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**UNIVERSITY OF NAIROBI
(UON)**



**COLLEGE OF HEALTH SCIENCES
SCHOOL OF PHARMACY.**

**AN INVESTIGATION ON THE PHYTOCHEMICAL COMPONENTS,
THE ANALGESIC AND ANTI INFLAMMATORY ACTIVITIES OF A
TRADITIONALLY USED PLANT EXTRACT.**

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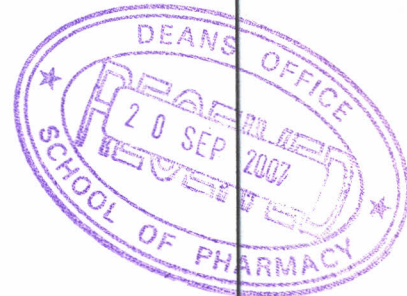
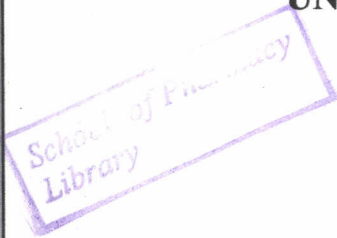
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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE AWARD OF A BACHELORS DEGREE IN
PHARMACY.

2007




DECLARATION

I declare that this dissertation is my original work and that to the best of my knowledge, it has not been presented for evaluation by anyone else in any other institution.

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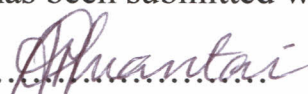
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DEDICATION.

To almighty God for life and good health.

To my late mother who believed in me and would have wished to see me grow into a responsible person, may the Almighty God rest her soul in an eternal place.

ACKNOWLEDGEMENTS.

I would like to thank the following persons:-

My supervisor, Prof.A.N.Guantai for the immeasurable assistance and guidance she accorded me during the course of this work.

My family members, especially my dad for his unwavering support in terms of resources and words of encouragement.

The technical staff, department of pharmacology and pharmacognosy especially Mr. Mwalukumbi and Mr. Maloba who assisted and guided me in my laboratory work.

ABSTRACT/SUMMARY

Inflammatory diseases including different types of rheumatic diseases like rheumatic fever and rheumatoid arthritis are a major world wide clinical concern. Such conditions are mainly managed using drugs like NSAIDS eg. Indomethacin and corticosteroids e.g. Dexamethasone which have notable side effects like gastrointestinal irritation and ulceration hence a major problem. There is therefore need for increased research and use of safer drugs for treatment of arthritis and herbal remedies have proved useful.

This study was aimed at assessing the anti-inflammatory activity of an herbal remedy used traditionally in management of arthritis and determine its analgesic activity and oral acute toxicity. Inflammation was induced using the formalin hind paw induced edema method with Indomethacin, Dexamethasone and DMSO as controls. Analgesic activity was studied using the hot plate method with morphine and normal saline as controls.

The results obtained identified anti-inflammatory activity but no analgesic activity. The herbal plant extract produced 40% inhibition of inflammation compared to 52% and 60% produced by Dexamethasone and Indomethacin respectively.

The plant extract thus has significant anti-inflammatory activity and thus is a good candidate for further studies in order to put it into more significant clinical use.

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LIST OF ABBREVIATIONS

UON	University of Nairobi.
RA	Rheumatoid Arthritis.
OA	Osteoarthritis.
DMSO	Dimethyl sulphoxide.
NSAIDS	Non steroidal anti-inflammatory drugs.
WHO	World Health Organization.
COX	Cyclo oxygenase enzyme.
V_i	Initial paw volume
V_f	Final paw volume.
ΔV_c	Change in paw volume for control.
ΔV_t	Change in paw volume for test.

CHAPTER 1: INTRODUCTION AND LITERATURE.

1.1 BACKGROUND

Arthritis usually involves some form of damage to joint or destruction of joint parts like articular cartilage, synovial tissue etc. There are more than one hundred different forms of arthritis. They are similar to each other in the symptoms they produce, which includes sore, stiff, inflamed, and painful joints. Beyond these common symptoms, the various forms of arthritis are quite different from each other. Most forms of arthritis can be subdivided into three major categories:

- 1) rheumatoid arthritis,
- 2) osteoarthritis, and
- 3) Gouty arthritis.

1.1.1 Rheumatoid Arthritis (RA)

Rheumatoid arthritis (RA) is an autoimmune disorder characterized by a chronic erosive inflammation in joints leading to the destruction of cartilage and bone. Other systemic problems throughout the body may also develop, including inflammation of blood vessels (vasculitis), the development of bumps (rheumatoid nodules) in various parts of the body, lung disease, blood disorders, and weakening of the bones (osteoporosis). In rheumatoid arthritis, the synovial membrane becomes severely inflamed. Usually thin and delicate, the synovium becomes *thick and stiff, with numerous infoldings on its surface.*

The membrane is invaded by white blood cells, which produce a variety of destructive chemicals. The cartilage along the articular surfaces of the bones may be attacked and destroyed, and the bone, articular capsule, and ligaments may begin to erode. These processes severely interfere with movement in the joint. ^[1]

RA exists all over the world and affects men and women of all races. In the United States alone, about two million people suffer from the disease. Women are three times more likely than men to have RA. About 80% of people with RA are diagnosed between the ages of 35 and 50. RA appears to run in families, although certain factors in the environment may also influence the development of the disease. ^[2]

The underlying event that promotes RA in a person is unknown. Given the known genetic factors involved in RA, some researchers have suggested that an outside event occurs and triggers the disease cycle in a person with a particular genetic makeup. In late 2001, researchers announced discovery of the genetic markers that predict increased risk of RA. The discovery should soon aid research into diagnosis and treatment of the disease. Recent research has also shown that several autoimmune diseases, including RA, share a common genetic link. In other words, patients with RA might share common genes with family members who have other autoimmune diseases like systemic lupus, multiple sclerosis, and others. ^[2]

1.1.2Osteoarthritis (OA)

In this case, cartilage begins to break down and wear away. It is no longer able to cushion the contact of bones with each other. One bone rubs directly on the other bone. It becomes very painful to move the joint. Scientists recognize two forms of OA: primary and secondary osteoarthritis. ^[3]

Primary OA is caused by abnormal stresses on healthy joints or by normal stresses on weakened joints. The joints most commonly affected by primary OA include the finger joints, hips and knees, the lower joints of the spine, and the big toe.

There is some evidence that primary OA is caused by genetic factors. Obesity is often a contributing factor. The heavier a person is, the greater the pressure on his or her joints. Finally, some researchers believe that primary OA may be caused by bone disease, liver problems, or other abnormal conditions in the body. ^[3]

Secondary OA is caused by a chronic (long-term) or sudden injury to a joint. Some factors that may contribute to the development of OA include:

- Physical trauma (shock), including sports injuries
- Repetitive stress associated with certain occupations, such as construction, assembly line work, computer keyboard operation, and hair-cutting.
- Repeated episodes of gout or other forms of arthritis.
- Poor posture or bone alignment caused by abnormal body development.

1.1.3 Gout (disease of the kings)

Throughout history, gout has been called the "disease of kings" because it can be caused by the over consumption of rich foods. Today, we know that gout is caused by the accumulation of uric acid crystals in a joint, most often the joint of the big toe.

Uric acid is produced in the body when proteins are broken down. It is water soluble and usually dissolves in urine then excreted from the body.

Some people, however, produce an unusually large amount of uric acid. Such a tendency is thought to be caused by genetic factors. When that happens, uric acid remains in the body, circulating through the bloodstream. Eventually, it is deposited as needle-like crystals in joints. These crystals cause friction when the joint is moved. The friction causes severe pain known as gout. ^[4]

1.2 Symptoms of arthritis.

All forms of arthritis share certain symptoms in common. These symptoms include pain, swelling, and stiffness in the joint. These symptoms may develop slowly over time or they may begin quite suddenly. After a period of time, joints may actually become deformed. Patients may find it difficult to straighten their fingers and toes, or their hands and feet may curve outward in an abnormal way. Eventually, a patient may lose the use of a joint entirely. ^[2]

Patients with RA often report other symptoms also. These symptoms include increased fatigue, loss of appetite and weight loss, and, sometimes, fever. RA may also be accompanied by the development of rheumatoid nodules.

Rheumatoid nodules are bumps that appear under the skin, in tissue covering the lungs and chest, or in the brain and spinal cord. These nodules can cause serious complications, including shortness of breath, poor blood circulation, gangrene (tissue decay), and damage to nerves. ^[2]

1.3 Diagnosis

Rheumatoid arthritis and osteoarthritis are usually both diagnosed based on a patient's history. This history typically includes an increasing occurrence of pain and stiffness in joints. The doctor can also examine the patient's affected joint for swelling, limitations on movement, pain, and a cracking sound that is sometimes heard with a damaged joint. ^[1]

There are no blood tests that strongly confirm the presence of arthritis. Many tests that can be used for RA are also positive for other disorders. Blood tests include a special test of red blood cells, the erythrocyte sedimentation rate, which is positive in nearly 100% of patients with RA. However, this test is also positive in a variety of other diseases. One test measures the amount of a chemical known as rheumatoid factor in a patient's blood.

Rheumatoid factor is an autoantibody found in about 66% of patients with RA. However, it is also found in about 5% of all healthy people and in 10–20% of healthy people over the age of 65. Rheumatoid factor is also positive in a large number of other autoimmune diseases and other infectious diseases. ^[4]

A long, thin needle can be inserted into a synovial joint to withdraw a sample of the synovial fluid for examination. In RA, this fluid has certain characteristics that indicate active inflammation. The fluid will be cloudy, relatively thinner than usual, with increased protein and decreased or normal glucose. It will also contain a higher than normal number of white blood cells. While these findings suggest inflammatory arthritis, they are not specific to RA. ^[2]

A good diagnosis for OA can sometimes be obtained from X rays or other imaging techniques. An X-ray photograph may show changes in the space between bones in a joint, indicating the presence of OA. ^[1]

1.4 Treatment

The first line of treatment for most forms of arthritis is medication to reduce inflammation, swelling, and pain. Aspirin, acetaminophen, and ibuprofen, are all effective in this regard. In fact, people with mild cases of arthritis can often control their condition satisfactorily simply with one of these drugs. ^[4]

In more severe cases of arthritis, stronger medications may be required. The most common of these is one of the corticosteroids. The corticosteroids are very effective in the treatment of pain, swelling, and inflammation. Other drugs called disease-modifying antirheumatic drugs. These include gold compounds, D-penicillamine, antimalarial drugs, and sulfasalazine. Methotrexate, azathioprine, and cyclophosphamide are all drugs that suppress the immune system and can decrease inflammation. Medications used to treat malaria can also be helpful. ^[2]

Rest and supportive devices may also be important in the treatment of arthritis. When the pain becomes too intense, patients may be advised to take to their bed and stay there until they experience relief. They may also be provided with various protective measures, such as neck braces and collars, crutches, canes, hip braces, and knee supports. [5]

Physical therapy can also be an important component of treatment programs. Physical therapists can teach patients how to exercise their affected joints. Exercise may reduce the rate at which the joints are worsening. It may increase the patient's balance, flexibility, and range of motion. Physical therapy can also consist of massage, moist hot packs, and soaking in a hot tub. [5]

In the most severe cases, surgery may be required. Some surgical techniques that can be used include: replacement of a damaged joint ,fusion (joining together) of spinal bones, scraping or removing damaged bone from a joint and removal of a bone chip to allow realignment of a joint.

1.5 Alternative Treatment

Some food supplements have been found to be effective in treating arthritis. One substance that is commonly recommended is a combination of glucosamine and chondroitin sulphate. This product is thought to help repair cartilage. . Nutritionists suggest that a vegetarian diet low in animal products and sugar may help to decrease both inflammation and pain from RA. Beneficial foods for patients with RA include cold water fish (mackerel, herring, salmon, and sardines) and flavonoid-rich berries (cherries, blueberries, hawthorn berries, blackberries, etc.). The enzyme bromelain, found in pineapple juice has also been found to have significant anti-inflammatory effects.

Other nutritional supplements that have been suggested include vitamins A, B, C, and E, and the minerals selenium and zinc. ^[6]

Traditional Chinese medicine emphasizes the use of various herbs for the treatment of arthritis. These herbs include: Turmeric (*Curcuma longa*) which contains curcuminoids, volatile oils, polysaccharides, free monosaccharides eg arabinose, fructose and galactose. Ginger (*Zingiber officinale*) which contains volatile oils, resinous matter, starch and mucilage. Feverfew (*Chrysanthemum parthenium*) contains sesquiterpene lactones eg parthenolide. Devil's claw (*Harpagophytum procumbens*) whose roots contain iridoid glycosides, flavonoids, various phenolic acids, triterpenes like oleanic acid and ursolic acids, aquinine and high concentration of glycoside sugars eg tachyons. Liquorices (*Glycyrrhizin glabra*) contains glycyrrhizin, glycyrrhizic acid, polysaccharides and B-sitosterol, Lobelia (*Lobelia inflata*) mainly contains alkaloids eg lobelanine, lobelidine and piperidineines. ^[5]

Naturopathic treatment may include hydrotherapy (water therapy), diathermy (deep-heat therapy), nutritional supplements, and various herbs; Hydrotherapy can help to greatly reduce pain and inflammation. Moist heat is more effective than dry heat, and cold packs are useful during acute flare-ups. ^[5]

Meditation, hypnosis, guided imagery, relaxation, and reflexology techniques have been used effectively to control pain. Stiff joints may also be loosened up with a warm sesame oil massage, followed by a hot shower to further heat the oil and allow entry into the pores. ^[2]

Acupressure and acupuncture have also been used for pain; work on the pressure points should be done daily in combination with other therapies. Bodywork can be soothing and is thought to improve and restore chemical balance within the body. Yoga has been used for RA patients to promote relaxation, relieve stress, and improve flexibility. A massage with rosemary and chamomile, or soaking in a warm bath with these essential oils can provide extra relief. ^[2]

RA is often connected with food allergies or intolerances. An elimination/challenge diet can help to decrease symptoms of RA as well as identify the foods that should be eliminated to prevent flare-ups and recurrences. Splints may be used to support and rest painful joints. Later, after inflammation has somewhat subsided, physical therapists may provide a careful exercise regimen in an attempt to maintain the maximum degree of flexibility and mobility. Joint replacement surgery, especially for the knee and the hip joints, is sometimes recommended when these joints have been severely damaged. Also surgery used to stop pain in a stiff joint, such as the ankle, is the fusion of the affected bones together (arthrodesis, or artificial anklylosis).^[4]

1.6 Plants contained in the herbal extract

1.6.1 *Nigella sativa*

Nigella sativa is an annual flowering plant which grows to 20-30 cm tall, with finely divided, linear (but not thread-like) leaves. The flowers are delicate, and usually coloured pale blue and white, with 5-10 petals. The fruit is a large and inflated capsule composed of 3-7 united follicles, each containing numerous seeds.

It is native to southwest Asia but grows in many areas of the world.

The seed is used as a spice. Frequently the seeds are referred to as black cumin; this is, however, also used for a different spice, *Bunium persicum*. The *Nigella sativa* seeds have many acclaimed medicinal properties such as bronchodilatory, hypotensive, antibacterial, antifungal, analgesic, anti-inflammatory and immunopotentiating and are universally accepted as a panacea.

Nigella sativa has been used for centuries, both as a herb and pressed into oil, by people in Asia, Middle East, and Africa for medicinal purposes. It has been traditionally used for a variety of conditions and treatments related to respiratory health, stomach and intestinal health, kidney and liver function, circulatory and immune system support, and for general overall well-being.

The seeds have been traditionally used in the Middle East and Southeast Asian countries to treat ailments including Asthma, Bronchitis, Rheumatism and related inflammatory diseases, to increase milk production in nursing mothers, to promote digestion and to fight parasitic infections. Its oil has been used to treat skin conditions such as eczema and boils and to treat cold symptoms. It mainly contains alkaloids and volatile oils. [7]

1.6.2 Malva verticilata

Malva verticilata belongs to the Malvaceae family and is variously called Mellow or musk mellow. It is an erect annual or biennial plant, 60-90 cm tall. Leaves are alternate, rounded, palmately lobed (5 or 7 lobed). The flowers are in dense axillary clusters with involucre bracts and white or purplish petals, about 1 cm long. The stamens are united in a tubular column, style branches are filiform. The fruit have discoid mericarps and are 1-seeded.

It is cultivated widely in Korea where it is known as A-wook and distributed in Eurasia. The seed is used and has reticuloendothelial system potentiating, anticomplementary, hypoglycaemic effect and contains mucilages, polysaccharides composed of beta-1, 3-linked D-galactose residues flavonoids and anthracyanidins eg. malvidin

Traditionally, it has been used in management of postpartum fever, poisoned dermatitis from contact with varnish tree, abortifacient. [7]

1.6.3 Caesalpinia volkensii

Caesalpinia volkensii is a perennial climbing shrub which is drought resistant and grows in many parts of Africa especially Ethiopia, Kenya, Tanzania and Uganda. It belongs to the Caesalpinoideae family. It is also known as Mkomwe (Swahili), mubuthi, muchuthi (kikuyu), msoo miba (shambaa).

Phytochemical investigation shows that the plant contains cyanogenetic glycosides, saponins, tannins, mucilage and anthocyanins.

Used for many common diseases like malaria. The extract is taken by pregnant women who have some pains, stomach troubles.

Roots are used as aphrodisiac by shambaa bonddei. [8]

1.6.4 *Wedelia calendulacea*.

It is a perennial herb which belongs to the expansive compositae family. Phytochemical investigation of *Wedelia calendulacea* reveals three diterpenes and a steroid. The diterpenes were identified as (-)-kaur-16-en-19-oic acid , 3x-tigloyloxykaur-16-en-19-oic acid , and 3x-angeloyloxykaur-16-en-19-oic acid . It is used in liver disorders, uterine hemorrhage and menorrhagia.

Osteoporosis in women occurs mainly due to estrogen deficiency following menopause. Studies indicate that isoflavones are estrogenic enough to promote bone formation.

Studies have shown that the ethanol extract of the plant indeed has a definite protective effect. [7]

1.6.5 *Strychnos heningisii*.

It is native among the kamba where it is called Muteta.

Phytochemical investigation reveals that the plant mainly contains alkaloids especially strychnine and brucinine in the seeds and other plant parts.

Traditionally the decoction from roots of the plant is drunk as a treatment of chest pains, internal injuries.

The fresh roots can be chewed for snake bite treatment. [8]

1.6.6 *Urtica massaica*

It belongs to the genus *Urtica* in Urticaceae family, which contains between 30-45 species of flowering plants commonly called the Nettles. The stinging hairs of most nettle species contain formic acid, serotonin and histamine; however recent studies implicate oxalic acid and tartaric acid.

Nettle is believed to be a galactagogue and a clinical trial has shown that the juice is diuretic in patients with congestive heart failure

Extracts of the plants can be used to treat arthritis, anemia, hay fever, kidney problems, benign prostate hyperplasia (BPH) and pain.

The macerated roots used for treatment of hepatic diseases.

1.6.7 *Mallotus rotundifolia*.

The trichomes and glands are used. It occurs as a dull reddish brown powder without odour. The characteristic globular glands contain red resin and radiating groups of unicellular curved trichomes. It contains the antihelminthic phloroglucinol derivatives rottlerin and isorottlerin, resins and wax. It is also used for treatment poultry diseases.

1.7 Experimental methods of induction of arthritis.,

1.7.1 Paw edema induced by carrageenan.

0.1 ml of 1% carrageenan in 0.9% NaCl is administered into the plantar surface of the right hind paw of the animals. The experimental groups, negative control group (2.5% DMSO and 2.5% tween 20), and positive control group (10 mg/kg indomethacin) are given either the control drug or test compounds orally, an hour prior to the administration of the carrageenan. Before injection of carrageenan, the average volume (V_0) of the right hind paw of each rat is calculated from 3 readings that do not deviate more than 3%. After injection of the phlogistic agent, readings (V_t) are obtained for each rat at 30, 60, 120, 180, 240, 300 and 360 min, with the aid of a Ugo Basil Plethysmometer. ^[9]

1.7.2 Paw edema induced by histamine and serotonin

Serotonin and histamine are used as the phlogistic agents. The inflammatory mediators are administered one hour after the injection of test preparations. Pyrilamine maleate (1 mg/kg) is used as the antagonist of histamine. The volume of paw edema is determined plethysmographically. ^[10]

1.7.3 Paw edema induced by formalin.

Acute inflammation can also be induced by subaponeurotic injection of 0.1 ml of 2% formalin one hour after oral administration of n-butanol fraction, diclofenac (5 mg/kg), or vehicle (solution of 2.5% DMSO and 2.5% tween 20). The volume of paw is determined one, two, and four hours following the injection of formalin. The changes in the volume of paw are measured plethysmographically. ^[10]

1.9 Rationale of study

Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis, and quinine. The World Health Organization (WHO) estimates that 80 percent of the world population presently uses herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all traditional medicine systems and a common element in Ayurvedic, homeopathic, naturopathic, traditional Chinese medicine and Native American Indian medicine. According to the WHO, 74% of 119 modern plant-derived pharmaceutical medicines are used in ways that correlated directly with their traditional uses

This study aims at scientifically rationalizing the use of phytomedicines already in use for management of arthritis. The activity of these preparations could be due to a single anti-inflammatory compound or could be due to synergistic effects of various components co-existing in plant or plant mixtures.

Objectives.

Main objective

To assess the anti-inflammatory activity of an herbal remedy used traditionally in management of arthritis.

Specific objectives.

- 1) To establish the phytochemical constituents of the plant extract.
- 2) To study any acute toxicity associated with administration of the plant extract.
- 3) To test for the anti-arthritic/anti-inflammatory effect of the plant extract.

CHAPTER 2 METHODOLOGY/EXPERIMENTAL.

2.0 Study area

Pharmacology and pharmacognosy laboratories,
School of Pharmacy,
UON.

2.1 Study population-the study is designed to be carried out on laboratory rats.

2.2 Materials and reagents

(i) Plant material.

The plant material was provided by my supervisor, Prof.A.Guantai as a crude drug powder.

(ii)Formalin 2% was used to induce inflammation, Morphine, Dexamethasone and Indomethacin were used as standards, and Dimethyl sulphoxide and normal saline (0.9% sodium chloride) were each used as solvent and control for the anti-inflammatory and analgesic testing respectively. Rats and mice were used as experimental animals.

Mercury manometer was used to determine the degree of inflammation; Hot plate was improvised and used to induce a pain stimulus.

VERY WILD

VERY WILD

2.3 Preparation of extracts

About 4 litres of water and 2 lemons sliced into 4 pieces were placed in a sizeable container Eg. sufuria and boiled covered for 7 minutes. The water was removed from fire and the drug poured in then covered tightly to infuse for 6-7 hours

The residue was sieved and squeezed to extract most of the active drug from the plant material. The volume of the extract obtained was then reduced by rotary evaporation before being freeze dried to a dry mass. This is dissolved in water to get the required concentration in order to obtain dose for the mice/rats.

VERY WILD

2.4 Oral acute toxicity testing.

12 mice were divided into 4 groups of 3 animals each. One group served as a control and was given 0.9%Nacl alone intraperitoneally orally; while the rest were given increasing doses of the aqueous herbal extract 15, 30 and 60mg/kg orally respectively. The doses were selected by calculating on basis of weight/kg of mice from the human dose.

2.5 Analgesic activity using hot plate method.

8 mice were selected according to their sex (male) and weight (25-30grams) and divided into 4 groups each consisting of two mice. The control group was treated to 2mg/kg normal saline IP. The second group was given morphine (50mg/kg) via the same route as a reference drug.

The remaining groups were treated with the plant extracts, 15mg/kg and 30mg/kg respectively. The mice were placed on a hot plate maintained at a constant temperature (550c) and observed at intervals of 5 minutes for 1 hour.

2.6 Anti-inflammatory activity.

The formalin-induced paw edema method was used. Indomethacin and Dexamethasone were suspended in DMSO while the plant extract is dissolved in water. The compounds were administered intraperitoneally in dose volumes of 0.5ml into male albino rats (180-210grams), one hour before injection of 0.1ml of 2% formalin into the sub plantar area of the left hind paw.

The doses employed were: Indomethacin 1.67, 3.00 and 5.00mg/kg of body weight, Dexamethasone 1.00, 2.00 and 4.00mg/kg of body weight and Compound X 10.5 and 21mg/kg of body weight. Control animals were given 0.5ml of the vehicle, 1 hour before the injection of Formalin. Before formalin injection, the initial paw volume (V_i) was measured using mercury displacement method. The volume of paw was determined one, two and four hours following the injection of formalin. Four hours after formalin administration, the final paw volume (V_f) was measured.

2.7 Phytochemical analysis.

2.7.1 Procedures

2.7.1.1 Alkaloids

The plant raw powder was dissolved in 0.2ml of 1%hydrochloric acid and to 0.1ml portions the following added

- i) one drop of Mayer's reagent.
- ii) one drop of dragendroffs reagent. ^[13]

2.7.1.2 Glycosides

About 1gm of the plant material was extracted with 10ml of 70%alcohol by heating on a water bath for 2 minutes, cooled and centrifuged. To the filtrate, 10ml of water and 5 drops of lead sub acetate were added, filtered and 10% sulphuric acid added drop wise until no precipitate was formed. It was then filtered and extracted with 2 successive 5ml portions of chloroform and the extracts combined. The filtrate was passed through a cotton plug and divided into two portions.

a) Test for unsaturated lactone ring in aglycone (kedde's test) ^[13]

The extract was evaporated to dryness and added with 1 drop of 90% alcohol and 2 drops of dinitrobenzoic acid in 90%alcohol.the solution was made alkaline with sodium hydroxide solution.

b) Test for deoxy sugar (Keller killian test) ^[13]

The extract was evaporated to dryness and added with 0.4ml of glacial acetic acid containing traces of ferric chloride. The solution formed was transferred to a test tube and 0.5ml of concentrated sulphuric acid added.

2.7.1.3 Tannins.

About 2gms of coarsely powdered extract were boiled with 20ml of water, cooled, and filtered and to 2ml portions added with few drops of ferric chloride,1ml solution of lead sub acetate and1ml solution of potassium dichromate. ^[14]

CHAPTER 3. RESULTS AND DISCUSSION

3.1 Phytochemical analysis.

3.1.1 Test for alkaloids

A slight white precipitate formed with one drop of Mayer's reagent and a yellow orange precipitate is produced with a drop Dragendroff's reagent confirming that the plant extract contains alkaloids.

3.1.2 Test for glycosides

The plant extract contains glycosides as it produced a positive Kedde's test; a brown – purple color was produced in Kedde's test.

3.1.3 Tests for Tannins.

A greenish black color was observed when a few drops of $FeCl_3$ were added to the plant extract and a buffy white precipitate was formed when 1 ml of lead sub acetate was added to a sample of the extract confirming the presence of tannins.

3.1.4 Test for saponins.

The plant extract formed persistent froth when shaken with water and hemolysed red blood cells suggesting the presence of saponin compounds in the plant extract.

3.2 Acute toxicity.

There was no toxicity observable when the recommended human therapeutic doses were administered. This included doses of 15, 30 and 60 mg/kg of body weight.

Therefore more sensitive procedures and different species should be used to get a better toxicological picture of the plant extract.

3.3 Analgesic potency using hot plate method.

TABLE 1: TABLE SHOWING RESPONSE OF MICE TO A PAIN STIMULUS OVER 1 HOUR AFTER INJECTION OF SOME COMPOUNDS.

KEY: NR-No response-The animal did not respond to stimulus (heat) within 30 seconds.

R-Response- animal responded to stimulus within 30 seconds or minutes.

		TIME(minutes)												
MICE	DRUG	1	5	10	15	20	25	30	35	40	45	50	55	60
Group 1 a	Normal Saline	R	R	R	R	R	R	R	R	R	R	R	R	R
B	Normal Saline	R	R	R	R	R	R	R	R	R	R	R	R	R
Group 2a	Morphine	R	R	R	R	R	R	R	NR	NR	NR	NR	NR	NR
B	Morphine	R	R	R	R	R	R	NR	NR	NR	NR	NR	NR	NR
Group 3a	Extract. 15mg/kg body wt.	R	R	R	R	R	R	R	R	R	R	R	R	R
B	Extract. 15mg/kg body wt.	R	R	R	R	R	R	R	R	R	R	R	R	R
Group 4a	Extract. 30mg/kg body wt.	R	R	R	R	R	R	R	R	R	R	R	R	R
B	Extract. 30mg/kg body wt.	R	R	R	R	R	R	R	R	R	R	R	R	R

Discussion.

Group 1.Normal saline-control.

The response to stimulus (hot plate) was observed through out the 1hour test period. This represents the expected observations on untreated normal individual/mice.

Group 2-Morphine.

The animals initially responded to stimuli but after some time, response diminished. After drug administration, time is required for drug to be transported to the site of action.

Explanation-Analgesic activity of morphine has spinal and supraspinal components.

Spinal-It acts in the substantia gelatinosa of dorsal horn to inhibit release of excitatory neurotransmitters from afferents carrying pain impulses. The release of substance P from the post synaptic action on the dorsal horn neurons inhibited by morphine hence temporary loss of pain sensation.

Supraspinal-sites involved include medulla, midbrain, limbic and cortical areas which may alter processing and interpretation of pain impulses as well as send inhibitory impulses through descending pathways to the spinal cord.

DEXAMETH

TEST PLAT

Group 3 and 4-Plant extract.

Response to stimulus was observed through out the period of investigation. This suggests that the plant extract has no analgesic activity or its analgesic potency cannot be detected using the hot plate method. However the plant extract contains components which individually have been used traditionally to relieve various types of pains eg. *Strychnos heningisii* and *caesalpinia volkensii*. The observed lack of analgesic activity could be due to very little analgesic activity undetectable using the method used or antagonistic effect of different components in the plant extract.

3.4 Anti-inflammatory activity.

TABLE 2: TABLE SHOWING VOLUMES OF MERCURY DISPLACED BY RAT PAW.

COMPOUND (DRUG)	DOSE Mg/Kg	VOLUME OF MERCURY DISPLACED (ML)					
		Before injection of formalin	Immediately after injection	After 1 hour	2 hours	3 hours	4 hours
INDOMETHACIN	1.67	0.14	0.16	0.18	0.18	0.16	0.15
	3.00	0.16	0.18	0.18	0.17	0.17	0.16
	5.00	0.12	0.17	0.18	0.16	0.15	0.14
DEXAMETHASONE	1.00	0.14	0.18	0.20	0.18	0.16	0.15
	2.00	0.14	0.18	0.20	0.18	0.16	0.16
	4.00	0.16	0.20	0.18	0.18	0.16	0.16
TEST (PLANT EXTRACT)	10.5	0.14	0.16	0.18	0.18	0.17	0.17
	10.5	0.15	0.17	0.18	0.17	0.16	0.16
CONTROL	10.0	0.14	0.18	0.18	0.17	0.17	0.17
	10.0	0.16	0.20	0.20	0.18	0.18	0.18

3.41 CALCULATIONS.

- $\Delta V_c = V_f - V_i$

- $\Delta V_t = V_f - V_i$

$$\% \text{ inhibition} = 100[1 - \Delta V_t / \Delta V_c]$$

TABLE 3: TABLE SHOWING CHANGES IN PAW VOLUME AND PERCENTAGE INHIBITION OF INFLAMMATION FOR TEST AND CONTROL DRUGS.

COMPOUND	INITIAL PAW VOLUME(V_i)	FINAL PAW VOLUME(V_f)	V_T OR V_C	$\Delta V_T/\Delta V_C$	% INHIBITION OF INFLAMMATION
CONTROL	0.150	0.175	0.025	0.00	00
HERBAL PREPARATION	0.150	0.165	0.015	0.60	40
DEXAMETHASONE	0.145	0.157	0.012	0.48	52
INDOMETHACIN	0.140	0.150	0.010	0.40	60

3.42 DISCUSSION

The control group showed the least percentage inhibition of inflammation. This was an indication of its lack of anti-inflammatory activity. The reduction in paw volume could be as a result of formalin elimination, metabolism or distribution to other tissues.

The herbal preparation showed considerable inhibition of inflammation compared to other drugs in clinical use. The herbal extract contains plant *Nigella sativa* which has anti-inflammatory activity. Its seeds have been used traditionally in the treatment of rheumatism and related inflammatory conditions like rheumatoid arthritis. It is therefore a potent anti-inflammatory agent.

Dexamethasone produced reduction in inflammation comparable to indomethacin's but greater than that produced by the herbal extract. It is a glucocorticoid agent with potent anti-inflammatory and immunosuppressive properties. Glucocorticoids induce the lipocortin-1 (annexin-1) synthesis, which binds to cell membranes preventing the phospholipase A2 from acting on its substrate arachidonic acid. This leads to diminished eicosanoid production. The COX enzyme expression is also suppressed, potentiating the effect. As a result, the two main products in inflammation, Prostaglandins and Leukotrienes are inhibited. Therefore glucocorticoids are widely used as drugs to treat inflammatory conditions such as arthritis or dermatitis.

Indomethacin caused the highest inhibition of inflammation among the compounds in study. It is a nonselective inhibitor of COX 1 and 2, enzymes that participate in prostaglandin synthesis from arachidonic acid. Prostaglandins are hormone-like molecules normally found in the body, where they have a wide variety of effects, some of which lead to pain, fever, and inflammation. Since indomethacin inhibits both COX-1 and COX-2, it inhibits the production of prostaglandins in the stomach and intestines which maintain the mucous lining of the gastrointestinal tract. Indomethacin, therefore, like other nonselective COX inhibitors, can cause peptic ulcers.

3.5 CONCLUSION.

The herbal extract was found to have potent anti-inflammatory activity (it caused 40 % inhibition of inflammation). This activity can be attributed to the presence of plant *Nigella sativa* in the plant extract which has anti-inflammatory activity. It was however not found to have analgesic potency from the study carried. This is despite containing plant species with some documented analgesic activity eg. *Strychnos heningsii* and *caesalpinia volkensii*.

3.6 RECOMMENDATIONS.

There is immense potential for the use of plant extracts as anti-inflammatory agents in medicine if intensive study is carried where activity is suspected. However it is important to note that with experimental models, the most pronounced anti-inflammatory activity or any stated activity may not necessarily be achieved with the normal therapeutic dose of starting plant material in humans ^[15]. In most cases further toxicity studies need to be undertaken in order to generate more useful information as concerns its toxicological profile. Clinical trials and stability tests on the plant preparation generally require further study.

Though preliminary results of the laboratory work were positive, the anti-inflammatory activity of the herbal preparation is not conclusive and requires further testing. The extract could also have analgesic activity which can be detected if more sensitive methods are used.

3.7 BIBLIOGRAPHY.

1. Day.R., Quin.D, Williams.K, Handel.M, Rheumatic Disorders, Clinical pharmacology (Melmon and Morelli` s) 4th edition. (2000) 645-670
2. [http://www.medicinenet.com/rheumatoid arthritis /article.htm](http://www.medicinenet.com/rheumatoid_arthritis/article.htm)
3. Lipsky.E.P, Rheumatoid arthritis, Harrison` s principles of internal medicine. 16th edition, volume II. (2006) 1968-1976.
4. Schlotzhauer, M. Living with Rheumatoid Arthritis. Baltimore: Johns Hopkins University Press, 1993.
5. [http://www.alternative medicinechannel.com/arthritis/tcmtreatment.shtml](http://www.alternative_medicinechannel.com/arthritis/tcmtreatment.shtml)
6. [http://www.arthritis.about.com/od/supplement/dietary supplements_information_uses_benefits_side effects.htm.](http://www.arthritis.about.com/od/supplement/dietary_supplements_information_uses_benefits_side_effects.htm)
7. Zohary.D,Hopf.M,Domestication of plants of the old world,3rd edition (Oxford university press,2000) p.36
8. Kokwaro.O.J. Medicinal plants of East Africa,2nd edition(1993) 128,158.235
9. Kamau.F.N,I.OKibwage,MuriukiG,Guantai,A.N,HoogMartens,E.Roets,C.Govaert ,Chepkony.H and.Busson.R.Estrogenic and anti-inflammatory activity of a steroidal indoxyl,The East and Central African journal of pharmaceutical sciences.vol.5(3), (December 2002) 44-48.
10. Theophile.D,Agathe.F.L,Nguelefack.T.B,Asongalem E.A, Anti-inflammatory activity of leaf extracts of Kalanchoe crenata,Indian journal of pharmacology,2006 (2) 115-119.
11. Hollander.J.L, Mc Carty.DJ Arthritis in animals, Arthritis and Allied condition,8th edition (1974) 195-207
12. Kannan K, Ortmann RA, Kimpel D. Animal models of rheumatoid arthritis and their relevance to human disease. *Pathophysiology*. 2005
13. Pharmacognosy practical book,department of pharmacology and pharmacognosy,U.O.N (2006).
14. Evans.W.C, General methods associated with the phytochemical investigation of herbal plants Trease and Evans-(Pharmacognosy) .15th edition. 2002 137-149.
15. Borgi.W., Ghedira.K., Chouchane.N. Anti-inflammatory and analgesic activity of zizyphus lotus root barks. *Fitoterapia* 78, (2007) 16-19.

16. Lum G, Aisenbrey G, Dunn M, Berl T, Schrier R, McDonald K (Jan 1977). "In vivo effect of indomethacin to potentiate the renal medullary cyclic AMP response to vasopressin." *J Clin Invest* 59 (1): 8-13.
17. Scherzer P, Wald H, Rubinger D, Popovtzer M (September 1992). "Indomethacin and sodium retention in the rat: role of inhibition of prostaglandin E2 synthesis." *Clin Sci (London)* 83 (3): 307-11.
18. . Betram G. Katzung, Non steroidal anti inflammatory drugs and Drugs used in Gout Basic and clinical pharmacology.9th edition. (2005) 576-599.
19. Winter.C.A,Risley.E.A,Nuss.G.W. Carrageenan induced edema in hind paws of rat as an assay for anti-inflammatory drugs.*Proc Soc Expt Biol Med* 1962;111:544-547.