ANTI-INFLAMMATORY EFFECT OF THE ETHANOL EXTRACT TURMERIC RHIZOME (Curcuma longa) USING ALBINO RATS

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UNIVERSITY OF BENIN
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A PROJECT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY (BIOLOGICAL SCIENCE TECHNIQUES), FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFULLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF A BACHELOR OF SCIENCE (B.Se. HONOURS) SCIENCE LABORATORY TECHNOLOGY.

NOVEMBER, 2025

CERTIFICATION

This is to certify that this research work was carried out by Miss Blessing Akaniyere ADODO (Miss) with matriculation number LSC2010039 of the Department of Science Laboratory Technology, Faculty of Life Sciences, and University of Benin, Benin City.

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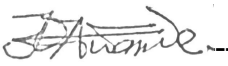

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CHAPTER ONE

1.0 INTRODUCTION

1.1 **Arthritis**

Arthritis basically means inflammation of the joints (Pirotta, 2010; Athanasiou *et al*., 2013). Even so, joint inflammatory disease is more like a clinical signs than a precise diagnosis. All conditions that have an impact on the joints are commonly referred to as having arthritis (Pirotta, 2010). These illnesses are included in the larger category of rheumatic diseases (Athanasiou *et al*., 2013). Arthritic pain is likely the most well-known type of pain in both primary and secondary care (Athanasiou *et al*., 2013; Pirotta, 2010). Arthritis is a condition in which a joint becomes inflamed. When joints become inflamed, they can cause stiffness, warmth, swelling, redness, and discomfort (Athanasiou *et al*., 2013). Gout, rheumatoid arthritis, osteoarthritis, psoriatic arthritis, lupus, and spondylitis are only a few of the many kinds of arthritis.

1.2 Most Common Types of Arthritis

1.2.1 Osteoarthritis

This type of arthritis affects a greater number of people than other types. As a result of the overuse of the joints, "wear and tear" develops (Jessar and Hollander, 1995). It ordinarily occurs with aging; however, it can also result from joint damage or obesity, which puts additional strain also on joints. Resulting in the loss of the body's shock-absorbing mechanism. Ligament gradually separates from the lubricant covering the ends of the bones (Jessar and Hollander, 1995). One example is the damage that being overweight can do to your knees.

1.2.2 Rheumatoid Arthritis

An autoimmune condition is RA. That implies that the immune system targets and harms various body parts, especially the joints (Montgomery and Poske, 1968). That prompts inflammation,

which can result in acute joint damage if not treated. Rheumatoid nodules are protuberances that appear on the skin in about one in five people with rheumatoid joint inflammation. These frequently appear over pressure-sensitive joint areas, such as the knuckles, elbows, or heels (Montgomery and Poske, 1968).

1.2.3 Psoriatic Arthritis

Psoriasis and arthritis are skin and joint inflammation in those with this illness (Fitzgerald *et al*., 2021). Psoriasis results in irregular, raised, inflamed, white, and red skin patches covered in scales. It typically affects the skin around the genital areas, the scalp, the navel, knees and as well as elbows. Just 10% to 30% of people with psoriasis will also develop psoriatic arthritis. (Fitzgerald *et al*., 2021). This kind of arthritis for the most part begins between ages 30 and 50, yet it can begin as soon as infancy. It's likewise commonly found amongst males and females. The skin infection (psoriasis) ordinarily appears first.

1.2.4 Gout

This is the accumulation of acid crystals from the uric acid in a joint. More often than not, it affects the big toe or other areas of the foot. Often resulting in waking up with an unexpected (sudden), piercing pain in the big toe following an evening of drinking. Be that as it may, medications, stress, or another sickness can likewise set off a gout assault (Jessar and Hollander, 1995).

1.3 Causes of Arthritis and Risk factors

The exact cause of many kinds of arthritis is unknown. Most types of arthritis are thought to be caused by immune system dysfunction, which makes the body target its own tissues inside the joints. This may be a hereditary characteristic (CDC, 2020). Other types of arthritis may be brought on by immune system problems or a metabolic disorder like gout.

1.3.1 Environmental factors

The following are a few examples of environmental factors that could contribute to arthritis formation: Joint stress is increased by obesity (CDC, 2020). activities that demand repeated joint movement. Arthritis can also develop in people who have already suffered joint damage, including injury through sports. And also smoking and don't get enough exercise, makes it more likely to get arthritis. Arthritis triggered by an infection is termed reactive arthritis (CDC, 2020). It's difficult to diagnose and can strike anyone at any age, but it's more common in children and teenagers. The duration of reactive arthritis might range from a few weeks to six months.

1.4 Treatment of Arthritis

For certain kinds of arthritis, there are therapies that can help lessen symptoms and prevent further joint damage. The type of arthritis present, the affected joints, and the symptoms present will decide the most appropriate treatment. Medicines such as pain relievers (painkillers), anti-inflammatory prescriptions, or anti-disease medications are among the options for treatment known as disease-modifying antirheumatic drugs, or DMARDs, which are used to treat inflammatory types of arthritis such as juvenile idiopathic arthritis, ankylosing spondylitis, and rheumatoid arthritis. NSAIDs, joint infusion, or lower dosage prednisone are all examples of nonsteroidal anti-inflammatory drugs (American College of Rheumatology Subcommittee on Rheumatoid Arthritis Guidelines, 2002). There are many available methods of managing pain and stress, including meditation. Extreme circumstances might necessitate medical surgical procedure to help repair damaged joints.

1.5 Arthritis Relationship with Inflammation

Inflammatory arthritis is the term utilized to define a collection of diseases instigated by an overactive compromised immune system that leads to inflammation (Stevenson, 2009). Many

types of these ailments manifest mostly with inflammation of the joints felt as pain in the joint and stiffness, however, inflammatory arthritis can likewise attack other connective tissues, such as the eyes, lungs, skin, heart, and other organs (Stevenson, 2009). Significantly, when inflammation acts on any area of the body it can bring about the irreversible harm.

1.6 Inflammation

An area of living, vascularized tissues will become inflamed in response to both endogenous and exogenous stimuli (Ferrero-Miliani *et al*., 2007). The term is gotten from the Latin "inflammare" which denotes to burn. Inflammation is essentially meant to localize and eradicate the disease-causing agent and to reduce tissue damage. Along these lines, inflammation is a physiologic (defensive) response to a wound.

1.7 Causes of Inflammation

The causative agent of inflammation are apparently causes of diseases such as:

1. Physical agents include mechanical wounds and injuries, variation in temperatures and pressure, radiation wounds
2. Chemical agents these include the expanding lists of drugs (medications) and toxins.
3. Biologic agents (infectious) such as parasites, viruses, bacteria, and fungi.
4. Immunological conditions such as autoimmunity, hypersensitivity reactions, and immunodeficiency states.
5. Metabolic and genetic conditions such as gout and diabetes.

(Kumar *et al*., 2004; Hall, 2011)

1.8 Plant used for treatment

In Nigeria, a lot of plants have been studied for their anti-inflammatory properties using various methods to carry this experiment. Examples of such plants include, *Ribes nigrum* (blackcurrant),

Oenothera biennis (Evening Primrose)*, Curcuma longa* (Turmeric)*, Zingiber officinale* (ginger), Harpagophytum procumbens (Devil's Claw) *Urtica dioica* (stinging nettle), *Rosmarinus officinalis*(rosemary), *Borago officinalis* (Borage), *Boswellia serrata* (Indian Olibanum), Rosa canina (Dog rose), *Uncaria tomentosa* (cat's claw), *Salvia officinalis, Persea americana* (Avocado), *Elaeagnus angustifolia* (Oleaster), *Vaccinium myrtillus* (bilberry) and *Olea europaea* (Olive) (Ghasemian *et al*., 2016; Jurenka, 2009; Srivastava and Mustafa, 1992; Deodhar *et al.,* 1980; Drozdov *et al*., 2012; Khalvat, 2005; Altman and Marcussen, 2001; Randall *et al*., 2000; Shakibaei *et al*., 2012; Riehemann *et al*., 1999; Roschek *et al*., 2009; Watson *et al*., 1993; Montserrat-de la Paz *et al*., 2014).

1.9 Statement of Problem

Inflammation has become a global problemaffecting the young, youth and the old. Most drugs discovered has various side effects ranging from ulceration and hypertension, hence the need for an effective anti-inflammatory drug without side effects.

1.10 Aim of Study

This study was aimed at investigating the on anti-inflammatory effect of the ethanol extract *Curcuma longa* (Turmeric rhizome) using albino rats.

1.11 Objectives of Study

The aim was achieved by the following objectives;

1. Extraction of *Curcuma longa* (Turmeric rhizome).
2. Determination of anti-inflammatory activity using carrageenan induced for paw edema model.
3. Determination of anti-inflammatory activity using egg albumen induced for paw edema model.
4. Evaluation of anti-inflammatory activity using a model of paw edema induced by formaldehyde.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Curcuma longa*

Curcuma longa, or turmeric is a perennial herb and member of the Zingiberaceae (ginger) family and is cultivated extensively in Asia mostly in India and China. The rhizome, the portion of the plant used medicinally, yields a yellow powder. Dried *Curcuma longa* is the source of turmeric, the ingredient that gives curry powder its characteristic yellow color. It has many names such as Curcum in the Arab region, Indian saffron, Haridra (Sanskrit, Ayurvedic), Jianghuang (yellow ginger in Chinese), Kyoo or Ukon (Japanese). (Goel *et al*., 2008).

Turmeric has been used in Asian cuisines for both its flavor and color and in the Chinese and Ayurvedic medicine particularly as an anti-inflammatory and for the treatment of jaundice, menstrual difficulties, hematuria, hemorrhage, and colic. It is official in the Pharmacopoeia of China as well as in other Asian countries such as Japan and Korea and its usage covers a wide range of health indications. In China it is ingested orally and applied topically for urticaria and skin allergy, viral hepatitis, inflammatory conditions of joints, sore throat and wounds.

Oral administration is the main route of administration for *Curcuma longa,* it can also be used topically and via inhalation (Ayurvedic tradition) or can be applied topically for the treatment of acne, wounds, boils, bruises, blistering, ulcers, eczema, insect bites, parasitic infections, hemorrhages and skin diseases like herpes zoster and pemphigus (Labban, 2014).

2.2 Chemical Composition of Turmeric

Turmeric contains protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%) and moisture (13.1%). The essential oil (5.8%) obtained by steam distillation of rhizomes has a-

phellandrene (1%), sabinene (0.6%), cineol (1%), borneol (0.5%), zingiberene (25%) and sesquiterpines (53%). Curcumin (diferuloylmethane) (3–4%) is responsible for the yellow colour, and comprises curcumin I (94%), curcumin II (6%) and curcumin III (0.3%). Demethoxy and bisdemethoxy derivatives of curcumin have also been isolated7 (Figure 1). Curcumin was first isolated8 in 1815 and its chemical structure was determined by Roughley and Whiting9 in 1973. It has a melting point at 176–177°C; forms a reddish-brown salt with alkali and is soluble in ethanol, alkali, ketone, acetic acid and chloroform (Chattopadhyay *et al*., 2004).

2.3 Active Constituents of Turmeric

The active constituents of turmeric are the flavonoid Curcuminoids which is a mixture of curcumin (diferuloylmethane), monodexmethoxycurcumin and bisdesmethoxycurcumin Curcumin makes up approximately 90% of the curcuminoid content in turmeric. Other constituents include sugars, proteins, and resins. The bestresearched active constituent is curcumin, which comprises 0.3-5.4% of raw turmeric (Heath *et al*., 2004).

Turmeric is comprised of a group of three curcuminoids: curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin (Fig.1), as well as volatile oils (tumerone, atlantone, and zingiberone), sugars, proteins, and resins. The Curcumin is a lipophilic polyphenol that is nearly insoluble in water but is quite stable in the acidic pH of the stomach. The phenolic groups in the structure of curcumin explain the ability of curcumin to eliminate oxygen-derived free radicals. The free radicals which can be eliminated by curcumin are hydroxyl radical, singlet oxygen, superoxide radical , nitrogen dioxide and NO (Labban, 2014). With regard to pharmacokinetic, studies have demonstrated that 40-85% of an oral dose of curcumin passes through the gastrointestinal tract unchanged. Due to its low rate of absorption, curcumin is often formulated with bromelain for increased absorption and enhanced antiinflammatory effect. This

review focuses on the medicinal and pharmacological properties of turmeric as anti-inflammatory, antioxidant, hepatoprotective, anticarcinogenic, antidiabetic, antimicrobial, antidepressant in addition to its use in cardiovascular disease, gastrointestinal and neurological disorders (Labban, 2014).

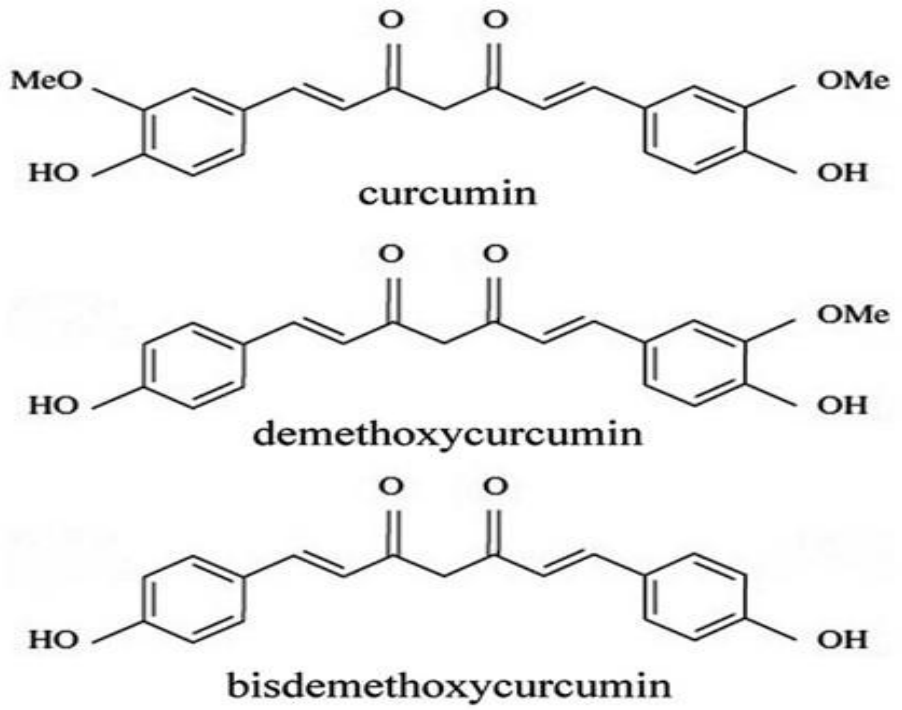

Figure 2.1: Structural formula of three curcuminoids ((Labban, 2014).

2.4 TAXONOMY

Turmeric was described as C. longa by Linnaeus and its taxonomic position is as follows:

Class	Liliopsida
Subclass	Commelinids
Order	Zingiberales
Family	Zingiberaceae
Genus	Curcuma

Species Curcuma longa

The wild turmeric is called *C. aromatica* and the domestic species is called *C. longa.* (Chattopadhyay *et al*., 2004).

Plate 2.1: Turmeric rhizome and powder (Debjit *et al*., 2009).

2.5 PHARMACOLOGICAL BENEFITS/MECHANISM OF ACTION OF TURMERIC

2.5.1 Anti-inflammatory benefits

Oral administration of curcumin in instances of acute inflammation was found to be as effective as cortisone or phenylbutazone. Oral administration of Curcuma longa significantly reduced inflammatory swelling (Cronin, 2003). C. longa's anti-inflammatory properties may be attributed to its ability to inhibit both biosynthesis of inflammatory prostaglandins from arachidonic acid, and neutrophil function during inflammatory states. Curcuminoids also inhibit LOX, COX, phospholipases, leukotrienes, prostaglandins, thromboxane, nitric oxide elastase, hyaluronidase, collagenase, monocyte chemoattractant protein-1, interferon inducible protein, TNF and interleukin-12. They also decrease prostaglandin formation and inhibit leukotriene biosynthesis via the lipoxygenase pathway (Bundy *et al*., 2004). An RCT investigated the effect of a combination of 480mg curcumin and 20mg quercetin (per capsule) on delayed graft rejection (DGR) in 43 kidney transplant patients. Of 39 participants who completed the study, two of 14 in

the control group experienced DGR compared to zero in either treatment group. Early function (significantly decreased serum creatinine 48 hours post-transplant) was achieved in 43% of subjects in the control group, 71% of those in the lowdose treatment group. Since the amount of quercetin in the compound was minimal, the majority of benefit is thought to be due to curcumin's anti-inflammatory and antioxidant activity. Likely mechanisms for improved early function of transplanted kidneys include induction of the hemeoxygenase enzyme, and pro-inflammatory cytokines, and scavenging of free radicals associated with tissue damage (Shoskes *et al*., 2005).

2.5.2 Antifertility activity

Petroleum ether and aqueous extracts of turmeric rhizomes show 100% antifertility effect in rats when fed orally43. Implantation is completely inhibited by these extracts111. Curcumin inhibits 5a-reductase, which converts testosterone to 5a-dihydrotestosterone, thereby inhibiting the growth of flank organs in hamster112. Curcumin also inhibits human sperm motility and has the potential for the development of a novel intravaginal contraceptive113 (Chattopadhyay *et al*., 2004).

2.5.3 Bacterial and Viral Infections

Test tube and animal studies suggest turmeric may kill bacteria and viruses. But researchers don't know whether it would work in people. The aqueous extract of turmeric rhizomes has antibacterial effect. Curcumin also prevents growth of Helicobacter pylori CagA+ strains in vitro. Both curcumin and the oil fraction suppress growth of several bacteria like Streptococcus, Staphylococcus, Lactobacillus, etc. Curcumin has been shown to have antiviral activity (Araujo *et al*., 2001). It acts as an efficient inhibitor of Epstein-Barr virus (EBV). Most importantly, curcumin also shows anti-HIV (human immunodeficiency virus) activity by inhibiting the HIV-1

integrase needed for viral replication. It also inhibits UV light induced HIV gene expression127. Thus curcumin and its analogues may have the potential for novel drug development against HIV (Verma *et al*., 2018).

2.5.4 Cardiovascular disorders

The antioxidants in turmeric also prevent damage to cholesterol, thereby helping to protect against atherosclerosis. In fact, the ability of the antioxidants in turmeric to decrease free radicals is similar to that in vitamins C and E. Since the antioxidant activities of turmeric are not degraded by heat (unlike most vitamins), even using the spice in cooking provides benefits. Animal studies show that curcumin lowers cholesterol and triglycerides, another fat that circulates in the blood stream and is a risk factor for cardiovascular disease. In a recent study of atherosclerosis, mice were fed a standard American diet, rich in refined carbohydrates and saturated fat, but low in fiber. Some of the mice, however, received this diet plus turmeric mixed in with their food. After four months on these diets, the mice that consumed the turmeric with their food had 20 percent less blockage of the arteries than the mice fed the diet without the turmeric. In another study, rabbits were fed turmeric plus a diet designed to cause atherosclerosis. Several risk factors for the disease were improved, including a decrease in cholesterol, triglycerides, and free-radical damage (Krup *et al*., 2013).

2.5.5 Anticarcinogenic properties

Animal research demonstrates inhibition at all three stages of carcinogenesis-initiation, promotion, and progression. During initiation and promotion, curcumin modulates transcription factors controlling phase I and II detoxification of carcinogens; down-regulates proinflammatory cytokines, free radical-activated transcription factors, and arachidonic acid metabolism vicyclooxygenase and lipoxygenase pathways; and scavenges free radicals. Studies involving

rats and mice, as well as in vitro studies utilizing human cell lines, have demonstrated curcumin's ability to inhibit carcinogenesis at three stages: tumor promotion, angiogenesis, and tumor growth. Turmeric and curcumin are also capable of suppressing the activity of several common mutagens and carcinogens in a variety of cell types in both in vitro and in vivo studies. (Labban, 2014). The anticarcinogenic properties of turmeric and curcumin are due to direct antioxidant and free-radical scavenging properties, as well as their ability to indirectly increase glutathione levels, thereby aiding in hepatic detoxification of mutagens and carcinogens, and inhibiting nitrosamine formation and Curcumin also induces apoptosis of cancer cells and it inhibits angiogenesis. The efficacy of curcumin or turmeric extract in reducing chemically-induced tumours was studied by. Application of both curcumin and turmeric extract during carcinogenesis and promotion resulted in less papilloma production, compared to controls. This indicates that both curcumin and turmeric extract produce their best properties during tumour promotion. The effect of dietary curcumin (0.2% and 1.0%) on 7,12dimethylbenz (a) anthracene (DMBA) and 12,0tetradecanoylphorbol-13-acetate (TPA)-promoted skin tumor formation was investigated by Limtrakul *et al*., (2001). They found a significant lower number of papillomas in the curcumin treated group compared to the control group. The enhanced expression of ras-p21 and fos-p62 oncogenes were decreased dose dependently in the curcumin treated group. The effect of Curcuma longa on myocardial apoptosis in experimentally induced myocardial ischemic-reperfusion injury was investigated by Mohanty *et al*., (2006). Curcuma longa demonstrated significant anti-apoptotic property, which might contribute to the observed preservation in cardioprotective properties and cardiac function. Azuine et al. investigated the protective effect of turmeric extract on chemically induced mutagenicity in Salmonella typhimurium strains and clastogenicity in mammalian bone marrow in female Swiss mice

(Labban, 2014).The anticarcinogenic properties were assessed in the benzo (a) pyrene induced forestomach neoplasia model. Aqueous turmeric extract exhibited antimutagenic activity against direct acting mutagens and also inhibited the mutagenicity of benzo (a) pyrene in Salmonella typhimurium strains. Treatment with the aqueous tumeric extract inhibited the development of fore-stomach tumors induced by benzo (a) pyrene significantly (Labban, 2014). These findings were all dose-dependent. There is some evidence that curcumin inhibits the activity of certain chemotherapy drugs. Research reveals curcumin decreased camptothecininduced death of cultured breast cancer cells and prevented cyclophosphamide-induced breast tumor regression in mice. Curcumin might also interfere with the absorption and efficacy of the chemotherapy drug irinotecan, which is used to treat colon cancer. On the other hand, curcumin may enhance the effects of some chemotherapy drugs. In a mouse xenograft model of human breast cancer, curcumin in conjunction with paclitaxel (Taxol) significantly inhibited breast cancer metastasis to the lung to a greater degree than either curcumin or paclitaxel alone (Labban, 2014).

2.5.6 Antivenom effect

Ar-turmerone, isolated from C. longa, neutralizes both haemorrhagic activity of Bothrops venom and 70% lethal effect of Crotalus venom in mice4. It acts as an enzymatic inhibitor of venom enzymes with proteolytic activities (Chattopadhyay *et al*., 2004).

2.5.7 Indigestion

Curcumin stimulates the gallbladder to produce bile, which some people think may help improve digestion. The German Commission E, which determines which herbs can be safely prescribed in Germany, has approved turmeric for digestive problems. And one double-blind, placebo-controlled study found that turmeric reduced symptoms of bloating and gas in people suffering from indigestion. Turmeric powder has beneficial effect on the stomach. It increases mucin

secretion in rabbits and may thus act as gastroprotectant against irritants. However, controversy exists regarding antiulcer activity of curcumin. Both antiulcer and ulcerogenic effects of curcumin have been reported but detailed studies are still lacking (Verma *et al*., 2018).

2.5.8 Hepatoprotective

The powder of the rhizome mixed with amla juice is used in jaundice. Corriliyum (Anjana) with Haridra, Red ochre (Gairika), and Amalaki (Emblica officinalis) cures jaundice. .Curcumin, the most common antioxidant constituent of Curcuma longa rhizome extract, was reported to enhance apoptosis of damaged hepatocytes which might be the protective mechanism whereby curcumin down-regulated inflammatory effects and fibrogenesis of the liver (Krup *et al*., 2013).The ethanolic extract of Curcuma Longa rhizomes showed a significant hepatoprotective effect when orally administrated in doses of 250 mg/kg and 500 mg/kg, and the protective effect was dosedependent. The main constituents of Curcuma longa rhizome ethanolic extract are the flavonoid curcumin and various volatile oils, including tumerone, atlantone, and zingiberene. The hepatoprotective effects of turmeric and curcumin might be due to direct antioxidant and free radical scavenging mechanisms, as well as the ability to indirectly augment glutathione levels, thereby aiding in hepatic detoxification. The volatile oils and curcumin of Curcuma longa exhibit potent antiinflammatory effects (Salama *et al*., 2013).

2.5.9 Antimicrobial properties

Turmeric extract and the essential oil of Curcuma longa inhibit the growth of a variety of bacteria, parasites, and pathogenic fungi. A study of chicks infected with the caecal parasite Eimera maxima demonstrated that diets supplemented with turmeric resulted in a reduction in small intestinal lesion scores and improved weight gain. Another study, in which guinea pigs were infected with eitherdermatophytes, pathogenic molds, or yeast, found that topically applied

turmeric oil inhibited dermatophytes and pathogenic fungi. Improvements in lesions were observed in the dermatophyte- and fungi-infected guinea pigs, and at seven days post-turmeric application the lesions disappeared (Labban, 2014).Curcumin has also been found to have moderate activity against Plasmodium falciparum and Leishmania major organisms.

Khattak *et al*., (2005) studied the antifungal, antibacterial, phytotoxic, cytotoxic and insecticidal activity of an ethanolic extract of turmeric. The extract showed antifungal activity towards Trichophyton longifusus and Microsporum canis and weak antibacterial activity against Staphylococcus aureus. Toxic activity was observed against Lemna minor. The Curcuma longa treated rabbit group showed a significant higher mean value for contraction of the wound compared to controls. Furthermore the wounds showed less inflammation and an increasing trend in the formation of collagen (Kundu *et al*., 2005).

2.5.10 Management of Obesity

People who would like to lose a couple of pounds or treat obesity and other similar condition can take benefits of turmeric powder which can be very helpful in keeping one's ideal body weight. The component in turmeric helps in boosting the flow of bile which is an essential element in the process of breaking down of dietary fats (Verma *et al*., 2018).

2.5.11 Neuroprotective activity

Curcuma oil significantly reduces the ill effect of ischemia by attenuating nitrosative and oxidative stress. Ischemia induces collapse of mitochondrial membrane potential, cytochrome c release, altering the Bax: Bcl-2 ratio and subsequently caspases activation led to induction of apoptosis in sequential fashion was reverse significantly by Curcuma oil. So there is an evidence for the high efficacy of Curcuma oil as a neuroprotective, with an excellent therapeutic window for the prevention of ischemic brain injury (Krup *et al*., 2013).

2.5.12 Dyspepsia and gastric ulcer

In a phase II clinical trial involving 45 subjects with endoscopically diagnosed peptic ulcers were given 600mg curcumin five times daily for 12 weeks. Ulcers were absent in 12 patients (48%) after four weeks, in 18 patients after eight weeks, and in 19 patients (76%) after 12 weeks (Labban, 2014). The remaining 20 patients, also given curcumin, had no detectable ulcerations at the start of the study, but were symptomatic-erosions, gastritis, and dyspepsia. Within 1-2 weeks abdominal pain and other symptoms had decreased significantly. Kim *et al*., (2005) investigated the protective effect of turmeric ethanolic extract against gastric ulcers by blocking H2 histamine receptors (H2R) of male Sprague-Dawley (pylorus-ligated) rats. The effect of Curcuma longa extract was compared to the properties of ranitidine. Curcuma was found to protect the gastric mucosal layer as effective as ranitidine. Orally administerd ethanolic extract inhibited gastric acid, gastric juice secretion and ulcer formation comparable to the properties of ranitidine. The antiulcer activity of an ethanolic extract of turmeric investigated. Administration of turmeric extract led to a significant decrease in ulcer index and acidity of stomach contents. Pretreatment with the turmeric extract reduced the intensity of ulceration. Hypothermic-restraint stress reduction of gastric wall mucus was inhibited by turmeric extract treatment and reduced the severity of lesions induced by various necrotizing agents (Labban, 2014).

2.5.13 Inflammatory bowel disease

Crohn's disease (CD) and ulcerative colitis (UC) are the two primary forms of inflammatory bowel disease (IBD). Holt *et al*., (2005) conducted a pilot study to examine the effect of curcumin therapy in patients with IBD who had previously received standard UC or CD therapy. Hematological and biochemical blood analysis, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) (the latter two inflammatory indicators), sigmoidoscopy, and biopsy were all

performed at baseline and at the study end (Labban, 2014). Crohn's Disease Activity Index (CDAI), CRP, ESR, hematological blood analysis, and kidney function was assessed in all patients at baseline and end of study. In the proctitis group all five patients improved by study's end as indicated by a global score, and all five subjects demonstrated normal ESR, CRP, and serologic indices of inflammation after two months. In the CD group, CDAI scores decreased by an average of 55 points, and CRP and ESR decreased in four of five patients. The authors concluded that curcumin plus standard therapy was more effective in maintaining remission than placebo plus standard UC treatment (Hanai *et al*., 2006; Debjit *et al*., 2009).

2.6 PHARMACOKINETIC STUDIES ON CURCUMIN

Curcumin, when given orally or intraperitoneally to rats, is mostly egested in the faeces and only a little in the urine. Only traces of curcumin are found in the blood from the heart, liver and kidney. Curcumin, when added to isolated hepatocytes, is quickly metabolized and the major biliary metabolites are glucuronides of tetrahydrocurcumin and hexahydrocurcumin. Curcumin, after metabolism in the liver, is mainly excreted through bile (Chattopadhyay *et al*., 2004).

2.7 CLINICAL STUDIES AND MEDICINAL APPLICATIONS OF TURMERIC AND CURCUMIN

Although various studies have been carried out with turmeric extracts and some of its ingredients in several animal models, only a few clinical studies are reported so far.

2.7.1 *Turmeric*

Powdered rhizome is used to treat wounds, bruises, inflamed joints and sprains in Nepal. In current traditional Indian medicine, it is used for the treatment of biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis. Data are also available showing that the powder, when applied as capsules to patients with respiratory disease, gives

relief from symptoms like dyspnoea, cough and sputum. A short clinical trial in 18 patients with definite rheumatoid arthritis showed significant improvement in morning stiffness and joint swelling after two weeks of therapy with oral doses of 120 mg/ day. Application of the powder in combination with other plant products is also reported for purification of blood and for menstrual and abdominal problems (Chattopadhyay *et al*., 2004).

2.7.2 *Curcumin*

In patients undergoing surgery, oral application of curcumin reduces post-operative inflammation. Recently, curcumin has been formulated as slow-release biodegradable microspheres for the treatment of inflammation in arthritic rats. It is evident from the study that curcumin biodegradable microspheres could be successfully employed for therapeutic management of inflammation (Chattopadhyay *et al*., 2004).

2.8 SAFETY EVALUATION WITH TURMERIC AND CURCUMIN

Detailed studies have been reported on the safety evaluation of the rhizomes of C. longa and its alcohol extract, curcumin. The major findings are presented below.

2.8.1 *Turmeric*

The average intake of turmeric by Asians varies from 0.5 to 1.5 g/day/person, which produces no toxic symptoms.

Male and female Wistar rats, guinea pigs and monkeys were fed with turmeric at much higher doses (2.5 g/kg body wt) than normally consumed by humans. No changes were observed in the appearance and weight of kidney, liver and heart. Also, no pathological or behavioural abnormalities were noticed and no mortality was observed (Chattopadhyay *et al*., 2004).

2.8.2 *Curcumin*

Curcumin was given to Wistar rats, guinea pigs and monkeys of both sexes at a dose of 300 mg/kg body wt. No pathological, behavioural abnormalities or lethality was observed. No adverse effects were observed on both growth and the level of erythrocytes, leucocytes, blood constituents such as haemoglobin, total serum protein, alkaline phosphatase, etc. Human clinical trials also indicate that curcumin has no toxicity when administered at doses of 1–8 g/day and 10 g/day (Chattopadhyay *et al*., 2004).

2.9 STATEMENT OF PROBLEM

Turmeric has been used in ayurvedic medicine since ancient times, with various biological applications. Although some work has been done on the possible medicinal applications, no studies for drug-development have been carried out as yet. Although the crude extract has numerous medicinal applications, clinical applications can be made only after extensive research on its bioactivity, mechanism of action, pharmacotherapeutics and toxicity studies. However, as curcumin is now available in pure form, which shows a wide spectrum of biological activities, it would be easier to develop new drugs from this compound after extensive studies on its mechanism of action and pharmacological effects. Recent years have seen an increased enthusiasm in treating various diseases with natural products. Curcumin is a non-toxic, highly promising natural antioxidant compound having a wide spectrum of biological functions. It is expected that curcumin may find application as a novel drug in the near future to control various diseases, including inflammatory disorders, carcinogenesis and oxidative stress-induced pathogenesis (Chattopadhyay *et al*., 2004).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Procurement of Sample

The *Curcuma longa* (Turmeric rhizome) were purchased in December 2023 from Ovbiogie market in Ovia South, Benin City, Edo state, Nigeria. The turmeric rhizome was authenticated by Prof. H.A. Akinnibosun a taxonomist in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, where a herbarium specimen number *UBH-P602* of the plant was deposited. The turmeric rhizome were first washed, cut into smaller pieces and air-dried at room temperature after purchase for 2 weeks then dried in an oven at 40^0C for 1 hour. The dried sample was then reduced to fine powder using an electric milling machine and stored in air tight containers for future use (Obaro-Onezeyi and Oshomoh, 2019).

3.2 Preparation of extract

Five hundred gram (500 g) of the powder was extracted with ethanol solvent using cold extraction method. The ground powdered leave was weighed and placed in a glass jar. 2.5 litres of ethanol solvent was poured into the glass jar to make ¾. The solution was macerated and then shake vigorously as often as possible. After 72 hours the solution was macerated and filter using a cheese-cloth. The resulting extract was concentrated to dryness using an oven at 40^0C. The percentage yield was calculated with reference to the dried powder used.

3.3 Preparation of stock solution

Twenty grams (20 g) obtained from the extract were dissolved in 100 ml of water from a distilled source daily to obtain a stock solution (200 mg/ml) from which dilutions were made and calculated doses administered to the animals during the experimental procedures (Oshomoh and Obaro, 2019).

3.4 Solvents/chemicals

Ethanol solvent, chloroform (supplied by Fharmatrends Nigeria Ltd), Sodium Chloride all of analytical standards.

3.5 Drugs

Indomethacin, were of pure samples and pharmaceutical standards.

3.6 Experimental Design

3.6.1 Acute Toxicity Study

Acute toxicity study was carried out by methods of OECD (Organization of economic co-operation development), 2008a guidelines. Six (6) mice (3 males and 3 females) were administered 5, 000 mg/kg per body weight of the extract and observed for 72 hours for possible signs of toxicity, mortality or morbidity.

3.7 Experimental animals

The animal house of the Phytomedicine unit in the Department of Plant Biology and Biotechnology, University of Benin, provided 60 male and female adult albino Wistar rats, with an average weight of 140-150 g. The animals were housed in wooden cages at room temperature and kept in standard laboratory environment with 12-hour cycles of light and darkness. Prior to the experimental study, the rats were given clean water and standard pelletized layers mash to acclimate for two weeks This study was approved by the Animal Ethnics Committee of the Faculty of Science Laboratory Technology with Reference Number, UNIBEN/FSLT/00044.

3.8 Systemic acute inflammation of the rat paw

The rat paw edema model (Winter *et al*., 1962) involves experimentally inducing acute edema in the right hind paw of the rats in order to assess the systemic effects of test substances on acute inflammation. By injecting phlogistic agents (such as fresh egg albumen or a 1 percent solution of carrageenan in normal saline) into the sub-plantar area of the paw, it is possible to cause an acute inflammatory response in rat paw records. The rat paw develops edema, which is seen as an intense pink swelling, as a result of the acute inflammatory response. The size of the paw is measured in terms of the volume at various intervals, using a vernier caliper. Other methods of measurement paw size include volume displacement from a measuring cylinder containing water, measurement of linear circumference of the paw for using a tape and measurement of paw thickness with a micrometer screw gauge or caliper.

In some instance, the animal is sacrificed and the inflamed paw amputated and weighed. However, the latter should be relegated to extremely necessary experimental needs. Anti-inflammatory substance abolished or reduce the extent of the swelling or edema when compared to the negative control. Adult rats of either sex are used in this experiment. The animals are randomly allotted into groups based on the dose levels of the test substance and appropriate controls (NSAIDs such as acetylsalicylic acid and indomethacin for positive control; the vehicle or suspending agents served as negative control). At time t = 0, the animal's right hind paw is measured for size. The test substance is administered. One hour after oral administration of test substance, the rat's right hind paw's subplanter region receives an injection of phlogistic agent measured at 0.1 milliliter. At various times (0.5, 1, 2, 3, 4 and 5h) after carrageenan injection, the paw size is measured again. Edema is measured as a rise in size of the paw size at 0 hours and at

various intervals following injection of a phlogistic agent. For each group, the relation (where paw volume is used) is used to calculate the inhibition level (percent) of edema.

Inhibition (%) =[1-(Vt/Vc)] 100

Where, Vt = average paw volume of the treated group,

Vc = average paw volume of the control group.

3.9 Chronic Inflammation

Evaluation of the effect of substances on chronic inflammation involves models using repeated administration of the test agent as well as inflammation sustained beyond 24 h. Inflammation becomes chronic when the assault on the body is not contained within the acute phase. Models that mimic pathological chronic inflammation have been developed and used in screening suitable substances for its management. These models include induction of arthritis in rats through formaldehyde, adjuvant induced arthritis, collagen adjuvant-induced arthritis, air pouch inflammation and cotton pellet granuloma tests.

3.9 .1 Arthritis - induced by formaldehyde in rats

Formaldehyde-induced arthritis in rats (Seyle, 1949) can be used to assess how test substances affect the chronic phase of inflammation. Formaldehyde edema has been shown to be mediated by histamine, 5-HT, substance P, and bradykinin and prostaglandins which are involved in the acute phase of inflammatory response. Sustenance of the edema beyond the initial 24 h may invoke induction of other humoral and cell- mediated responses such as lymphocyte accumulation and proliferation, indicative of chronic inflammation. Due to the ability of formaldehyde to cause necrosis of the tissues of the paw, it is also thought that necrosis amplifies the later stages of the formaldehyde edema through activation of kinin, coagulation, complement and fibrinolytic systems and the mediator release from passenger leukocytes that are dying or

dead. The arthritic foot appears swollen and painful initially but wanes within 1-3 days to prolong and further sustaining the arthritics, the paw is re-inflamed on day 3 with formaldehyde injection. The experiment typically lasts for 10 days.

The experiment uses adult rats of either sex. The animals are divided into five (5) groups at random, negative control (Distilled Water), extract treated and indomethacin (positive control) groups. On day 1 of the experiment, we use a Veneer caliper to measure the animal's right hind paw or volume (by displacement) and the test substance was administered (orally). One hour after oral administration of test substance, arthritis is induced through injection administered sub-plantar at 0.1 ml of 2 % v/v formaldehyde solution. After 4 hours, the paw's volume or size is once more measured. On day 2, the animal is treated once and the size (or volume) of the arthritics foot measure. On day 3, after treatment and measurement of the arthritic foot size (or volume), arthritis is re induced by formaldehyde injection. From day 4- 7, the animal is treated daily and the size (or volume) of the arthritic foot measured. Changes in the size or volume of the arthritics foot is used as a measure of arthritics. The overall result of the anti-arthritic treatment is quantified by the area under the curve (AUC) of the time-course of the arthritic event, which represents the global response of edematous to arthritis from formaldehyde. The inhibition level in percentage of arthritis is determined using the relation, and the AUC is determined using the trapezoidal rule:

Inhibiton (%) = [1 – (AUCt / AUCc)] 100

Where, AUCt = AUC of the treated group

AUCc = AUC of the control group.

3.10 Administration of extract

Extract was freshly prepared every morning and administered orally to rats by carefully inserting an orogastric tube into the oral cavity of the rats. The animals were grouped into three categories groups; carrageenan induced for paw edema ([a]), egg albumen induced for paw edema ([b]) and formaldehyde induced for paw edema ([c]), consisting of 5 animals each.

Group I (Negative control) – Distilled Water (2 ml/kg)

Group II- Positive control (10 mg/kg indomethacin)

Group III, IV and V (50, 100 and 200 mg/kg of the extract respectively after acute toxicity study).

Throughout the period of administration, food and water were given to the rats.

3.11 Statistical analysis

Every value is presented as Mean ± Standard Error of Mean (SEM). Using the UK's Graph Pad Prism 8.2 software, one-way ANOVA was used to analyze the data. $P \leq 0.01$ was used to define significance for differences.

CHAPTER FOUR

4.0 RESULT

4.1 Acute Toxicity Study

Acute toxicity study revealed that there was no sign of toxicity, mortality or morbidity after administration of 5000 mg/kg per body weight of the extract and observed for 72 hours (Table 4.1).

4.2 Carrageenan induced for paw edema

Rat treated with the two doses of ethanol extract of turmeric rhizome exhibited significant decreases in paw size, which when compared to the negative control, were significant (Figure 4.1).

4.3 Egg albumen induced for paw edema

Rat treated with the two doses of ethanol extract of turmeric rhizome exhibited significant decreases in paw size, which when compared to the negative control, were significant (Figure 4.2).

4.4 Formaldehyde induced for paw edema

Rat treated with the two doses of ethanol extract of turmeric rhizome exhibited significant decreases in paw size, which when compared to the negative control, were significant (Figure 4.3).

Table 4.1: Acute effect of single dose of 5000 mg/kg of ethanol extract of turmeric rhizome administered to albino mice after 72 hours of administration.

Group(s)	Dose (mg/kg)	Cognition	Agility	Signs of Toxicity such as: Grooming, nausea, writhing, salivation, and diarrhoea	Mortality after 72 hours of administration
Control	2 ml/kg	Normal	Normal	None	0/6
EETR	5000	Normal	Normal	None	0/6

EETR= Ethanol Extract of Turmeric Rhizome

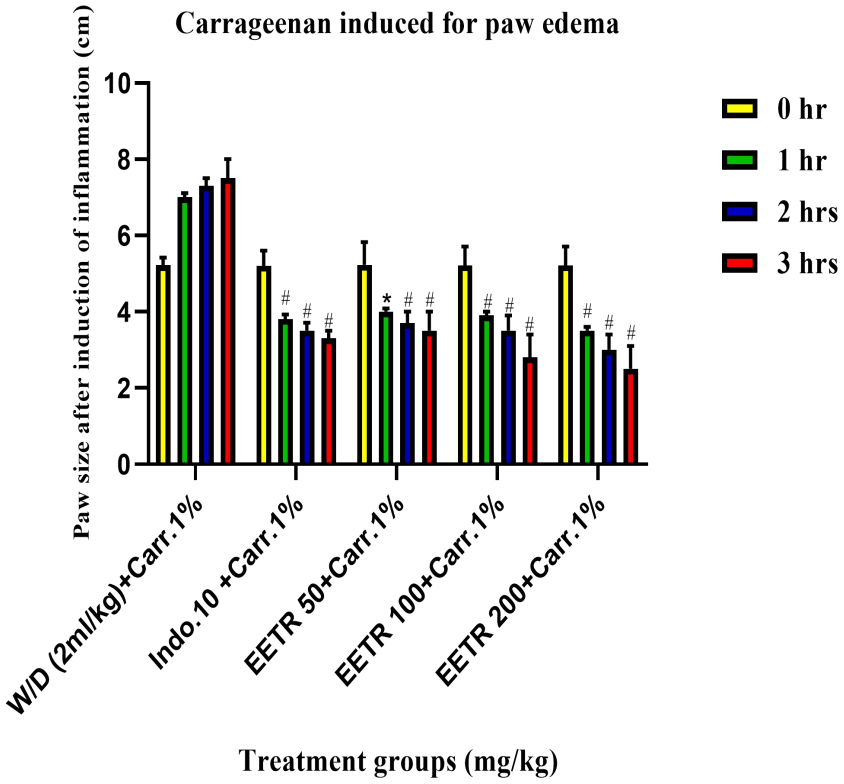

Figure 4.1: Effects of ethanol extract of turmeric rhizome (EETR) (50, 100 and 200 mg/kg) and indomethacin (10 mg/kg) on carrageenan induced for paw edema. Results are Expressed as mean ± S.E.M (n=5). * =P≤0.01 # =P≤0.001 as compared to control (Distilled Water (DW)) group

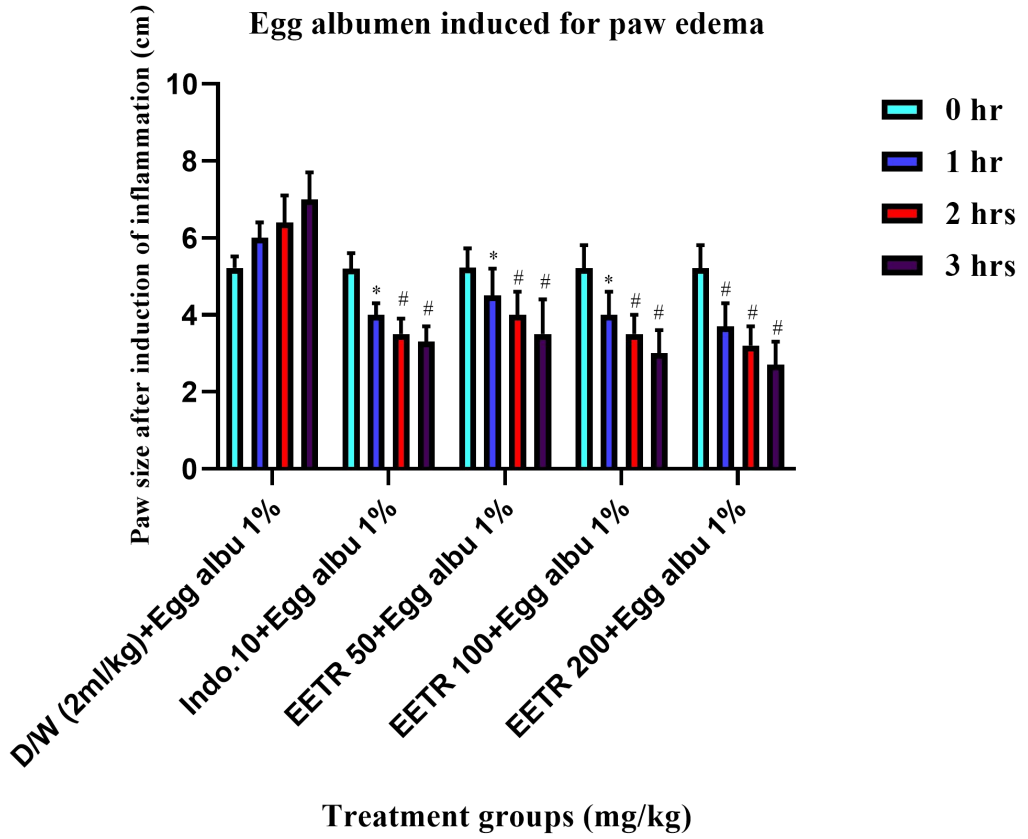

Figure 4.2: Effects of ethanol extract of turmeric rhizome (EETR) (50, 100 and 200 mg/kg) and indomethacin (10 mg/kg) on egg albumen induced for paw edema. Results are Expressed as mean ± S.E.M (n=5). * =*P≤0.01*, # =*P≤0.001* as compared to control group.

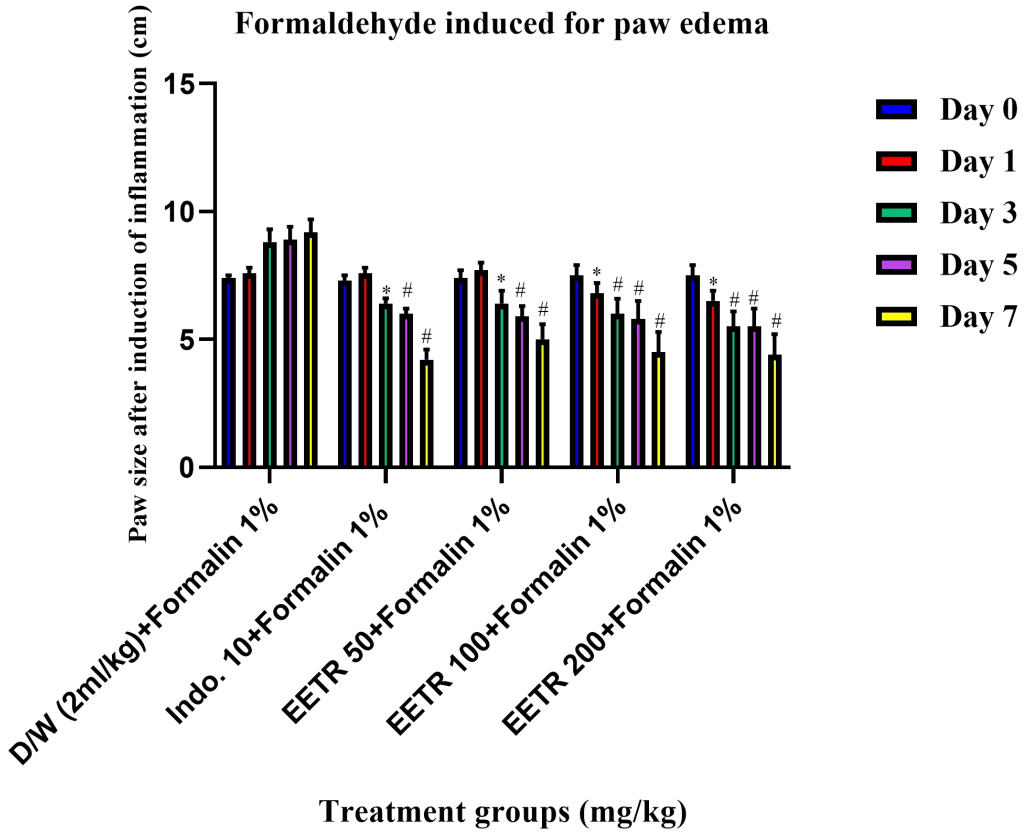

Figure 4.3: Effects of ethanol extract of turmeric rhizome (EETR) (50, 100 and 200 mg/kg) and indomethacin (10 mg/kg) on formaldehyde induced for paw edema. Results are Expressed as mean ± S.E.M (n=5). * =*P≤0.05*, # =*P≤0.01* as compared to control group.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Acute Toxicity Study

Acute toxicity study revealed that there was no sign of toxicity, mortality or morbidity after administration of 5000 mg/kg per body weight of the extract and observed for 72 hours.

5.2 Carrageenan induced for paw edema

A well-established method of inflammation that is acute in nature. This involves a wide range of inflammatory mediators in the development of paw edema caused by carrageenan has been widely used to assess the anti-edematous properties of products obtained natural (Mansouri *et al*., 2015). However, it is well known that the inflammation caused by carrageenan in the hind paw is largely caused by neutrophil infiltration (Gilligan *et al*., 1994). The Carrageenan were induced in rat for paw edema. The ethanol extract of turmeric rhizome (EETR) (50, 100 and 200 mg/kg) were administered at different time interval off 0, 1, 2 and 3 hours, when the treatment and standard group were compared to that of control group were great enough to show significant decrease in paw size with a dosage of 50, 100 and 200 mg/kg for the ethanol extract of turmeric rhizome (EETR) and 10 mg/kg of indomethacin as the standard drug, while distilled water treated group showed no decrease in contrast to the extract and indomethacin treated groups. This reduction of paw edema size corroborates the results of Mansouri *et al*. (2015), which revealed in his study that ellagic acid at a dosage of (5.26–14.76) mg/kg causes decrease in paw size in carrageenan induced paw edema. The phytochemical ellagic acid which is synthesized naturally from *Punica granatum* as ellagic acid glycosides or as ellagitannins in plants. It has been demonstrated that ellagic acid inhibits the enzymes prostaglandin-endoperoxide synthase and nitric oxide synthase, thereby preventing the release of nitric oxide and prostaglandin E2.

They are in charge of producing the prostaglandin and thromboxane enzymes that cause inflammation in the body (Ricciotti and FitzGerald, 2011). The site of an inflammation is treated with the release of prostaglandins and thromboxane.

The ethanol extract of turmeric rhizome (EETR) in this study at doses of 50, 100 and 200 mg/kg demonstrated a reduction in the size of the paw. The glycosides present in the ethanol extract of turmeric rhizome (EETR) are thought to be responsible for the reduction in paw size. These glycosides work by inhibiting the synthase of prostaglandins and nitric oxide from acid of arachidonic, which is released out of plasma membrane through phospholipases, and by blocking the activities and production of the prostaglandins and thromboxane enzymes.

5.3 Egg albumen induced for paw edema

In this study, the egg albumin in rat paw edema method was used to analyze the ethanol extract of turmeric rhizome (EETR) for anti-inflammatory activity. However, egg white proteins had special protein and nullify inflammatory attributes (Andersen, 2015), so more investigation is required to identify the protein fraction that functions as an inductor of edema. Egg albumen test was carried out on induced paw edema in rats. The ethanol extract of turmeric rhizome (EETR) was administered to the rat with a dosage of 50, 100 and 200 mg/kg and measured for a period of 0, 1, 2 and 3 hours. The result obtained from the treatment and standard group when contrasted with that of control (distilled water) group revealed significant decrease in paw size, while distilled water treated group showed increase in paw size. This reduction in paw edema size corroborates the results of Akindele *et al*. (2015), which evaluated *Thuja occidentalis* hydroethanolic leaf extract with a dosage of 50, 100, 200, 400 mg/kg for a period of 0.5, 1, 1.5, 2, 2.5, 3 hours indicated statistically substantial inhibitory activity on the growth of rat paw edema in the egg albumen-induced test, with the anti-inflammatory action as it most noticeable at

the third phase. These results imply that the extract hinders the secretion and/or activities of compounds that are vasoactive such as kinins, serotonin, histamine and as well as prostaglandins. Given that the discharge of prostaglandin-like substances is linked to the late stage of carrageenan-induced edema, it is suggested that the impact of secretion and/or activity prostaglandins is highly associated (Hunskaar *et al*., 1985) and is comparable to clinically effective anti-inflammatory drugs such as the steroidal and non-steroidal drugs (Di Rosa *et al*., 1971). On the development of edema in rat paw brought on by albumin of egg, *Peperomia pellucida* also had a great enough inhibitory activities. This supports one of the mechanisms by which the extract produced its observed anti-inflammatory action: inhibition of histamine and serotonin secretion and/or activities.

5.4 Formaldehyde induced for paw edema

Formaldehyde test is mostly usage is to assesses nullify inflammatory activities of drugs. Formalin causes pain, but this irritation also causes a localized inflammatory response, which includes pain. In fact, a formalin injection also results in oedema and increased vascular permeability (Hong and Abbott 1995). In this current study, Formaldehyde test were carried out on rats to induce paw edema. The ethanol extract of turmeric rhizome (EETR) was administered to the rat with a dosage of 50, 100 and 200 mg/kg and measured for a period of 0, 1, 2 and 3 hours. When the treatment and standard groups were contrasted to the control groups, they revealed significant decreases in paw sizes. The decrease in paw size is in support of the study by Akindele *et al*., (2015), who revealed in his study that the *Thuja occidentalis* hydroethanolic leaf extract with a dosage of 50, 100, 200 and 400 mg/kg with a duration of 0.5, 1, 1.5, 2, 2.5 and 3 hours showed significant decrease in paw edema by impeding the activities of the enzymes prostaglandins, which causes inflammatory responses. The result gotten in the formaldehyde test,

revealed that the extract significantly impede the effect on inflammation by inhibiting the activities of enzymes arachidonic acid to prostaglandins and thromboxane, that are crucial in controlling physiological systems like inflammatory and immune reactions. Prostaglandins are small potent inflammatory mediators that are created by the secretion of arachidonic acid from the membrane phospholipids by the phospholipase A2 family (Fattahi and Mirshafiey, 2012). Thus resulting in a decrease in the paw size through decrease of vascular permeability which is similar to the indomethacin inhibition of inflammation.

FINDINGS

The following are the findings of the experiment;

1. No sign of toxicity, mortality or morbidity after administration of 5000 mg/kg per body weight of the ethanol extract of turmeric rhizome (EETR) and observed for 72 hours.
2. The ethanol extract of turmeric rhizome (EETR) revealed anti-inflammatory activities and thus, at therapeutic levels with an EC_{50} range of 50 to 200 mg/kg, may be helpful in the treatment for arthritis and other inflammatory ailments.

RECOMMENDATION

It is recommended that the ethanol extract of turmeric rhizome (EETR) should be included as a remedy for the management of inflammations at a concentration of 50 to 200 mg/kg. And also, be subjected to clinical trials.

CONCLUSION

The ethanol extract of turmeric rhizome (EETR) possesses vital phytochemicals which are not only useful in arthritis but may be also useful in the management of other inflammations.

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APPENDIX

Positive Control

$$1 - \frac{VT}{VC} \times 100$$

$$1 - \frac{3.3}{3.2} \times 100 = 1 - 0.4600 \times 100 = 54\%$$

FOR 100 mg/kg

$$200\ mg/kg = 1 - \frac{2.9}{7.2} \times 100 = 1 - 0.40 \times 100 = 60\%$$

FOR 200 mg/kg

$$200\ mg/kg = 1 - \frac{2.8}{7.2} \times 100 = 1 - 0.39 \times 100 = 61\%$$

$$C = 1 - \frac{7.2}{7.2} \times 100 = 1 - 1 \times 100 = 99\%$$