EFFECT OF ETHANOL EXTRACT OF *Tetrapleura tetraptera (Schumach. & Thonn.)* TAUB (FABACEAE) STEM BARK ON MONOSODIUM GLUTAMATE-INDUCED FIBROID IN SPRAGUE DAWLEY RATS (PREVENTIVE AND CURATIVE TREATMENT).

BY

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DEPARTMENT OF PHARMACOGNOSY,
FACULTY OF PHARMACY,
UNIVERSITY OF BENIN,
BENIN CITY,
NIGERIA.

SEPTEMBER, 2023.

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACOGNOSY, FACULTY OF PAHRMACY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHARMACY (PHARM.D) OF THE UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

SEPTEMBER, 2023.

CERTIFICATION

This is to certify that this work was successfully carried out BY ONYEBUCHI ESTHER CHINAZA, Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin city, in partial fulfilment of the requirement for the award of the degree of Doctor of Pharmacy (Pharm.D) of the University of Benin, Benin City, Edo State, Nigeria.

_____ _____

Dr. (Mrs.) Rose O.Imade **DATE**

(Supervisor)

_____ _____

Dr. (Mrs.) Josephine Omose Ofeimun. **DATE**

(Head of Department)

DEDICATION

This work is wholly dedicated to God Almighty for his love, grace, guidance and provision and to my parents, who didn't give up on my education.

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ACKNOWLEDGEMENT

My greatest appreciation goes to Almighty God for his grace that has kept me thus far and for making this project a success.

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ABSTRACT

Uterine fibroids also known as leiomyomas are the most common benign tumors of the female reproductive tract which is mostly seen in women of reproductive age. *Tetrapleura tetraptera* is mostly used in herbal medicine for the management and/or control of a wide range of human ailments, which includes arthritis and other inflammatory conditions. This study aimed at evaluating the anti-fibroid potential of the ethanol extract of the stem bark of *Tetrapleura tetraptera* plant on monosodium glutamate induced fibroid in Sprague-Dawley rats. The objective of the study was to evaluate the ability of the extract to reduce Monosodium glutamate induced increase in cholesterol, total protein and estradiol respectively. The study also assessed the effect of the extract in ameliorating leiomyoma formation through histological studies.

The stem bark of *Tetrapleura tetraptera* were dried, pulverized by milling, extracted using soxhlet apparatus and concentrated using a water bath. The percentage of the extract obtained from the bark of *Tetrapleura tetraptera* was 21.27% after extraction.

For the preventive treatment the rats were divided into five groups (A, B, C, D, and E), of five rats each. Group A (control) received only food and water. In other to stimulate uterine fibroid, groups B, C, D, and E were given 800 mg/kg of MSG for 30 days. Then, groups C, D, and E were also given 100, 200, and 400 mg/kg of T. tetraptera stem bark extract, respectively alongside the MSG, once daily for 30 days. For the curative treatment; Five rats divided into groups labelled A,B,C,D and E respectively were used. Group A (control) received only food and water. In other to stimulate uterine fibroid, groups B, C, D, and E were given 800 mg/kg of MSG for 30 days. Then, groups C, D, and E were also given 100, 200, and 400 mg/kg of the extract respectively once daily from the 31st day for another 30 days after which the animals

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were sacrificed on the 61st day. All administration was done by the means of oral gavage. The animals were sacrificed, blood samples were collected and assessed. Histopathology studies of the uterus in addition to serum total protein, total cholesterol and estradiol levels were determined. Significant increase in cholesterol and estradiol levels were observed in MSG-treated animals in relation to the groups treated with the extract in both treatment groups. There was no significant difference in the protein levels when different concentrations groups were compared to MSG group ($p>0.05$). The extract prevented and also led to a mild reversal of these biomarkers in the curative group of this study. MSG also resulted in endometrial epithelium distortion and lamina propria fibrosis while the extract ameliorated the distortion observed in the uterus. These findings suggest that T. tetraptera stem bark extract contains bioactive constituents that are useful in having a more preventive effect than curative effect in the management of fibroid as it reduces serum hormone levels which play roles in the etiology of uterine fibroid.

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Deleted[PHub5]: **ABSTRACT**

Uterine fibroids also known as leiomyomas are the most common benign tumors of the female reproductive tract which is mostly seen in women of reproductive age.*Tetrapleura tetraptera* is mostly used in herbal medicine for the

CHAPTER ONE.

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1.0 INTRODUCTION

The oldest form of healthcare is herbal medicine, which has been used for decades in both developing and developed nations. Primitive people have relied on nature for food, shelter, clothing, and medicine to cure illnesses. These humans distinguished useful herbs with beneficial effects from those that were inactive or toxic, and their understanding of plant-based drugs has gradually advanced and been passed down, setting a foundation for many traditional medicine systems (Kunle *et al*, 2012). Approximately 50,000 plant species are claimed to have therapeutic characteristics in literature (Barboza *et al*, 2009). Thus, the basis of modern medicinal drugs such as aspirin, morphine, digitoxin and quinine were synthesized through scientific validation of herbal medicine (Wachtel-Galor *et al* ,2011).

Herbal or Traditional medicine is the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness". There are many different systems of traditional medicine, and the philosophy and practices of each are influenced by the prevailing conditions, environment, and geographic area within which it first evolved (WHO 2005).

Over the years the use of traditional medicine has provided us with valuable formulas on the selection, preparation and application of herbal remedies. The same vigorous method clinically and scientifically must be implemented to verify the effectiveness and safety of curative products, to be viable alternative to western medicine (Pal *et al*, 2003).

Herbal medicines include active components such as plant parts or materials in a processed or raw form along with certain excipients, such as dilutions, solvents, or preservatives (Bent 2008). These active compounds add to the plants' aroma, flavor, and color while also defending them against harm and disease. They are referred to as phytochemicals in science, and there are various classes of them, including saponins, flavonoids, glycosides, tannins, alkaloids, and terpenoids (Saxena *et al,* 2013). As an illustration, aromatic plant species with medicinal essential oils that have antimicrobial, stomach-soothing, and antispasmodic effects are the core component of herbal sedatives and stomachic mixes. Combinations for diarrhea and stomach ulcers often contain plant species with high tannin content since they typically have antibacterial, astringent, and anti-inflammatory properties (Ndhlala *et al,* 2011; Cano *et al,* 2004).

People use herbal medicine for different reasons and they include;

i. Herbal medicine is widely used due to cultural acceptance and the fact that plant medicines have been used for ages (Wassie *et al*, 2015).

ii. Compared to expensive western medicine, herbal treatment is still affordable (Maroyi, 2013).

iii.The rural population in overpopulated nations, like India, has almost no access to modern treatment; as a result, they are forced to rely on herbal medicine for their essential medical requirements (Pandey *et al*, 2013).

iv.Traditional medicines are widely perceived as natural and safe, that is, not toxic (Cohen *et al*, 2010)

v. Due to the advancement of scientific evaluation, herbal medicines' quality has improved (Qazi *et al*, 2016)

vi. To help alleviate the symptoms of long-lasting or fatal conditions such diabetes, cancer, sickle-cell anemia, HIV/AIDS, and malaria (Barnes *et al,* 2017; NHS 2008).

The challenges facing the use of herbal medicine include;

i. In some countries, toxicological assessment of herbal medicine and associated products are not employed before placing them in the market thereby causing adverse effects such as childhood blindness from the use of traditional eye medicine (Ekor ,2014), with several herbs, including ephedra and echinacea, nausea and sometimes vomiting might happen. Teas made from herbs have been linked to gastrointestinal, hematologic, and cardiac issues as well as diarrhea (Cui *et a*l ,2016)

ii. Hundreds of ingredients can be found in a single plant's herbal medicine, and combined preparations can have many times that many. It would take a long time to separate each active component from each herb (WHO,2005)

iii. The majority of nations lack the necessary infrastructure to enact laws governing manufacturing quality standards and practices. Consequently, customers would always have access to dangerous herbal products (Ekor ,2014)

iv. The lack of a reliable source for high-quality herbal resources and their formulations is one of the main issues facing the market for herbal drugs (Verma *et al*, 2008)

v. The quality and therapeutic value of medicinal plants can be greatly influenced by a number of variables, including temperature, the usage of fresh plants, light exposure, nutrients, water availability, the time and season of harvest, the technique of harvesting, drying, packing, storing, and transporting raw herbal material, etc (Calixto ,2000)

vi.It is unknown whether most herbal products are effective, safe, and of high quality, which raises questions about the safety of these natural medications (Ekor M. 2014).

vii. The concern regarding the safety of herbal medicinal products is growing, as there is a need for scientific and clinical examination of herbal medicine (Ekor ,2014)

About 121 pharmaceutical products have been developed in the last ten years based on knowledge of herbal therapy (Verma *et al* 2008). At least 25% of current medications, including aspirin, picrotoxin, and many more, are said to be originated from plants. These synthetic equivalents were developed based on plant-derived prototype chemicals (Msomi *et al*, 2017).

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Plants will increasingly be used in medicine as a source of therapeutic agents in the future due to the rising acceptance of plant-derived pharmaceuticals (Verma *et a*l, 2008). This has greatly enhanced the trade in herbal medicines internationally and attracted a number of pharmaceutical firms, including multinationals (Calixto ,2000). Scientific confirmation of the use of these plants has expanded due to WHO's interest in recording ethnic groups' use of medicinal herbs. People will be better informed about the efficacy and security of the treatment as a result (Qazi, 2016) The regulation of herbs has helped herbal products get better, but more adjustments are needed to promote and enhance high-quality research (Bent,2008)

Since the beginning of human history, people have used plants, herbs, and ethnobotanicals to treat illnesses and promote health. The foundation of contemporary medicine today is made up of plants and other natural resources, which also significantly influence how commercial drug preparations are made today. Some people prefer to treat themselves using herbal remedies. Others supplement traditional medications with the usage of botanicals. Herbal medicine is a vital component of traditional medicine, which is the only system of healthcare that is accessible or inexpensive in many underdeveloped nations. Whatever the cause, those who use herbal remedies should be certain the items they purchase are safe and contain what they claim to, whether this is a specific herb or a specified quantity of a certain herbal component. Information on dose, contraindications, and efficacy should also be provided to consumers based on science. To do this, a global harmonization of law is required to direct the ethical manufacturing and distribution of herbal medicines. If a plant has enough scientific support to support its use, then suitable legislation should permit this use to encourage its use so that the advantages can be achieved for the promotion of public health and the treatment of disease.

1.1 UTERINE FIBROID

Uterine fibroid, often referred to as uterine leiomyoma, are abnormal growths that appear in the uterus of a woman who is of reproductive age. It is the most common of all pelvic tumours in women (N.Bhatla, 2001). They are tumors made of smooth muscle and connective tissue from the myometrium or muscular outer layer of the uterus (Ke *et a*l ,2013).These tumors are benign neoplasms and are not predicted to lead to cancer (Orellana *et a*l, 2022). They are present in premenopausal women, and post-menopause, they are seen to retreat (Ali *et al,* 2021). According to estimates by 60% of women in reproductive age are affected and 80% of women will contract the disease at some point in their lives (Myers *et al* 2012). They can be found in premenopausal

women and are observed to regress post-menopause (Ali *et al, 2*021). It is estimated that 60% of reproductive-aged women are affected, and 80% of women develop the disease during their lifetime (Myers *et al* ,2012)

It grows in various locations on and within the uterine walls or in the uterine cavity, hence it could be 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)

1.2 TYPES AND CLASSIFICATION OF UTERINE FIBROID

According to the International Federation of Gynecology and Obstetrics (FIGO), uterine fibroids are categorized into eight different subtypes (Table 1). The FIGO categorization also has a type 8, which includes lesions on extrauterine locations such as the cervix or broad ligament (Gomez *et al*, 2021). Subtypes are determined by the position of the myoma in relation to the endometrial cavity.

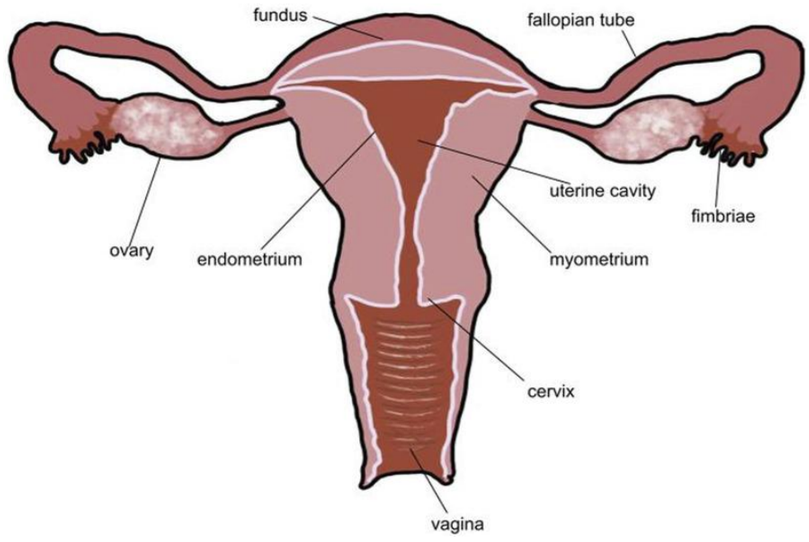

FIGURE 1: Typical healthy uterus.

In a healthy uterus, there are no lesions. The endometrium is a thin layer that surrounds the uterine cavity and myometrium. Both fallopian tubes and ovaries are present. The uterine cavity is empty. No part of the uterus is distended or disformed.

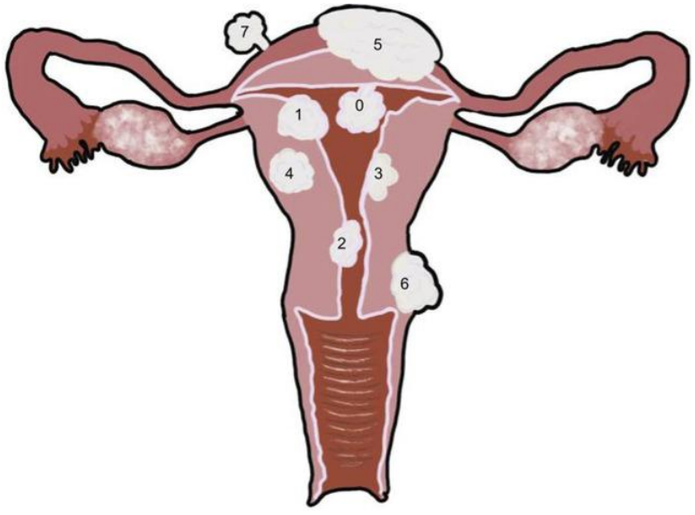

FIGURE 2: Uterus with multiple fibroid types

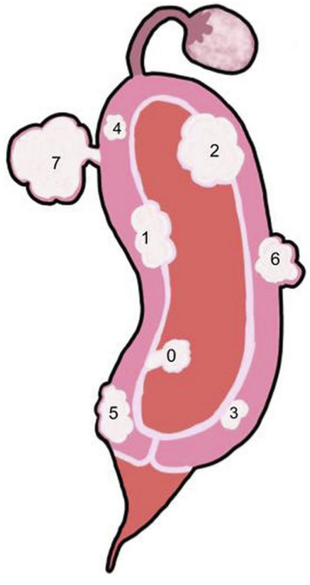

FIGURE 3: Side view of the uterus with multiple fibroid types

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-Key: See Table 1.

0: Pedunculated submucosal, 100% of the fibroid is in the uterine cavity.

1: Submucosal, greater than 50% of the fibroid is within the myometrium and the other portion is distorting the endometrium and uterine cavity.

2: Submucosal, less than 50% of the fibroid is within the myometrium and the majority is distorting the endometrium and uterine cavity.

3: Intramural, the fibroid is within the myometrium but touches the endometrium, and it does not distort the uterine cavity.

4: Intramural, the fibroid is completely within the myometrium.

5: Intramural, the fibroid is predominantly within the myometrium with less than 50% extending outside of the myometrium.

6: Subserosal, greater than 50% of the fibroid is located outside of the myometrium.

7: Pedunculated subserosal, 100% of the fibroid is outside of the myometrium.

TABLE 1.0: Classification of uterine fibroids by FIGO.

FIGO	Subtype	Positioning
0	Submucosal - Subtype 0	100% endometrial cavity or intracavity
1	Submucosal - Subtype 1	> 50% intramural
2	Submucosal - Subtype 2	< 50% intramural
3	Intramural	In contact with the endometrium
4	Intramural	100% intramural
5	Intramural	Subserosal >50% intramural
6	Subserosal	Subserosal <50%
7	Subserosal	Pedunculated

1.2 Classification of uterine fibroids by anatomical positioning

A.**Submucosal**:

Ieiomyomas that cause intramural distortion or reside within the uterine cavity are submucosal fibroids (Figures 1, 2).Submucosal fibroids were found to lower fertility rates and are also likely to be symptomatic, as they can lead to intermenstrual bleeding and hemorrhage (Puri *et al,* 2014). Submucosal fibroids can negatively impact the implantation rates of assisted reproductive technology (ART) because the uterine cavity is occupied (Guo X. C. and Segars, 2012).

B. Intramural:

Intramural fibroids reside in the myometrium cavity without distorting the endometrial cavity (Wilde & Scott-Barrett, 2009) (Figures 3,4, and 5). Intramural myomas impact the establishment of early pregnancy .They also produce significantly lower pregnancy rates, implantation rates, and ongoing pregnancy/live birth rates and even significantly higher rates of spontaneous abortion (Pritts *et al*, 2009). This effect on implantation is seen even when the fibroid does not reach the uterine cavity (Zepiridis *et al.,* 2016)

C.Subserosal

Subserosal fibroids reside predominantly outside the myometrium (Klatsky *et al.*, 2008) (Figures 5 and 6). Subserosal myomas have been found to impact the establishment of early pregnancy (Pritts *et al,* 2009) However, they have been associated with a very minimal effect on fertility (Zepiridis *et al*., 2016). Women with subserosal fibroids were found to have no significant differences from those without fibroids (Pritts *et al,* 2009). Subserosal fibroids tend to be asymptomatic unless they are large, which can cause substantial pressure or pain (Gomez et al, 2021)

D.Pedunculated

Fibroids of the final subtype do not reside in a specific location. Pedunculated fibroids can occur both within and outside the uterine cavity (Klatsky *et al,* 2008), and they are attached to the uterus by a vascular stalk (Gomez *et al,* 2021) (Figure 1 and 7). These fibroids are likely to be asymptomatic unless they are torsioned (Divakar, 2008; Wilde and Scott-Barrett, 2009), but they can also become symptomatic if they grow and begin to push on other masses or detach and

become parasitic to the pelvis (Gomez *et al*., 2021). Parasitic myomas are rare cases where a pedunculated subserosal myoma detaches from the uterus and develops an alternative blood supply from other sources, such as the omental or mesenteric vessels (Cucinella *et a*l, 2011).

This distinction of fibroids is very important as they cause different symptoms and might need a different access during surgery.

1.3 SIGNS AND SYMPTOMS OF UTERINE FIBROID

According to a study, up to 70% of fibroids are asymptomatic and may be unintentionally identified during radiologic treatments used to treat other conditions (Levens et al, 2009). As a result, they receive less clinical attention, and fibroid tumors frequently go undiscovered. Women who have symptoms frequently express discomfort during their periods, abnormally heavy bleeding from the uterus (which can cause anemia), enlargement of the lower abdomen, frequent urination, pain during sexual activity, lower back pain, complications during pregnancy and labor, and reproductive issues like infertility. Typically, the location and size of the fibroids determine how severe these symptoms will be (Hart *et al,* 2001;Surrey *et al,* 2001)

1.4 RISK FACTORS OF UTERINE FIBROID

Although the true aetiology of fibroid is unknown, there are known risk factors for developing uterine fibroids and these includes;

Age (women between the ages of 20 and 44 are more at risk), inactivity, and consumption of certain chemical compounds, such as monosodium glutamate (MSG), which is present in a broad variety of foods, cause uterine fibroid by raising levels of estrogen, cholesterol, and total protein (Obochi *et al,* 2009). ethnic origin; African-American women are more prone than Caucasian women to develop uterine fibroids; regular use of soy milk (Wise *et al*, 2005), excessive vitamin

E levels (Ciebiera *et al* 2018), altered reproductive tract microbiome (Baker *et al* ,2018), nulliparity, hypertension, late menopause, early menarche, family history of fibroids, hormonal factors, and some lifestyle habits like smoking, stress, alcohol, and caffeine consumption (Pavone *et al,* 2018), a lack of vitamin D (Mohammadi *et al* ,2020) exposure to endocrine-disrupting substances (such as plasticizers and organophosphate esters) (Lee *et a*l ,2022) unfavorable environmental exposures during early life (Yang *et al* ,2018) menarche at a young age (Dragomir *et al*, 2010)

1.5 PATHOPHYSIOLOGY OF UTERINE FIBROID

Despite being so common, very little is understood about the precise causes and molecular mechanisms that control the development, growth, and regression of fibroid (Segars *et al* 2014) Several pathways and mechanisms, including sex hormones, stem cells, glucocorticoids, growth factors, cytokine signaling, extracellular matrix remodeling, and epigenetic factors, have been identified through numerous in vitro and in vivo studies (Segars *et al,* 2014 ; Moravek MB *et al.* 2014). Another peculiar characteristic of fibroids is their biological heterogeneity even among those within the same woman's uterus, as shown by different patterns of growth over time (Laughlin-Tommaso SK, 2018). These factors emphasize the demand for more individualized and fibroid-specific therapies.

Some causes of uterine fibroid are discussed below;

Genetic modifications

Myometrium cells are converted into leiomyoma tumor-forming stem cells during the development of fibroid (Bulun, 2013) Because each fibroid growth is a distinct occurrence, several genotypes might arise in a single patient. Several studies point to specific genetic mutations that lead to the development of fibroids, specifically the "mediator subunit 12 (MED12), High-mobility group AT-hook 2 (HMGA2), Collagen alpha-5(IV) chain / Collagen alpha-6(IV) chain (COL4A5/COL4A6), First apoptosis signal (FAS) or fumaratehydratase (FH) genes" (Eggert *et al,* 2012; Segars *et al,* 2014). According to studies, the mediator complex subunit 12 (MED12) gene has the greatest mutations (affecting up to 85% of patients) (MakinenN *et al* 2011) On chromosome X, there is a gene called MED12, which produces the mediator subunit 12 protein. Most women with fibroid whose chromosomal alterations have been observed had mutations to MED12. These modifications might include simple or complex deletions or rearrangements, as well as clonal chromosomal anomalies. In addition to MED12. HMGA2 is another frequent modification that has been identified in fibroids with chromosomal abnormalities. High mobility group AT2 hook proteins are encoded by HMGA2. The HMGA2 locus has been targeted and elevated in certain fibroid chromosomal rearrangement situations. About 80%–90% of fibroids with chromosomal abnormalities are caused by MED12 and HMGA2, however these two mutations are mutually exclusive (Markowski *et al*., 2012; Bulun, 2013). Additionally, about 40% of women with these tumors have chromosomal abnormalities in "trisomy 12, translocation involving chromosomes (t12; 14) (q14-q15; q23-q24), deletions on

chromosome 7 (q22q32), 3q and 1p, and rearrangements of 6p21, 10q22, and 13q21-q22." (Hodges *et al,* 2002)

•Inflammation

Inflammation has also been associated with the growth of fibroid. In leiomyoma tissues, Protic et al. found a high concentration of CD68 (cluster of differentiation 68)-positive macrophages and inflammatory cells. They discovered that leiomyomas and the tissues around them contained significantly more CD68 macrophages than in the distant myometrium. Additionally, they discovered a significant number of inflammatory cells in early-stage cellular leiomyomas, establishing a connection between inflammation and leiomyomas (Protic *et al,* 2016).As a result of excessive wound healing brought on by the inflammatory response, fibrotic illnesses including uterine fibroids are linked to altered extracellular matrix pathology (Zannoti *et al*., 2021).

• Particulate Matter

It has also been discovered through research that the prevalence of clinically symptomatic uterine fibroids is linked to prolonged exposure to particulate matter 2.5 (dust, ash, forest fires, pollen, and soot).More investigations are required to confirm these results in other populations because there hasn't been much study done on the connection between air pollution and the growth of uterine fibroid (Lin CY *et al* 2019).

•Extracellular matrix (ECM):

Extracellular matrix (including collagen, proteoglycan, fibronectin) is the substance that binds cells collectively, much like mortar does between bricks. Fibroids become fibrous due to an

increase in ECM. Additionally, ECM stores growth factors and influences cellular biology (Johnson, 2022). Studies have demonstrated that the volume and composition of the extracellular matrix, as well as the increased deposition of aberrant collagen, glycoproteins, laminins, and fibronectins, are both associated to the growth of fibroids. These alterations cause mechanical stress, which is then turned into chemical signals in the cells through a process called mechanotransduction, which ultimately has an impact on gene expression and protein synthesis. Recent studies further indicate that improper mechanical signaling occurs in fibroid cells, as shown by the decreased death of abnormal cells and the creation of a stiff extracellular matrix that encourages fibrosis (Saima Rafique *et al* ,2017)

•**Hormones**:

Hormones based on cholesterol have been demonstrated to affect the formation of tumors (Chimento *et al* ,2019) Such hormones include progesterone, estradiol, and vitamin D3. Estradiol and progesterone cooperate to keep tumor cells viable. Progesterone is vital to the growth of fibroids, as it works to proliferate cells and maintain their rapid growth and estradiol increases the availability of progesterone receptors on the cells and allows for more sensitivity to progesterone, thus increasing development (Ishikawa *et a*l, 2010; Reis *et al.,* 2016). Also a biologically active form of vitamin D3, 1,25-Dihydroxyvitamin D3, has been shown to decrease tumor proliferation [Halder *et al.*, 2012]. In a study conducted between black and white women using an assay of 25-hydroxyvitamin D (25(OH)D), which is a commonly recognized marker of vitamin D, researchers were able to determine the status of vitamin D in women. The results showed that only 10% of black women and 50% of white women had sufficient vitamin D levels,

and women with sufficient vitamin D levels were 32% less likely to have fibroids than women who were deficient (Baird *et al*., 2013)

1.6 DIAGNOSIS OF UTERINE FIBROID

Using a combination of physical exam and ultrasound helps doctors determine the presence of fibroids (Sarkodie *et al*., 2016; Igboeli *et al,* 2019). The determination can also be made using the patient's medical history and laboratory tests. Additional tests to detect specific fibroids, such as a hysterogram and an ultrasound of the uterus, may be done.

In the diagnosis of fibroid;

-A medical history is important. In this case, the doctor inquires about any present illnesses and discomforts that might be connected to fibroids, as well as any common disorders associated to the symptoms of fibroid. The questions are designed to assess the severity of the symptoms related to pain, irregular bleeding, and bowel and bladder issues.

-Palpation is another way of diagnostics. A doctor will palpate your body to evaluate your health. Palpating or feeling the abdomen and pelvis to look for common fibroids symptoms such a solid uterine lump, an enlarged belly, or other anomalies is the technique. Large myomas on the front and rear of the uterus are frequently seen during this procedure. Smaller fibroids are challenging to find with basic palpation. Generally speaking, this test does not cause any pain but discomfort may be felt if other sources of inflammation are present, such as infection or endometriosis

An ultrasound is also used if more verification is required. It maps and measures fibroids and uses sound waves to create an image of your uterus to validate the diagnosis. In order to obtain

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images of your uterus, a doctor or technician will either place the ultrasound transducer within the vagina or move the transducer across the abdomen.

Lab tests could be recommended if one experiences unusual menstrual bleeding in order to rule out other possible reasons. They may consist of a complete blood count (CBC) to see if one have anemia from ongoing blood loss and additional blood tests to rule out thyroid issues or bleeding diseases.

Magnetic resonance imaging (MRI) is an imaging technology used to identify different kinds of tumor in order to see in greater detail , the size and location of fibroids and choose the best course of therapy. Women with bigger uteruses or those who are perimenopausal most frequently undergo an MRI.

Hysterosonography, also known as a saline infusion ultrasonography, employs sterile salt water (saline) to expand the uterine cavity, making it simpler to obtain images of submucosal fibroids and the lining of the uterus in women who are trying to get pregnant or who are experiencing excessive monthly bleeding.

In hysterosalpingography, a dye is used to make the uterine cavity and fallopian tubes more visible on X-ray images. If infertility is an issue. In addition to revealing submucosal fibroids, this test can assist in determining whether the fallopian tubes are blocked or open.

In hysteroscopy, a small illuminated telescope called a hysteroscope is inserted into the uterus through the cervix by a doctor for this examination. The doctor then injects saline into the uterus, expanding the uterine cavity and allowing the doctor to examine the walls of the uterus and the openings of the fallopian tubes (Louie, 2022)

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1.7 TREATMENT OR MANAGEMENT OF UTERINE FIBROID.

There are many different ways to treat uterine fibroid; there is no one method that works best. They include:

1.7.1 Watchful waiting

Numerous women with uterine fibroids either don't have any symptoms at all or very minor symptoms that they may tolerate. If that's the situation, the best course of action might be watchful waiting. Cancerous tumors are not fibroids.fibroids rarely interfere with pregnancy Following menopause, when levels of reproductive hormones decline, they typically grow slowly, if at all, and tend to shrivel (Louie , 2022)

1.7.2 Medications

Medications for uterine fibroids target the hormones that control your menstrual cycle to relieve symptoms including pelvic discomfort and heavy monthly flow. While they don't get rid of fibroids, they might get smaller.These drugs consist of:

•Gonadotropin-releasing hormone (GnRH) agonists.:

GnRH agonists include leuprolide, goserelin and triptorelin. In order to exert its effects, GnRH agonists must first bind to the GnRH receptor, which results in an initial rise in gonadotropin production. Then, the GnRH agonist desensitizes the receptor, which results in a reduction in the release of gonadotropin. As a result, the estrogen level is subsequently decreased since the ovary's ability to be stimulated by gonadotropin is diminished. In addition, it was discovered that GnRH agonists directly inhibited fibroid proliferation (Khan *et al*., 2010). Currently GnRH agonists can be used to treat uterine fibroid prior to surgery. However due to the numerous

reported negative effects such as severe hot flashes, its use is often restricted to 6 months (Lethaby,2017)

GnRH antagonist:

It works by competing for GnRH receptors, thereby lowering the levels of estrogen and progesterone as a result. It does not cause the initial rise of follicle-stimulating hormone and luteinizing hormone, unlike GnRH agonist. In 2020, the Food and Drug Administration (FDA) approved the use of elagolix, a GnRH antagonist, in conjunction with estradiol and norethindrone acetate for the treatment of uterine fibroid (FDA, 2020) Relugolix, the most recent GnRH antagonist, is undergoing clinical trials for the treatment of uterine fibroid and offers the benefit of once-daily dose as opposed to twice-daily dosing with elagolix (Al-Hendy *et al*., 2021). Relugolix has been given the go-ahead to be sold in Japan in 2019 as a treatment for uterine fibroid symptoms (Markham, 2019). The addition of estradiol and norethindrone acetate (add-back therapy) is necessary to reduce the GnRH antagonist's hypoestrogenic side effects, which are identical to those of GnRH agonist (Schlaff *et a*l ,2020). FDA recommends against using elagolix for longer than 2 years due to the risk of bone loss and fractures [FDA, 2020]

Selective progesterone receptor modulator (SPRM):

This is a combination of agonist and antagonist activity on the progesterone receptor. The two most commonly used SPRM are mifepristone (pure antagonist) and ulipristal acetate (UPA), which have been shown to be successful in treating uterine fibroid. Prior to surgery, UPA was initially used for three months to reduce the fibroid volume. Recently, however, it is used in patients who refuse to have their uterus removed (Pazzaglia *et al*., 2017; Rozenberg et al.,

2017].UPA's progesterone antagonist effect on the endometrium may result in unopposed estrogen activity, which could lead to an increase in cell proliferation (De Milliano *et a*l, 2017). Due to the high incidence and severity of liver toxicity, with more than 900,000 women who were given with UPA for uterine fibroid requiring liver transplant, its use has been suspended until further studies have been done on the drug (Ekanem and Talaulikar, 2021)

Aromatase inhibitor:

This class of drug is used to prevent the enzyme aromatase from converting androstenedione to estrogen, which would enhance cell proliferation and fibrosis.The two aromatase inhibitors that have been widely studied for uterine fibroid are letrozole and anastrozole.In a randomized controlled trial comparing the effects of aromatase inhibitor and GnRH agonist on uterine fibroid, it was discovered that both interventions can reduce the myoma volume and symptoms, but aromatase inhibitors have the advantage of having a quick onset of action and not causing the initial flare associated with GnRH agonistagonist (Parsanezhad *et al*., 2010). Concerns with aromatase inhibitors revolve around their hypoestrogenic side effects, which include hot flashes and bone loss. In contrast, it has been noted that the use of aromatase inhibitors reduced hot flushes when compared to the use of a GnRH agonist, although the side effects of aromatase inhibitors are often minor and occur more frequently with continued usage.But a Cochrane Systematic Review found that there wasn't enough proof to recommend the use of aromatase inhibitors in uterine fibroid (Song *et al.*, 2013)

Antifibrinolytics:

Tranexamic acid which is a type of anti fibronytics is used to reduce painful menstrual cycles, this nonhormonal medicine is taken only on heavy bleeding days to help reduce the amount of blood lost during monthly periods. Common side effects include unusual bleeding or bruising, difficulty breathing with exertion, and weakness or fatigue (Louie, 2022)

Anti-inflammatory drugs:

Pain relievers known as nonsteroidal anti-inflammatory drugs (NSAIDs), which are not hormonal treatments, may be useful in reducing fibroids-related pain but not the associated bleeding. Examples of the pain relievers include ibuprofen and naproxen (Louie, 2022)

Hormonal contraceptives

Oral contraceptives can help control heavy menstrual bleeding and menstrual pain but they don't reduce fibroid size. This includes intrauterine devices (IUDs), a long-acting method of birth control that can reduce heavy monthly bleeding brought on by fibroids and distributes a little amount of hormone into the uterus. It does not reduce or eliminate fibroids (Louie, 2022)

Iron supplements:

Fibroid can cause heavy monthly flow and anemia, a disorder that arises when there aren't enough red blood cells to provide enough oxygen to tissues throughout the body. To increase red blood cell production and treat common anemia symptoms like weakness and exhaustion, a woman with fibroid may be advised to take over-the-counter iron supplements (Louie, 2022)

Noninvasive procedure: Focused ultrasound surgery (FUS)

Focused ultrasonic surgery (FUS) using an MRI is a non-surgical, outpatient method for treating uterine fibroids that doesn't involve cutting open the uterus.it is carried out when one is within an MRI scanner that has a high-energy ultrasound transducer for therapy. The images show the doctor exactly where the uterine fibroids are located. The ultrasonic transducer concentrates sound waves (sonications) into the fibroid at the chosen site, where they cause small patches of fibroid tissue to heat up and be destroyed. Researchers are learning more about the effectiveness and safety of FUS over the long term. However, studies to date indicate that FUS for uterine fibroids is both safe and effective (Louie, 2022)

Minimally invasive procedures

Without removing fibroids surgically, certain methods can eliminate uterine fibroids. They include:

•Uterine artery embolization :

The arteries supplying the uterus are injected with tiny particles (embolic agents), which starve the fibroids of blood and cause them to shrink and die. This method has the potential to decrease fibroids and lessen the problems they bring on. If the ovaries or other organs' blood flow is disturbed, complications could result. Research reveals that risk of transfusion is significantly decreased, and consequences are comparable to those seen with surgical fibroid therapies (Louie, 2022)

•Radiofrequency ablation:

Radiofrequency energy is used in this therapy to eliminate uterine fibroids and constrict the blood arteries that feed them. Laparoscopic and transcervical procedures are both capable of performing this. The fibroids are frozen using a similar technique known as cryomyolysis.With laparoscopic radiofrequency ablation (Acessa), also known as Lap-RFA, the doctor makes two tiny incisions in the belly and inserts a thin viewing device (laparoscope) with a camera at the tip. The doctor then finds the fibroids that need to be treated using the laparoscopic camera and a laparoscopic ultrasound instrument.

After locating the fibroid, the doctor inserts a number of tiny needles into it using a specialized tool. The fibroid tissue is heated by the needles and is subsequently destroyed. When a fibroid is destroyed, its texture rapidly changes, going from being hard like a golf ball to soft like a marshmallow. The fibroid continues to diminish during the following three to twelve months, which helps to alleviate symptoms. Lap-RFA is seen by clinicians as a less invasive alternative to hysterectomy and myomectomy since there is no cutting of uterine tissue.After 5 to 7 days of recovery, most women who undergo the operation can resume their normal activities. Ultrasound guidance is also used in the transcervical, or via the cervix, method of radiofrequency ablation (Sonata), to identify fibroids (Louie, 2022)

• Laparoscopic or robotic myomectomy:

A myomectomy involves the removal of the fibroids while keeping the uterus intact. If there are only a few fibroids, a laparoscopic or robotic treatment which uses slender instruments inserted through small incisions in the abdomen to remove the fibroids from the uterus is used . By

mortillating—the breaking of larger fibroids into smaller pieces—which can be accomplished inside a surgical bag or by extending one incision to remove the fibroids, larger fibroids can be removed through smaller incisions. A tiny camera linked to one of the equipment allows the abdomen to be seen on a monitor. The robot-assisted myomectomy gives a magnified, three-dimensional image of the uterus, thus giving a greater control, flexibility, and dexterity than is feasible with certain other methods (Louie, 2022)

Hysteroscopic myomectomy

If the fibroids are submucosal (confined within the uterus), this treatment might be a possibility. Instruments introduced via the cervix and vagina into the uterus are used to access and remove fibroids (Louie, 2022)

Endometrial ablation.

In this procedure, a specialized tool is placed into the uterus which uses heat, microwave energy, hot water, or electric current to damage the lining of the uterus thus, either terminating menstruation or lessening the amount of blood that comes out during a period. Abnormal bleeding can usually be stopped with endometrial ablation. Submucosal fibroids can be removed during hysteroscopy for endometrial ablation; however this doesn't apply to fibroids outside the uterus' internal lining. After endometrial ablation, it is unlikely that a woman would become pregnant; however, ectopic pregnancy must be avoided by using birth control.

Any procedure that doesn't remove the uterus carries the potential for the development of new fibroids and the resulting symptoms (Louie, 2022)

1.7.3 Traditional surgical procedures

Traditional surgical options include:

Abdominal myomectomy:

An open abdominal surgery might be done to remove the fibroids if one has numerous, very large, or very deep fibroids. Many women who are advised that hysterectomy is their only option can get an abdominal myomectomy instead. However, surgical scarring may impair a woman's ability to conceive in the future.

Hysterectomy

In this procedure, the uterus is removed. It is still the only treatment for uterine fibroids that has been shown to be permanent. The ability to have children is lost after a hysterectomy. The surgery triggers menopause and the decision to utilize hormone replacement treatment if one also chooses to have their ovaries removed. Most uterine fibroid patients may be able to opt to maintain their ovaries (Louie, 2022; Lisa Rapaport and K.S 2023)

1.7.4 Other treatments of fibroids

1.7.4.1 Complementary and alternative treatments:

Numerous complementary and alternative therapies for uterine fibroids can assist to ease symptoms including back pain and discomfort from excessive bleeding. Some complementary therapies include;

Turmeric: Also known as *Curcuma longa*, is a rhizomatous herbaceous perennial plant from the ginger family. *Curcuma longa* was included as one of the herbs in the tumor-shrinking decoction, which is currently under phase-III clinical trials (ClinicalTrials.gov, 2014; Cheng *et al.,* 2019) It

has been documented that turmeric extract can protect the uterine myometrium against oxidative damage-induced uterine fibroid (Eze-Steven, 2019).Reactive oxygen species (ROS) can cause leiomyoma smooth muscle cells to multiply, and they are also required for MAPK1/MAPK3(Mitogen-activated protein kinase 1/3] signaling pathway, which also causes leiomyoma smooth muscle cell proliferation (Mesquita *et al.*, 2010). Since turmeric has antioxidant properties and protects the uterine myometrium, it may be utilized to treat or prevent uterine fibroid.

Green tea: The ability of green tea also known as *Camellia sinensism* to exert anti-uterine fibroid activity is mainly attributed by epigallocatechin gallate (EGCG), one of the main active constituents in green tea. In a randomized controlled experiment, green tea extract (45% epigallocatechin gallate, or EGCG) significantly reduced the fibroid size in women with symptomatic uterine fibroid as compared to the placebo group after taking 800 mg of green tea extract for the duration of 4 months.In addition, green tea extract considerably reduced the severity of fibroid symptoms when compared to a placebo, with no negative side effects found (Roshdy *et al*., 2013).

Strawberry (*Fragaria x Ananassa*): Strawberry extract has proven as a treatment and/or preventative for uterine fibroid. In leiomyoma cells, anthocyanin-rich strawberry extract has been shown by Islam et al to cause apoptosis, inhibit glycolysis, and drastically lower levels of the ECM proteins like collagen 1A1, fibronectin, and versican. According to the authors, increased formation of reactive oxygen species (ROS) and dead cells may be the mechanism by which strawberry extract triggers apoptosis. However, the production of ROS was reduced instead, and the percentage of apoptotic and dead cells in the normal myometrial cells did not

differ significantly. This could have suggested that strawberry extract might only target the uterine fibroid cells while keeping the normal myometrial cells in a homeostatic state, resulting in fewer side effects from its use (Islam *et al* , 2017)

Dietary Therapy: According to research, populations that consume more red meat, including beef, ham, and alcohol, have higher rates of uterine fibroids. A woman's risk of developing uterine fibroids increases by more than 50% if she drinks one beer or more each day.Red meats and other high-energy foods that contribute to the pathogenesis of fibroids should be avoided, whereas foods high in flavonoids, oily fish, green vegetables, citrus fruits, soya, and broad beans that prevent the pathogenesis of fibroids should be encouraged (Sacks *et al,* 2001).

Acupuncture: This is a form of complementary alernative medicine treatment that uses small needles applied at specific sites on the body to relieve chronic pain. It acts on a variety of therapeutic targets involved in the pathophysiology and symptomatology of fibroids.It is a successful treatment for dysmenorrhea, chronic pelvic inflammatory illness, and dysfunctional bleeding (The British Acupuncture Council, 2015; Cho S-H and Hwang E-W, 2010)

Stress reduction: Activities that encourage relaxation, such as yoga, tai chi, meditation, massage and deep breathing techniques, may lessen the pain and discomfort brought on by fibroids. All of these techniques are generally regarded as effective (Harvard health publishing, 2020)

Cognitive behavioral therapy (CBT): This is a talking treatment or therapy that can help one manage difficulties or problems by altering the thoughts and behaviors of a person. A wide range of medical issues, including fibroids, can be relieved by using this popular form of talk

therapy. CBT makes it simpler to manage pain by assisting in reframing one's thoughts around their health and its symptoms (APA 2017 ;NHS 2022)

Exercise: According to certain research, physical activity, particularly when it results in a healthy body weight, can stop the growth of fibroid tumors. Exercise can lower blood levels of insulin and circulating sex hormones (Souza MJ,2003; Jasienska G and Ellison PT,2004) hence reducing proliferative effects that may be brought on by these factors. Exercise may also affect estrogen metabolism, perhaps resulting in less estrogenic metabolic products being produced, but this has not been proven in recent investigations (Atkinson *et al* , 2004; Campbell *et al,* 2005).To assess these potential fibroids processes, more study will be needed.

1.7.4.2 Herbal treatment:

Some herbal medications have been reported to be effective in lowering the size of fibroids and relieving symptoms. They include;

Polyherbal Guizhi Fu Ling Wan (GZFLW):

GZLFW is a formula made up of five herbs in a 1:1:1:1:1 (g/g) ratio. These five herbs are: *Cinnamomi ramulus, Poria, Semen persicae, Paeoniae radix* and *Moutan cortex* (Xiao *et al,* 2012). Numerous research that looked into how GZLFW affected leiomyoma cells discovered that GZLFW can decrease cell viability and proliferation in a dose-dependent way (Shen *et al*., 2016; Lee *et al*., 2019). Additionally, a meta-analysis showed that GZLFW and mifepristone together were more efficient at reducing fibroid volume than mifepristone alone. Additionally, GZFLW can alleviate the dysmenorrhea symptoms in uterine fibroid Patients without any major side effects (Chen *et al*., 2014)

Polyherbal Lichong Decoction:

Lichong decoction (LD) is another Chinese herbal formulation that has been studied for its effect on uterine fibroid. The herbs included in this decoction includes 9 g of the root of *Astragalus mongholicus* Bunge [Fabaceae], 6 g of root of *Codonopsis pilosula* (Franch.), 6 g of rhizome of *Atractylodes macrocephala* Koidz (Asteraceae), 15 g of rhizome of *Dioscorea opposita* Thunb (Dioscoreaceae), 12 g of root of *Trichosanthes kirilowii Maxim (*Cucurbitaceae), 12 g of rhizome of *Anemarrhena asphodeloides* Bunge [Asparagaceae], 9g of rhizome of *Sparganium stoloniferum* (Buch.-Ham. Ex Graebn.), 9 g of rhizome of *Curcuma phaeocaulis* Valeton (Zingiberaceae) and 9 g of Endothelium *coreneum Gigeriae* (chicken gizzard). This decoction was traditionally formulated for the treatment of uterine fibroid by strengthening the healthy Qi (the circulating life force whose existence and properties are the basis of much Chinese philosophy and medicine) ,enhancing blood flow, and eliminating disease-causing pathogens (Wang et al, 2016)

Polyherbal *Sparganii rhizoma* and *Curcumae rhizoma:*

Studies have demonstrated and proved the antiangiogenic activity of Curcumae rhizoma essential oil and also its ability in inhibiting cell proliferation. Additionally, it has been shown that *curcumae rhizoma* inhibits the expression of matrix metallopeptidase 2. (MMP-2) , which may also support its usage in the treatment of uterine fibroid (Chen *et al,* 2011). When *Sparganii rhizoma* was also studied in mice, it was observed that there was a significant decrease in the fibroblast growth factor-1 (FGF-1) and Vascular endothelial growth factor (VEGF) level, suggesting that *Sparganii rhizoma* may have an effect on angiogenesis (Sun *et al,*2011). When both of these herbs are administered together (known as CRSR, CR:SR = 1:1) in the treatment of uterine fibroid, the uterine mass was found to be greatly reduced with a significantly lower

progesterone and estradiol level. Additionally, CRSR was discovered to alter a number of ECM-associated genes, which may explain its capacity to reduce uterine mass (Yu *et al,* 2019). Another independent study revealed a decreased expression of fibroblast activation protein, a collagen component of the ECM and Transforming growth factor beta (TGF-b), supporting the concept that CRSR has an impact on the ECM (Feng *et al.,* 2021).

1.8 Literature review of the plant: *Tetrapluera tetraptera*

T. tetraptera, a popular medicinal plant belonging to the family Fabaceae (formerly Leguminosae: Mimosoideae), is commonly known as Aridan is generally found in the lowland forest of many tropical African countries, and known to have fruit that consists of fleshy pulp and small, brownish-black seeds, a characteristic fragrant and pungent aromatic odor(Aladesanmi, 2007). *T. tetraptera* is a perennial plant with dark green leaves that is typically found in the West, Eastern, and Central sub-regions of the continent of Africa rain forest belt. *T. tetraptera* fruit is a good source of calcium, phosphorus, potassium, zinc, iron, salt, and vitamins as well as phytochemicals such tannins, phenolic compounds, saponins, alkaloids, steroids, flavonoids, phlobatannins, and terpenoids *(*Erukainure *et al.* 2017*). T. tetraptera* is widely prescribed in tropical African traditional medicine to treat a range of human disorders, including schistosomiasis, diabetes mellitus, asthma, arthritis, and other inflammatory conditions. (Ojewole and Adewunmi 2004). In the eastern part of Nigeria, soups are frequently made from the plant since it is edible (Akintola *et al*. 2015).

1.8.1 Taxonomy of Tetrapleura tetraptera

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Genus: Tetraplura

Species: tetraptera

Botanical name: *Tetraplura tetraptera* (Schumach. & Thonn.) Taub. Fabaceae

Tetrapleura tetraptera plant is commonly known as Aridan or Aidan among the Yoruba ethnic group of South Western Nigeria, Abogolo among the Igala people of north central Nigeria, and Dawo among the Hausa people of Northern Nigeria(Aladesanmi, 2007).

1.8.2 Morphology

Tetrapleura tetraptera Shumach & Thonn Taub, Fabaceae, often referred to as Aridan (fruit), is a 30-meter-tall, sturdy, perennial tree with a single stem. It has smooth/rough, grey/brown bark and glabrous(smooth, having a surface lacking hairs, bristles, and glands) young branchlets. The fruit has dark brown, four-winged pods that measure 12.25 x 3.5 x 6.5 cm and contains a yellow/pink bloom with white racemes. The lowland forests of tropical Africa are where it is

most frequently found. The fruit is made up of a luscious pulp and tiny, brownish-black seeds(Adetunji ,2006)

The common stalk is 15-30 cm long and has a little groove on the upper surface. The leaves are sessile, glabrous or minutely hairy. The leaflets on each side of the pinna stalk are alternate, 6-12 mm long, 12-25 mm wide, slightly elongated, elliptic or slightly obovate, rounded at both ends, the apex occasionally very slightly notched, the base typically unequal, practically glabrous, with slender stalks about 2 mm long; lateral nerves are indistinct, running at a wide angle to the prominent midrib (Orewa *et al*,2009)

Figure 4: Picture showing *Tetrapleura tetraptera* pod

1.8.3 Traditional uses

In Tropical African traditional medicine, the fruit of *Tetrapleura tetraptera* (Taub) is frequently employed for the management and/or control of a variety of human illnesses, such as arthritis and other inflammatory disorders, asthma, diabetes mellitus, wound healing, hypertension, epilepsy, schistosomiasis and post partum care (Ojewole *et al*, 2004)

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1.8.4 Pharmacological activities of *Tetrapleura tetraptera*

Anti tuberculosis:

In order to establish the plant's anti tuberculosis effect, the leaves of *Tetraptera tetraptera* were collected, weighed, dried, and ground into a powder. Methanol was then extracted from the powder, and the extract was tested against isolates of multidrug resistant *Mycobacterium tuberculosis* (Izebe *et al*,2020)

Antistaphylococcal activity

Acetone and methanol fruit extracts from *Tetraptera tetraptera* are effective against methicillin- and multidrug-resistant *staphylococci*. Standard antibiotics included nitrofurantoin, gentamicin, amoxicillin, and augmentin in the study (Morenike *et al,*2002)

Hypolipidaemic property

Albino rats received *T. tetraptera* aqueous extract five times each week for eight weeks in a row. The amount of total cholesterol, LDL cholesterol, triglycerides, and HDL cholesterol in the blood of the experimental rats was measured at the conclusion of the eighth week. When compared to hypercholesterolemic rats not receiving treatment, *T. tetraptera* greatly reduced the body weight growth brought on by cholesterol, and blood and total cholesterol levels dropped by

almost 50%. When compared to hypercholesterolaemic rats, LDL-cholesterol considerably lowered in the extract-treated wistar rats (Sarah *et a*l., 2010).

Anti-inflammatory and hypoglycaemic property

For the purpose of inducing pain and diabetes in mice, fresh egg albumin-induced pedal oedema and streptozotocin (STZ) were utilized, respectively. For referencing and comparison, diclofenac and chlorpropramide were used. A considerable, dose-related reduction in inflammation was obtained by *T. tetraptera* extract. Additionally, both fasted diabetic and fasted normal rats' blood glucose levels were significantly reduced by the plant extract in a dose-dependent manner (Ojewole and Adewunmi, 2004).

Moluscidal property

In one study, a methanolic extract of *T. tetraptera* fruits produced the rare sapogenin 27-hydroxyolean-12 (13)-en-28-oic acid, echinocystic acid-3-0-sodium sulfate from the stembark, and umbelliferone and ferulic acid from the leaves and branches, respectively. Aridanin and three of its olean-12-en-28-oic acid derivatives were also isolated from the fruits. According to Adetunji (2006), all the chemicals extracted from the fruits or other portions were highly molluscicidal toward the Biomphalaria glabrata snails, which carry schistosomiasis.

Antihelminthic property

A study was conducted to ascertain the anthelminthic effects of *T. tetraptera'*s aqueous extract (fruit and stembark) against Pheretima posthuma worms. After around 200 minutes of exposure, the worms were able to be paralyzed and killed by the aqueous extract of *T. tetraptera* fruit and

stem bark. After around 300 minutes of exposure to the worm, the ethanol extract was able to paralyze it, but it did not result in the worm's death (Ahmed, 2021)

Antimicrobial properties

Staphylococcus aureus, Streptococcus pneumonia, Streptococcus pyrogens, Klebsiella pneumonia, and *Candida krusei* were some of the bacteria and fungi isolates against which the ethanol extract of *T. tetraptera* was evaluated. The ethanol extract was able to inhibit the growth of this microbial isolates.Based on this findings, it was concluded that the stem bark and leaf crude ethanol extracts both possessed potent anti-bacterial and anti-fungal properties (Okwute *et al.*, 2016).

Anxiolytic property:

The aridanin found in *T. tetraptera*'s fruit was extracted from the plant and tested to see if it had any anxiolytic properties. The effects of aridanin, which were induced at doses of 5 and 10 mg/kg, i.p., 30 min beforehand, were expressed as an increase in the number of entries and time spent in the open arms and a decrease in the number of entries and time spent in the closed arms. This effect was inhibited by flumazenil, a Gamma amino butyric acid receptor blocker, demonstrating that aridanin acts via the GABA pathway. (Aderitigbe and others, 2010)

Antiproliferative property:

The Methanolic extract of *T. tetraptera* was investigated to see if it has antiproliferative activity toward cancer cells in the search for chemotherapeutic drugs with fewer severe and unpleasant side effects. Leukemia and breast cancer cells were evaluated for resistance to the methanolic

extract. The Jurkat cancer cells were more responsive to the extract's antiproliferative action (>100 g/mL) than the MCF-7 cancer cells were (Rosebud *et al*. 2021).

Wound healing property

T. tetraptera aqueous extracts were produced, and petroleum jelly was used as the ointment's foundation. When this ointment was applied topically to a wound on wistar rats, the wound healing rate and epithelization time significantly improved (Olimaram *et al*., 2020)

Anagelsic and anticonvulsant property

When mice were given *T. tetraptera*'s extract at doses ranging from 50 to 800 mg/kg intraperitoneally (i.p.), the analgesic effects were dose-dependent, substantial ($p < 0.05–0.001$), and they were able to diminish pain caused in mice using electrical and chemical approaches. Anticonvulsant substances like diazepam and phenobarbitone were utilized as benchmarks for comparison when evaluating the substance's anticonvulsant abilities (Oyewole, 2005).

Antimalarial property

T. tetraptera, when given daily at a dose of 300-900 mg/kilogram body weight, revealed significant ($P < 0.05$) blood schizonticidal action. *Tetraptera* fruit ethanol extract shown invivo antiplasmodial activity in Plasmodium berghei infected mice (Okokon *et al*,2007)

1.9 AIMS AND OBJECTIVES OF THE STUDY.

Aim: The aim of the study is to investigate the anti fibroid potential of ethanol extract of the stem barks of *Tetrapleura tetraptera* plant on monosodium glutamate induced lyeomyoma in Sprague Dawley rat using preventive and curative method.

Objective:

1. To evaluate the ability of the extract to reduce monosodium glutamate induced increase in cholesterol

2. To evaluate the ability of the extract to reduce monosodium glutamate induced increase in total protein.

3. To evaluate the ability of the extract to reduce monosodium glutamate induced increase in estradiol

4. To assess the effect of the extract in ameliorating leiomyoma formation through histological studies.

CHAPTER TWO

2.0 MATERIALS AND METHOD

2.1 Materials

2.1.1 Equipments.

The equipment used include: Soxhlet apparatus, digital weighing balance, electric milling machine, Thermo-regulated water bath, refrigerator, oven, semi auto analyser, centrifuge, microscope, semi- automated rotary microtome, microplate reader and E2 Accubind ELISA kits. Porcelain dishes, glass jar, mortar, pestle, orogastric tube, plastic cages, measuring cylinders, beakers, ink marker, distilled water, oestradiol , cholesterol and total protein test kits,

2.1.2 Consumables

Syringes, sample bottles, dissecting kit, micro pipettes.paraffin wax, Cryovials, automatic pipette, test tubes, test tube rack

2.1.3 Chemicals/reagents

Chloroform, Ethanol, Monosodium glutamate, Cholesterol biuret reagent, Total protein biuret reagent, Total protein standard, Cholesterol standard, formaldehyde solution , xylene, DPX (Dibutylphthalate Polystyrene Xylene) and bouin's solution.

2.2. Methodology

2.2.1 Plant material collection and authentication.

The plant (*Tetrapleura tetraptera*) bark was collected along Agbegi - Odofin village road, ikire Osun state, Nigeria, in January, 2023. It was authenticated by Dr. Odewo Akinniyi Samuel at Forest Research Institute Ibadan, Oyo State, Nigeria. A voucher specimen was deposited at the institute and the hebarium number FHI 113604 was issued.

2.2.2 Preparation of Plant Material.

Foreign matters in the collected *Tetrapleura tetraptera* barks were removed and the barks were spread on a clean under shade for air drying at room temperature until completely dried. The air dried plant material was then milled into coarse powder using modern laboratory electric milling machine. The powdered plant material was weighed and stored in airtight container for further use.

2.2.3 Extraction of Plant Material.

The powdered plant material (889.1g) was extracted with ethanol (95%). The extraction was carried out in batches, with the aid of a soxhlet apparatus at 60ºC. The obtained plant extract was concentrated using a thermo-regulated water bath. Thereafter, the semi-solid plant extract obtained was weighed and stored in the refrigerator at 4ºC until it was needed.

2.2.4 Phytochemical test

Phytochemical test was done to confirm the absence or presence of plant metabolites.

2.2.4.1 General tests for glycosides

To a little quantity of the powdered plant material of *T. tetraptera* was added 20 mL of distilled water in a beaker. The content of the beaker was heated on a boiling water bath for 15 minutes and then stirred with a glass rod, filtered hot and allowed to cool.

a. Molisch's test

To 2 mL filtrate obtained above, a drop of 10% alcoholic solution of alpha-naphthol was added. The tube was inclined at an angle of 450C and 2mL of concentrated sulphuric acid was cautiously poured down the side of the test tube to form a layer below the extract without mixing.

A deep violet ring formed at the interface of the two liquids indicated the presence of carbohydrate.

b. Fehlings test

To 2 mL of the filtrate obtained above, 1 mL each of Fehling's solutions A and B were added. It was boiled gently on a water bath for 5 mins. Formation of a reddish-brown precipitate indicated the presence of reducing sugars.

2.2.4.2 Tests for Saponins

To 2 g of the powdered plant material, 20 mL of distilled water was added in a beaker and boiled gently for 15 minutes. It was then filtered and allowed to cool for 10 minutes. About 2 mL of the filtrate was diluted with water (2 mL) and shaken vigorously. Persistent frothing indicated the presence of saponins.

2.2.4.3 Tests for phenolic compounds

About 2g of the powdered plant material was extracted with 20 mL of distilled water in a beaker and was boiled gently for 15 minutes. It was then filtered and allowed to cool for 10 minutes.

a. Tests for tannins

To 3 mL of the filtrate obtained above, a drop of 15% Ferric chloride solution was added. A blue black or greenish precipitate indicated the presence of tannins.

b. Test for flavonoids

To 3 mL of the filtrate obtained above, 2 mL of dilute sodium hydroxide solution and 2 mL of concentrated hydrochloric acid solution were added.

2.2.4.4 Test for steroids (Salkwoskis test for steroidal nucleus)

About 0.2 g of the ethanol extract was dissolved in 2 mL of chloroform. About 2 mL of concentrated sulphuric acid was cautiously added into the test tube with the test tube inclined at an angle of 450 to form a lower layer. A reddish-brown colour observed at the interface of the liquids indicated the presence of steroidal nucleus.

2.2.4.5 Test for alkaloids

About 2g of the powdered plant material of *T. tetraptera* 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 1 mL of Mayers, Dragendorffs, Wagners and Hagers alkaloidal reagents, respectively. Formation of characteristic coloured precipitates (reddish brown for Dragendorffs and Wagners; cream coloured for Mayers and yellow colour for Hagers reagents) indicated the presence of alkaloids(pcg manual 2005)

2.3 Animal study

2.3.1 Source of Laboratory Animals.

Adult non pregnant female Sprague Dawley rats weighing within the range of 100 to 200g were used for this study. 62 Sprague Dawley rats were procured from the animal house in

pharmacology department and were maintained in the department of pharmacology animal house, University of Benin. The animals were kept in separate clean plastic cages with wood shavings as bedding, and were maintained under hygienic and well ventilated condition. They were fed with standard palletized animal chow (chukun Feed, Ibadan) and clean water. They were housed at normal room temperature of 27 +/-3°C, with 12 hours light and 12 hours dark cycle. The animals were allowed to acclimatize for two weeks.

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2.3.2 Ethical Clearance.

All experiments were conducted in accordance with the guidelines of the Animal Ethical Committee of the Faculty of Pharmacy University of Benin.

2.4 Acute Toxicity test

This method has two phases which are phases 1 and 2 respectively using Lorke's method

Phase 1

Nine animals were used in this phase. The nine animals were divided into three groups of three animals each. Each group of animals were administered different doses (10, 100 and 1000 mg/kg) of *Tetrapleura tetraptera* stem bark extract. The animals were placed under observation for 24 hours to monitor their behavior as well as if mortality will occur.

Phase 2

This phase involved the use of three animals, which were distributed into three groups of one animal each. The animals were administered higher doses (1600, 2900 and 5000 mg/kg) of *Tetrapleura tetraptera* stem bark extract and then observed for 24 hours for behavior as well as mortality (lorke, 1983)

2.4 EVALUATION OF THE ANTI FIBROID ACTIVITIES OF THE ETHANOL EXTRACT OF *Tetrapleura tetraptera* stem bark.

2.4.1 Preventive Experiment

The stem barks of *Tetrapleura tetraptera* were dried, pulverized by milling, extracted using soxhlet apparatus and concentrated using a water bath. The rats were divided into five groups (A, B, C, D, and E), of five rats each. Group A (control) received only food and water. In order to stimulate the formation of uterine fibroids, groups B, C, D, and E were given 800 mg/kg of MSG for 30 days. Then, groups C, D, and E were given 100, 200, and 400 mg/kg of *T. tetraptera* stem bark respectively alongside with the extract, once daily for 30 days. All administration was done once daily by the means of oral gavage using an orogastric tube. (Koffuo *et a*l, 2013)

2.4.2 Curative Experiment

The rats were divided into five groups (A, B, C, D, And E), of five rats each. Group A (Control) received only food and water. In an effort to stimulate the formation of uterine fibroid, group B was given 800 Mg/Kg of MSG for 30 days and thereafter stopped, with normal rat chow continued till day 61. Groups C, D and E were also given 800mg/ kg of MSG for 30 days to induce fibroid, then from the 31st day, groups C, D, and E were given 100, 200 and 400 Mg/Kg Of *T. tetraptera* stem bark extract respectively, once daily for another 30 days. All administration was done by the means of oral gavage. On the 61st day, the animals were sacrificed, blood samples were collected and the uterus excised to be assessed (Koffuo *et al*., 2013).

2.5 Specimen collection

At the end of 60 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

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2.5 Specimen

supernatant which was carefully collected into labeled hematocrit tubes. Total plasma cholesterol, total plasma protein and plasma estradiol were determined. The uterus was surgically removed and transferred into sterile tissue bottles containing Bouin's solution for histopathology tests.

2.6 Biochemical Assay

Total protein and total cholesterol were determined using a semi-auto biochemistry analyzer which works on the principle of photoelectric colorimetry to measure a specific chemical composition in a sample.

2.6.1 Biochemical assay of Total cholesterol

This assay was carried out using the semi-automated chemistry analyser (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 microtube marked 'blank' ,1000µL of cholesterol buirret 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 the remaining samples (A1 to M5) in their respective tubes. All tubes were incubated for 10 minutes at 37°C. 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.

2.6.2 Biochemical assay of Total protein

This assay was carried out using the semi-automated chemistry analyser (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 microtube marked 'blank' ,1000µL of protein buirret reagent was added. 20µL of standard total protein and 1000µL of the reagent were added to and properly mixed in a tube marked "standard". 20µL of sample A1 and 1000µL of Total protein reagent were added to and properly mixed in a tube labelled 'A1' .The same procedure was carried out for the remaining samples (A1 - M5) in their respective tubes. All tubes were incubated for 10 minutes at 37°C. 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.

2.6.3 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 in the principle of solid phase 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 after which they were swirled gently for about 30 seconds and then incubated for 30 minutes followed by the addition of 50µL of estradiol enzyme reagent to all the wells and incubated for 90 minutes at room temperature. The contents of the microplate were then discarded by decantation and the

plates were dried with absorbent paper afterwards. 350µL of the wash buffer was added and the content was decanted thrice. 100µL of substrate was added and incubated for 20 minutes, then 50µL of the stop solution was added to each well and gently mixed for 20 minutes. The absorbance was read at 450nm within 15 minutes of adding the stop solution. The concentration of the estradiol in the sample was extrapolated from a dose response curve.

2.7 Histology studies

The tissues were fixed by placing them in 10% neutral buffer formalin solution. The uterus was further dissected to select appropriate area for examination and were placed in suitably labelled 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 (Dibutyl phthalate Polystyrene Xylene) and subsequently viewed under the microscope and photographs taken.

2.8 Statistical Analysis.

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

CHAPTER THREE

3.0 Results

3.1 Percentage Yield

The percentage of the extract obtained from the bark of *Tetrapleura* tetraptera was 21.27% after extraction.

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3.2 Acute and Delayed Toxicity Test

There were no obvious CNS or autonomic nervous system disorders. No diarrhoea. No alterations in behavior were noticed. Feeding and the sleep/wake cycle were both normal. Body weight did not alter significantly.Also, during the study, no deaths occurred.

3.3 Phytochemical Screening

The results of the various phytochemical tests showed the presence of glycosides, saponins and steroids. A summary of the results is shown in Table 2.0

Table 2.0: Phytochemical tests and results for the powdered *T. tetraptera*

Tests	**Observation**	**Inference**
Molischs Test	Purple ring observed at the interface of the 2 liquids.	Carbohydrate present
Fehlings Test	Reddish- brown precipitate formed.	Reducing sugar present
Saponins	Persistent frothing observed.	Saponin present
Tannins	Presence of blue black or greenish precipitate	Tannin present
Flavonoids	Yellow coloured solution that turned colourless on adding acid observed	Flavonoids present
Molischs Test	Purple ring observed at the interface of the 2 liquids	Carbohydrate present
Salkowskis test.	Reddish brown ring at the interface was observed .	Steroidal nucleus present
Test for Alkaloids Dragendorffs reagent	Reddish brown colour precipitate was observed	Alkaloid present.
Wagners reagent	Reddish brown colour precipitate was observed	Alkaloids present.
Hager's reagent.	yellow colour precipitate was observed	Alkaloids present
Meyer's reagent	Light cream precipitate was observed	Alkaloid present

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Test 3.4 Biochemical Analysis.

Preventive group

The result of the biochemical analysis in total cholesterol shows that there was a significant difference between the MSG group and the control group as $P < 0.05$ while the extract groups of 100 mg/kg, 200 mg/ kg and 400 mg / kg showed no significant difference ($P > 0.05$) between them and the control group but mean values of 57.40mg/dl, 60.60 mg/dl and 38.0mg/dl respectively.

For total protein analysis, there was no significant difference between the MSG group and the control group as $P > 0.05$ although the MSG increased the total protein to 10.73. Also there was no significant difference ($P > 0.05$) between the different doses of the extract and the control group but a dose dependent reduction in the total protein mean.

For estradiol analysis, there was a significant difference between the MSG group and the control group as $P < 0.05$ while the extract groups of 100 mg/kg, 200 mg/ kg and 400 mg / kg showed no significant difference ($P > 0.05$) between them and the control group but a dose dependent reduction in their mean.

Curative group;

In the cholesterol analysis, there was a significant difference between the MSG group and the control group as $P < 0.05$ while the extract groups of 100 mg/kg, 200 mg/ kg and 400 mg / kg showed no significant difference ($P > 0.05$) between them and the control group but mean values of 74.20 mg/dl, 62.00 mg/dl and 64.40 mg/dl respectively.

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For total protein analysis, there was no significant difference between the MSG group and the control group as P >0.05 although the MSG increased the total protein to 98.46. Also there was no significant difference (P> 0.05) between the different doses of the extract and the control group but gave values of 87.46g/dl, 88.76g/dl and 94.14g/dl respectively

For estradiol analysis, there was a highly significant difference between the MSG group and the control group as P < 0.01 while the extract groups of 100 mg/kg, 200 mg/ kg and 400 mg / kg showed no significant difference (P> 0.05) between them and the control group but a dose dependent reduction in their mean.

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From the result of the total protein group, there was a significant difference in the between the MSG group and the control group in the preventive treatment method while there was no significant difference between the MSG group and the control group in the curative treatment method. Also the 100mg/kg,200 mg/ kg and 400 mg / kg also showed no significant difference when compared to the control group.

From the result of the estradiol group, both the preventive and curative treatment group showed no significant difference when the MSG and the different doses of the extract were compared to the control group.

3.4.1. Table 3.0: Biochemical assay of Total Cholesterol(mg/dl).

Group	Preventive Mean ± SEM	Curative Mean ± SEM
Control	45.8 ± 6.42	61.2 ± 7.58
MSG (800mg/kg)	71.4 ± 5.87*	88.8 ± 5.93*
100mg/ kg	57.40 ± 3.44	74.20 ± 7.08
200 mg/kg	60.60 ± 7.10	62.00 ± 4.39
400 mg/ kg	38.0 ± 2.95	64.40 ± 9 62

The results are expressed as mean ± SEM and n=5

* indicates significance compared to negative control. *P < 0.05,

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3.4.2. Table 4.0: Biochemical assay of Total Protein(g/dl)

Group	Preventive Mean ± SEM	Curative Mean ± SEM
Control	9.50 ± 0.48	91. 84 ± 2.23
MSG (800mg/kg)	10.73 ± 1.25	98.46 ± 4.61
100mg/kg	9.12 ± 0.58	87.46 ± 3.18
200mg/kg	10.32 ± 0.45	88.76 ± 3.00
400mg/kg	9.43 ± 0. 19	94..14 ± 2.59

Values are expressed as mean ± SEM, n=5, P > 0.05 compared to negative control.

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3.4.3. Table 5.0: Biochemical assay of estradiol (PG/ml)

Group	**Preventive** **Mean ± SEM**	**Curative** **Mean ± SEM**
Control	9.44 ± 2.01	9.66 ± 1.84
MSG(80mg/kg)	17.99 ± 2.42*	20.22 ± 2.65**
100mg/kg	9.53 ± 1.43	12.89 ± 1.91
200mg/kg	8.86 ± 1.84	11.22 ± 2.24
400mg/kg	8.75 ± 1.75	10.32 ± 1.05

Values are expressed as mean ± SEM, n=5, P < 0.05 compared to negative control.

**P < 0.01 which shows high significance difference compared to negative control.

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3.5. Histological studies result

Preventive group;

In figure 5 which shows the control group, the rat uterus is composed of normal tissue architecture. The uterine cavity is surrounded by endometrial lining and stroma surrounded by supporting endometrial glands. In figure 6, the rat uterine wall given 800 mg/kg MSG only is composed of proliferating bundles of smooth muscle fibers dispersed in haphazard manner and supported by thick collagenous stroma which suggests the beginning of the formation of leiomyoma cells. In figure 7 which shows the rat uterus given MSG and 100mg/ kg extract shows endometrial cavity , plump endometrial stromal cells and endometrial gland. Figure 8 shows rat given MSG and 400mg/ kg stem bark extract shows that the uterine cavity has endometrial glands , stroma containing plump cells and blood vessels which shows that the extract was able to prevent leiomyoma.

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Curative group;

Figure 9 shows rat uterus control which has endometrial lining, endometrial stroma glands.Figure 10 shows rat uterus given 800mg/kg MSG for 30 days with endometrial stroma overtaken by bands of spindle cells arranged in haphazard manner supported by collagenuous stroma. Figure 11 shows a rat uterus given 100mg/kg stem bark plus MSG which shows mild bundles of spindle cells arranged in haphazard manner, supported by collagenuous stroma. Figure 12 shows a rat uterus given 400mg/kg stem bark plus MSG which shows thick bundles of smooth muscle cells supported by collagenuous stroma.

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Preventive Treatment group:

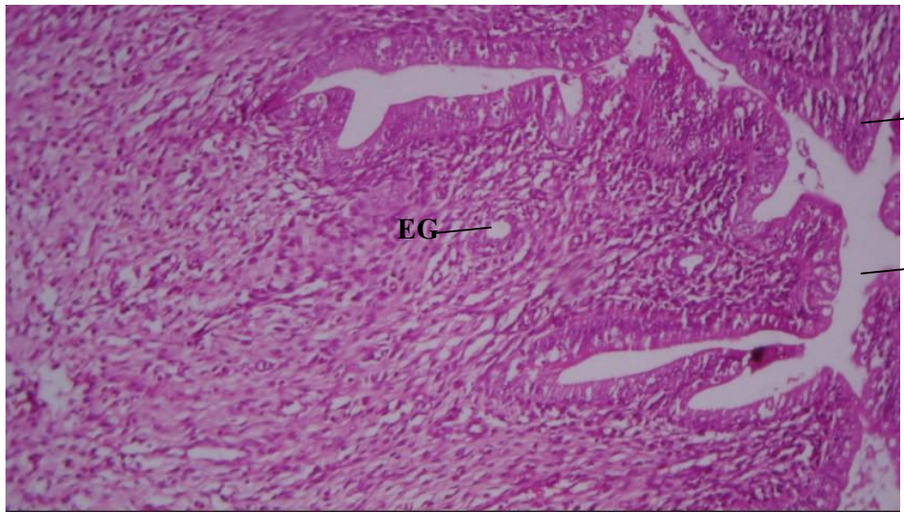

Figure 5. Rat uterus Control:

Composed of normal tissue architecture. uterine cavity (UC), surrounded by endometrial lining (EL), endometrial stroma (ES) supporting the endometrial glands (EG): BF x 100

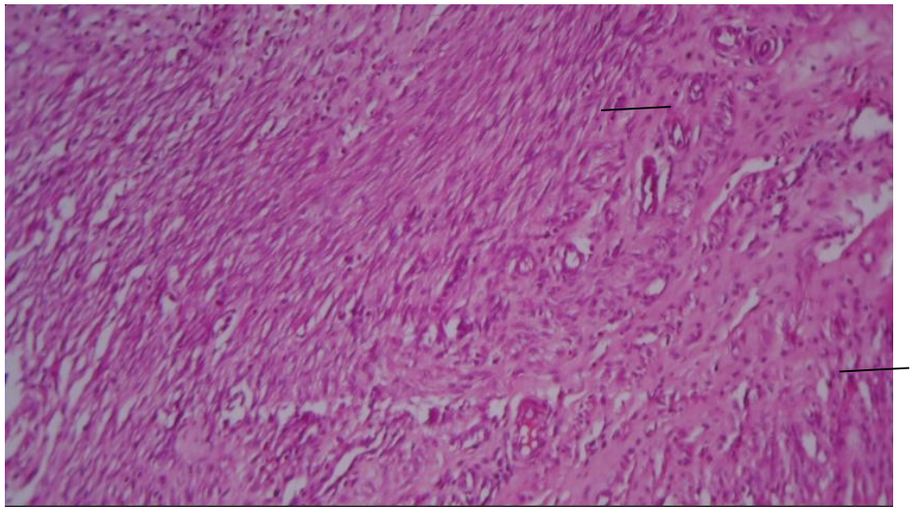

Figure 6: Rat uterine wall given MSG only:

Rat uterine wall given MSG only, composed of proliferating bundles of smooth muscle fibres (SF) disposed in haphazard manner and supported by thick collagenous stroma (ST): BF x 100

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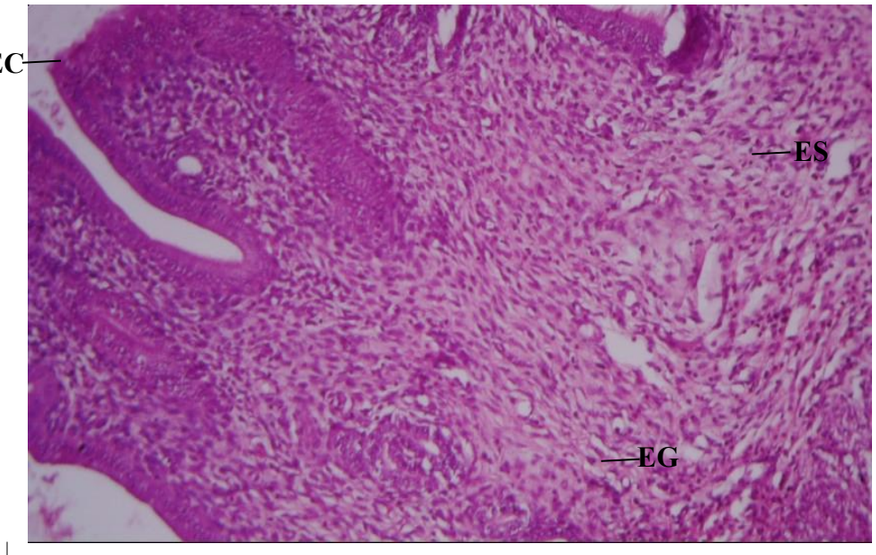

Figure 7: Rat given MSG + 100mg :

Rat given MSG + 100mg Extract showing endometrial cavity (EC), plump endometrial stromal cells (ES), endometrial gland (EG).BF x 100

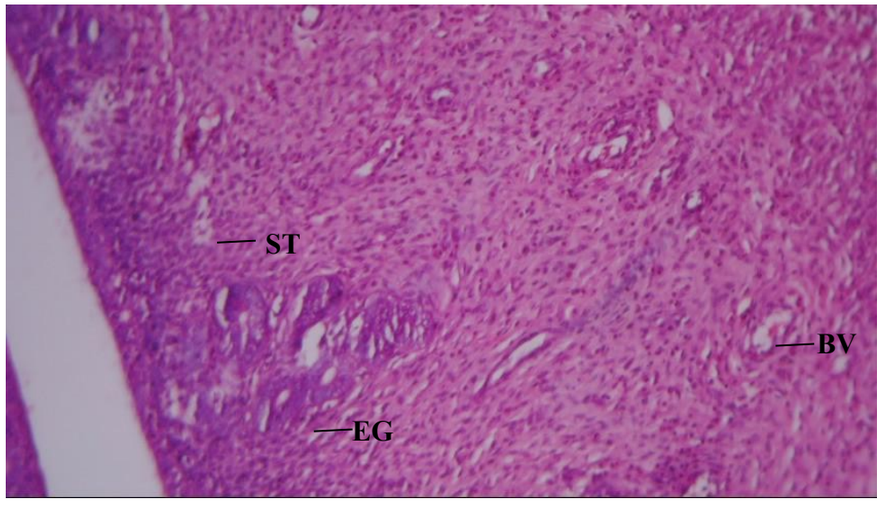

Figure 8: Rat given MSG + 400mg stem extract:

uterine cavity (UC), endometrial glands (EG), stroma (ST) containing plump cells and blood vessels (BV): H&E x 100

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Curative Treatment group

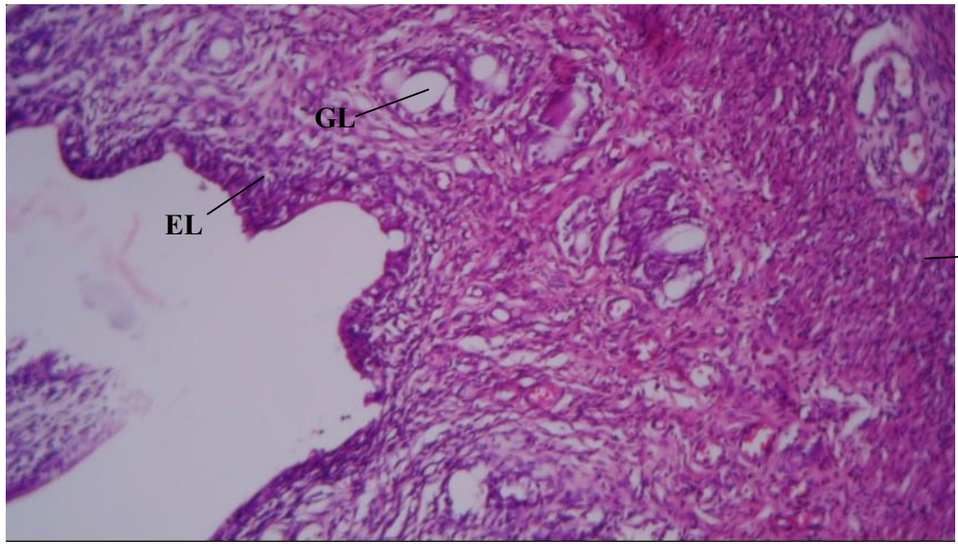

Figure 9:. Rat uterus control:

Endometrial lining (EL), endometrial stroma (ES), glands (GL). H&E x 100

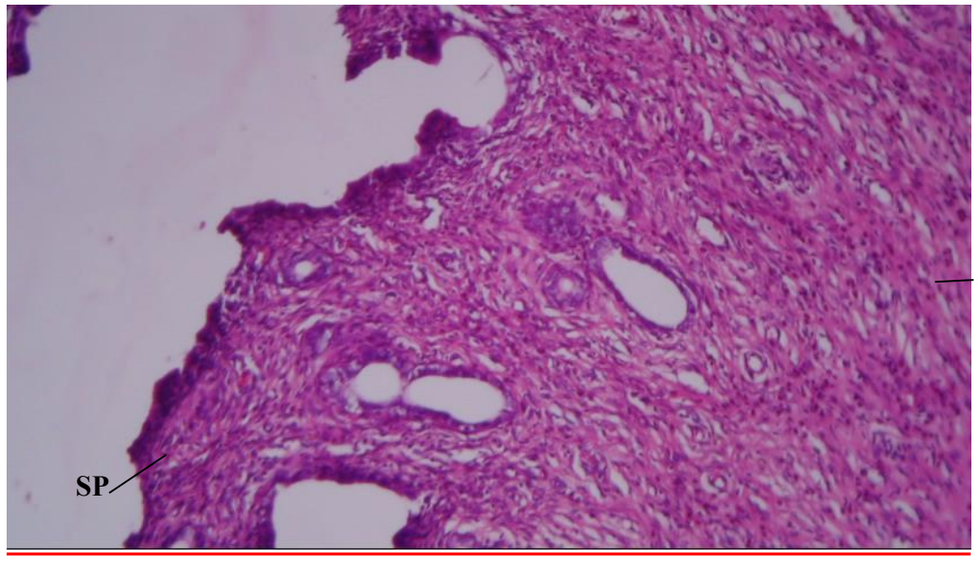

Figure 10: uterus given 800mg/kg MSG for 30 days:

Endometrial stroma overtaken by bands of spindle cells (SP) arranged in haphazard manner, supported by collagenuous stroma (CS).H&E x 100

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Plate 6

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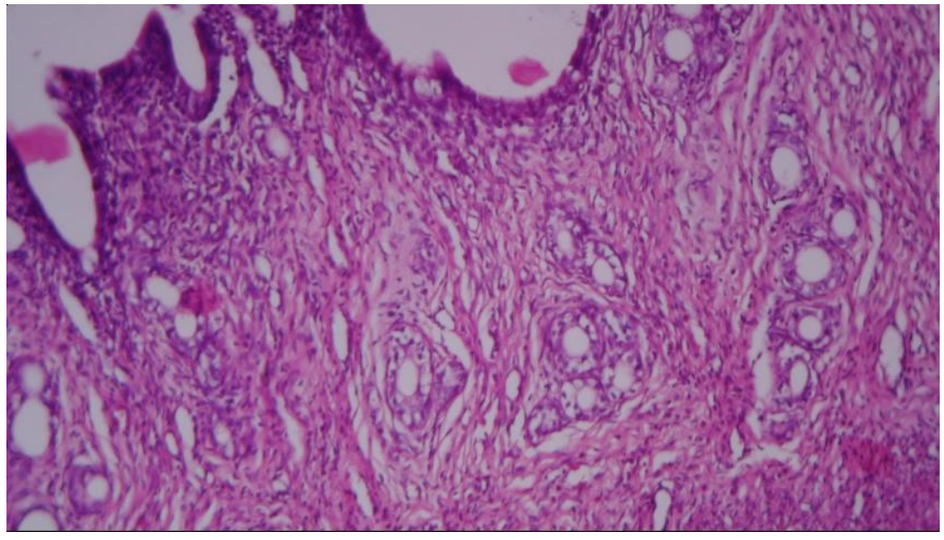

Figure 11: Rat uterus given 100mg/kg stem bark + MSG

SP

Mild bundles of spindle cells (SP) arranged in haphazard manner, supported by collagenuous stroma (CS). H&E x 100

CS

Figure 12: Rat uterus given 400mg/kg stem bark + MSG:

Thick bundles of smooth muscle cells (SM), supported by collagenuous stroma (CS). H&E x 100

CHAPTER FOUR

SM

4.0. DISCUSSION

CS

This study evaluated the safety margins as well as the preventive and curative effects of the ethanolic extract of *Tetrapleura tetraptera* stem bark extract on monosodium glutamate-induced uterine fibroid in Sprague Dawley rats. The study also examined how the extract affected plasma protein, cholesterol, and estradiol.

The result of the acute toxicity test indicated that oral administration of *T. tetraptera* stem bark extracts up to the highest dose of 5000 mg/kg induced no mortality, sedation, diarrhoea, change

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in gait or body posture, decreased locomotory activity and hyperactivity in the rats. This shows that the extract has a large margin of safety. (Jorke 1983).

From the result obtained in the preventive method for total cholesterol, there was a significant difference between the MSG group and the control group as $P < 0.05$. This shows that MSG increases serum cholesterol. The different doses of the extract groups showed no significant difference ($P > 0.05$) between them and the control group but mean values of 57.40mg/dl, 60.60 mg/dl and 38.0mg/dl respectively.

Cholesterol exists in two different stages, an inactive (phosphorylated) stage and an active state (dephosphorylated). In the presence of monosodium glutamate, the enzyme, 3-hydroxyl-3-methoxylglutamyl-COA reductase, HMGR, is activated. This enzyme catalyzes the desphosphorylation of the inactive cholesterol, leading to the formation of the active cholesterol (Obochi et al, 2009). This enzyme is more active in the dephosphorylated state of cholesterol (Bernard et al, 2002). This mechanism therefore leads to an increased production of cholesterol. This mechanism is what led to the increased cholesterol level that was seen in the blood samples of the rats administered MSG. In the presence of monosodium glutamate, there was an increase activation of the enzyme aromatase, which catalyzed the conversion of testosterone to estradiol, and the aromatization of estradiol's ring A. This boosts the activity of the enzyme, leading to an increase in estradiol synthesis. An increase in these three factors; Protein levels, cholesterol, and estradiol, leads to the development of fibroids (Obochi *et al,* 2009).

In total protein analysis, there was no significant difference between the MSG group and the control group as $P > 0.05$ in both the preventive and curative treatment methods. However, the MSG increased the total protein to 10.73 in the preventive method. This shows that MSG increases serum total protein. Also there was no significant difference ($P > 0.05$) between the

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different doses of the extract and the control group but a dose dependent reduction in the total protein mean. This also shows that the extract was able to reduce the total protein level. In the curative treatment method, MSG increased the total protein to 98.46. There was also no significant difference (P> 0.05) between the different doses of the extract and the control group but gave mean values of 87.46g/dl, 88.76g/dl and 94.14g/dl respectively. This shows that the extract mildly reduced the total protein level in the curative group.

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There are specific proteins associated with uterine fibroids, and in the presence of monosodium glutamate, the production of these proteins increases. This is because of the activation of transcriptional enhancer and promoter regions used to regulate expression of genes associated with protein development, this is done by activating RNA polymerase, and enhancing its activity. This enhanced RNA polymerase's capacity to identify the nucleotide of the protein at the initiation stage, this will then lead to an increase in protein synthesis involved in fibroid (Obochi et al, 2009). Thus, an increase in protein levels was observed when the blood sample obtained from the rats were analyzed.

In the preventive method, there was a significant difference between the MSG group and the control group as $P < 0.05$ for plasma estradiol. This showed that MSG was able to increase estradiol levels by the method described below. Concomitant treatment of the MSG and the different doses of the extract together showed no significant difference (P> 0.05) as the extract was able to lower the elevated plasma estradiol in a dose dependent manner. In the curative treatment method, there was a highly significant difference between the MSG group and the control group as $P < 0.01$ while all doses of the extract treatment showed no significant difference (P> 0.05) between them and the control group but a dose dependent reduction.

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The ability of *Tetrapleura tetraptera* to lower increased levels of estradiol may be linked to its ability to inhibit the enzyme aromatase, which is involved in the manufacture of estradiol from cholesterol and is responsible for aromatizing androstanedione and testosterone to estrogens. Another possibility is that it could also be an agent that promotes the metabolism of estradiol by inducing liver microsomal enzymes.*T. tetraptera* could also contain phytochemicals which could exert its effect as gonadotropin releasing hormone (GnRH) agonists which downregulates or decrease expression of GnRH receptors on the anterior pituitary upon continuous stimulation. This would therefore result in less estradiol being produced. The decrease in estradiol could be as a result of the reduction in cholesterol biosynthesis (Bernard et al., 2002).

T. tetraptera was shown to phytochemically contain phytosterol, alkaloid, glycosides, saponins, and triterpenoids, even though a potential pharmacological mechanism has been hypothesized. Studies have demonstrated that glycosides and phytosterols lower plasma cholesterol. According to the European Food Safety Authority (EFSA), if a person consumes 1.5 to 2.4 g of plant sterols and stanols daily, their blood cholesterol will typically decrease by 7 to 10.5% within the first 2-3 weeks. Additionally, phytosterols have been proven to lower plasma LDL cholesterol and cholesterol absorption (Koffuor *et al*,2013).

The histological examination of the tissues in the rats that took MSG only, showed proliferating bundles of smooth muscle fibres disposed in haphazard manner and supported by thick collagenous stroma which are the beginning of leiomyoma formation.The rats given MSG alongside 100mg extract showed normal endometrial cavity, plump endometrial stromal cells and endometrial gland with reduced proliferative cells. Rats given MSG alongside 400mg stem bark extract showed normal endometrial cavity, normal uterine cavity, normal endometrial glands, normal stroma containing plump cells and blood vessels with no proliferative cells.This

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indicates an efficacy in the extract in preventing fibroid. In the curative group, rats given 100 and 400mg /kg extract from the 31st day showed mild proliferative spindle leiomyoma. This implies that the extract has a mild effect in curing the fibroid(Koffuor *et al*,2013).

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4.1 Conclusion

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In conclusion, the ethanolic stem bark extract of *Tetrapleura tetraptera* significantly decreases elevated levels of plasma cholesterol, estradiol and mildly reduced total protein which play roles in the etiology of uterine fibroid thus suggesting its efficacy as an anti- fibroid treatment method. The results of this study shows that the ethanol extract of *T. tetraptera* stem bark has potential to prevent fibroid rather than curing it, as it's effect was more in the preventive assay than in the curative.

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However, more research is required to determine the pharmacologically active component of the plant that is responsible for the observed activities as well as to identify the precise mechanism(s) of action of the extract.

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APPENDIX

Preventive Treatment:

Total Cholesterol (mg/dl):

Sample	Control	MSG(800mg/dl)	100mg/ dl	200mg/dl	400mg/dl	Average
A1	46	71	57	76	32	45.75

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A2	28	61	51	63	36	71.5
A3	45	85	50	38	41	57.50
A4	68	84	69	74	33	60.6
A5	42	56	60	52	48	38

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Total Protein(g/dl):

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Sample	Control	MSG (800mg/dl)	100 mg /dl	200mg/dl	400mg/dl	Average
B1	9.5	9.35	9.1	10.04	9.4	9.50
B2	9.47	9.19	10.03	8.91	9.98	10.73
B3	11.24	10.04	7.31	9.93	8.9	9 .12
B4	8.34	9.39	10.63	11.65	9.17	10.32
B5	8.94	15.68	8.51	10.69	9.68	9.43

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Estradiol (pg/ml):

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Sample	Control	MSG(800mg/dl)	100mg/dl	200mg/dl	400mg/dl	Average
C1	6.5	19.04	14.16	5.35	6.58	9.44
C2	7.53	15.95	8.17	13.87	5.77	17.99

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C3	5.73	25.8	10.16	6.99	14.96	9.53
C4	10.73	10.86	5.4	5.4	6.13	8.86
C5	16.72	18.3	9.75	12.7	10.29	8.75

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Curative Treatment.

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Total cholesterol (mg/dl)

Sample	**Control**	**MSG(800 mg/dl)**	**100mg/dl**	**200mg/dl**	**400mg/dl**	**Average**
D1	89	96	53	62	64	61.2
D2	61	102	72	47	43	88.8
D3	61	90	68	61	43	74.2
D4	47	67	83	66	85	62
D5	48	89	95	74	87	64.5

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Total protein (g/dl)

Sample	Control	MSG(800 mg/dl)	100mg/dl	200mg/d	400mg/dl	Average
E1	88	88.7	83	80.2	97.7	91.84
E2	90.9	88.6	79.6	98.5	84.5	98.46
E3	97.1	97	84.8	89.6	93.6	87.46
E4	86.3	111	94.3	89.9	99.2	88.76
E5	96.9	107	95.6	85.6	95.7	94.14

Estradiol (pg/ml):

Sample	Control	MSG(800 mg/dl)	100mg/dl	200mg/dl	400mg/dl	Average
F1	5.5	12.4	12.4	6.8	11.1	9.66
F2	11.8	16.5	17.7	13.2	13.1	20.22
F3	11.6	26.9	15.2	13.9	10.4	12.89
F4	14.3	20.5	12.88	16.98	6.61	11.22
F5	5.1	24.8	6.26	5.21	10.4	10.32

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