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Effect of Garlic Extracts on Monosodium Glutamate (MSG) Induced Fibroid in Wistar Rats

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Abstract: Effect of garlic extracts on MSG induced fibroid in wistar rats was studied. Fifteen rats were randomly assigned into 3 study groups. The animals in Group 1 (the control) received a placebo of 5.0 mL distilled water via gastric intubation. The animals in the Groups 2 and 3 were treated with 100 mg MSG/kg, or a combination of 100 mg MSG/kg + 100 mg garlic/kg, respectively in a total volume of 5.0 mL vehicle. However, the animals in Group 3 were treated with MSG only for 30 days before the commencement of treatment with garlic extracts. The fibroid was confirmed by myometry. The experiment lasted for 60 days. One day after the final exposure, the animals were euthanized by inhalation of overdose of chloroform. Blood was collected by cardiac puncture into EDTA sterilized sample bottles. Serum was prepared by centrifugation (6000 x g, 30 min) and used for the analysis of serum total protein, estradiol (estrogen and serum total cholesterol. The results showed that Monosodium Glutamate (MSG) alone increased total protein, cholesterol and estradiol (estrogen), which in turn, induced fibroid in the rats. However, treatment with garlic extracts near-completely abrogated/mitigated any effects that have been induced by the MSG alone. It appears that Garlic extracts acted to remove catabolic wastes from the pelvic cavity and from uterine and ovarian tissue, thereby accelerating metabolism and lymph drainage; promoted the sloughing-off tissues; corrected imbalances of estrogen metabolism associated with excessive catechol estrogens and elevated inflammatory prostaglandins. It also appears that garlic extracts stimulated the secretion of gonadotrophins and ovarian hormones and inhibited proliferation of cancer cells at the levels of the pituitary gland; promoted the exit of cells from the golgi phase of the cycle; promoted the unliganded estrogen receptors ability to transducer growth signals from other pathways, leading to apoptosis of fibroid or tumour cells. The results of this study may offer the possibility of treating women with fibroids for extended periods of time without the need for surgery or hormone add-back.

Key words: Monosodium Glutamate (MSG), Garlic, leiomyomas, fibroid and cancer

INTRODUCTION

Nutritional status is a major factor controlling fertility in humans. Poor nutrition results in delayed puberty, aberrant estrous cycles, lowered conception rates, reduced birth weight and ovarian follicular growth (Bernard *et al*., 2002). Endocrine and metabolic signals that's regulate follicular growth are also expected to influence oocyte development either through changes in hormones/growth factor concentrations in follicular fluid or via granulose-oocyte interactions (Benagiano *et al*., 1992; Ignar-Trowbridge *et al*., 1993; Newton *et al*., 1994; Bernard *et al*., 2002). In addition, high levels of high degradable proteins as well as increasing plasma ammonia concentration of ammonia in bovine follicular fluid (Everitt *et al*., 1995; Bernard *et al*., 2002).

Diets high in proteins feed cancerous and non-cancerous growth in the body. Foods that are promoting coagulation of cells such as dairy products can promote production of estrogen. Estrogen has been reported to feed cancerous growth such as fibroid (Ross *et al*., 1986). Also, refined sugar and starches, peharp complex carbohydrate and foods containing growth hormones, drugs and chemicals can feed fibroids (Adamson, 1992; Newton *et al*., 1994). However, hormones free foods and low fat diets such as minerals, vitamins, vegetables, eggs, should be encouraged. These foods are necessary for cell development, nerve function, aid in body cleansing, alkalinize and purify the blood; nourishes the thyroid gland with natural ions, fight cancer and proliferation of cancer cells by supporting the immune system with a multitude of antioxidants.

Fibroid or leiomyomas are benign tumours f the uterus (Howe *et al*., 1995). They grow in various locations on and within the uterine walls itself or in the uterine cavity. Symptoms of fibroids include pelvic pain, irritation bowels, low back pain and severe menstrual bleeding, leading to anemia (Ross *et al*., 1986; Adamson, 1992; Everitt *et al*., 1995; Howe *et al*., 1995; Bernard *et al*., 2002).

Fibroids are hormones dependant, thriving on estrogen, reaching their peak during ovulating and just before the commencement of menstrual period and also increases during pregnancy when Gonatrophins-Releasing Hormones (GnRH) is at its highest (Johansen *et al*.,

1988; Adamson *et al*., 1992; Donnez *et al*., 1992; Everitt *et al*., 1995; Benagiano *et al*., 1992; Cohen *et al*., 1994). Synthetic chemicals such as Polychlorinated Biphenyls (PCBs) can introduce estrogen-like hormones into the body thereby increasing the size of the fibroid (Sadan *et al*., 1987; Fuschs-Young *et al*., 1996).

Diseases related to estrogen include breast and uterine diseases, including cancers, fibroid, premenstrual syndrome, reproductive dysfunctions such as infertility or lactation suppression (Wilson *et al*., 1980; Fuschs-Young *et al*., 1996). High level of estrogen has been reported to be the most common cause of fibroid and painful menstruation (Szekeres, 1996; Bernard *et al*., 2002). Monosodium Glutamate (MSG) is a salt of glutamate, synthesized from L-glutamic acids and used as a flavour enhancer in foods; binder and filler for nutritional supplements, in prescription drugs, intravenous fluids given in hospitals and in the chicken pox vaccines (Ikonomidou and Turski, 1995; Rodriguez *et al*., 1998; Eskes, 1998). Glutamate occurs naturally in virtually all foods, including meat, fish, poultry, breast milk and vegetables, with vegetables tending to contains proportionally higher levels of free glutamate (as MSG). Various processed and prepared foods such as traditional seasonings sauce and certain restaurant foods contain significant levels of free glutamate (as MSG), both from natural sources and from added monosodium glutamate (Rodriguez *et al*., 1998; Eskes, 1998). Monosodium Glutamate (MSG) causes reduction in the secretion of growth hormones, leading to stunted growth and irreversibility in obesity, excessive weight, essentially due to accumulation of excess fats in adipose tissue (Ikonomidou and Turski, 1995; Eskes, 1998; Rodriguez *et al*., 1998; Parson and Warring, 1998). Arising from high cholesterol levels leading to cardiovascular diseases and endocrinological disorder (Eskes, 1998).

Garlic (*Allium sativum*) has been used as spice in foods and for medicinal purposes-shown to have antibiotic, antiviral and antifungal qualities (Yamasaki *et al*., 1991; Reuter *et al*., 1996; Silvam, 2001). Garlic exhibits a broad antibiotic spectrum against gram-positive and gram-negative bacteria. Other therapeutic effects of garlic include lowering of cholesterol levels, blood pressure, cancer prevention, immune system boosting and treatment of infections such as athlete's foot and ring worm and antioxidant effects as well as anti-asthmatic and anti epileptic effects (Reuter *et al*., 1996; Silvam, 2001).

The composition of garlic include sulphur containing allicin, Diallyl Disulphide (DADS) and Diallyll Trisulphide (DATS),Which are responsible for most of garlic's pharmacological properties, while the non-sulphur composition of garlic include allixin, flavonoids, saponins and fructans (Reuter *et al*., 1996; Silvam, 2001). Allicin is mainly responsible for the pungent odour of garlic (Silvam, 2001) and is produced from an inert chemical in raw garlic called alliina derivative of cysteine by the action of an enzyme, allinase in the presence of pyridoxal phosphate (Silvam, 2001). Garlic produces the allicin to protect itself from bacteria and other diseases and antioxidant (Reuter *et al*., 1996). Garlic also contains minerals and vitamins, which are an important parts of its health benefits.

Recently, women between the ages of 20-35 are more proned to the development of fibroids. This may be attributed to several factors, including exposure to industrial chemical such as polychlorinated biphenyls, poisons (estrogen replacement therapy), diets high in proteins, or foods that promote co-agulation of cells and refined sugars. Women are widely exposed to these diets, perhaps to replace lost nutrients usually experienced during menstrual periods and or their desire for cured foods, such as soyabean products, dairy products and fatty snacks, which contains several food additives. These foods lodge in fatty tissues and mimic the activity of estrogen and fibroids thrive on high levels of estrogen. This situation has aroused considerable medical interest and has been considered a public health problem. This current study focused on the assessment of how garlic and ginger extracts could impact upon MSG induced fibroid in animals exposed daily to garlic and ginger extracts.

MATERIALS AND METHODS

Experimental animals: Fifteen wistar rats weighing 170-300 g were obtained from disease free stocks maintained in the animal house of the Department of Biochemistry at the College of Medical Sciences, University of Calabar, Nigeria. The animals were randomly assigned into two study groups on the basis of average body weight and litter origin. Each rat in a study group was individually housed in a stainless cage with plastic bottom grid and a wire screen top. The animal's room was adequately ventilated and kept at a room temperature and relatively humidity of 29±2°C and 40-70%, respectively, with a 12 h natural light-dark cycle. Animals were fed *ad libitum* with water and rats chew (Live stock feeds Ltd, Calabar, Nigeria) Good hygiene was maintained by constant cleaning and removal of feces and spilled from cages daily. All animals experiments were approved by the Animal Care and use committee of the Medical College, University of Calabar, Nigeria.

Treatment of regimen: All rats received daily treatment with their test solutions for a period of 60 days. All treatments were conducted between the hours of 9.00-10.00 am. The rats in group 1 (control) received a placebo of 5.0 mL distilled water via gastric intubation while the animals in groups 2 and 3 were treated with 100 mgMSG/KG + 100 mg garlic/kg, respectively as part of the 5.0 mL used for gastric intubation.

Preparation of monosodium glutamate: Synthetic glutamate [Monosodium glutamate (MSG) was obtained from a major Ajino motto distribution shop (Calabar, Nigeria) for use in the study. A stock solution was prepared by dissolving MSG granules in 500 mL distilled water. From this and based on the animals weight that morning, the 100 mg/kg dosages were administered to the animals in groups 2 and 3 as part of the 5.0 mL volume used for gastric intubation.

Preparation of Garlic (*Allium sativum*): Fresh garlic cloves (*Allium sativum*) were obtained from the Marian Market (Calabar, Nigeria) for use in the study. A stock solution was prepared by dissolving finely ground cloves of garlic in 500 mL distilled water and kept overnight. The garlic extract was then filtered using cheese cloth. From this and based on the animals weight that morning, the 100 mg/kg dosages were administered to the animals in groups 3 as part of the 5.00 mL volume used for gastric intubation.

Sample preparation: One day after the final exposure. The animals were euthanized by inhalation overdose of chloroform. Blood was collected by cardiac puncture into EDTA sterilized sample bottles. Serum was prepared by centrifugation (6000xg, 30 min) and used for the analysis of serum total serum estradiol (estrogen) serum total protein and serum lipid profile.

Determination of estradiol (estrogen): Estradiol was determined with modification of an Enzyme Immunoassay (EIA) described by Meyer *et al.* (1997). Briefly, 4 mL of serum samples were adjusted to pH of 3.5 with acetic acid and extracted with 12 mL of diethyl ether (pH 3.5), evaporated and re-extracted with diethyl ether (pH 3.5). The residue was dissolved in 12 mL of assay buffer (40 m M PBS, 0.1% BSA, pH 7.2) and pooled, resulting in 3.2 mL in PBS (pH7.5) after evaporation, the sample was dissolved in 12 mL of 100% methanol. The content of estradiol in 4ml of each serum was analyzed in triplicate by an enzyme immunoassay described by Meyer *et al.* (1997). This analyte was identifying by retention time (11.4 min) and the specific antigen-antibody reaction. Calibration curve of the EIA was prepared in 40% methanol. The working interval ranged from 0.15 pg (80% displacement of labeled antigen) to 7.2 pg (20% displacement of labeled antigen of estradiol per 4 mL).

Determination of serum total cholesterol: Serum total cholesterol was determined with the method of Brown and Goldstein (1984). In this assay, cholesterol was extracted from the serum with ethanol. The extract was then reacted with a solution of $FeCl_3$ dissolved in phosphoric acid and the resulting colour was read in a spectrophotometer at 550 nm against a reaction blank. Briefly, 0.1 mL of the serum was pipetted into test tubes and 10 mL of absolute ethanol was added to each tube and mixed rapidly on a vortex mixer for 10 sec the tubes were centrifuged for 5 min at full speed in a clinical centrifuge. The extracts were carefully transferred to clean test tubes. Then, 2.0 mL of the clear solution of the extracts were pipetted into new test tubes. The blank received 2.0 mL distilled water. Then, 2.0 mL of the colour reagent (diluted 40 mL iron stock solution to 500 mL with conc. H_2SO_4 and dispensed with automatic dispenser) was slowly added to all test tubes including the blank and mixed by gentle swirling. The iron stock solution was prepared by dissolving 5.0 g $FeCl_3$. $6H_2O$ in 200 mL conc. H_3PO_4. The cholesterol working standard solution was prepared by adding 2.0 mL cholesterol stock solution (0.1 mg/mL cholesterol standard) to 98 mL absolute ethanol. The tubes were then covered with parafilm and allowed to stand at room temperature for 30 min and the absorbance read at 550 nm in 6400/6405 spectrophotometer against the reaction blank. The average mg/mL value for total cholesterol in serum was calculated. The concentration of the unknown was calculated using the ratio formula:

[A550nm unknown: A550nm Standard x Conc. of Std. x 100]
[A550nm/A550nm Standard x Conc. of Std. x 100] = mg /dL

Determination of serum total protein: Serum total protein was determined by the Biuret method described by Gornall *et al.* (1949). Briefly, 0.5 mL of the serum sample solution was pipetted into test tubes and 1.0 mL distilled water added to bring the volume to 1.5 mL in each tube. Tube 1 (the blank) received 1.5 mL distilled water. The suspension was mixed and 0.2 mL of 5% sodium deoxycholate (DOC) in 0.01N KOH was added and mixed to make the suspension more soluble. Then, 1.5 mL of biuret reagent (1.50 g $CuSO_4$. $5H_2O$, 6.0 g sodium potassium tartrate and 300 mL of 10% NaOH per liter) was added (including the blank). The tubes were mixed in a vortex mixer and incubated at 37^{0}C for 15 min and the absorbance read at 540 nm against the blank (tube 1) in a 6400/6405 spectrophotometer (Jenway, Essex, England). The concentration of the standard Bovine Serum Albumin (BSA) was 2 mg/mL.

Statistical analysis: Data collected were expressed as means±Standard Deviation (SD) and the student 't' test were used for analysis. Values of $p<0.05$ were regarded as significant.

RESULTS

Table 1 present the results of the treatments on serum total protein levels in rats. The results showed that there

Table 1: Effect of the treatment on serum total protein levels in the rats

Treatment group (N)	Serum total protein (mg/mL)
Control	10.68±0.48
MSG only	37.34±1.37*
MSG + Garlic	10.93±0.51#

N = Number of rats per group = 5. Values are expressed as mean±Standard Deviation (SD). * = significantly different from control at p<0.05. # = significantly different from MSG only

Table 2: Effect of the treatment on serum total cholesterol in the rats

Treatment group (N)	Serum total cholesterol (mg/dL)
Control	18.94±0.62
MSG only	46.73±1.42*
MSG + Garlic	19.08±0.71#

N = Number of rats per group = 5. Values are expressed as mean±Standard Deviation (SD). * = significantly different from control at p<0.05. # = significantly different from MSG only at p<0.05

Table 3: Effect of the treatment on serum total estrogen (estradiol) levels in the rats

Treatment group (N)	Serum total estrogen levels (pg/mL)
Control	98.87±2.64
MSG only	216.72±4.98*
MSG + Garlic	99.16±2.65#

N = Number of rats per group = 5. Values are expressed as mean±Standard Deviation (SD). * = significantly different from control at p<0.05. # = significantly different from MSG only at p<0.05

was a significant (p<0.05) increase 60.9% in the levels of serum total protein, in the MSG treated host when compared to those seen in the controls. There was no significant difference noted in the MSG + Garlic-treated group (i.e., 2.3%). However, relative to the MSG only animals, this value was significantly lower, i.e., comparatively decreased by 70.73%.

Table 2 presents the results of treatments on serum total cholesterol. The results showed that treatment with MSG only led to significantly great increases 146.73% in the values of cholesterol relative to those measured in the control hosts. While there was no significant (p<0.05) in values of MSG + Garlic-treated animals relative to those seen in the controls. However, relative to the MSG only animals, these values were significantly lower, i.e., comparatively decrease by 59.17%.

Table 3 summarizes the effects of the treatment on the estrogen (estradiol) levels in the rats. The results showed that there was significant (p<0.05) increased 119.2% in the levels of estrogen (estradiol), in the MSG treated hosts relative to those levels in the controls. There was no significant (p<0.05) difference 0.29% noted in the MSG + Garlic treated group. However, relative to the MSG only animals, this value was significantly lower, i.e., comparatively decreased by 54.4%. Because the abrogate/mitigate any effects that have been induced by the MSG alone.

DISCUSSION

In this study, MSG alone increased the levels of total protein, cholesterol and estradiol (estrogen), which had led to induction of fibroid in the rats. The effects of MSG on protein levels could be attributed to the activation of transcriptional promoter and enhancer elements used for the control of gene expression, which promoted the ability of RNA polymerase to recognize the nucleotide at the initiation stage, thereby increased protein synthesis. The effect of MSG on cholesterol levels could be attributed to the activation of the enzyme, 3-hydroxyl-3-methoxylglutamyl-COA reductase, HMGR, which catalyzed the rate limiting step of cholesterol synthesis (i.e., conversion of HMG-COA to mevalonate), by covalent modification, which converted the phosphorylated state (inactive) to dephosphorylated state (active).

The enzyme is most active in the dephosphorylated sate (Bernard *et al*., 2002). This in turn, increased the activity of HMGR, resulting in increased cholesterol synthesis. The activation of HMGR through dephosphorylation also increased the levels of insulin, which stimulated the removal of phosphates from the cells and thereby activated HMGR activity, resulting in increased cholesterol synthesis (Verkauf, 1993; Wilson *et al*., 1980; Bernard *et al*., 2002). The effects of MSG on estradiol (estrogen) levels could be attributed to the activation of the enzyme, aromatese, which catalyzed the conversion of testosterone to β-estradiol and aromatization of ring A of β-estradiol, which increased the activity of the enzyme, resulting in increased estradiol synthesis.

However, treatments with Garlic extracts near-completely abrogated/mitigated any effects that have been induced by the MSG alone. Though, the mechanisms of action of this extracts may not be known but it appears that the Garlic extracts acted to remove catabolic wastes from the pelvic cavity and from uterine and ovarian tissues thereby accelerated metabolism and lymph drainage and promoted the sloughing-off of wasted tissues; corrected imbalances of estrogen metabolism associated with excessive catechol estrogen and elevated inflammatory prostaglandins. It also appears that the Garlic extracts stimulated the secretion of gonadotrophins and overian hormones and inhibited proliferation of cancer cells, resulting in apoptosis of the cancer cells (Wilson *et al*., 1980; Howe *et al*., 1995; Bernard *et al*., 2002). The effects of Garlic extracts on protein levels could be attributed to inhibition of RNA polymerase at the level of transcription, resulting in reduced gene expression, leading to reduced protein synthesis. Also, the effects of Garlic extracts on cholesterol levels could be attributed to the activation of cAMP signaling pathway, which increased the levels of cAMP, which activated cAMP-dependent protein kinase, PKA.

The activated PKA then phosphorylated phosphoprotein phosphatase inhibitor, PP1-1 (Bernard *et al*., 2002) and

increased its activity. An increase in activity of PP1-1 then inhibited the activity of HMGR, leading to reduced cholesterol synthesis. This effect also activated glucagons and adrenaline, which increased the levels of cAMP and acted opposite to insulin. The basic function of insulin and glucagons is to control the availability and deliver of energy to all cells of the body (Bernard *et al*., 2002).

The effects of Garlic extracts on estrogen levels could be attributed to inhibition of the enzyme, aromatase, which prevented aromatization of ring A of estradiol (estrogen) thereby preventing mechanisms involving modulation of cell proliferation (Wilson *et al*., 1980), by covalently binding to the estrogen receptors and promoting estradiol in exerting its growth stimulatory effects (Howe *et al*., 1995; Everitt *et al*., 1995). Generally, the growth of fibroid arising from uterus smooth muscle cells is modulated by circulating steroid hormones and has been associated with periods of increased estrogen secretion. This increased growth response has commonly been attributed to a hypersensitive state of tumour cells to estrogen (Verkauf, 1993), indicating that estrogen receptors have been over expressed in myomas with respect to adjacent myometrium (Bernard *et al*., 2002). Therefore, the ability of estrogen to modulate the growth dynamics of uterine fibroid cells occurs by mechanisms involving modulation of cells proliferation (Wilson *et al*., 1980).

The growth of fibroid during periods of increased estrogen secretion, such as pregnancy, is primarily due to cellular hypertrophy, resulting in increase in intracellular volume (Fisher *et al*., 1994). Fibroid growth is similarly stimulated by estrogen and affected by hormonal changes during menstrual cycle (Friedman *et al*., 1990). However, in fibroids, this hormone, estrogen, appears to stimulate cell proliferation as well as cellular hypertrophy (Black *et al*., 1994; Fuschs-Young *et al*., 1996).

Current non-surgical management of fibroids relies on reducing circulating levels of overian hormones with the use of Gonadotrophin-Releasing Hormones (GnRH) agonist (Verkauf, 1993). Such strategies result in the regression of fibroids during treatments by creating a hypoestrogen state through desensitization of signaling pathways within the hypothalamic-pituitary axis, resulting in bone loss and increase in blood lipid levels due to the reduced levels of circulating estrogen (Johansen *et al*., 1988; Dawood *et al*., 1989). This effect increases the risk for early-onset osteoporosis and cardiovascular diseases that precludes the long term use of these drugs. After the cessation of therapy, regrowth of tumours usually occurs when normal fluctuations involved in the menstrual cycles are reestablished (Friedman *et al*., 1990; Adamson *et al*., 1992).

The mechanisms of action of the antiestrogens and endocrine manipulation involve competitively binding to the estrogen receptors and preventing estradiol from exerting its growth stimulatory effects (Howe *et al*., 1995; Everitt *et al*., 1995) and increasing latency and decreasing mean tumour size (Howe *et al*., 1995), which inhibited the secretion of gonadotrophins and overian hormones at the level of the pituitary gland (Everitt *et al*., 1995). These therapies do not result in apoptotic cell death because they involve inhibition of cell proliferation by blocking the exit of cells from the golgi phase of the cell cycle (Wilson *et al*., 1980) and this helps to explain the observed rapid regrowth of these tumours after cessation of treatment. The effects could result from binding of components to unique antiestrogen sites on tumour cells and blocking of the unliganded estrogen receptors ability to traduce growth signal from other pathways and these effects appear to be tissue specific (Newton *et al*., 1994; Howe *et al*., 1995). The inability of hypoestrogenism to induce cell death emphasizes the need for improved modalities of treatment for uterine fibroid-perhaps herbal tonic therapy, which offers the possibility of treating women for extended periods of time (without side-effects accompany treatment)-without the need for surgery or hormone add-back. In addition, the fact that transformed myometrial cells (cell lines) appear to remains competent for the apoptosis could be instrumental in the development of novel therapeutic techniques for the treatment of uterine fibroids.

Conclusion: In conclusion, the results from this study, have shown that MSG increased the levels of total protein, cholesterol and estrogen (estradiol), which had led to increased proliferation of fibroid cells and that the proliferation of fibroid cells was sensitive to the availability of estrogen. However, treatments with garlic extracts near-completely abrogated/mitigated any effects that have been induced by the MSG alone. It appears that Garlic extracts acted to remove catabolic waste from the pelvic cavity and from uterine and ovarian tissues thereby accelerated metabolism and lymph drainage and promoted the sloughing-off of tissues; corrected imbalances of estrogen metabolism associated with excessive catechol estrogens and elevated inflammatory prostaglandins. It also appears that Garlic extracts stimulated the secretion of gonadotrophins and ovarian hormones and inhibited proliferation of cancer cells. Because Garlic extracts activated the secretion of gonadotrophins and ovarian hormones at the pituitary gland; promoted the exit of cells from the golgi phase of the cell cycle; promoted the unliganded estrogen receptor ability to transducer growth signals from other pathways, leading to apoptosis of fibroid cells, this mixture may offer the possibility of treating women with fibroids for extended periods of time without the need for surgery or hormone add-back.

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