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A study of the effect of an ethanolic extract of femitol on uterine fibroid in laboratory model

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Abstract

Uterine fibroids are becoming a common pelvic benign tumor among women of reproductive age in Ghana. This study aimed at evaluating the anti-fibroid property of femitol on MSG-induced uterine leiomyoma in female Sprague-Dawley rats. Total plasma cholesterol, plasma estradiol concentrations and uterus weight to body weight ratio were determined in normal rats, rats in which MSG (800 mg/kg) and an ethanolic extract of femitol (80, 160 and 400 mg/kg) had been administered concurrently for 30 days, as well as pretreatment with 800 mg kg MSG treatment for 30 days and treated with femitol (80, 160 and 400 mg/kg) for 30 days. Acute and delayed toxicity tests were carried out to establish the safety for use of the extract by cage-side observation, assessing hematological, liver and kidney function. Histopathological studies on the liver and kidney were also performed. Total plasma cholesterol and estradiol were significant elevation ($P \leq 0.001$) together with an elevation of uterus weight to body weight ratio by 71.55% on treating normal female rats with 800 mg kg-1 MSG. Preventive and curative treatment of MSG-treated animals with 80 and 160 mg/kg femitol significantly decreased ($P \leq 0.001$) the elevated plasma cholesterol and estradiol with a subsequent lowering of uterine/body weight ratio. Pictures showed decrease uterine cell proliferation. Biochemistry results indicated no significant changes in liver and kidney functions. Histological studies showed no significant femitol-treatment damage in the liver and kidney. Findings suggest that femitol has antifibroid activity and is safe to use

Keywords: Deforestation • Physical change • Global warming • Climate policies • Forest laws

Introduction

Uterine leiomyoma commonly termed uterine fibroid, a benign smooth muscle neoplasm, is the most common (approximately 50%of fertile women) and constitutes a significant reproductive threat in women worldwide. 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. Some risk factors that increase the chance of uterine leiomyoma are age (women between the ages of 20-44 are at greater risk), diet (refined sugar starches, and foods containing growth hormones have been shown to increase the risk) obesity; ethnicity (African American women stand a higher chance to develop uterine leiomyoma than their Caucasian counterparts), lack of exercise and the ingestion of some chemical compounds like monosodium glutamate (MSG) found in a wide range of foods, where it has flavor enhancing effects. Furthermore, certain drugs that have the potential to elevate the levels of cholesterol, total protein and estradiol put the individual at a higher risk of developing uterine leiomyoma [1].

A lot of women who develop uterine fibroids may not show symptoms, the consequence of of which will lead to little clinical attention. The few who develop symptoms usually complain of abnormal uterine bleeding, feeling of fullness in the pelvic area, pain the lower back, severe complications in pregnancy and labour, as well as infertility. It is noteworthy that whether or not these symptoms are severe is dependent on the size and location of the tumor. Although the pathogenesis of uterine fibroid is not fully understood, several theories have been put forward. One theory asserts that high levels of the hormones - progesterone and estrogen lead to an elevated rate of mitosis that can contribute to the formation of myoma. Estrogen and progesterone have been reported to enhance the growth of the tumor, as part of the continuous menstrual cycling. This is further supported by the clinical observations that show that uterine leiomyoma occurs very rarely before the first occurrence of menstruation and regress following menopause. They also reported that estrogen feeds cancerous growth such as fibroid. Another hypothesis supports an inherent abnormality in the myometrium of individuals who develop uterine fibroids. The basis of this hypothesis is the finding of significantly high levels of Estrogen Receptors (ER) in the myometrium of fibroid uteri. Genetic basis has also been

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suggested by some researchers based on familial and ethnic predisposition.

There are limited treatment options available for this condition. Usually, hysterectomy and myomectomy are suggested by doctors. Hormone replacement therapy is only effective for 6-12 months due to the adverse effects that result from prolong usage. The adverse effects, cost and the time related symptomatic remedy that orthodox medicines offer necessitates better options of treatment. It is on this note that a herbal fibroid dissolver product, Femitol, has been made by Milyash Herbs Ltd, Ghana. Femitol is composed of Anthocleista nobilis, Vernonia amygdalina, Alstonia boonei, Persea americana, Heliotropium indicum, and Angelica sinensis.

Employing the uterine leiomyoma inducing effects of MSG, the anti-fibroid effects as well as the safety of femitol were investigated. The aim would be specifically achieved by; assessing both the preventive and curative effects of an ethanolic extract of femitol on monosodium glutamate-induced uterine hyperplasia in Sprague-Dawley rats. The liver, kidney and blood will also be assessed.

Materials and Methods

Non-pregnant female Sprague-Dawley rats weighing 180-200 g were obtained from the Animal House of the Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana. They were housed in groups of ten (10) in stainless steel cages with wood shavings as the bedding material. The cages were adequately ventilated and kept at a room temperature and relative humidity of 24-28oC and 60-70% respectively, with a natural light-dark cycle. The animals were fed with commercial pellet rat feed (Agricare Ltd, Tanoso, Kumasi) and clean water. The processes and techniques used during the experiment were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health Services Publication) [2].

Preparation of femitol extract

A 1 kg quantity of the powder from the capsules of femitol was extracted with 70 % ethanol by cold maceration technique for 48 hours. The extract was concentrated using a rotary evaporator (Rotavapor R-215, BUCHI Labortechnik AG, Flawil, Switzerland) at 60℃ to yield a syrupy mass which was subsequently dried at 40°C, in a hot air oven. The solid mass obtained (yield: 12.44%), labeled as femitol extract (FE), was reconstituted in normal saline for dosing in this study.

Dosing of experimental animals

Dosing of the preparation in this study was done based on information given by the manufacturers of femitol. SD rats were grouped (n=10) and by gavage. Dosing was once daily over the experimental period at a volume of 10 ml/kg body weight. Individual dose volumes were calculated based on the animal's most recent recorded body weight. The oral route of administration was used because it is the intended human exposure route.

With five in a group (n=10), female SD rats were put into five groups. Group a (Control) was a no treatment group. Groups B, C, D, and E were treated with 800 mg/kg MSG and FE (80, 160, and 400 mg/kg) concurrently for a 30-day period after which total plasma cholesterol and plasma estradiol were determined. The uterus was harvested, pictures taken, and uterus weight to body weight were determined.

In this study, female SD rats were treated with 800 mg/kg MSG for 30 days in an attempt to induce the development of uterine hyperplasia. Induced rats were put into four groups (B-E) with ten per group. Group A was a no treatment group (Control). Groups B, C, D, and E were then treated with 80, 160, and 400 mg/kg FE for 30 days. Plasma estradiol concentrations were determined. The uterus was harvested, and uterus weights to body weight ratios were determined.

Determination of plasma estradiol concentration

A day after the experimental period the animals were euthanized. Blood was collected from the jugular vein into MediPlus K3 EDTA tubes. Plasma was obtained by centrifugation at 3220 rpm for 20 min. 17β–estradiol was assayed using the fortress 17β–estradiol assay kit.

With all plasma controls and reagents at room temperature, 25 uL quantities each of these were dispensed into their respective wells. Fifty microliters (50 uL) of estradiol biotin reagent was added to each of wells and swirled gently for 30 s to mix after which 50 uL of estradiol enzyme reagent was added to each of the wells and mixed after incubating at 28°C for 30 min. The wells were then covered with a foil and incubated at 28°C for 90 min. When incubation had been complete, the foil was removed, and the content of wells aspirated using an automatic washer (Rayto RT-3100, China) with each well washed three times with 350 uL wash Buffer. After this 100 uL of working substrate solution was dispensed into each well in the same order and incubated for 20 min at room temperature. 50 uL stop solution was added into all wells in the same order, swirled gently to mix for 20 s. The absorbance of the specimen was measured at 450 nm using a microplate reader within 30 min after addition of the stop solution. The values of the samples were obtained from a graph constructed using the standards.

Hematological analysis

After the 30-day treatment period, bloods from sacrificed rats were collected into MediPlus K3 EDTA tubes for hematological analysis using a hematological analyzer.

After the 30-day treatment period, bloods from sacrificed rats were collected into plain tubes were allowed to stay undisturbed for 45 minutes to clotting. The clotted samples were spanned at 3000 rpm for 15 minutes in a centrifuge. Plasma obtained from the samples was transferred into sample tubes, labeled. Using reagents produced by Fortress, Fortress Diagnostics, UK and a semi-automated chemistry analyzer (URIT 810, Guangzhou Shihai Medical Equipment Co., Ltd, China), plasma levels of Aspartate Aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP), Gamma-Glutamyl Transferase (GGT), Total Bilirubin (TBIL) and Direct Bilirubin (DBIL) were determined. For kidney function, the following were measured.

Determination of uterus weight to body weight ratio

The female rats were weighed before they were sacrificed and dissected to obtain the intact uterus, fallopian tubes and ovaries. The wet organs weights were taken and the organ weight to body weight ratio was calculated.

Sprague-Dawley rats were observed hourly for up to 24 hours (acute) and subsequently for 14 days for possible delayed toxicity after FE was administered orally to 5 groups of rats (n = 6) at doses of 80, 160, 240, 400 and 800 mg/kg. The control group was administered with 2 ml/kg distilled water. The time of onset, intensity, and duration of these symptoms, if any, was recorded. Hematological parameters as well as liver and kidney function were monitored biochemically. The histology of the liver and kidney was observed in all treatment groups for possible pathological changes.

All results are presented as mean ± SEM. Data was analyzed using one-way analysis of variance (ANOVA). When ANOVA was significant, multiple comparisons between treatments were done using Newman Keuls post hoc test when comparing control with treatment groups. GraphPad Prism for Windows Version 5 (GraphPad Software, San Diego, USA) was used for all statistical analyses.

Results

Total plasma cholesterol

There was very significant elevation [31.58% (P ≤ .001)] in plasma cholesterol after MSG-induction of uterine hyperplasia which did not happen when FE was administered concurrently with MSG. All doses of FE in curative treatment however decreased elevated plasma cholesterol to normal i.e levels prior to MSG induction (Figure 1) [3].

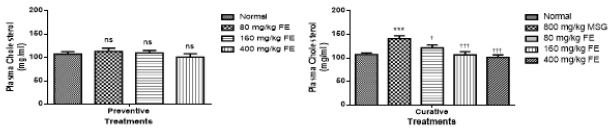

Figure 1. The preventive and curative effects of the ethanolic extract of Femitol (FE) on uterus weight in normal female Sprague-Dawley rats and those pre-treated with 800 mg/kg MSG. Values plotted are Mean ± SEM (n=10). Significance: Normal vs MSG treatment ns P>0.05; *** P≤ 0.001.

Total Plasma Estradiol

Concurrent treatments with 800 MSG and FE at various doses did not have any significant (P > 0.05) effect on plasma estradiol in normal rats. Pretreatment of normal rats with MSG however caused very significant (64.8%; P ≤ 0.001) elevation of plasma estradiol. FE dose-dependently decreased elevated plasma estradiol to normal.

Uterus Weight to Body Weight Ratio

Concurrent administration of FE and MSG did not result in significant changes (P>0.05). in uterine weight. A 800 mg/kg MSG treatment of rats caused significant, dose-dependent increases (71.55%, P ≤ 0.001) in uterus weight to body weight ratio. Curative treatments with 80 -400 mg/kg FE significantly decreased (P ≤ 0.001) in elevated organ weight to body weight ratio. Uterus size therefore increased very significantly with MSG treatment; as seen in representative photographs of isolated uteruses taken during the study (Figure 3, Table 1).

- Plate A: Normal uterus of a Sprague-Dawley rat
- Plate B: 800mg/kg MSG-treated Sprague-Dawley rat uterus
- Plate C: 800mg/kg MSG-Treated Sprague-Dawley rat uterus treated with 80 mg/kg FE
- Plate D: 800mg/kg MSG-Treated Sprague-Dawley rat uterus treated with 160 mg/kg FE
- Plate E: 800mg/kg MSG-Treated Sprague-Dawley rat uterus treated with 400 mg/kg FE

Acute and Delayed Toxicity Test

There was no observable CNS/ autonomic nervous system disorder. No behavioural changes were observed. Feeding and sleep/ wake cycle was normal. There was no significant change in body weight. Any other observations were considered common findings which could not be associated to FE treatment. No mortality occurred during the study. Histopathological studies indicated not detrimental changes of the liver and kidney architecture.

Acute and Delayed Toxicity Test

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Parameters measured	FE treatment				
	Control	80 mg/kg	160 mg/kg	400 mg/kg	800 mg/kg
AST (U/L)	9.90 ± 2.31	10.69 ± 1.2	10.25 ± 1.8	10.34 ± 1.7	10.45 ± 1.9
ALT (U/L)	151.64 ± 5.62	172.50 ± 14.6	165.4 ± 8.9	158.9 ± 6.5	155.3 ± 8.2
ALP (U/L)	1129.9 ± 297.5	941.2 ± 212.2	1055 ± 165.4	966.4 ± 189. 4	1089 ± 278
GGT (U/L)	3.44 ± 1.65	4.72 ± 2.30	4.45 ± 1.82	3.56 ± 1.85	4.22 ± 1 93
TBIL (µmol/L)	0.44 ± 0.15	0.52 ± 0.11	0.49. 0.13	±. 0.55 ± 0.14	0.59±0.16
DBIL (µmol/L)	0.17 ± 0.92	0.16 ± 0.24	0.18 ± 0.86	0.16 ± 0.16	0.17 ± 0.65
IBIL (µmol/L)	0.27 ± 0.36	0.36 ± 0.51	0.29 ± 0.67	0.39 ± 0.72	0.32 ± 0.48
ALB (g/l)	35.5 ± 0.59	37.3 ± 0.68	38.2 ± 0.55	34.1 ± 0.64	33.9 ± 0.79
GLO (g/l)	12.1 ± 0.70	14.1 ± 1.25	11.3 ± 1.10	14.9 ± 1.06	12.6 ± 0.85
TP (g/l)	48.2 ± 0.37	51.6 ± 0.94	49.2 ± 0.92	43.0 ± 2.57	45.9 ± 0.51

Table 1. Liver function test parameters measured of controls.

The values are mean ± SEM (n = 5). There were no ignificant differences (p>0.05) between the normal and FE treated groups and the control were determined using One-Way Analysis of Variance followed by Dunnet's Multiple Comparison's Test. Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Gamma-Glutamyltransferase (GGT), Total Bilirubin (TBIL), Direct Bilirubin (DBIL) and Indirect Bilirubin (IBIL), Albumin (ALB), Globulin (GLO), Total Protein (TP).

- Plate A: Normal liver
- Plate B: 80mg/kg FE-treated liver
- Plate C: 160 mg/kg FE-Treated liver
- Plate D: 240mg/kg FE-treated liver
- Plate E: 400 mg/kg FE treated liver
- Plate F: 800 mb/kg FE treated liver

FE treatment					
	Control	**80 mg/kg**	**160 mg/kg**	**400 mg/kg**	**800 mg/kg**
Creatinine (µmol/l)	37.20 ± 0.45	38.6 ± 0.81	36.74 ± 0.40	36.45± 0.72	35.7 ± 0.51
Urea (mmol/l)	15.04 ± 0.56	9.6 ± 1.05	8.5 ± 0.58	10.1 ± 1.65	10.6 ± 0.39
Sodium (mmol/l)	143.82 ± 1.51	143.1 ± 2.52	144.42 ± 3.26	1.41.8 ± 2.10	140.2 ± 1.08
Potassium (mmol/l)	8.92 ± 0.40	8.84 ± 0.67	9.3 ±0.17	8.96 ± 0.81	10.3 ± 0.51
Chloride (mmol/l)	111.92 ± 1.25	114.4 ± 2.56	109.12 ± 3.26	112.5 ± 1.05	111.6 ± 1.84

Table 2. Kidney function test parameters measured of controls.

The values are mean ± SEM (n = 5). There were no ignificant differences (p>0.05) between the normal and FE treated groups and the control were determined using One-Way Analysis of Variance followed by Dunnet's Multiple Comparison's Test.

- Plate A: Normal kidney
- Plate B: 80mg/kg FE-treated kidney
- Plate C: 160 mg/kg FE-Treated kidney
- Plate D: 240mg/kg FE-treated kidney
- Plate E: 400 mg/kg FE treated kidney
- Plate F: 800 mb/kg FE treated kidney

Discussion

This study aimed at establishing anti-fibroid activity of femitol and its safety for use by assessing both preventive and curative effects of an ethanolic extract of femitol on monosodium glutamate-induced uterine hyperplasia in Sprague-Dawley rats. The study continued to assess the effect on liver and kidney function and histology and also on the cellular composition of blood. Per the findings, it was ascertained that Femitol extract prevented the development of uterine hyperplasia and reduced the size and hence the weight of the proliferated uterus to almost normal. This was possibly as a result of its ability to reduce elevated estradiol and cholesterol.

Femitol's ability to reduce elevated estradiol could possibly be attributed to inhibition of the enzyme aromatase responsible for aromatization of androstenedione and testosterone to estrogens in the biosynthesis of estradiol from cholesterol. It could also be a liver microsomal enzyme inducer that enhances estradiol metabolism. Femitol 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 then decrease estradiol production.

The decrease estradiol could be as a result of the reduction in cholesterol biosynthesis. There was a very significant elevation ($P \leq .001$) in cholesterol level by treating normal female rats 800 mg/kg MSG. Results indicate that there was a significant reduction in plasma cholesterol associated with a curative treatment (than a preventive treatment) with FE.

A rise in total plasma cholesterol is usually attributed to the activation of the enzyme 3-Hydroxyl-3-Methoxyl Glutamyl-CoA Reductase (HMGR). By covalent modification from its phosphorylated (inactive) state to dephosphorylated (active) state, HMGR catalyzes the conversion of HMG-CoA to mevalonate, the rate limiting step of cholesterol synthesis. The activation of HMGR further increases insulin levels which stimulate the removal of phosphates from the cells leading to increase activity of HMGR and resultant increase in cholesterol synthesis. The cholesterol lowering effect of FE could possibly be attributed to decreased levels of dephosphorylated HMGR (active form). FE could also be activating glucagon and epinephrine that negatively affects cholesterol biosynthesis [5].

MSG treatment resulted in an increase in estradiol which is specific in uterine cell proliferation. Estradiol binds to ERα receptors in the uterus, forming a complex that interacts with DNA of the nucleus to activate transcriptional promoter and enhancer elements responsible for control of gene expression. This allows binding of RNA polymerase II and subsequent initiation of transcription which produces proteins that leads to increase proliferation of the cells of the uterus and ovaries. The reversal of MSG induced hyperplasia by femitol could be due its activity of reducing cholesterol and biochemical markers of protein synthesis. Uterine proliferation has also been linked to periods of estrogen secretion because of their increase response to estradiol. Decreased levels of estradiol by effect of femitol would decrease uterine cell proliferation. Phytosterols have exhibited encouraging results in inhibiting cancer. There have been positive results even on estrogen-dependent cancer lines. The evidence that sitosterols and stigmasterols have anti-estrogenic activity is building up. A study found estrogen receptor expression to be down-regulated with doses of beta-sitosterol glycosides (BSSG) compared to estradiol control.

A 800 mg/kg MSG treatment of rats caused significant increases (71.55%, $P \leq 0.001$) in uterus weight to body weight ratio. Uterus size also increased very significantly (1.87 fold) with MSG treatment. Treatments with femitol significantly decreased ($P \leq 0.001$) the elevated organ weight to body weight ratio. An increase in weight and size of an organ usually indicates hyperplasia (cell proliferation).

Endometrial hyperplasia may represent an early neoplastic process which can lead to uterine leiomyoma.

No mortality occurred during the safety study. Daily clinical observations recorded were considered common findings in laboratory rats which could not be associated to FE treatment. There were no secretions from the eye, ear, nose, anus, and external genitalia, no “wasting”, audible “chattering”, alopecia, and pallor in the eyes. The mice were not lethargic, they fed well and had normal formed stool. There were no ocular findings, decreased motor activity and neurological conditions. There was no significant test article effect on body weight.

Conclusion

Observations made at hourly and daily for 14 days is a convenient for an acute and delayed toxicity study because most of the observable toxic symptoms occur within the first 1-2 hours of drug administration. The “no effect on body and skin” observed suggests that FE may not have caused hypersensitization and neurogenic inflammation which results in rats scratching, licking, or bite their skin in response to the atopy. The product may not cause autonomic nervous system hypereflexia because lacrimation, miosis, rhinorrhoea, salivation, urination, defecation, and labored breathing which are signs of muscarinic hyperactivity did not occur.

Observations showed no CNS excitation or depression, muscle relaxation effects as well as pain and inflammatory effects (realized as writhing, change in gait and body posture and decreased locomotory activity). The product did not seem to have any “wasting effect”. There was no pallor in the eyes (symptom of anaemia). No death recorded implies that the lethal dose was less than 1000 mg/kg when given as a single dose.

Femitol significantly prevented MSG-induced uterine hyperplasia and decreases elevated levels of cholesterol and estradiol, as well as uterus size and weight suggesting its efficacy as an anti-fibroid agent. Within limits Femitol is safe to use.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Reference

1. Martinez, Carlos M., Carla Ibanez, and Juan M. Corpa. "Simultaneous Uterine Leiomyoma and Endometrial Hiperplasia in a White-Nosed Monkey (Cercopithecus Nictitans). First Case Report." *An Vet Murcia* 26 (2010): 61-68.
2. Obochi, G. O., S. P. Malu, M. Obi-Abang, Y. Alozie, and M. A. Iyam. "Effect of Garlic Extracts on Mono Sodium Glutamate (MSG) Induced Fibroid in Wistar Rats." Pak J Nutrit 8 (2009): 970-976.
3. Eskes, T. K. A. B. "Neural tube defects, vitamins and homocysteine." Euro J Pediat 157 (1998): 139-141.
4. Rodriguez, M. C., J. A. Obeso, and C. Warren Olanow. "Subthalamic Nucleus Mediated Excitotoxicity in Parkinson's Disease: A Target for Neuroprotection." Ann Neurol 44 (1998): 175-188.
5. Person, R. B., and R. H. Warring. "*In Vitro* effect of the cysteine Metabolism homocysteic acid." *Neurotoxicol* 19 (1998): 599-603.

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