ANTI FIBROID ACTIVITY OF THE ETHANOL EXTRACT OF *TETRAPLEURA TETRAPTERA* PODS (SCHUMM. AND THONN.) TAUBERT (FABACEAE-MIMOSOIDEAE)

BY

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FACULTY OF PHARMACY

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A PROJECT REPORT SUBMITTED IN PARTIAL FULFILMENT FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHARMACY (PHARM.D) IN THE

DEPARTMENT OF PHARMACOGNOSY

FACULTY OF PHARMACY

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CERTIFICATION

This is to certify that this project work was carried out by AGOREYO OGHENEMARO VICTORY with the matriculation number PHA1505891 under the supervision of Dr (Mrs.) Rose O. Imade.

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DEDICATION

This project work is dedicated to God Almighty for his grace, provision sustenance and guidance through the course of this program.

Also, to my beautiful parents, Prof and Prof (Mrs.) Fred Agoreyo as well as my siblings; Miracle and Favour.

ACKNOWLEDGEMENT

I am forever grateful to God the father, Son and Holy spirit who has constantly been with me right from when I was in my mother's womb till this day and has been leading me on the right path to go.

My profound gratitude goes to my supervisor Dr (Mrs) Rose O. Imade for her support and patience through the course of this project.

My thanks go to the non-academic staff in the Department of Pharmacognosy, Mr. Kingsley Uzzo, Mrs. Adeshina, Mr. Anozie for providing a comfortable environment for me to work.

Special thanks to my ever-supportive parents for always caring, loving and providing for me and for their incessant prayers and words of advice.

Big thanks to my lovely aunty, Mrs. Helen A. and her family for all the emotional support.

I appreciate my friends, Theresa, Justice, Ezomo, Udeme, Ehis, Eju, Faustina, Rachel, Obruche, Catherine, Ade bakes, Dami, Adeshina, Valerie, Jude, Sammy, Cyril for their endless support during the course of this project.

TABLE OF CONTENT

Chapter Four

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Uterine leiomyoma commonly termed uterine fibroid, a benign smooth muscle neoplasm, constitutes a significant reproductive threat in women worldwide. It is a benign monoclonal tumor of the smooth muscle cells found in the uterus.

Tetrapleura tetraptera pod has various ethnomedicinal properties which are used against different diseases and ailments which may include convulsion, leprosy, inflammation and rheumatic pains.

This research work was carried out to determine preventive effects on fibroid activity of the ethanol extract *of T. tetraptera* pods using female wistar rats as test subject.

The anti-fibroid activity of the plant was ascertained by inducing fibroids in female wistar rats using 800mg/kg concentration of Monosodium glutamate and concomitant administration of 100, 200 and 400mg/kg of plant extract. Hormonal assays and histopathology investigations were then carried out after a 30-day period.

The plant extract showed anti-fibroid activity by reducing major markers for fibroid development including cholesterol, total protein and estradiol levels. The histology results also displayed the plant's ability to prevent fibroid growth as MSG resulted in endometrial epithelium distortion and lamina propria fibrosis with plant extract ameliorating the distortion observed in the uterus. This study establishes the ability of *T. tetraptera* pods to preventing MSG-induced uterine leiomyoma.

CHAPTER ONE

1.0 INTRODUCTION

In ancient times, to get a cure for their disease, the ancient man had to look into wildlife, his surroundings and nature in general. The beginning of the medicinal plants use was based on instinct, as is the case with animals (Stojanoski, 1999).

The oldest write up of medicinal plants, and its use for drug preparations has been found on a Sumerian clay slab from Nagpur, it is approximately 5000 years old. It was made up of 12 recipes of drug preparation referring to over 250 various plants, a few of them were alkaloidal such as poppy, henbane, and mandrake (Kelly, 2009).

In modern pharmacy, almost all pharmacopoeias in the world—Ph Eur 6, USP XXXI, BP 2007, prescribe plant drugs of real medicinal value. There are countries (the United Kingdom, Russia, Germany) that have pharmacopoeias strictly for herbal products. This is different in practice, as a much higher number of unofficial drugs are always used. The application of herbal drugs is based on the experiences of popular medicine (traditional or popular medicine) or on the new scientific research and experimental results (conventional medicine). Many medicinal plants are administered by self-medication or at the recommendation of a clinical specialist. Their usage can either be independently or sometimes in combination with orthodox drug. For an effective, safe and complete therapy to be achieved, information on the correct diagnosis of the infection/disorder/disease and correct information of the specific medicinal plants, i.e. the pharmacological components of the plant are very important. For a plant to be used for clinical therapy, it should meet all requirements for pharmaceutical quality of drugs (Petrovaska, 2012).

1.1 Uterine Fibroids

Uterine leiomyoma commonly termed uterine fibroid, a benign smooth muscle neoplasm, is the most common (approximately 50% of fertile women) and constitutes a significant reproductive

threat in women worldwide. (Crum *et al.,* 2003; Martinez *et al.,* 2010). It is a benign monoclonal tumor of the smooth muscle cells found in the uterus. It grows in various sites on and within the uterine walls or in the uterine cavity. It is hence described as subserosal, submucosal or intramural fibroids. It can be of any size and shape ranging from the size of a pea to an average-sized water melon (Obochi *et al.,* 2009).

A lot of women who develop uterine fibroids may not show symptoms, the consequence of which will lead to little clinical attention. The few who develop symptoms usually complain of abnormal uterine bleeding, feeling of fullness in the pelvic area, pain the lower back, severe complications in pregnancy and labor, as well as infertility. It should be noted that whether or not these symptoms are severe is dependent on the size and location of the tumor (Hart, *et al.,* 2001; Surrey *et al.,* 2001).

1.2 Pathophysiology of Uterine Fibroids

The pathophysiology of uterine leiomyoma is uncertain (Okolo, 2008). Therefore, therapeutic approaches have been empirical. Studies have indicated a possible role of growth factors in uterine leiomyoma amongst other factors

The most important aspect of the etiology of fibroids remains unknown. Several theories have been advanced. One hypothesis suggests that increased levels of estrogen and progesterone result in increased mitotic rate and may play a part in myoma formation by increasing the likelihood of somatic mutations (Rein, 2000). Other studies detected an increase in estrogen concentration (estradiol levels), resulting presumably from inadequate conversion of estradiol to estrone (Pollow *et al.,* 1978).

Another theory favors an inherent peculiarity in the myometrium of those who develop fibroids, based upon the finding of significantly increased levels of Endoplasmic Recticulum in the

myometrium of fibroid uteri (Richards and Tiltman 1996). A predisposing genetic factor has been suggested by others on the basis of ethnic and familial predilections (Marshall *et al.,* 1997)

Estrogen has been traditionally proposed as the major cause of uterine leiomyoma growth. This theory has been based in part upon the clinical observations that fibroids occur only after puberty, develop during the reproductive years, may enlarge during pregnancy and frequently regress following menopause. (Cramer *et al.,* 1992; Parazzini *et al.,* 1996a; Ross *et al.,* 1986).

Several growth factors and their receptors have now been identified in both myometrium and leiomyoma. Those that have received the most attention in the literature include transforming growth factor (TGF)-β, bFGF, epidermal growth factor (EGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF) (Ciarmela *et al.,* 2011).

1.3 Classification of uterine *Fibroid*s

Most of the numerous classifications of myomas in literature take into account the degree of intramural extension and/or uterine cavity distortion. (Dolmans *et al.,* 2019)

The most widely accepted classification system is the one set by the International Federation of Gynecology and Obsterics (FIGO). Uterine leiomyoma is classified based on its position in the uterus. According to the FIGO fibroid classification, there are nine (9) types of fibroids, assigned numbers ranging from 0-8. (Munro *et al.,* 2011). These numbers describe the location of the fibroid relative to the three main layers of the uterus; the inner lining of the uterine cavity(endometrium), the smooth muscle of the uterine wall (myometrium) and the outer surface of the uterus (serous membrane). (Cook *et al.,* 2010). Under FIGO classification system, uterine fibroids are described as either pedunculated, submucosal, intramural or subserosal.

- Pendunculated fibroids grow on small stalks inside of the uterus or outside of the uterus.

- Intramural fibroids are located within the uterine wall. A study of 96 Italian women with fibroids suggested Intramural fibroids as the most prevelant type identified on transvaginal ultrasound. (Lippman *et al.,* 2004)
- Submucosal fibroids are a type of intramural fibroid where part of the fibroid projects into the uterine cavity and another part resides in the smooth muscle of the myometrium. Generally, submucosal fibroids are not as prevalent as subserosal or intramural. (Lippman *et al.,* 2004)
- Subserosal fibroids grow on the outside of the uterus.

Table 1: FIGO Fibroids Classification (Lippman *et al.,* 2004)

Fibroid Type	**Description**
Type 0	Pedunculated, intracavitary
Type 1	Submucosal, <50% intramural
Type 2	Submucosal, ≥50% intramural
Type 3	Contact with endometrium, 100% intramural
Type 4	Intramural
Type 5	Subserosal, ≥50% intramural
Type 6	Subserosal, <50% intramural
Type 7	Subserosal, pedunculated
Type 8	Other (e.g., cervical, parasitic

1.4 Risk factors

- The major risk factors associated with the development of fibroids include age, race, endogenous and exogenous hormonal factors, obesity, uterine infection, lifestyle, racial

differences, diet, use of oral contraceptives, vitamin D deficiency, food additive consumption.

- The impact of many of these factors has been attributed to their effects upon estrogen and progesterone levels or metabolism. Several studies have shown an inverse relationship between parity and the risk of fibroids (Lumbiganon *et al.,* 1996; Parazzini *et al.,* 1996a; Ross *et al.,* 1986; Samadi *et al.,* 1996).
- An increase with age in the prevalence of fibroids during the reproductive years has been demonstrated by several epidemiologic studies (Marshall *et al.,* 1997).
- Studies have found an association between obesity and an increased incidence of uterine leiomyomas. In a prospective study from Great Britain (Ross *et al.,* 1986), the risk of fibroids increased approximately 21% for each 10-kg increase in body weight; similar results were obtained when the body mass index (BMI) was analyzed rather than weight.
- A reduced risk of fibroids requiring surgery in postmenopausal patients (Parazzini *et al.,* 1988; Ross *et al.,* 1986; Samadi *et al.,* 1996) could be due to tumor shrinkage in the absence of hormonal stimulus following the menopause.
- The inverse correlation between smoking and fibroids has been commonly attributed to an anti-estrogenic effect of cigarette smoking (Parazzini *et al.,* 1988)

1.5 Signs and symptoms

Many women who have fibroids don't have any symptoms. In those that do, symptoms can be influenced by the location, size and number of fibroids.

In women who have symptoms, the most common signs and symptoms of uterine fibroids include:

- Heavy menstrual bleeding
- Menstrual periods lasting more than a week

- Pelvic pressure or pain
- Frequent urination
- Difficulty emptying the bladder
- Constipation
- Backache or leg pains
- abnormal uterine bleeding
- dyspareunia
- infertility. (Williams, 2017)

1.6 Diagnosis

The following diagnostic tests can be used to detect fibroids and also rule out other conditions:

Laparoscopic techniques which involve inserting a small, lighted tube into a small incision in the abdomen has found major progress and has been proven by randomized trails (Gordon *et al.,* 1989).

A non-invasive technique, magnetic resonance imaging (MRI)-guided focused Ultrasound surgery has been approved by Medicine Agency of the European Union (EMA) in 2002. It consists of noninvasive thermoablative technique, combining anatomic details' visualization through MRI, with the therapeutic potential of high-intensity-focused ultrasound waves capable of passing through the abdominal wall (Jenne *et al.,* 2012).

It is necessary for patients to undergo routine pelvic examination as it is the most effective way of detecting fibroids as a result of its lack of symptoms.

1.7 Management of Uterine Fibroid

Various medical therapies are now available for women with uterine fibroids, although, each therapy has its own advantages and disadvantages.

Another option is a vaginal hysterectomy, which is the approach that most people prefer. In this method, a surgeon will remove the uterus through the vagina. A vaginal hysterectomy may not be possible if the uterus or fibroid is too large to fit through the vagina. Individuals who undergo an open hysterectomy may have a longer recovery time, this is why it is usually recommended for those whose fibroids are very large or significantly interfere with their quality of life. People who have other reproductive health issues, such as endometriosis may find that a hysterectomy provides significant relief from fibroids and other symptoms.

Nonsteroidal anti-inflammatory drugs (NSAIDs), may be effective in relieving pain related to fibroids, but have no effect on the bleeding associated with the disease.

1.8 Alternative Medicine

In 2014, about 1503 racially and ethnically diverse women who had fibroids were selected and treated using complementary and alternative medicine which included exercise (45%), diet (34%), herbs (37%), and acupuncture (16%). Participants reported significant symptom improvement and few side effects with these interventions. (Jacoby *et al.,* 2014)

Some herbal drugs have been found to be quite effective in reducing the volume size of fibroids, and causing symptomatic relief. One of these herbal preparations is the Guizhi Fuling Formula. This formula contains a combination of medicinal herbs, these herbs are Guizhi Fuling Formula consists of five herbs: *Ramulus Cinnamomi, Poria, Semen Persicae, Radix Paeoniae Rubra or Radix Paeoniae Alba, and Cortex Moutan* (Zhang, 2022). Research shows that when this formula was combined with mifespritone, there was a reduction in fibroid volume and uterine size (Chen *et al.,* 2014).

Huoxue sanjie, a plant usually found in china, a decoction was made out of it, and tested in 150 women with symptomatic fibroid, it was found that it could cause shrinkage of fibroids, as reported by the participants in these trials (Feng *et al.,* 2019).

1.9 Fabaceae Family

The Fabaceae or Leguminosae, commonly known as the legume, pea, or bean family, is a large, economically and medicinally important family of flowering plants. Products of plants of this family were reported for their cytotoxicity against human cancer cells. The bark methanol extract from *Guibourtia tessmannii* Harms Leonard, harvested in Cameroon, also showed antiproliferative activity, with an IC50 of 13.1 µg/mL against MCF-7 and 8.8 µg/mL against the human cervical cancer cells HeLa. (Kuete *et al.,* 2013).

The vast majority of trees, shrubs, and herbaceous plants belonging to this family have significant economic value (Hickey and King, 1997). Leguminosae family consists of three subfamilies: Caesalpinioideae, Mimosoideae, and Papilionoideae (Schrire *et al.,* 2005).

The leguminosae have a wide variety of growth forms including trees, shrubs or herbaceous plants or even vines or lianas. The herbaceous plants can be annuals, biennials or perennials, without basal or terminal leaf aggregations. Many legumes have tendrils. They are upright plants, epiphytes or vines. The latter support themselves by means of shoots that twist around a support or through cauline or foliar tendrils.

The Leguminosae family consists of plants possessing anti-bacterial properties like *Cassia fistula, Tephrosia hamiltonii, Glycyrrhiza glabra and Cassia senna* which showed highest antibacterial activity against different strains of bacteria (Ahmed *et al.,* 2018).

Other plants possess antitumor activities and they include; *Acacia niclotica, Arachis hypogaea, Cajanus Cajan, Crotalaria juncea, Glycine max, Mimosa pudica, Psoralea escenta and Trifolium pretense* (Velusamy *et al.,* 2016).

1.10 The genus: Tetrapleura

Tetrapleura has two species, they include;

Tetrapleura tetraptera

Tetrapleura chevalieri

Tetrapleura tetraptera has molluscicidal, cardio-vascular, neuromuscular, hypotensive, anti-convulsant, molluscicidal, trypanocidal, hirudinicidal, schistomiasis control, anti-ulcerative, ectoxicity, anti-inflammatory properties.

Tetrapleura chevalieri is used as wood.

1.12 Species: *Tetrapleura tetraptera*

1.12.1 Botanical description of *T. tetraptera*

Taxonomy

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order: Fabales

Family: Fabaceae

Subfamily: Caesalpinioideae

Clade: Mimosoid clade

Genus: *Tetrapleura*

Species: *T. tetraptera*

The local names of T. tetrapleura are Oshosho, Obogolo (Igbo), Aridan, Aid an (Yoruba), Eseyeseye, Ighimiaka (Edo)

The tree can grow up to g 20-25 m in height, with a girth of about 1.2-3 m. The stem bark is fairly smooth, greyish brown and thin. Leaves are sessile, glabrous or minutely hairy with a common stalk some 15-30 cm long and slightly channeled on the upper phase. Fruit has been described as being persistent, hanging at the ends of branches on stout stalks 25 cm long. The fruit appears green when still tender but on maturity and ripening, it is shiny, glabrous, dark-purple-brown some 15- 25 cm long by about 4-5 cm broad (depending on size), with four longitudinal, wing like ridges nearly 2.5-3.0 cm broad. Two of these wings are woody while the other two are soft and are used for food, drinks and medicine, the small, black, hard and flat seeds are hidden in the pods (Burkill HM,1985)

Figure 1: Picture of *Tetrapleura tetraptera* pods

1.12.2 Ethnomedicinal uses of *Tetrapleura tetraptera*

Studies have shown the use of *T. tetraptera* in the management and/or control of arthritis and other inflammatory conditions, as well as in adult-onset, type-2 diabetes mellitus in some Yoruba-speaking communities of South-Western Nigeria. (Ojewole *et al.,* 2004).

The fruits are used locally in Nigeria in flavouring, in creams and in soaps. An infusion of the whole fruit is usually taken by recuperating patients to bathe in order to get relief from feverish conditions, for use as an enema, for constipation and as an emetic (Akintola *et al.,* 1980).

The plant has many traditional medicinal uses mainly in the management of convulsion, leprosy, inflammation and rheumatic pains. Infusion of the whole fruit is taken as a recuperative tonic (Ojewole and Adesina 1983).

1.12.3 Reported pharmacological activities on *Tetrapleura tetraptera*

Aqueous extract of *T. tetraptera* was evaluated for its antidiabetic activity. The extract produced dose-dependent, significant reductions ($P < 0.05–0.001$) in the blood glucose concentrations of both fasted normal and fasted diabetic rats. (Ojewole *et al.,* 2004)

The ethanolic extract of *T. tetraptera* at dose of 900mg/kg, showed an antiplasmodial effect on mice infected with plasmodium. It showed a significant schizonticidal effect on mice that have been infected with the parasite for 4-days, and the ones that have been infected for a longer period. The effect was compared to a control group where chloroquine at 5mg/kg was used in treating mice infected with the same parasite (Okokon *et al.,* 2012).

Aqueous extract of *T. tetraptera* was evaluated for its anti-convulsant properties. The aqueous extract significantly delayed the onset of seizures, similar to the standards. It was also discovered that it also delayed the onset of induction of seizures induced by picrotoxin (Ojewole, 2005).

T. tetraptera fruit aqueous extract was found to possess analgesic effects. Pain was induced in mice, chemically and thermally. Using morphine, diclofenac as standard anagelsics, the extract was also used in comparison, to see if it has significant anagelsic properties. *T. tetraptera* fruit aqueous extract produced dose-dependent, significant ($p < 0.05–0.001$) analgesic effects against thermally and chemically induced pain in mice (Ojewole, 2005).

Aqueous extract of *T. tetraptera* showed anxiolytic properties in a study. The extract was adminisitered to albino mice at 5 and 10 mg/kg, it induced anxiolysis 30 minutes after administration, which was measured by the length of time spent with the paws of the mic closed. Paw closing is an indicator of sedation in mice. When flumazenil, a GABA receptor blocker, was administered 15 minutes before the administration of aqueous extract of *T. tetraptera*, it blocked it anxiolytic action. This shows that aqueous extract works through the GABA pathway (Aderigbe *et al.,* 2010).

In a study aimed to determine the anti-trypanosomal and anthelminthic properties of aqueous and ethanolic extract of *T. tetraptera, t*he aqueous extract exhibited marked antitrypanosomal and antihelminthic properties, while the ethanolic extract exhibited mild properties (Obeng *et al.,* 2021).

Aqueous, ethanolic and petroleum ether extracts of *T. tetraptera* were investigated for their antibacterial and antifungal activity. The bacteria isolates used were *Esherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa* while the fungi were *Aspergillus, Mucor and Rhizopus* species. Antimicrobial sensitivity of the extracts showed marked antimicrobial properties, the petroleum ether extract of *T. tetraptera* showed inhibitory activity against the test isolates (Ebana *et al.,*2016)

1.12.4 Reported phytochemical constituents of *Tetrapleura tetraptera*

The qualitative phytochemical screening carried out on the aqueous and ethanol extracts of pulp, seeds and whole fruit of *T. tetraptera* showed the presence of flavonoids, alkaloids, tannins, saponins, steroids, sterols and phenols in either one in both extracts (Akintola *et al.,* 2015).

1.13 Aim and Objectives

The aim of this study is to evaluate the antifiboid property of the ethanol extract of *Tetrapleura tetraptera* pod on Monosodium glutamate (MSG) induced uterine leiomyoma in female wistar rats using preventive methods

The objectives of this study are to:

I. Extract the pods of *T. tetraptera*

II. Assess the ability of *T. tetraptera* pods to shrink fibroid

III. Assess the ability of the extract to regulate oestrogen levels

IV. Assess the ability of the extract to regulate cholesterol levels and

V. Assess the ability of the extract to regulate total protein levels

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Laboratory Equipment

Mindray Centrifuge

Fridge

Soxhlet apparatus

Semi-automated chemistry analyzer (Mindray BA-88A Reagent system)

Mindray MW-12A microplate washer

Mindray MR-96A microplate reader

2.1.1 Laboratory materials

Beakers

Test tubes

Porcelain dishes

Filter papers

Cotton wool

Oven

Water bath

Jars

Stirrer

Suction pipette

Conical flask

Syringes

Ethylenediaminetetraacetic acid (EDTA bottles)

Orogastric tube

Round bottom flask

Dissecting Forceps

Scissors

Animal cages

Gloves

Microliter tubes

Timer

Disposable hematocrit tubes,

2.1.2 Reagents

Monosodium glutamate

Cholesterol biuret reagent

Total protein biuret reagent

Total protein standard

Cholesterol standard

2.1.3 Solvents

Ethanol

Chloroform

Distilled water

2.2 Plant collection and preparation

The pods of *Tetrapleura tetraptera* plant were harvested in Ibadan and authenticated at the Forest Research Institute of Nigeria (FRIN) in January 2022. The pods were assigned a voucher number; FHI 110614.

The pods were sun dried for a week and then ground into smooth fine powder and extracted using ethanol. *T. tetraptera* pods (1.2kg) were extracted using a soxhlet apparatus after which the filtrates were concentrated to a solid mass over a water bath.

2.3 Preliminary phytochemical screening for *Tetrapleura tetraptera* pods

The tests were carried out to test the presence of alkaloids, carbohydrates, reducing sugars, tannins, flavonoids, and saponins using standard methods.

2.3.1 Test for Carbohydrates

To about 2ml aqueous solution of *T. tetraptera* pods, 2 drops of 10% alcoholic solution of alpha naphthol was added in a test tube. The test tube was inclined at an angle of 45^{o} and 2ml of concentrated sulphuric acid was carefully added to form a layer and observed for the presence or absence of a violet ring at the interface between the solution and the acid.

2.3.2 Test for reducing sugars

About 2ml of mixed Fehling's solution (1ml of A and B) was boiled with 2ml of aqueous solution of *T. tetraptera* pods and the colour changes were observed.

2.3.4 Test for tannins

A drop of 15% Ferric chloride solution was added to an aqueous solution of *T. tetraptera* pod extract.

2.3.5 Test for saponins

Powdered sample of *T. tetraptera* pod was extracted by heating with 20ml distilled water, filtered whilst hot and allowed to cool. 5ml of the filterate was then shaken vigorously with water.

2.3.6 Test for flavonoids

An aqueous solution of the sample extract of *T. tetraptera* pod was obtained, few drops of sodium hydroxide was added, followed few drops of hydrochloride.

2.3.7 Test for alkaloids

Powdered sample of *T. tetraptera* pod was extracted with 15 mL of 1% solution of sulphuric acid by heating on a water bath. It was then filtered and allowed to cool for 10 minutes. About 2 mL each of the filtrate was transferred into four test tubes labelled A, B, C and D; and these were tested with 2 drops of Mayer's, Dragendorff's, Wagner's and Hager's alkaloidal reagents, respectively.

2.3.8 Test for Anthracene derivatives (Borntrager's test)

About 2g of the powdered sample of *T. tetraptera* pod was extracted with 15 mL chloroform in a test tube by heating gently for 3 minutes. The extract was then filtered with a filter paper. 2.5 mL dilute ammonia solution was added to 5ml of the filterate.

2.3.9 Test for Cyanogenic glycosides

Powdered sample of *T. tetraptera* pod was placed in a test tube and was mixed with 5 mL of water. A sodium picrate test paper was inserted into the test tube which was stoppered immediately. The tube was immediately placed in a boiling water bath.

2.4 Sources of female wistar rats

Non-pregnant female wistar rats weighing 160-200g were obtained from the Animal House of the Department of Anatomy, Faculty of Basic Medical Sciences, University of Benin, Nigeria. They were housed in groups of five (5) in stainless steel cages with wood shavings as the bedding material. The cages were kept at ambient temperature and were properly aired. The animals were fed with commercial pellet feed and clean water. Ethical care and handling were observed at all times, and the study was approved by University of Benin, Faculty of Pharmacy Ethical Committee. Rats were separated into five groups totaling 25, with five rats in each group, for the purpose of this study.

2.5 Determination of preventive anti-fibroid effects on female wistar rats

2.5.1 Experimental design

Female wistar rats were placed in five groups of five rats each. Dosing was done once a day through the oral route. Group A (Control) was the no treatment group and was administered only water and feed. Groups B, C and D, were treated with 800 mg/kg MSG and *T. tetraptera* (100, 200, and 400 mg/kg) concurrently. Group E was treated with 800mg/kg Monosodium Glutamate (MSG) only. All treatments were given concurrently for a 30-day period. The animals were sacrificed on the 31st day and blood collected to determine total plasma cholesterol, protein and plasma estradiol. The uteruses were harvested, pictures taken, and uterus weight to body weight determined. All results gotten were recorded.

2.5.2 Specimen collection

At the end of 30 days of treatment period, the rats were anesthetized in a chloroform saturated chamber, sacrificed and dissected. Blood was collected through cardiac puncture into labeled sample bottles and centrifuged at 3000rpm for 10 minutes to obtain the blood serum as the supernatant which was carefully collected into labeled hematocrit tubes. The uterus was surgically removed and ovaries separated from the uterus, both were transferred into sterile tissue bottles containing Bouin's solution for histopathology tests.

2.5.3 Biochemical assay of Total cholesterol

This assay was carried out using the Semi-automated chemistry analyzer (Mindray BA-88A Reagent system) and the AGAPPE test kit.

The total cholesterol kit was programmed on the semi-automated biochemistry analyzer using the information on the instruction manual. In a micro tube marked 'blank', 1000µL of cholesterol biuret reagent was added. 10µL of standard cholesterol and 1000µL of the reagent were added to and properly mixed in a tube marked 'standard'. 10µL of sample A1 and 1000µL of cholesterol reagent were added to and properly mixed in a tube with the label 'A1'. The same procedure was carried out for remaining samples (A1-E5) in their respective tubes. All tubes were incubated for 10 minutes at 37^0C. After incubation, the content of the blank tube was aspirated into the flow cell of the analyzer to measure the absorbance, after which the content of the standard tube was also aspirated into the flow cell to measure the absorbance. Reaction mixture for each sample was then aspirated into the flow cell to measure the absorbance. The value of absorbance for each tube was recorded accordingly.

2.5.4 Biochemical assay of Total protein

This assay was carried out using the Semi-automated chemistry analyzer (Mindray BA-88A Reagent system) and the AGAPPE test kit.

The total protein kit was programmed on the semi-automated biochemistry analyzer using the information on the instruction manual. In a micro tube marked 'blank', 1000µL of cholesterol biuret reagent was added. 20µL of standard cholesterol and 1000µL of the reagent were added to and properly mixed in a tube marked 'standard'. 20µL of sample A1 and 1000µL of cholesterol reagent were added to and properly mixed in a tube with the label 'A1'. The same procedure was carried out for remaining samples (A1-E5) in their respective tubes. All tubes were incubated for 10 minutes at 37^0C. After incubation, the content of the blank tube was aspirated into the flow cell of the analyzer to measure the absorbance, after which the content of the standard tube was also aspirated into the flow cell to measure the absorbance. Reaction mixture for each sample was then aspirated into the flow cell to measure the absorbance. The value of absorbance for each tube was recorded accordingly.

2.5.5 Biochemical assay of Estradiol

This assay was carried out using the Microplate Reader (mindray MR-96A), microplate washer (Mindray MW-12A) and E2 AccuBind ELISA Kits. This test works on the principle of solid_ohase enzyme-linked immunosorbent assay. The microplate wells for each serum reference calibrator, control and animal specimen were formatted. 25µL of sample was pipetted into corresponding well. 50µL of estradiol biotin reagent was added to all wells and plates were swirled gently for about 30 seconds and then incubated for 30 minutes followed by addition of 50µL of estradiol enzyme reagent to all the wells and incubation for 90mins at room temperature. The contents of the microplate were then discarded by decantation and the plates were dried with absorbent paper afterward. 350µL of the wash buffer was added and the content was decanted thrice. 100 µL of substrate was added and incubated for 20 mins, then 50 µL of the stop solution

was added to each well and gently mixed for 20 minutes. The absorbance was read at 450nm within 15 mins of adding the stop solution. The concentration of estradiol in sample was extrapolated from a dose response curve.

2.5.6 Histology studies

The tissues were fixed by placing them in formaldehyde solution. The uterus was further dissected to select appropriate area for examination and were placed in suitably labeled cassettes. Clearing was done using xylene and then infiltrated by paraffin wax. Paraffin wax-embedded tissues were subsequently processed into ultra-thin section of 5 microns using a semi-automated rotary microtome, dried overnight and subjected to hematoxylin and eosin staining. Stained on DPX and subsequently viewed under the microscope and photographs taken.

2.6 Statistical Analysis

The results obtained from the various experiment above were expressed as the mean ± standard error of the mean (S.E.M). Comparison between the treatment groups and control was carried out using one-way analysis of variance (ANOVA) followed by Dunnet post hoc test. Analysis and data presentation were done using GraphPad Prism version 8.0.2. Results were considered significant where $P < 0.05$.

CHAPTER THREE

3.0 RESULTS

Preliminary phytochemical examination of the powdered pods of *T. tetraptera* revealed the presence of tannins, flavonoids, saponins, carbohydrate, alkaloids and cyanogenic glycosides.

The yield of the ethanol pod extract of *Tetrapleura tetraptera* was 27.52%.

3.1 Result of preventive effect of the ethanol extract of *T. tetraptera* pod on serum cholesterol levels in female wistar rats

T. tetraptera pod extract demonstrated concentration dependent effect on cholesterol levels.

An average cholesterol level of 107.50 ± 4.21 mg/dL was obtained for the control group. The MSG only group experienced a spike in cholesterol level recording a value of 135.00 ± 4.22 mg/dL. Groups treated with 100, 200 and 400mg/kg ethanolic extract of *T. tetraptera* recorded cholesterol levels of 123.30 ± 2.2 mg/dL,112.20 ± 3.34 mg/dL and 111.60 ± 4.69 mg/dL respectively. A significant difference was observed with groups treated with 200 and 400mg/kg concentration compared with the MSG only group ($P<0.01$). Also, a significant difference was observed with the control group compared with the MSG only group ($p<0.001$).

No difference was observed between the control and 200,400mg/kg groups ($P>0.05$)

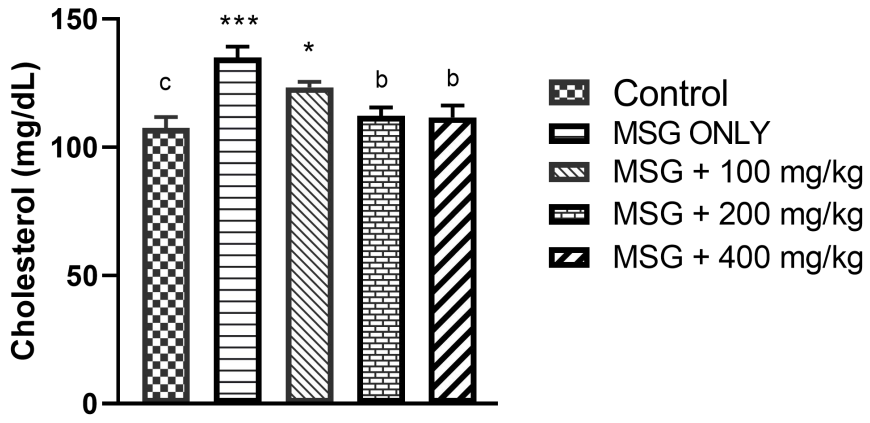

Figure 2: The preventive effects of the ethanol extract of *T. tetraptera* on total plasma cholesterol in female wistar rats pre-treated with 800 mg/kg MSG

Control group *p<0.05, ***p<0.001, against MSG only group ^{b}p<0.01, ^{c}p<0.001

3.2 Result of preventive effect of the ethanol extract of *T. tetraptera* pod on total protein levels in female wistar rats

T. tetraptera pod extract demonstrated concentration dependent effect on total protein levels

An average total protein level of 6.90 ± 0.07 g/L was determined for the control group. The MSG only group experienced a spike in total protein level recording a value of 8.20 ± 0.26 g/L. Groups treated with 100, 200 and 400mg/kg ethanolic extract of *T. tetraptera* recorded total protein level of 8.01 ± 0.39 g/L, 7.26 ± 0.23 g/L, 7.40 ± 0.14 g/L respectively showing decrease away from the MSG group. A significant difference was observed with control and group treated with 200mg/kg concentration compared with the MSG only group ($P<0.01$). There was also a significant difference observed with MSG only group and group treated with 100mg/kg concentration compared with the control group ($p<0.01$).

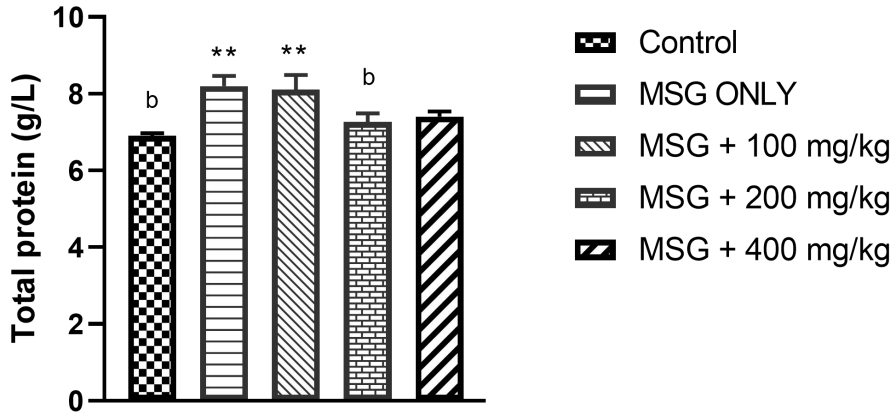

Figure 3: The preventive effects of the ethanol extract of *T. tetraptera* on total plasma protein in female wistar rats pre-treated with 800 mg/kg MSG

Control **p<0.01, against MSG only group; [b]p<0.01.

3.3 Result of preventive effect of the ethanol extract of *T. tetraptera* pod on estradiol levels in female wistar rats

T. tetraptera pod extract demonstrated concentration dependent effect with estradiol levels.

An average estradiol level of 43.39 ± 3.48 pg/mL was determined for the control group. The MSG only group experienced a spike in estradiol levels recording a value of 108.00 ± 1.88 pg/mL. Groups treated with 100, 200 and 400mg/kg ethanolic extract of *T. tetraptera* recorded 79.93 ± 3.32 pg/mL, 68.65 ± 3.3 pg/mL, 63.84 ± 2.06 pg/mL estradiol levels respectively showing reduction compared to the MSG group. A significant difference was observed with control and group treated with 100, 200 and 400mg/kg concentration compared with the MSG only group ($P<0.01$). There was also a significant difference observed with MSG only group and Control group ($P<0.001$).

Groups treated with 100, 200 and 400mg/kg also showed signigicant difference compared to the control group ($P<0.01$).

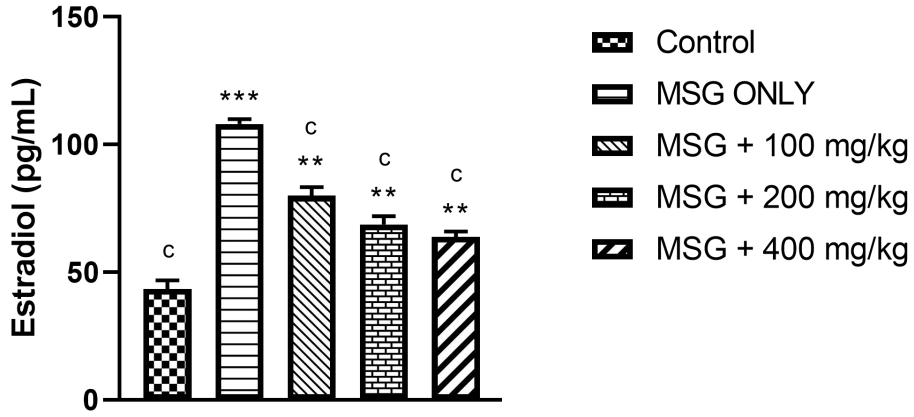

Figure 4: The preventive effects of the ethanol extract of *T. tetraptera* on estradiol levels in female wistar rats pre-treated with 800 mg/kg MSG

Control group; **p<0.01, against MSG only group; ᶜp<0.001

3.4 Photomicrographs of the uteri of female wistar rats

Gut section of the uterus showed normal tissue architecture: endometrial stroma, endometrial lining and uterine cavity. When the female wistar rats were treated with monosodium glutamate (MSG) only, the sections showed thick bands of bundles of smooth muscle fibres arranged in haphazard fashion and crisscrossing the endometrial glands and stroma which is characteristic of Leiomyoma uteri.

Simultaneous administration of graded doses of *T. tetraptera* (100mg, 200mg, 400mg) and monosodium glutamate showed amelioration of the proliferating fibroid

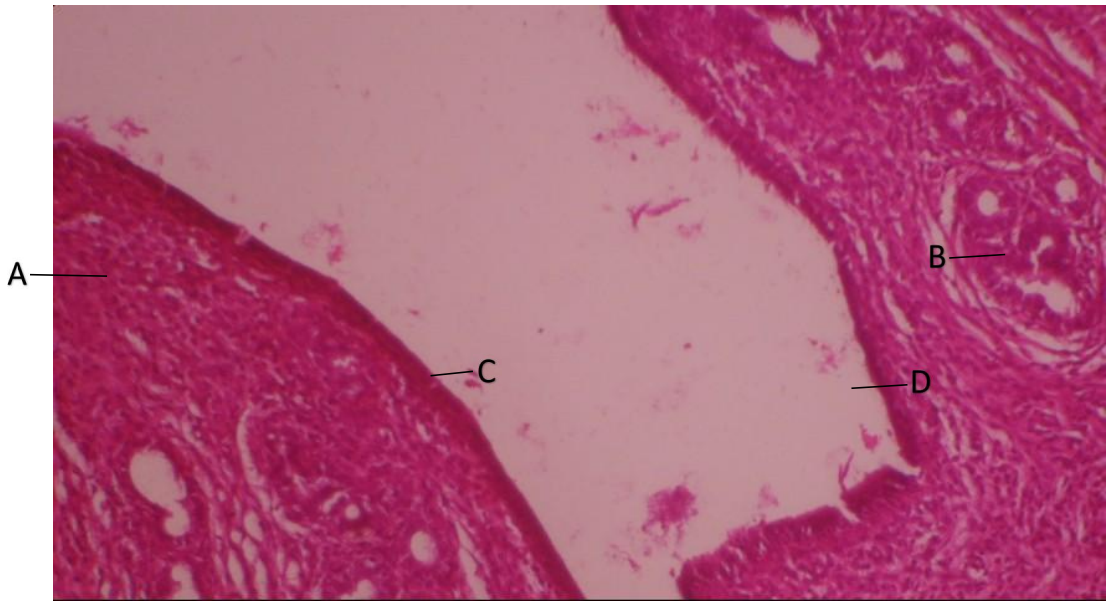

Figure 5: Rat uterus. Control. Composed of: A, endometrial stroma, B. endometrial glands, C. endometrial lining and D. uterine cavity (HandE x 100)

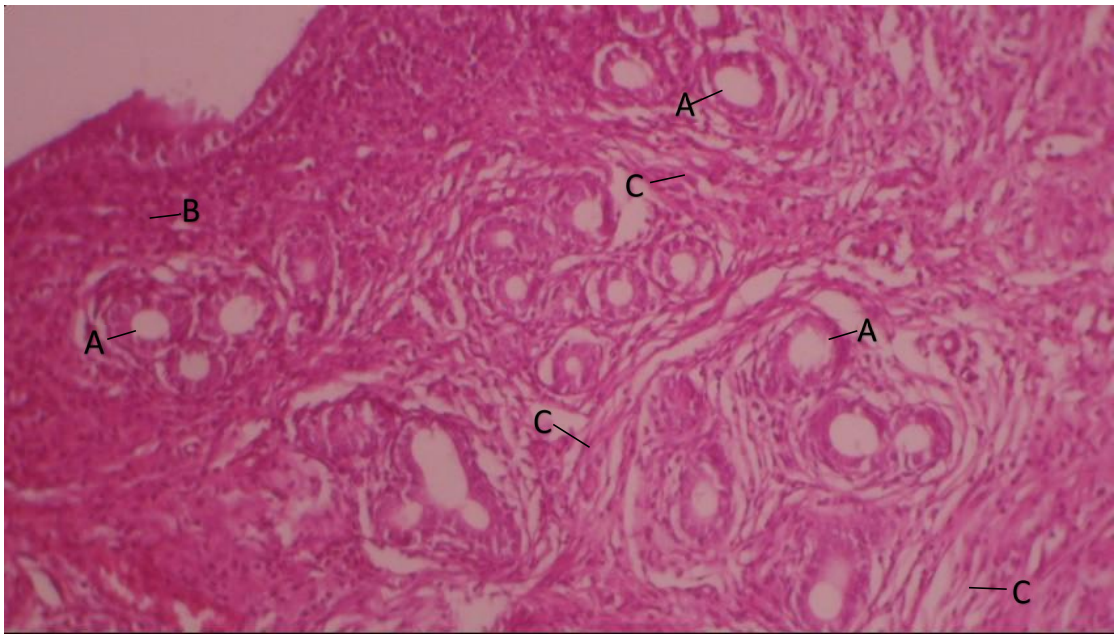

Figure 6: Uterus of rat given 800mg/kg mono sodium glutamate (MSG) only showing: A. endometrial glands and B, stroma surrounded by C, thick bands of smooth muscle fibres arranged in haphazard pattern (HandE x 100)

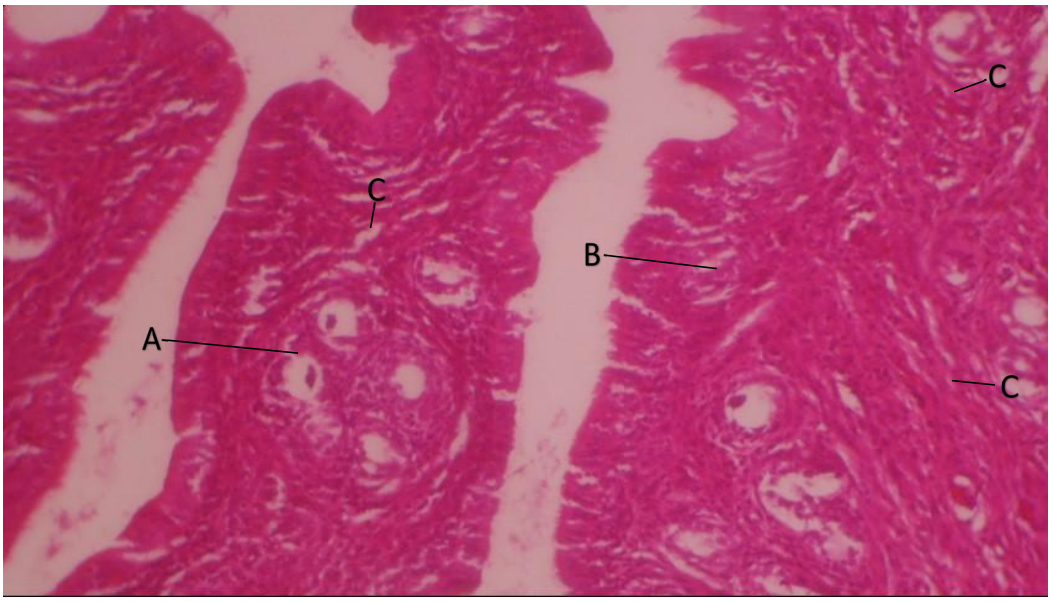

Figure 7: Uterus of rat given 100mg/kg Extract + 800mg/kg mono sodium glutamate (MSG) showing: A. endometrial glands, B. stroma surrounded by C. Fairly reduced bands of smooth muscle fibres arranged in haphazard pattern (HandE x 100)

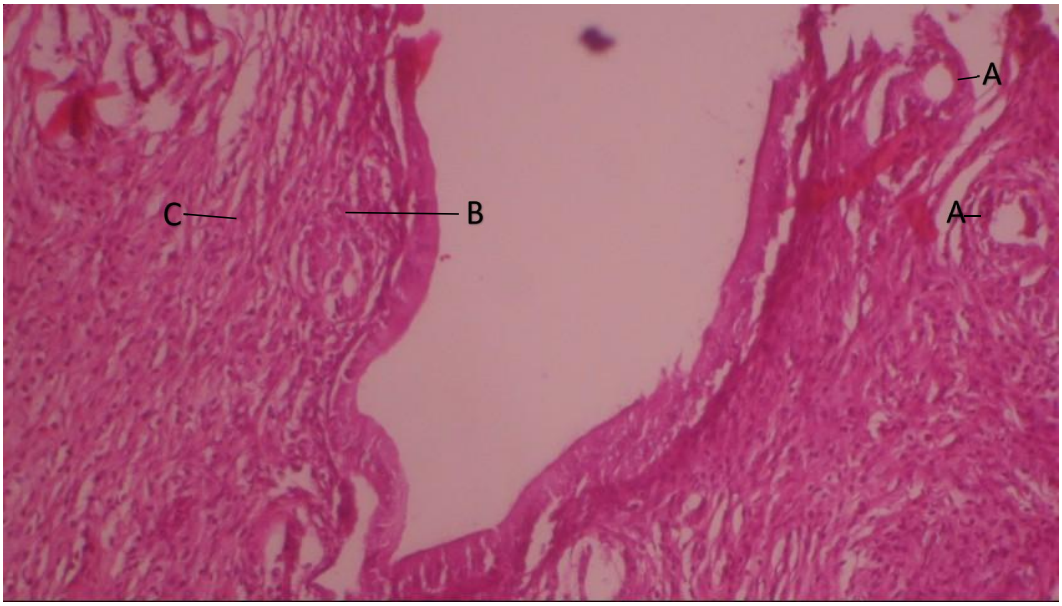

Figure 8: Uterus of rat given 200mg/kg Extract + 800mg/kg mono sodium glutamate (MSG) showing: A, endometrial glands, B, stroma surrounded by remarkably reduced bands of smooth muscle fibres (HandE x 100)

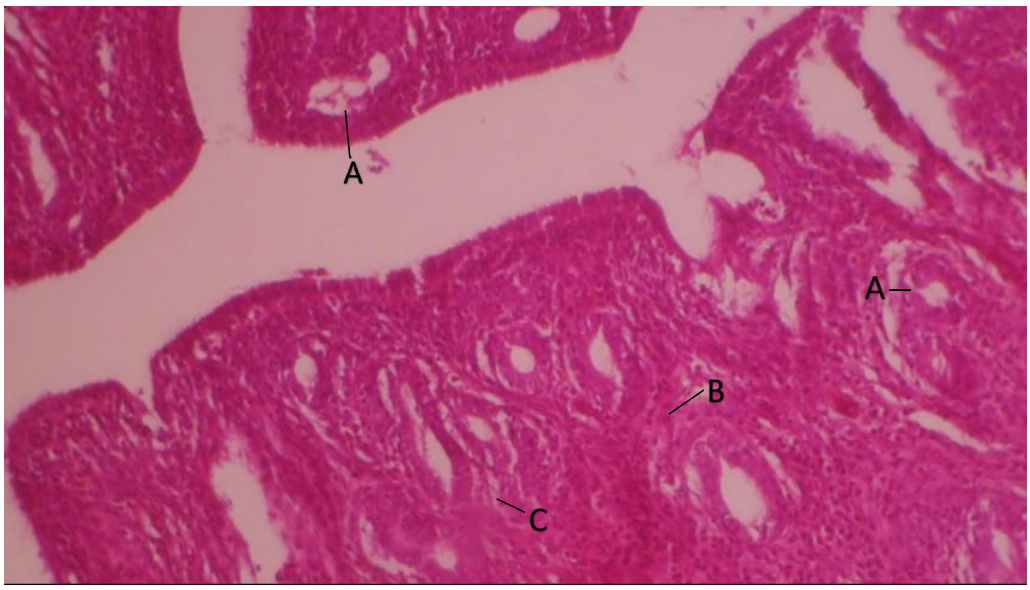

Figure 9: Uterus of rat given 400mg/kg Extract + 800mg/kg mono sodium glutamate (MSG) showing: A, endometrial glands, B, endometrial stroma surrounded by markedly reduced bands of smooth muscle fibres (HandE x 100)

CHAPTER FOUR

4.0 Discussion

This study investigated the potential benefits of *Tetrapleura tetraptera* pods in the prevention of uterine fibroids. Ovarian steroid hormones have been shown to be significant molecular markers associated with the growth and development of uterine fibroid. Estrogen has been considered the major factor with possibilities of progesterone and its receptors playing a key role in the growth and development of uterine fibroid (Cermik *et al.,* 2002).

Monosodium glutamate led to an increase in the levels of estrogen by activating aromatase. Aromatase is an enzyme that catalyzes estrogen synthesis by converting testosterone to β-estradiol and subsequent aromatization of β-estradiol (Obochi *et al.,* 2009).

Estradiol is specific in uterine cell proliferation (Wilson and Gisvold, 2004). Estradiol binds to ERα receptors in the uterus and forms a complex that interacts with DNA of the nucleus to activate transcriptional promoter and enhancer elements responsible for control of gene expression. This allows binding of RNA polymerase II and subsequent initiation of transcription which produces proteins that leads to increase proliferation of the cells of the uterus and ovaries. (Bernard *et al.,* 2002).

Monosodium glutamate (800mg/kg) also led to an increase in serum total cholesterol levels and total protein. Cholesterol is an important precursor in the biosynthesis of many steroid hormones which are powerful signal molecules regulating a couple of functions in living organisms (Payne *et al.,* 2004). A rise in total plasma cholesterol is usually attributed to the activation of the enzyme 3-hydroxyl-3-methoxylglutamyl-CoA reductase (HMGR) by covalent alteration from its phosphorylated state to dephosphorylated state. The phosphorylated state usually referred to as the inactive state and dephosphorylated state; active state. HMGR catalyzes the conversion of HMG-CoA to mevalonate: this is the rate limiting step of cholesterol synthesis. The activation of

HMGR leads to a further increase in insulin levels which stimulate the removal of phosphates from the cells leading to increase activity of HMGR and resultant increase in cholesterol synthesis (Bernard *et al.,* 2002).

Concentrations of 100, 200 and 400mg/kg were used because *T. tetraptera* plant has been reported to been reported to be non-toxic at oral doses as high as 5000mg/kg (Bonsou *et al.,* 2022)

Co-treatment with Monosodium glutamate and *T. tetraptera* pod extract led to decreased cholesterol and total protein levels. Result showed a significant difference with the control group compared with the MSG only group ($p<0.001$) indicating formation of fibroids due to increased cholesterol levels. Significant difference was also observed with groups treated with 200 and 400mg/kg concentration compared with the MSG only group ($P<0.01$) which is indicative of the extract's ability to reduce formation of induced fibroid. No significant difference was observed between the control and 200,400mg/kg groups ($P>0.05$) which shows reduction of cholesterol levels by extract to that of the control group.

Total protein levels had a significant difference with control group compared with the MSG only group ($P<0.01$) which indicates formation of fibroids. There was also a significant difference observed with 200mg/kg concentration compared with the MSG only group ($p<0.01$) which is indicative of the plant extract to reduce formation of induced fibroids.

The cholesterol lowering effect *T. tetraptera* of could be attributed to decreased levels of dephosphorylated HMGR and activation of glucagon and epinephrine that negatively affects cholesterol biosynthesis (Bernard *et al.,* 2002).

Co-treatment with Monosodium glutamate and *T. tetraptera* pod extract also led to a decrease in estrogen levels. The result showed a significant difference with control group compared with the MSG only group ($P<0.001$). This indicates a formation of fibroid in the MSG group. There was

also a significant difference observed with MSG only group and groups treated with 100, 200 and 400mg/kg extract ($P<0.001$). This is indicative of the ability of the plant to reduce formation of induced fibroid as a result of reduction in estradiol levels.

Proliferation of Uterine Leiomyoma has been linked to periods of estrogen secretion because of their increase response to estradiol. Decreased levels of estradiol by *T. tetraptera* would decrease uterine cell proliferation. One of the major possible mechanisms of action of *T. tetraptera* pod extract against uterine fibroid growth and development can be attributed to the inhibition of aromatase which leads to prevention of estrogen synthesis (Obochi *et al.,* 2009)

Further histology studies showed the effect of *T. tetraptera* on Uterine cell proliferation. Gut section of the uterus showed normal tissue architecture: endometrial stroma, endometrial glands, endometrial lining and uterine cavity. When the female wistar rats were treated with 800mg/kg monosodium glutamate (MSG) only, the sections showed thick bands of bundles of smooth muscle fibres arranged in haphazard fashion and crisscrossing the endometrial glands and stroma which is characteristic of Leiomyoma uteri.

Simultaneous administration of graded doses of *T. tetraptera* (100mg, 200mg, 400mg/kg) and monosodium glutamate showed a stepwise amelioration of the proliferating fibroid lesion in a dose dependent manner with the high dose showing the most potent effect.

T. tetraptera pod has been reported to contain flavonoids, alkaloids, tannins, saponins, steroids, sterols and phenols in their extracts (Akintola *et al.,* 2015).

The health benefits associated with saponins are lowering of the cholesterol levels in the body, stimulation of immune system, reduction of bone loss and cancer risk (Podolak, *et al.,* 2010). This further explains the cholesterol lowering effect seen with the extract.

Flavonoids has been found to have many antioxidant activities and some of these are; anti-allergic, antimicrobial, anti-diarrheal, anti-cancer, anti-inflammatory and antiviral (Cazarolli *et al.,* 2008).

The anti-oxidant capacity of phenols play an important role in chronic disease prevention, due to their ability to prevent oxidative damage caused by reactive oxidant species to vital molecule such as DNA, lipids and protein (Hollman, 2001).

4.1 Conclusion

The ethanol extract of *Tetrapleura tetraptera* pods possess anti-fibroid properties and can be used as prophylactic against fibroid development.

REFERENCES

Aderibigbe O.A, Iwalewa O.E, Adesina K.S. and Agboola I.O. (2010). Anxiolytic effect of aridanin isolated from Tetrapleura tetraptera in mice. International Journal of Biological and Chemical Sciences, 4(5).

Ahmed W.O, Boakye Y.D, Appiah A.T, Djameh G.I, Boamah D, Adu F. (2021). Anti-trypanosomal and anthelminthic properties of ethanol and aqueous extracts of Tetrapleura tetraptera Taub. Veterinary Parasitology. Volume 294. 109449

Akintola, O.O, Bodede A.I. and Ogunbanjo O.R. (2015). Nutritional and medicinal importance of Tetrapleura tetraptera fruits (Aridan). African journal of science and research, 6(4): 33-38.

Alistair R.W. Williams, Conceptualization, writing – Original Draft Preparation, Writing – Review and Editing

Bernard S.A, Gray T.W, Buist M.D, Jones B.M, Silvester W, Gutteridge G. and Smith, K. (2002). Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. New England journal of medicine, 346(8): 557-563.

Bonsou I.N, Mbaveng A.T, Nguenang G.S, Chi G.F, Kuete V. and Efferth T. (2022). Cytotoxicity, acute and sub-chronic toxicities of the fruit extract of Tetrapleura tetraptera (Schumm. and Thonn.) Taub. (Fabaceae). BMC Complementary Medicine and Therapies, 22(1): 1-15.

Burkill H.M. (1985). The useful plants of West Tropical Africa, 2nd edition. Royal Botanic Gardens, Kew, UK/The Whitefriars Press Ltd, UK.

Cazarolli L.H, Zanatta L, Alberton E.H, Bonorino-Figueiredo M.S.R, Folador P, Damazio R.G, Pizzolatti M.G. and Barreto-Silva F.R.M. (2008). Flavonoids: prospective drug candidates. Mini reviews in medicinal chemistry, 8(13): 1429-1440.

Cermik D, Arici A, and Taylor H.S. (2002) Coordinated Regulation of HOX Gene Expression in Myometrium and Uterine Leiomyoma. Fertility and Sterility, 78, 979-984.

Chen N.N, Han M, Yang H, Yang G.Y, Wang Y.Y, Wu X.K. and Liu J.P. (2014). Chinese herbal medicine Guizhi Fuling Formula for treatment of uterine fibroids: a systematic review of randomised clinical trials. BMC complementary and alternative medicine, 14(1), pp.1-10.

Ciarmela P, Soriful I.M, Fernando M.R, Peter C.Y, Felice P, Wylie V, and Mario C. (2011) Growth factors and myometrium: biological effects in uterine fibroid and possible clinical implications.

Cook H, Ezzati M, Segars J.H, McCarthy K. (2010). The impact of uterine leiomyomas on reproductive outcomes. Minerva Ginecol.;62(3):225-36.PMID:20595947;PMCID: PMC4120295.

Crum, C.S, Lester and R. Cotran (2003). The female genital system and breast. Robbins Basic Pathology, p. 679-717.

De Leo V, Morgante G, La Marca A, Musacchio M.C, Sorace M, Cavicchioli C. (2002) A benefit-risk assessment of medical treatment for uterine leiomyomas. Drug Safety 25:759–779.

Dolmans M.M, Donnez J, and Fellah L, (2019). Uterine fibroid management: Today and tomorrow. Journal of Obstetrics and Gynaecology Research, 45(7) :1222-1229.

Ebana R.U.B, Edet U, Ekanemesang U, Ikon G, Etok C. and Edet A. (2016). Antimicrobial activity, phytochemical screening and nutrient analysis of Tetrapleura tetraptera and Piper guineense. Asian Journal of medicine and Health, 1(3): 1-8.

Fabio P, Eva N, La Vecchia C, Chatenoud L, Elena R, and Guarnerio P. (1996). Reproductive Factors and Risk of Uterine Fibroids.

Feng J, Wang J, Zhang Y, Jia L, Zhang D, Zhang J, Han Y. and Luo S. (2021). The efficacy of complementary and alternative medicine in the treatment of female infertility. Evidence-Based Complementary and Alternative Medicine.

Gordon A.G, Magos A.L (1989). The development of laparoscopic surgery. Baillieres Clinical Obstetrics and Gynaecology 3(3): 429–449.

Hart R, Khalaf Y, Yeong C.T, Seed P, Taylor A. and Braude P. (2001). A prospective controlled study of the effect of intramural uterine fibroids on the outcome of assisted conception. Human reproduction, 16(11): 2411-2417.

Otimanam H, Adedayo A.T, Ngozi A, Onwuka, Usen W. (2020). Cutaneous Wound Healing Activity of Herbal Ointment containing Tetrapleura tetraptera Fruit Extract. Nigerian journal of pharmaceutical and applied science research.9(2).1-7

Hollman, P.C.H, (2001). Evidence for health benefits of plant phenols: local or systemic effects?. Journal of the Science of Food and Agriculture, 81(9): 842-852.

Islam M.S, Protic O, Giannubilo S.R, Toti P, Tranquilli A.L, Petraglia F, Castellucci M. and Ciarmela P. (2013). Uterine leiomyoma: available medical treatments and new possible therapeutic options. The Journal of Clinical Endocrinology and Metabolism, 98(3): 921-934.

Jacoby V.L, Jacoby A, Learman L.A, Schembri M, Gregorich S.E, Jackson R. and Kuppermann, M. (2014). Use of medical, surgical and complementary treatments among women with fibroids. European Journal of Obstetrics and Gynecology and Reproductive Biology, 182: 220-225.

Jenne J.W, Preusser T, Günther M. (2012). High-intensity focused ultrasound: principles, therapy guidance, simulations and applications. Zeitschrift für Medizinische Physik. ;22(4):311–322.

John O.A. (2005). Analgesic and anticonvulsant properties of Tetrapleura tetraptera (Taub) (Fabaceae) fruit aqueous extract in mice. Phytotherapy research.19(12).1023-1029

John A.O, Adewunmi C.O. (2004). Anti-inflammatory and hypoglycaemic effects of Tetrapleura tetraptera (Taub) fruit aqueous extract.Journal of Ethnopharmacology . 95(2-3) Pages 177-182,

Jude E.O, Aniekan E.U, Bassey S.A. (2012). Antimalaria activity of ethanolic extract of Tetrapleura tetraptera fruit. Journal of Ethnopharmacology.111(3). 537-540

Kelly K. (2009) History of medicine. New York: Facts on file

Kuete V, Viertel K. and Efferth T. (2013). Antiproliferative potential of African medicinal plants. In Medicinal plant research in Africa (pp. 711-724). Elsevier.

Lethaby A, Vollenhoven B, Sowter M. (2000). Pre-operative GnRH analogue therapy before hysterectomy or myomectomy for uterine fibroids. Cochrane Database Syst Rev CD000547.

Lippman, Sheri and Warner, Marcella and Samuels, Steven and Olive, David and Vercellini, Paolo and Eskenazi B. (2004). Uterine fibroids and gynecologic pain symptoms in a population-based study. Fertility and sterility. 80. 1488-94. 10.1016/S0015-0282(03)02207-6.

Lumbiganon P, Rugpao S, Phandhu-fung S, Laopaiboon M, Vudhikamraksa N. and Werawatakul, Y. (1996). Protective effect of depot-medroxyprogesterone acetate on surgically treated uterine leiomyomas: a multicentre case-control study. BJOG: An International Journal of Obstetrics and Gynaecology, 103(9): 909-914.

Lynn M, Marshall, Spiegelman D, Robert L, Barbieri M.D, Marlene B, Goldman S, Joann E, Manson M.D, Graham A, Colditz M, Walter C, Willett M.D, and Hunter D.J. (1997). Variation in the Incidence of Uterine Leiomyoma Among Premenopausal Women by Age and Race

Marret H, Fritel X, Ouldamer L, Bendifallah S, Brun J.L, De Jesus I. (2012). Therapeutic management of uterine fibroid tumors: updated French guidelines. European Journal for Obstetrics and Gynecology and Reproductive Biology; 165:156–164.

Marshall L.M, Spiegelman D, Barbieri R.L, Goldman M.B, Manson J.E, Colditz G.A, Willett W.C. and Hunter D.J. (1997) Variation in the incidence of uterine leiomyoma among premenopausal women by age and race. Obstetrics and Gynecology, 90(6): 967-973.

Martínez C.M, Ibáñez C. and Corpa J.M. (2010). Simultaneous uterine leiomyoma and endometrial hyperplasia in a white-nosed monkey (Cercopithecus nictitans). First case report. Animal Vetinary (Murcia), **26**: p. 61-68.

Munro M.G, Critchley H.O, Broder M.S, Fraser I.S. (2011). FIGO Working Group on Menstrual Disorders. FIGO classification system (PALM-COEIN) for causes of abnormal uterine bleeding in nongravid women of reproductive age. International Journal for Gynaecology and Obstetrics; 113:1–2.

Obeng A.W, Boakye Y.D, Agana T.A, Djameh G.I, Boamah D. and Adu, F. (2021). Anti-trypanosomal and anthelminthic properties of ethanol and aqueous extracts of Tetrapleura tetraptera Taub. Veterinary Parasitology, 294, p.109449.

Obochi G.O, Malu S.P, Obi-Abang M, Alozie Y, Iyam M.A. (2009). Effect of garlic extracts on monosodium glutamate (MSG) induced fibroid in Wistar Rats. Pakistan Journal of Nutrition 8(7):970-76. DOI: 10.3923/2009.970.976.

Ojewole J.A, Adewunmi C.O. (2004). Anti-inflammatory and hypoglycaemic effects of Tetrapleura tetraptera (Taub) [Fabaceae] fruit aqueous extract in rats. Journal of Ethnopharmacology ;95(2-3):177-82.

Okolo S. (2008). Incidence, aetiology and epidemiology of uterine fibroids. Best Practice and Research Clinical Obstetrics Gynaecology. Aug;22(4):571-88.

Parazzini F, Negri E, La-Vecchia C, Chatenoud L, Ricci E. and Guarnerio P. (1996). Reproductive factors and risk of uterine fibroids. Epidemiology: 440-442.

Parazzini F, La-Vecchia C, Negri E, Cecchetti G. and Fedele L. (1988). Epidemiologic characteristics of women with uterine fibroids: a case-control study. Obstetrics and gynecology, 72(6): 853-857.

Payne A.H. and Hales D.B. (2004). Overview of Steroidogenic Enzymes in the Pathway from Cholesterol to Active Steroid Hormones. Endocrine Reviews, 25,
947-970.

Petrovska, Department of Pharmacognosy, Faculty of Pharmacy, Cyril S.S and Methodius University, Vodnjanska 17, 1000 Skopje, The Republic of Macedonia

Petrovska B.B. (2012). Historical review of medicinal plants' usage. Pharmacognosy Review.6(11):1-5.

Lumbiganon P, Rugpao S, Phandhu-fung S, Laopaiboon M, Vudhikamraksa N, Yuthapong W. (1996). Protective effect of depot-medroxyprogesterone acetate on surgically treated uterine leiomyomas: a multicentre case-control study.

Podolak I, Galanty A. and Sobolewska D. (2010). Saponins as cytotoxic agents: a review. Phytochemistry Reviews, 9(3): 425-474.

Pollow K, Sinnecker G, Boquoi E. and Pollow B. (1978). In Vitro Conversion of Estradiol-17β into Estrone in Normal Human Myometrium und Leiomyoma.

Qin J, Yang T, Kong F, Zhou Q. (2013). Oral contraceptive use and uterine leiomyoma risk: a meta-analysis based on cohort and case-control studies. International Journal of Gynaecology and Obstetrics 288:139–148.

Rein M.S, (2000). Advances in uterine leiomyoma research: the progesterone hypothesis. Environmental health perspectives, 791-793.

Reissmann T, Diedrich K, Comaru-Schally A.M, Schally A.V. (1994). Introduction of LHRH-antagonists into the treatment of gynaecological disorders. Human Reproduction 9:769.

Richards P.A. and Tiltman A.J, (1996). Anatomical variation of the oestrogen receptor in the non-neoplastic myometrium of fibromyomatous uteri. Virchows Archiv, 428(6): 347-351.

Ross R, Malcolmcpike, Martinpvessey, Dianabull, Davidyeates (1986) Risk factors for uterine fibroids: reduced risk associated with oral –contraceptives

Ebana R.U, Edet U.O, Ekanemesang U.M, Ikon G.M, Etok C.A, and Edet A.P. (2016). Antimicrobial Activity, Phytochemical Screening and Nutrient Analysis of Tetrapleura tetraptera and Piper guineense. Asian Journal of Medicine and Health 1(3): 1-8.

Sabry M, Al-Hendy A. (2012). Innovative oral treatments of uterine leiomyoma. International Journal of Gynaecology and Obstetrics. 2012:943635.

Samadi A.R, Lee N.C, Flanders W.D, Boring J.R and Parris, E.B. (1996). Risk factors for self-reported uterine fibroids: a case-control study. American journal of public health, 86(6): 858-862.

Sayed G.H, Zakherah M.S, El-Nashar S.A, Shaaban M.M. (2011). A randomized clinical trial of a levonorgestrel-releasing intrauterine system and a low-dose combined oral contraceptive for fibroid-related menorrhagia. International Journal of Gynaecology and Obstetrics 112:126–130.

Schrire B.D, Lavin M, and Lewis G.P. (2005). Global distribution patterns of the Leguminosae: insights from recent phylogenies. Biologiske skrifter, 55, 375-422.

Socolov D, Blidaru I, Tamba B, Miron N, Boiculese L, Socolov R. (2011). Levonorgestrel releasing-intrauterine system for the treatment of menorrhagia and/or frequent irregular uterine bleeding associated with uterine leiomyoma. European Journal of Contraceptive and Reproductive Health Care 16:480–487.

Stojanoski N. (1999). Development of health culture in Veles and its region from the past to the end of the 20th century. Veles: Society of science and art.

Surrey E.S, Lietz A.K and Schoolcraft W.B. (2001). Impact of intramural leiomyomata in patients with a normal endometrial cavity on in vitro fertilization–embryo transfer cycle outcome. Fertility and sterility, **75**(2): 405-410.

Velusamy B, Kaliyaperumal S. and Raju A. (2016). Collection and data-mining of bioactive compounds with cancer treatment properties in the plants of fabaceae family. International Journal of Pharmaceutical Sciences and Research, 7(5), p.2065.

Williams A.R, (2017). Uterine fibroids–what's new? F1000Research, 6.

Wilson C.O, and Gisvold O. (2004) Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry (11th edition), Philadelphia: Lippincott William and Wilkins. 768, 770, 775.

Zhang Y, Meng W, Lin W.L, Yeung W.F, Lee Y.P.E, Zhang Z.J, Rong J, and Lao L. (2022). Randomized double-blind trial comparing low dose and conventional dose of a modified traditional herbal formula; Guizhi Fuling Wan in women with symptomatic uterine fibroids. Journal of Ethnopharmacology, 283, p.114676.

APPENDIX

Average hormonal assay values of cholesterol, total protein and estradiol

	Cholesterol (mg/dL)	Total protein (g/L)	Estradiol (pg/mL)
Control	107.50 ± 4.21	6.90 ± 0.07	43.39 ± 3.48
MSG ONLY	135.00 ± 4.22	8.20 ± 0.26	108.00 ± 1.88
MSG + 100 mg/kg	123.30 ± 2.2	8.01 ± 0.39	79.93 ± 3.32
MSG + 200 mg/kg	112.20 ± 3.34	7.26 ± 0.23	68.65 ± 3.3
MSG + 400 mg/kg	111.60 ± 4.69	7.40 ± 0.14	63.84 ± 2.06

Results are expressed as Mean ± S.E.M