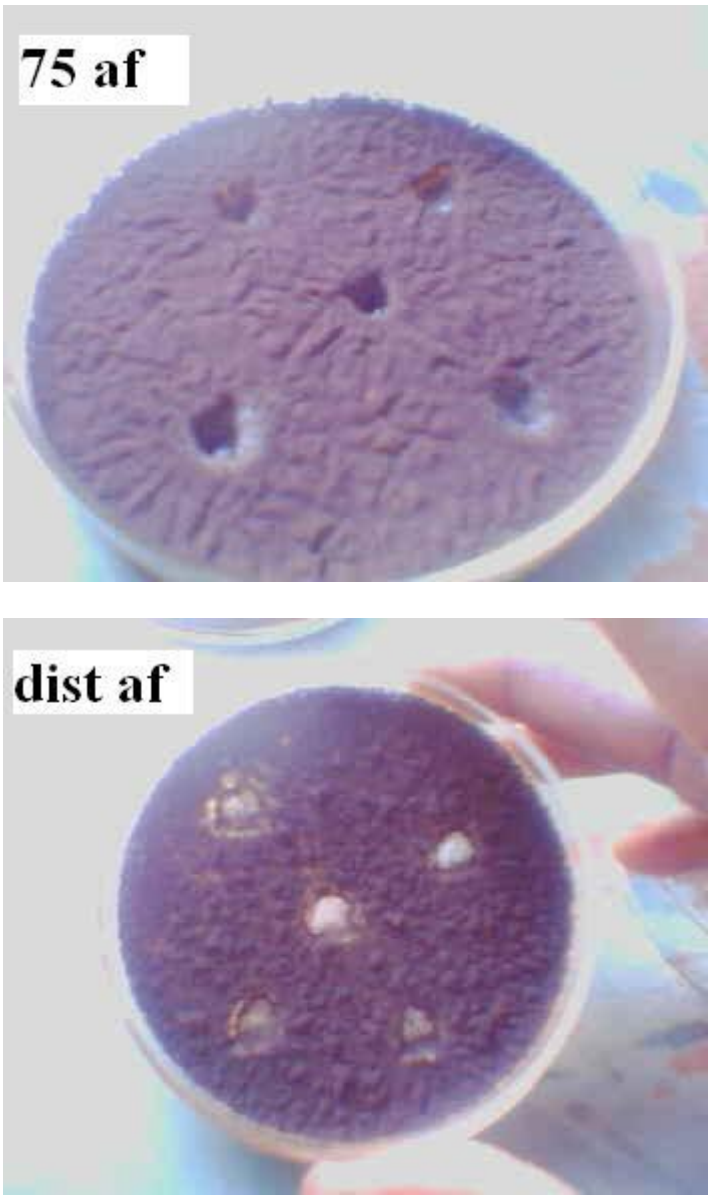
Appendix 24; Effect of *M. balsamina* (100 mg) on *Aspergillus fumigatus.* (af = *A. fumigatus*; dist. = distilled water)



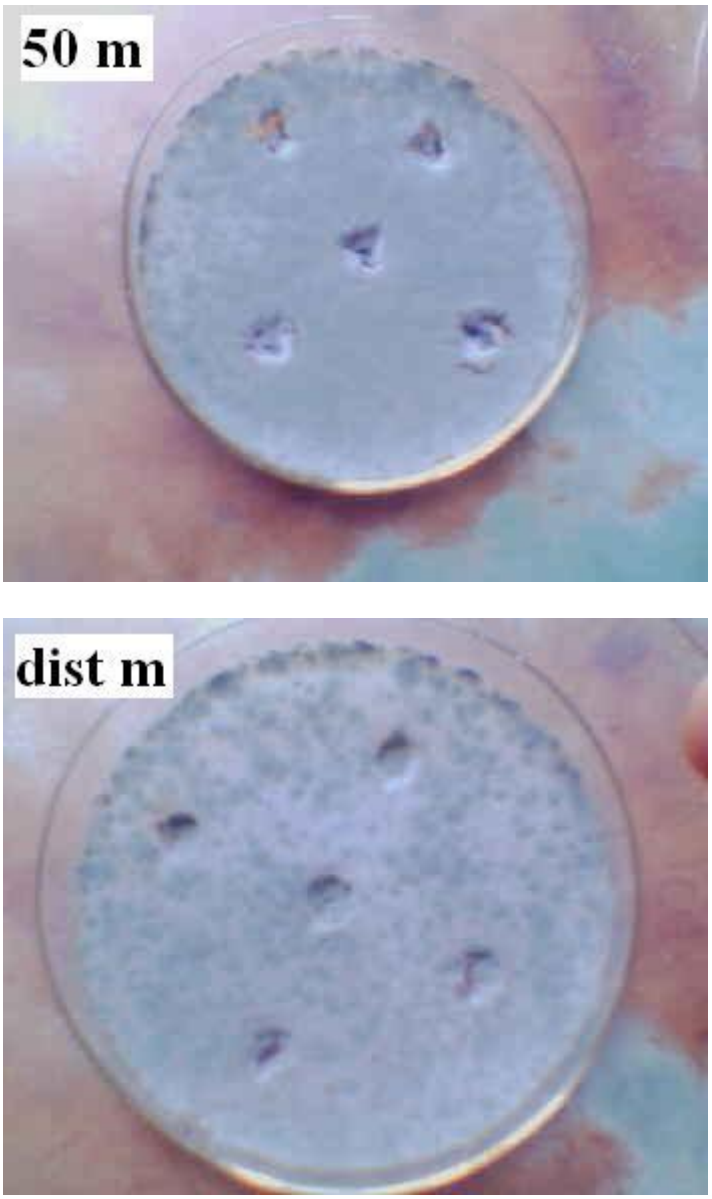
Appendix 25; Effect of *M. balsamina* (75 mg) on *M. pusillus.* (m = *M. pusillus*; dist. = distilled watar)



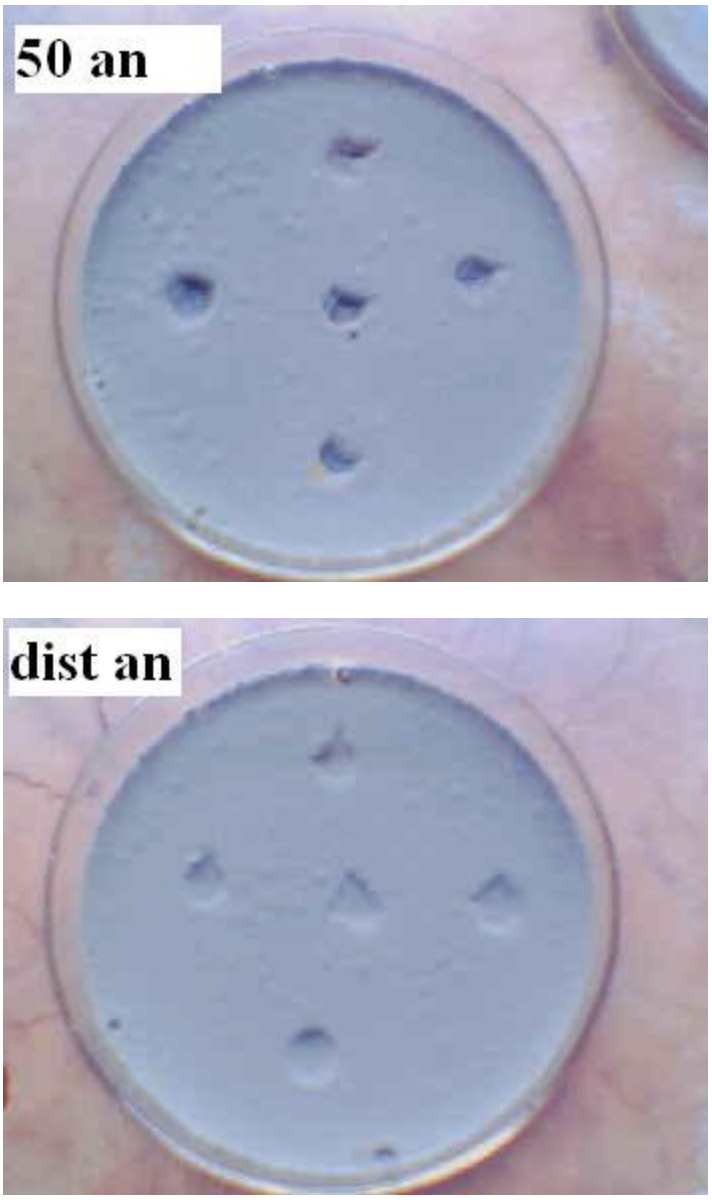
Appendix 26; Effect of *M. balsamina* (75 mg) on *Aspergillus niger.* (an = *A. niger*; dist. = distilled water)



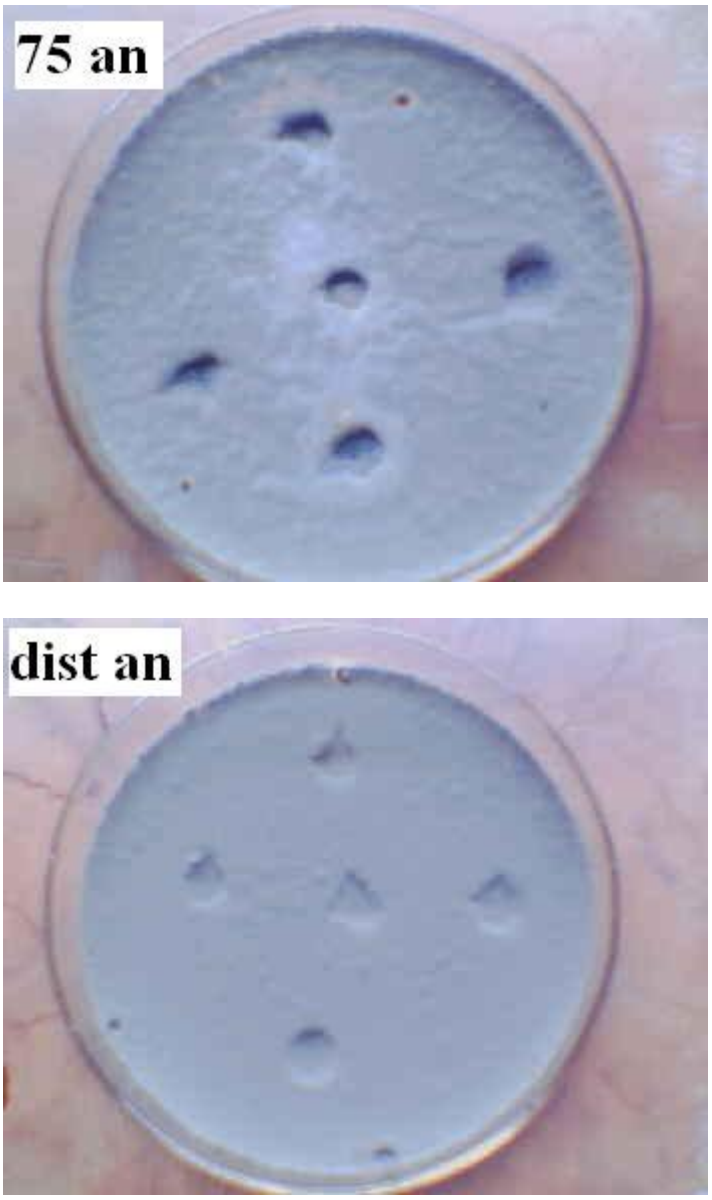
Appendix 27; Effect of *M. balsamina* (75 mg) on *Aspergillus fumigatus.* (af = *A. fumigatus*; dist. = distilled water)



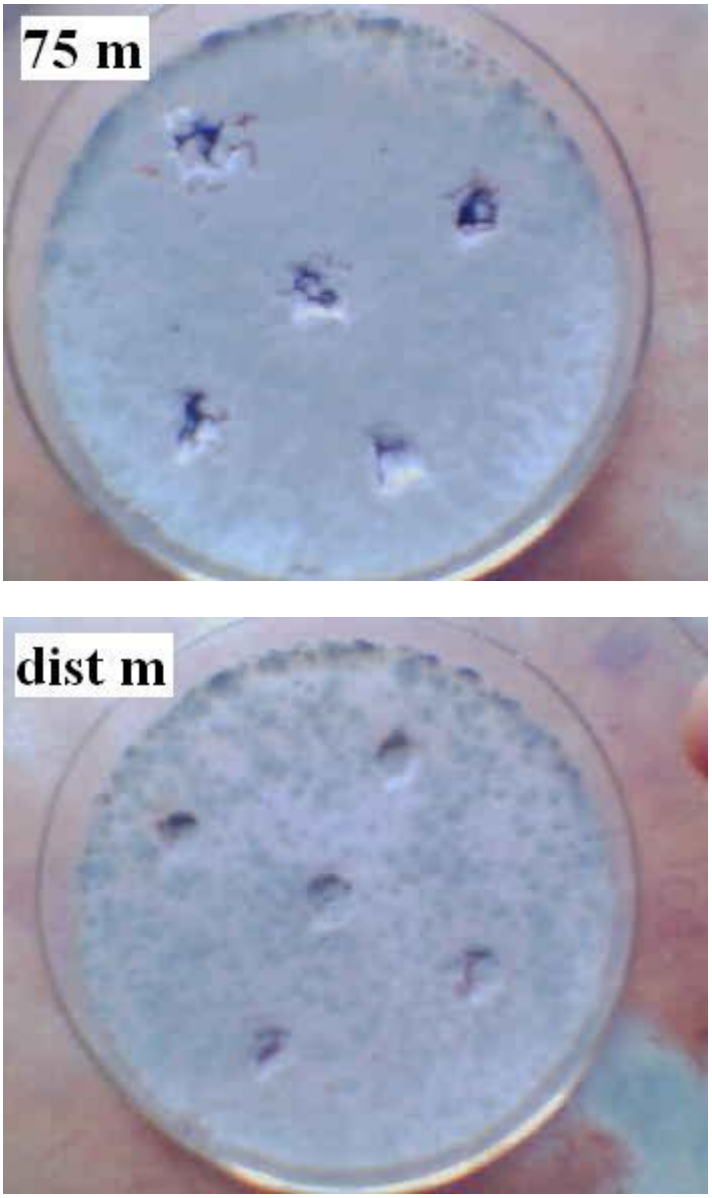
Appendix 28; Effect of *M. balsamina* (50 mg) on *M. pusillus.* (m = *M. pusillus*; dist. = distilled water)



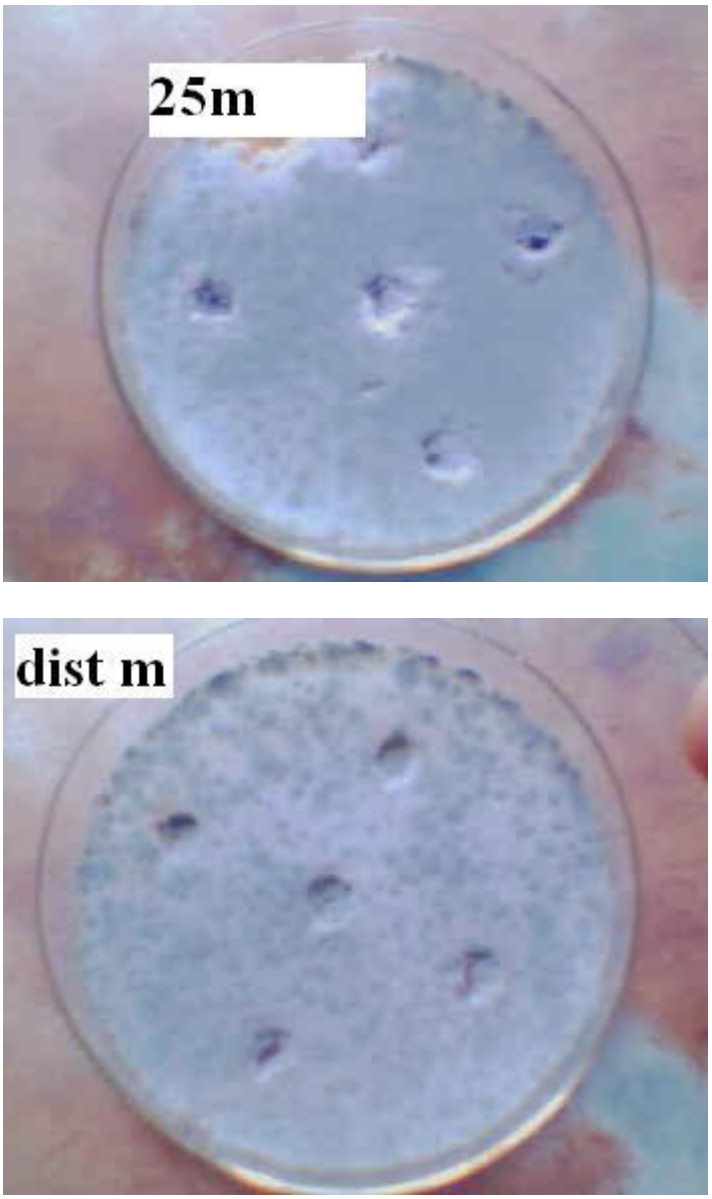
Appendix 29; Effect of *M. balsamina* (50 mg) on *A. niger*. (an = *A. niger*; dist. = distilled water)



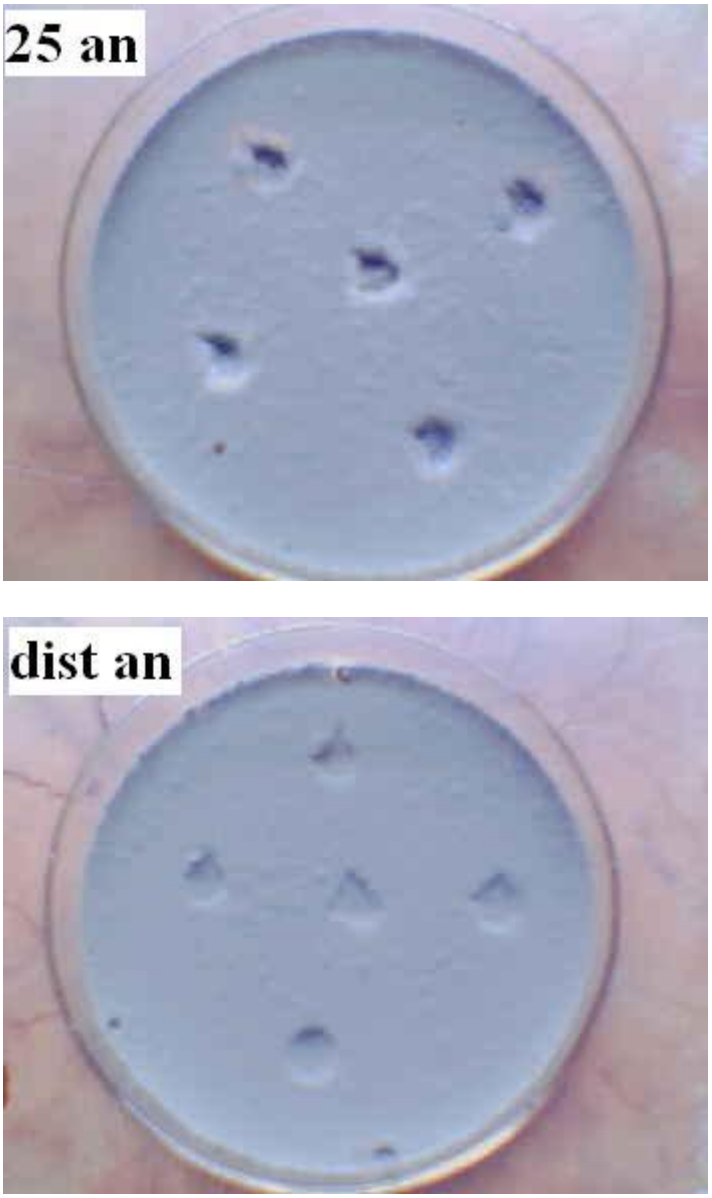
Appendix 30; Effect of *M. balsamina* (75 mg) on *A. niger*. (an = *A. niger*; dist. = distilled water)



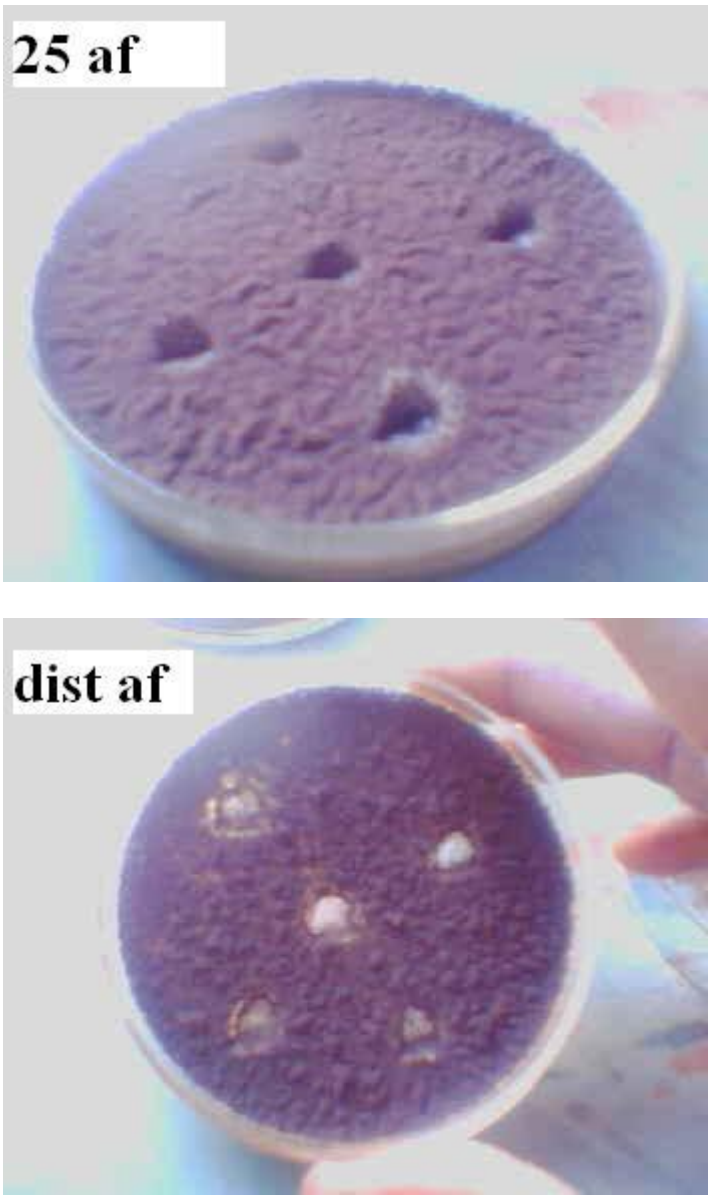
Appendix 31; Effect of *M. balsamina* (75 mg) on *M. pusillus.* (m = *M. pusillus*; dist. = distilled water)



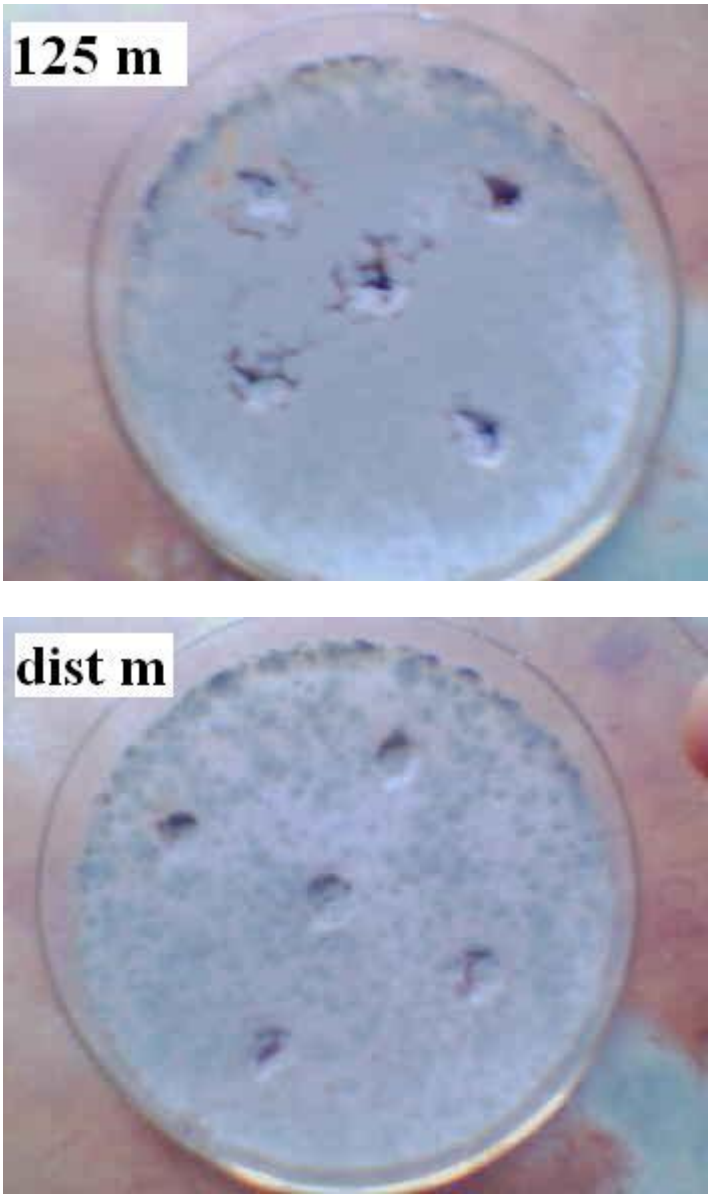
Appendix 32; Effect of *M. balsamina* (25 mg) on *M. pusillus.* (m = *M. pusillus*; dist. = distilled water)



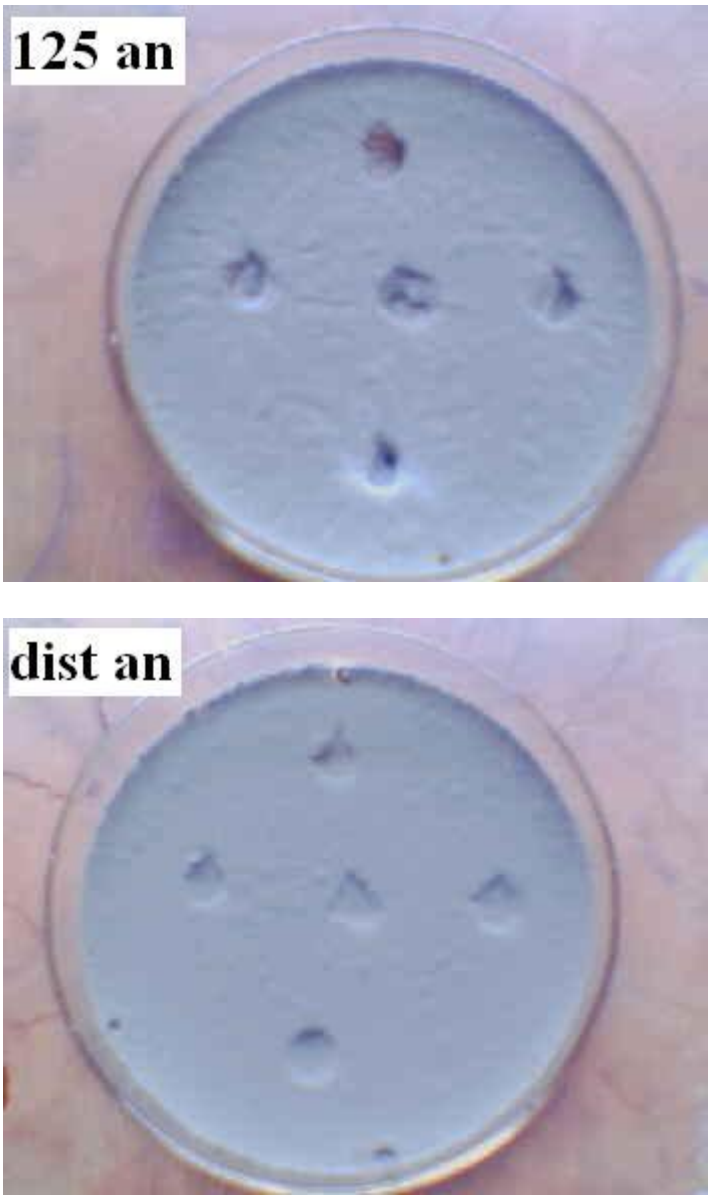
Appendix 33; Effect of *M. balsamina* (25 mg) on *A. niger*. (an = *A. niger*; dist. = distilled water)



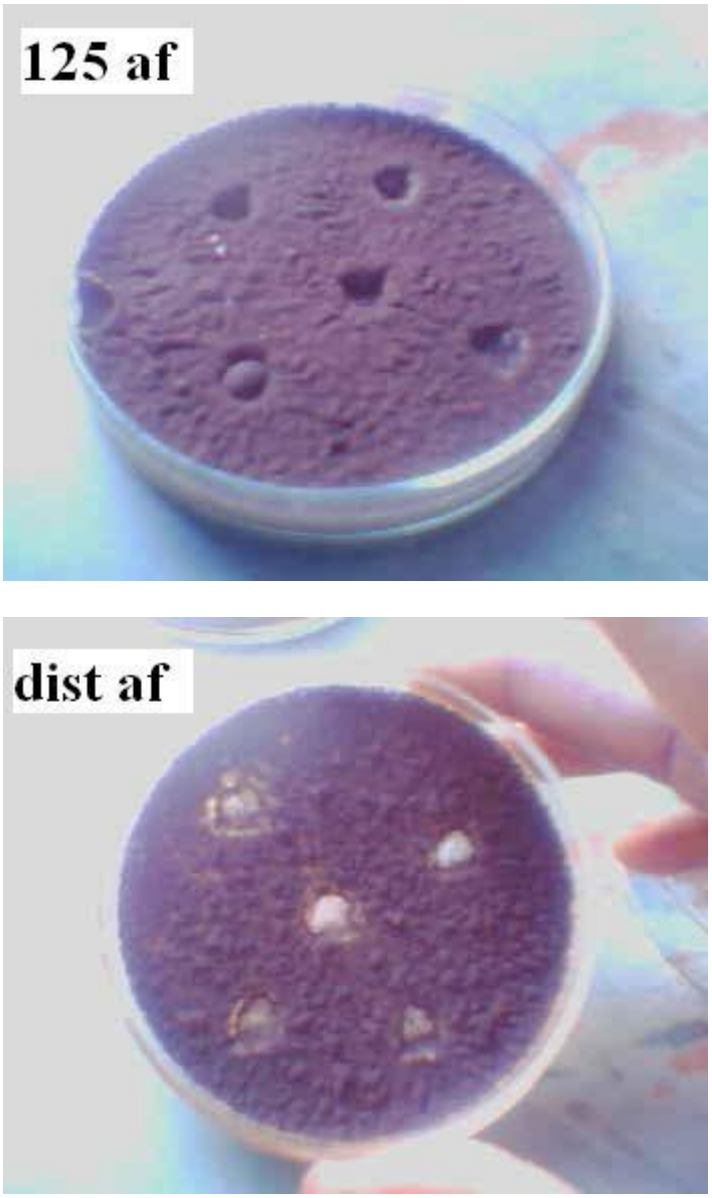
Appendix 34; Effect of *M. balsamina* (25 mg) on *A fumigatus.* (af = *A. fumigatus*; dist. = distilled water)



Appendix 35; Effect of *M. balsamina* (125 mg) on *M. pusillus.* (m = *M. pusillus*; dist. = distilled water)



Appendix 36; Effect of *M. balsamina* (125 mg) on *A. niger*. (an = *A. niger*; dist. = distilled water)

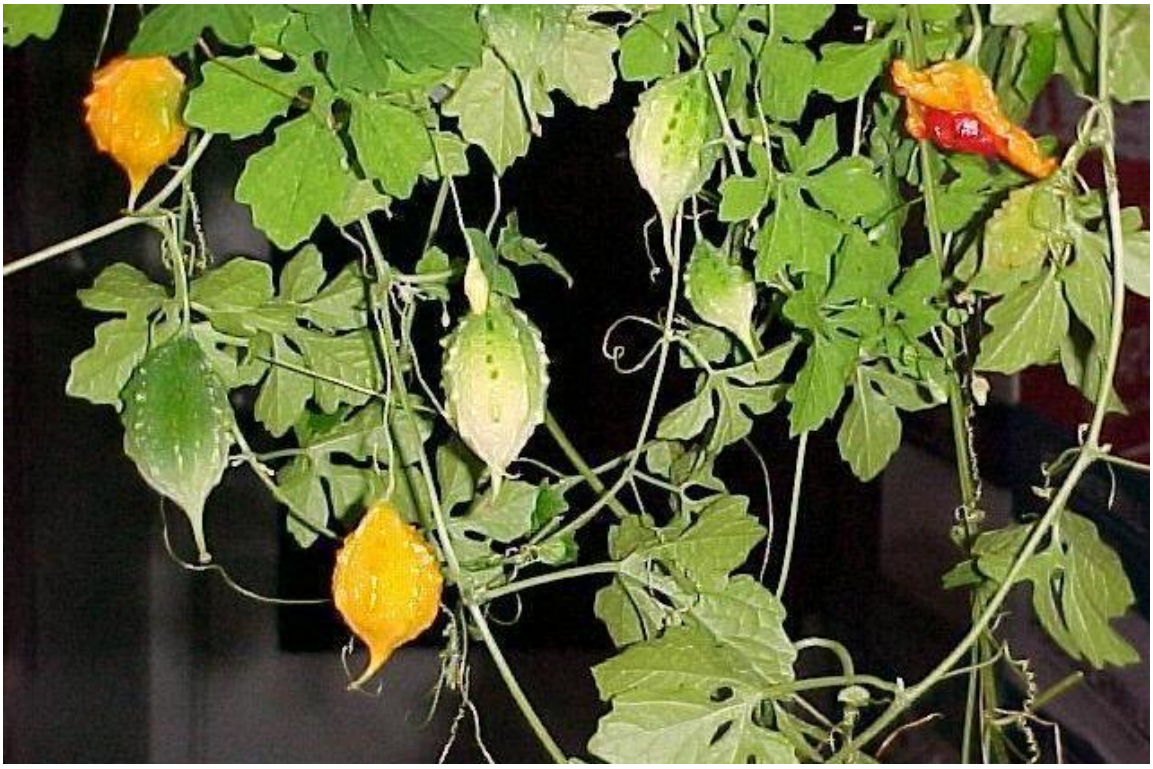


Appendix 37; Effect of *M. balsamina* (125 mg) on *Aspergillus fumigatus.* (af = *A. fumigatus*; dist. = distilled water)

Appendix 38. Pictures of *Momordica balsamina.*

Appendix 39; *M. balsamina* fruit and flower

Appendix 40; *M. balsamina* leaves and flower



Appendix 41; *M. balsamina* ripe and unripe fruits