

Original Research Article

Macroscopic & Microscopic molecular-associated treatments of Monosodium Glutamate-induced uterine fibroid via aqueous extract of ginger rhizomes: A study on adult female wistar rats

Abstract:

Background: Uterine fibroids are benign (non-cancerous) tumours that develop in the uterus. They have been and still are a major reproductive threat to women of child-bearing age especially to those of the African race. The present study involved the administration of Monosodium Glutamate (MSG) to induce uterine fibroid and the protective, preventive and curative effects of aqueous ginger extract on this as seen physically, gross morphologically and histologically

Aim: To determine the effects of aqueous ginger extract, as seen macroscopically and microscopically with underlying molecular consequences, on Monosodium Glutamate (MSG)-induced uterine fibroid in adult female wistar rats

Study design: Experimental and Statistical analysis

Place and Duration of Study: Department of Anatomy, Benjamin S. Carson School of Medicine, Babcock University, Ilishan-Remo, Ogun State, Nigeria between January 2017 and May 2017

Methodology: Acclimatization lasted for 10 days following procurement, after which oral administration of Monosodium Glutamate (MSG) and Aqueous ginger extract ensued to determine the prophylactic, protective and curative effects of ginger on MSG-induced uterine

fibroid in adult female wistar rats. Administration lasted for 50 days, after which the experimental animals were sacrificed via cervical dislocation, gross anatomical images were captured, uterus was fixed in 10% formosaline for histological analysis, and molecular studies were also carried out on the uterus to assay for MMP2

Results: Results showed that MSG-induced uterine fibroid had abnormal gross morphological effects, histo-pathological, amongst other hazardous effects, however the prophylactic, protective and high-dosed treatment with ginger extracts nearly completely to completely mitigated these effects.

Conclusion: The results of this study may contribute greatly to knowledge and may offer a non-surgical therapy of treating women with fibroids

1. INTRODUCTION

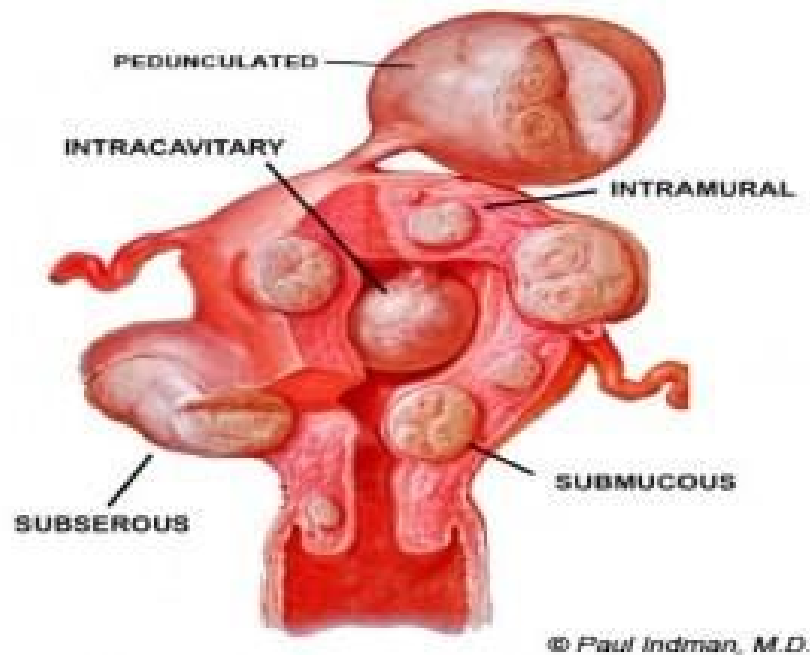
Uterine fibroids are monoclonal, non-malignant tumors that develop in the uterus and consist of large amounts of extracellular matrix that contain collagen, fibronectin, and proteoglycan (Sankaran & Manyonda, 2008). They grow in various locations on and within the uterine walls or in the uterine cavity and are therefore described as subserosal, submucosal or intramural fibroids. These tumors can be of any size and shape ranging from the size of a pea to an average-sized water melon (Obochi et al., 2009). With an estimated incidence of 20%–40% in women during their reproductive years, uterine fibroids are the commonest benign uterine tumors, (Wallach & Vhalos, 2004; Crum et al., 2004; Martínez et al., 2010), and may also be referred to as myoma, leiomyoma, leiomyomata, and fibromyoma, or simply fibroid. Also, they occur most frequently in women of childbearing age. In 2013, it was estimated that 171 million women were affected (Global Burden of Disease Study 2013, 2015). Historically, fibroids were called “uterine stone” in the period of Hippocrates in 460-375 B.C., (Nilo & Baracat, 2007). They were then called “scleromas” during the second century of the Christian period. In 1860 and 1863 respectively, Rokitsky & Klob coined and introduced the term “fibroid”. In 1854, Virchow, a German pathologist demonstrated that they were composed of smooth muscle cells. He also introduced the word, “myoma” (Siskin, 2009).

It is note-worthy to state that the growth and location are the main factors that determine if a fibroid leads to symptoms and problems (Wallach & Vhalos, 2004). A small lesion can be symptomatic if located within the uterine cavity while a large one on the outside of the uterus may go unnoticed. According to their location, uterine fibroids are classified as submucosal, intramural or subserosal (Vitiello & McCarthy, 2006).

Subserosal fibroids: are located underneath the peritoneal surface of the uterus and can become very large, giving the uterus a knobby appearance as they develop under the outside covering of the uterus and expand outward through the wall. They do not affect the woman's menstrual flow, but can cause pelvic pain, back pain and generalized pressure. As stated,

although this type may not have adverse effects on the woman, they, pedunculated as they are, can detach from the uterus to become a parasitic leiomyoma.

Intramural fibroids: develop within the wall of the uterus and expand inward, increasing the size of the uterus. They make the uterus feel larger than normal in gynecologic internal



examinations

Sub-mucosal fibroids: are just beneath the lining of the uterus. They are the least common, but tend to cause the most problems (even small lesions in this location may lead to cases of very heavy bleeding for prolonged periods and infertility).

Cervical fibroids are located in the wall of the cervix (referred to as the neck of the uterus). Also, rarely, fibroids are found in the supporting structures (round ligament, broad ligament, or uterosacral ligament) of the uterus that also contain smooth muscle tissue.

Histologically, leiomyoma in the uterus consists of whorled/fascicular patterns of bland smooth muscle fibres that are separated by well-vascularized connective tissue. These smooth muscle fibers resemble the muscle cells of the normal myometrium and are embedded in a fibrous stroma. Also, there is abundance of well vascularized Extracellular Matrix in uterine fibroids. The smooth muscle cells in uterine fibroids are elongated with eosinophilic or occasional fibrillar cytoplasm and distinct cell membranes, also they contain fairly large and conspicuous nuclei. (Bulun, 2013).

It has been reported that Estrogen and Progesterone, through their up-regulation of TGF- β 3 and down-regulation of p53 increases cell proliferation and survival and enhances extracellular matrix formation through induction of fibrosis which is characterized by resistance to apoptosis leading to the persistence of cells, and secretion of collagen and other ECM components by those cells leading to abundant disposition of highly cross-linked, disoriented, and often widely dispersed collagen fibrils. Also, the stiffness of ECM that surrounds the cells depends on the amount of cross-linking of the newly secreted altered

collagen. ECM stiffness have been reported to contribute to fibroid growth. (Leppert et al., 2004). Amyloid deposition is usually also characterized in uterine fibroids conditions. (Liu et al., 2012).

The growth of uterine fibroids takes place due to an increase in cell proliferation and deposition of the ECM (Walker and Stewart, 2005). Uterine fibroids contain abnormal deposition of ECM components that play important role in the pathogenesis (Stewart et al., 1994; Sozen and Arici, 2002; Malik and Catherino, 2007). The degradation of ECM is an important characteristic of development, morphogenesis, tissue repair and remodeling.

Although various types of proteinases are involved in ECM degradation, the major enzymes are considered to be matrix metalloproteinases (MMPs) (Visse and Nagase, 2003). They are designated matrixins that hydrolyze components of the Extracellular Matrix

Now acknowledged as key players in the regulation of both cell–cell and cell–extracellular matrix interactions, matrix metalloproteinases (MMPs) are involved in modifying matrix structure, growth factor availability and the function of cell surface signalling systems, with consequent effects on cellular differentiation, proliferation and apoptosis. They play central roles in many biological processes including embryogenesis and morphogenesis, angiogenesis, wound healing, tissue repair and remodelling in response to injury and in the progression of diseases such as atheroma, arthritis, cancer, tissue ulceration and cardiovascular disease.

Because of their wide spectrum of activities and expression sites, the elucidation of their potential as drug targets in disease or as important features of the repair process will be dependent upon careful analysis of their role in different cellular locations and at different disease stages. There are 24 MMPs in humans, although the activities of most MMPs are very low or negligible under normal physiological condition. Their activities are primarily regulated by tissue inhibitors of matrix metalloproteinases (TIMPs), and thus the balance between MMPs and TIMPs are critical for the ultimate ECM remodelling in the tissue (Nagase et al., 2006). Novel approaches to the specific regulation of individual MMPs in different contexts are also being developed.

The MMP family of proteases has been characterized into several subgroups based on their ability to specifically degrade various interstitial matrix and basement membrane components, and those are collagenase, gelatinases, stromelysins, membrane-type (MT)-MMPs and several others (Nagase et al., 2006).

Gelatinases (MMP-2 and MMP-9) digest gelatin via their fibronectin type II repeats that binds to gelatin/collagen, and they can also digest a number of ECM molecules including type IV, V and XI collagens, laminin and others. MMP-2, but not MMP-9 can also digest collagens I, II and III in a similar manner to the collagenases (Aimes and Quigley, 1995; Patterson et al., 2001). Gelatinase A (MMP-2) and gelatinase B (MMP-9) belong to this sub-group. Both enzymes have three repeats of a fibronectin type II motif inserted into the catalytic domain. They share similar proteolytic activity and degrade denatured collagens, gelatins and a number of ECM molecules including native type IV, V and XI collagens, laminin and aggrecan core protein. MMP-2 digests native collagen types I, II and III in a similar manner to the collagenases (Aimes and Quigley, 1995; Patterson et al., 2001), but

MMP-9 does not. However, the collagenolytic activity of MMP-2 is much weaker than MMP-1 or other collagenases in solution. Nevertheless, because proMMP-2 is recruited to the cell surface and activated by the membrane-bound MT-MMPs, it may accumulate pericellularly and express reasonable local collagenolytic activity. It may also act as a 'collaborator' activity, digesting collagenase-clipped collagen to smaller fragments because those fragments denature at body temperature 37°C (Murphy et al., 1985). MT-MMPs (MMP-14, MMP-15, MMP-16 and MMP-24) are activated intracellularly and active enzymes are expressed on the cell surface. All MT-MMPs can activate pro-MMP-2. The MMP family of proteases is involved in the degradation of fibrillar collagen (Butler et al., 1997; Gioia et al., 2007). Soluble collagen type I is enzymatically digested by collagenases such as MMP-1, MMP-8, MMP-13 (Chung et al., 2004; Inada et al., 2004), MMP-14 (Ohuchi et al., 1997), gelatinase A (MMP-2) (Aimes and Quigley, 1995) and gelatinase B (MMP-9) (Allan et al., 1995). MMP-3 functions as an activator of other MMPs such as MMP-1 and MMP-9. One study has shown that fibroid growth is associated with increased activity of MMP-2 (Wolanska et al., 2004). An additional report demonstrated that MMP-1, MMP-2, MMP-3, MMP-9, TIMP-1, TIMP-2 and collagenase types I and III were highly expressed in uterine fibroids and myometrium during the progesterone-dominant secretory phase of the menstrual cycle (Douet et al., 1997). The expression of MMP-1, MMP-2 and MMP-3 are elevated in larger uterine fibroids (Wolanska et al., 2004). MMP-2 and MMP-9 are the key enzymes that degrade major collagen in ECM component (Zucker et al., 1993). Increased activities of MMP-2 and MMP-9 have been associated with increasing tumor metastases in various human metastatic cancers (Mook et al., 2004). Most MMPs are secreted as inactive proenzymes and their proteolytic activities are regulated by TIMPs (Woessner, 1994). TIMPs inhibit the enzymatic activity by binding to the C-terminal catalytic domain of MMPs (Lambert et al., 2004). TIMP-1 and TIMP-2 are known to bind MMP-9 and MMP-2, respectively (Olson et al., 2000). Thus, the activities of MMPs are regulated by the expression levels of TIMPs. One study showed that MMPs and TIMPs are expressed in various normal tissues that undergo tissue remodeling, and their overexpression in certain pathological conditions is associated with extensive ECM degradation (Matrisian, 1992).

MSG: Monosodium Glutamate (MSG) is a salt of glutamate, synthesized from L-glutamic acids and used as a flavour enhancer in foods (Ikonomidou & Turski, 1995). Various studies have reported the toxicity of MSG in humans and experimental animals (Eweka & Om'Iniabohs, 2011; Dixit et al., 2014; Hamza & Al-Harbi, 2014). Monosodium glutamate has been used to induce uterine fibroids in experimental animals (Obochi et al., 2009; Koffuor et al., 2013).

Ginger is the common name for *Zingiber officinale* Roscoe, a plant that has been reported to be one of the more commonly used spices in the world (Koh et al., 2009). The most commonly consumed part of ginger is the rhizome, or the vertical portion of the root and studies have shown that ginger extracts had different pharmacological effects such as

anti-inflammatory (Young et al., 2005), anti-oxidant (Stoilova et al., 2007), and anti-tumor effects (Shukla & Singh, 2007). Some active compounds of ginger include:

- Gingerol, which has been reported to have anti-oxidant, antitumor, anti-inflammatory activities, amongst others.
- Paradol also has anti-oxidant and anti-cancerous effects
- Shogoal, according to researches done by Park et al., 2006 has Anti-oxidant and anti-inflammatory activities. [6]-shogaol showed anticancer activities through the inhibition of cell invasion reduction of matrix metalloproteinase-9 expression, anti-proliferation activity and anti-invasion (Ling et al., 2010; Choudhury et al., 2010; Weng et al., 2010)

2. MATERIALS AND METHODS:

2.1. STUDY AREA: The study was carried out at the department of Anatomy, Benjamin S. Carson School of Medicine, Babcock University, Ilishan-Remo, Ogun State. Molecular studies were carried out at CBDD, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

2.2. PROCUREMENT AND ADMINISTRATION OF MONOSODIUM GLUTAMATE (MSG): MSG was used to induce uterine fibroid in adult female wistar rats as it mimics many aspects of the disease in the experimental animal. The solid form of MSG in form of vedan was obtained from a major seasoning shop at Agric Bus-stop, Ikorodu, Lagos State. The solid MSG was dissolved in distilled water to produce the desired concentration of 900mg/kg body weight and stored in the refrigerator below (-4°C). On administration, the dissolved MSG was 'fed' orally to the experimental animals through orogastric intubation as instructed by table 1.1. The median lethal dose of MSG is 15,000mg/kg (Walker et al., 2000)

2.3. PROCUREMENT AND ADMINISTRATION OF GINGER EXTRACT: Ginger extract was used as a prophylactic, protective and curative agent in the present study. Fresh ginger rhizomes were purchased from **Kaduna State Ginger Processing Company**. They were washed in distilled water and ground in distilled water for 1 minute using a kitchen blender. The ground mixture was filtered through a fine cotton cloth and the aqueous extracts was administered orally to the animals in various grades (i.e., 500mg/kg, 900mg/kg, 1,700mg/kg) as instructed by the table 1.1

2.4. EXPERIMENTAL ANIMALS' HUSBANDRY AND DESIGN: After receiving ethical approval from Babcock University Health Research Ethics Committee (BUHREC), Forty nine (49) healthy, non-pregnant adult female wistar rats (*Rattus norvegicus*) weighing between 190-230g were obtained from, housed, and cared for at the Babcock University animal house, Babcock University, Ilishan-Remo. The animals were housed in suitable, clean and dry animal holding facility in well-ventilated plastic cages at room temperature under natural light and dark cycles while unnecessary restraint and overcrowding was avoided as rats were split into random groups of seven. Bedding were ensured to be non-allergenic,

dust-free, inedible, absorbent, non-toxic and free of pathogenic organisms, and were changed regularly to prevent the build-up of pathologic ammonia. Rats were properly fed to ensure adequate growth and development and prevent malnutrition-induced disease conditions. Drinking water was made available and accessible. A good housing measure was observed as recommended by the Animal Research Review Panel (ARRP, 2002). Also, the body weight of the animals were taken on the first day of administration, on the 25th day and on the 50th day using a sensitive Camry weighing balance so as to access weight gain and/or loss in each group

After the animals were allowed to acclimatize for a period of 10 days with free access to feed and water, with seven animals in each group, the following treatment regimen was followed to achieve the aim of the present study

Table 1.1. Table showing treatment regimen design for control and experimental groups

GROUP	Treatment schedule	Rationale
Group A	A placebo of water	Control group
Group B	Animals will be treated orally with 900mg/kg body weight of MSG only for 25 consecutive days	(Negative control group) To induce Uterine fibroid
Group C	Animals receiving 900mg/kg body weight of ginger extract only for 25 consecutive days	A placebo for ginger extract
Group D	Animals receiving 900mg/kg body weight of ginger extract orally for 25 days, followed by oral administration 900mg/kg of MSG for 25 consecutive days	Prophylactic group
Group E	Animals receiving oral administration of 900mg/kg of MSG + 900mg/kg body weight of ginger extract for 25 consecutive days	Protective group
Group F	Animals receiving oral administration of 900mg/kg of MSG for 25 consecutive days, followed by oral administration of low dose ginger (500mg/kg) extract for 25 consecutive days	(Curative group 1) To reverse uterine fibroid induced by MSG
Group G	Animals receiving oral administration of 900mg/kg of MSG for 25 consecutive days, followed by oral administration of high dose ginger (1,700mg/kg) extract for 25 consecutive days	(Curative group 2) To reverse uterine fibroid induced by MSG

2.5. CONFIRMATION OF THE PRESENCE OF UTERINE FIBROID: For each of the animals in groups A, F and G, blood tests were carried out on the 25th day to confirm the presence of fibroids before administration of ginger extract. Biochemical assays on serum estradiol, serum progesterone were carried out as they have been confirmed to be notable markers for uterine fibroid (Obochi et al., 2009; Zia et al., 2012; Koffour et al., 2013).

2.6. SACRIFICE OF EXPERIMENTAL ANIMALS AND ORGAN HARVEST

One day after the last administration, the experimental animals were weighed and then sacrificed by cervical dislocation after blood collection through the orbits. The skin and peritoneum of the abdomen were opened after cleansing with 70% ethanol, the uterus was excised carefully using a pair of dissecting scissors and forceps, weighed and fixed in 10% formosaline after a little part has been sectioned for molecular assays. Proper disposal of carcasses was ensured.

2.7. RELATIVE ORGAN WEIGHT (ROW) AND GROSS ORGAN MORPHOLOGY

Just before sacrifice, the animals were measured and after the sacrifice, following tissue excision, organ weight of the uterus was also measured using an air-tight AWS (American Weigh Scale)- 1kg digital scale so as to obtain ROW which was calculated as the whole organ (uterus) weight divided by the whole body weight as a means of evaluating the development and size of a particular organ relative to the whole body; this was employed to evaluate organ mass relative to the whole body mass across animal groups [Uterus ROW= Uterus weight/ Body weight]. Also pictures of the uterus were taken in JPEG format to depict gross morphological images of the fibroid and non-fibroid uteri.

2.8. METHODS: TISSUE PROCESSING FOR HISTOLOGICAL PROCEDURE

2.8.1. HAEMATOXYLIN AND EOSIN FOR GENERAL HISTOARCHITECTURE OF UTERUS

The harvested tissue samples were immersion-fixed in 10% formo-saline at room temperature after which they were dehydrated in ascending grades of alcohol (70%, 95%, 100%, and 100%) and cleared with two changes of xylene for one hour, 30 minutes each. Thereafter, the cleared tissues were then transferred into two changes of molten paraffin wax I and II for one and half hour each for infiltration process; the tissue blocks were then serially sectioned at 6µm thickness using a microtome. Strips of sections were gently lowered into the surface of a warm water bath at 40°C. The floated sections were mounted on egg albumin-coated microscopic slides, and put in an oven maintained at 60°C for 30 minutes to fix the tissue firmly on the slide. Thereafter, the slides were dewaxed with two changes of xylene and hydrated with decreasing alcohol concentration and then immersed in water for 5 minutes. Regressive staining and counter staining of the sectioned tissues then followed, with Ehrlich's hematoxylin and with Eosin respectively. Tissues were washed in tap water and dehydrated by rinsing in increasing concentration of alcohol and then xylene-I. They were then placed in

xylene-II until mounting done by placing a drop of mountant DPX (A mixture of Distyrene, a Plasticizer, and Xylene) on top of the sections and the cover slip was applied.

2.8.2. PERIODIC ACID SCHIFF (PAS) FOR MUCOPOLLYSACCHARIDES IN THE UTERUS

The PAS reaction in tissue sections is useful for the demonstration of mucopolysaccharides.

Reagents:

The Sections were deparaffinized and hydrated to water to deionized water, the slides were then immersed in 0.5% periodic acid solution for 5 minutes at room temperature and rinsed in several changes of distilled water. Then the slides were then immersed in Schiff's Reagent for 15 minutes at room temperature and+ washed in running tap water for 5 minutes. Also, the slides were counterstained in Haematoxylin Solution for 90 seconds, after which they were rinsed in running tap water and then dehydrated in graded alcohols, cleared in xylene and mounted in xylene based mouting media and coverslipped.

2.8.3. CONGO RED STAINING FOR AMYLOID IN THE UTERUS

Modified Highman's Congo Red Staining Protocol for Amyloid (IHC world, 2011):

Tissue sections were deparaffinized and hydrated to water, after which they were stained in congo red solution for 15-20 minutes at room tempearture and then rinsed in distilled water. Through about 5 to 10 dips, the tissue sections were differentiated in alkaline alcohol solution and rinsed in tap water for 1 minute. The sections were counterstained with Gill's hematoxylin for 30 seconds and rinsed in tap water for 2 minutes. Dehydration ensued through 95% alcohol and 2 changes of 100% alcohol for 3 minutes each. The sections were cleared in 2 changes of xylene for 3 minutes each and mounted with resinous mounting medium.

2.8.4. MASSON TRICHOME FOR COLLAGEN DETECTION

Staining procedure

After mordanting the tissue sections in Bouin's solution at a temperature of 60°C for 1 hour, they were then microwaved 1 minute, and allowed to stand 15 minutes. They were washed in running tap water for 5 minutes to remove the picric acid and then immersed in Weigert's working hematoxylin for 10 minutes, blued in running tap water for 5 minutes, and rinsed in distilled water. The tissue sections were immersed in Biebrich scarlet for 5 minutes, and thereafter rinsed in distilled water. They were immersed in Phosphotungstic acid for 10 minutes after which the solution was discarded. The tissue sections were transferred directly into Aniline blue for 5 minutes and rinsed in distilled water and immersed in 1% Acetic acid for 1 minute after which the solution was discarded and the sections were rinsed in distilled water. Dehydration, clearing, and coverslip then ensued respectively.

2.8.5. MOLECULAR ASSAY: GENE PROFILLING AND EXPRESSION OF MMP-2

The procedure include:

RNA Isolation using the RNA Snap method with the implementation of the RNA Snap kit whose components include: 18mM EDTA (Ethylenediaminetetraacetic acid), 0.025% SDS (Sodium Dodecyl Sulfate), 95% Formamide, 1% 2-mercapto-ethanol

RNA Quantification via the use of a spectrophotometer after the machine has been calibrated by means of distilled deionized water (ddH₂O).

Reverse transcription of RNA to cDNA with the use of Reverse transcriptase enzyme

Gene amplification was done using Polymerase Chain Reaction after specific cocktail containing 2.5UL of master mix, 0.5UL of forward primer and 0.5UL of reverse primer of the MMP-2 was added. (FORWARD 5'-3': CCACGTGACAAGCCCATGGGGCCCC REVERSE 5'-3': GCAGCCTAGCCAGTCGGATTGATG)

Gel electrophoresis: Following gene amplification, tracking dye was added to each amplicon (the resultant amplified genes), each of which was then placed in solidified agarose gel to be used for electrophoresis on a fixed electrophoresis tank and moved to the UV transilluminator for gene expression to be observed (The amount of DNA that moves towards the positive pole is the expressed gene)

3. RESULTS AND DISCUSSION

3.1. MEAN BODY WEIGHT (MBW)

The mean body weights of the animals were calculated as final body weight + initial body weight/2.

Group A: Control Group B: MSG only Group C: Ginger only
Group D: Ginger → MSG Group E: Ginger + MSG
Group F: MSG → Ginger (Low dose) Group G: MSG → Ginger (High dose)

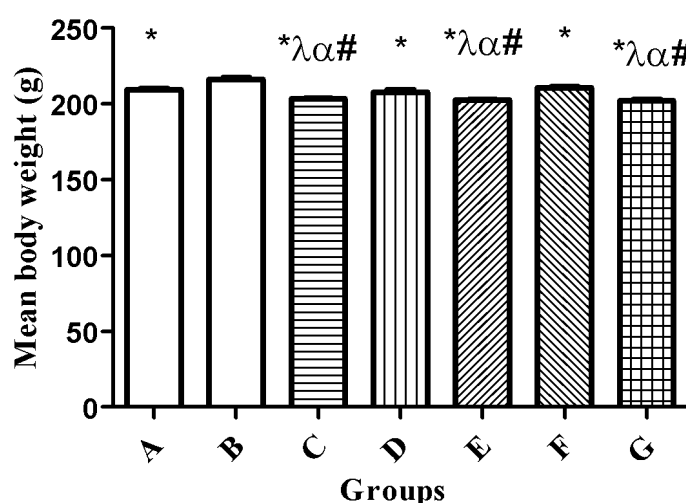


Fig 1.0 Bar graph showing Mean Body Weight of animals across groups

*=P<0.05 when compared with Group B

λ=P<0.05 when compared with Group F

α=P<0.05 when compared with Group A

#=P<0.05 when compared with Group D

The results obtained from the analysis of mean body weight across the groups revealed that the group administered with MSG only had the highest mean body weight when compared with the other groups (Fig 1.0). This can be attributed to the fact that with fibroid growth, there is increase in weight of the 'host' (Johnson, 2016) and may also imply that MSG

increases body weight over time although the risk of weight gain attributed to MSG intake was modest, as seen when compared with the control group. This weight gain could have been as a result of evidence that shows that MSG stimulates the pancreas to produce about three times the normal amount of insulin it produces (Golden, 2011). This unnatural amount of insulin then converts the sugar in the blood into fat very efficiently. Dr. Russell Blaylock in his book *Excitotoxins..The Taste that Kills*, reports that in animal studies MSG creates a lesion in the hypothalamus that correlates with abnormal development, including obesity. Studies have also suggested that increased food intake induced by MSG through leptin resistance and insulin action may also lead to an increase in weight gain as MSG might interfere with signalling system in the body that regulate appetite, and also lead to overproduction of hormone leptin which regulates appetite and metabolism (He et al., 2008; Marcus, 2011), although the present study did not monitor food intake levels over the experimental periods. Also, the mean body weight in groups C, E, G each held little or no significant difference from that of the control group, but showed significant decrease when compared with groups B, D, F. These groups (C, E, G) had lower mean body weight, this was as a result of administration of ginger extract. The effect of ginger in reducing body weight may most likely be due to the inhibitory action of ginger on absorption of dietary fats by inhibiting its hydrolysis and as a result may decrease the adipose tissue weight (Gerald et al., 2008). Also, this may be attributed to the fact that ginger suppresses cortisol production (as elevated level of cortisol may contribute to weight gain). These findings are in conjunction with researches on ginger done by Saravanan et al. in 2004.

3.2. RELATIVE ORGAN WEIGHT (UTERUS WEIGHT)

ROW (g)= Uterus weight/Body weight

Group A: Control Group B: MSG only Group C: Ginger only

Group D: Ginger → MSG Group E: Ginger + MSG

Group F: MSG → Ginger (Low dose) Group G: MSG → Ginger (High dose)

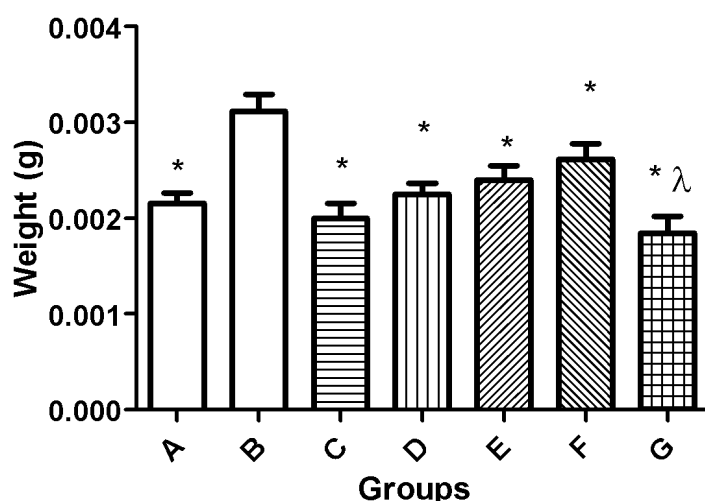


Fig 1.1. Bar graph showing Relative Organ Weight of animals across groups

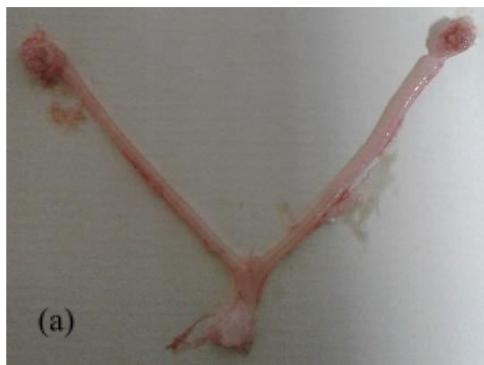
*= $P < 0.05$ when compared with Group B

$\lambda = P < 0.05$ when compared with Group F

There was observed and statistical significant increase in the relative organ weight of the animals in group B in comparison with group A (Fig 1.1). This could be attributed to fibroid growth in that group, induced by MSG administration. Fibroids do not only grow apart from the uterus, but also grow cause the uterus to increase (Kido, 2003). This abnormal growth of the uterus and outgrowth on the uterus hence caused the organ weight of the uterus to be increased in the group induced with uterine fibroid. It is also important to note that an increase in weight and size of the uterus usually indicates hyperplasia (cell proliferation), an early neoplastic process and a marker for uterine fibroid. Estradiol, which increases in this present study, is specific in uterine cell proliferation and binds to $ER\alpha$ receptors that are abundant in the uterus of the induced animal, forming a complex that interacts with DNA of the nucleus to activate transcriptional promoter and enhancer elements responsible for control of gene expression. This hyperplasia effect suggests that increased MSG produces a corresponding increase in synthesise of these biochemical markers that enhance proliferation process of the uterus.

Ginger extract then played its role in reversing this effect in groups D, E, F, G. As there was significant statistical decrease in the relative organ weight of the uterus specimens in these groups, therefore implying that ginger may have played a pivotal role in reducing organ weight and/or shrinking the growing tumor (Johnson, 2016).

3.3. GROSS ORGAN MORPHOLOGY OF UTERUS SPECIMENS



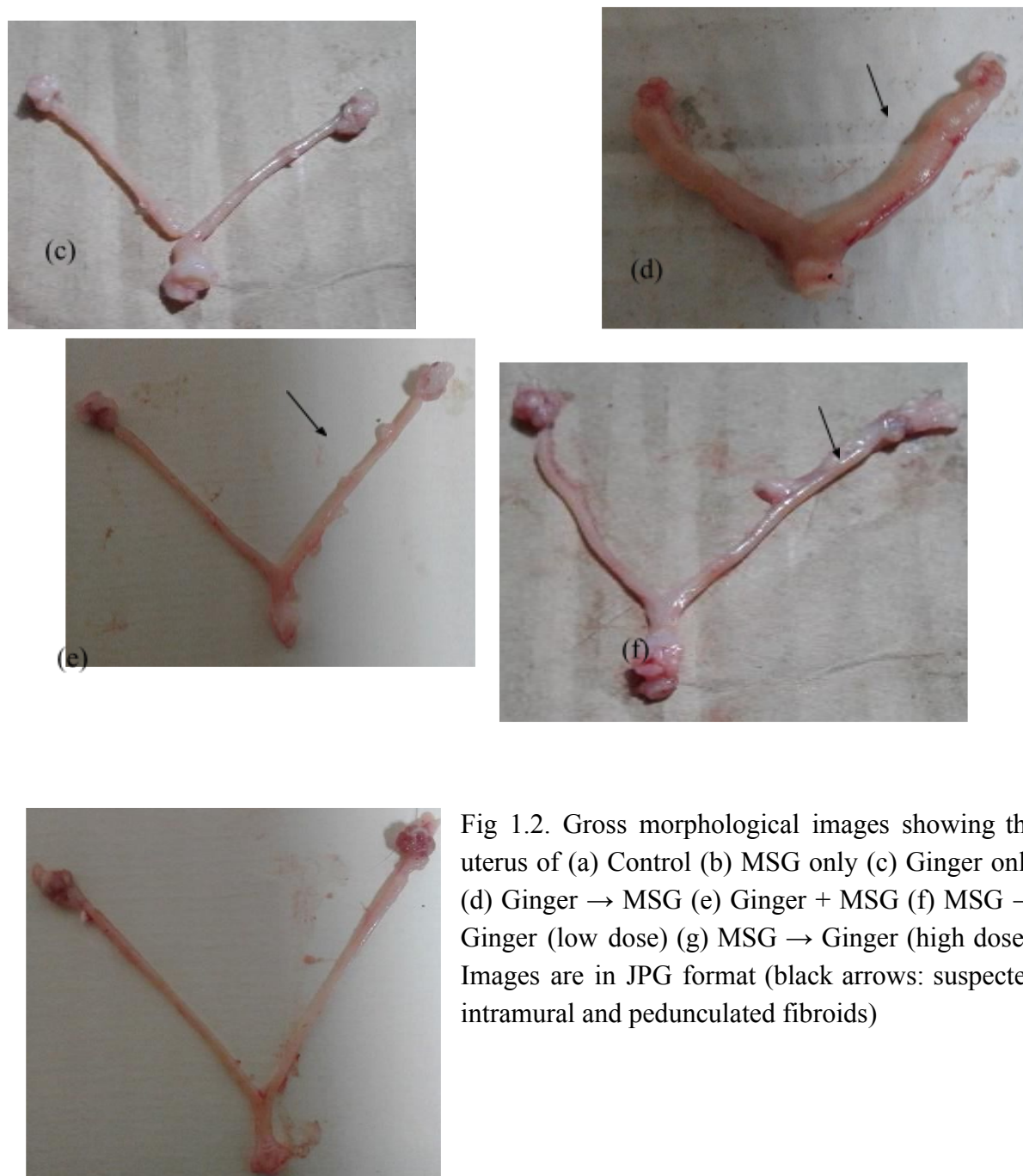


Fig 1.2. Gross morphological images showing the uterus of (a) Control (b) MSG only (c) Ginger only (d) Ginger → MSG (e) Ginger + MSG (f) MSG → Ginger (low dose) (g) MSG → Ginger (high dose). Images are in JPG format (black arrows: suspected intramural and pedunculated fibroids)

G

Gross morphologically, there was observable growth of pedunculated and submucous uterine fibroids in the specimen representative of group B, confirming the induction of tumour growth by Monosodium Glutamate (MSG). Generally, circulating steroid hormones modulates the growth of fibroid arising from uterine smooth muscle cells, which has been associated with periods of increased estrogen secretion. This increased growth response has commonly been attributed to a hypersensitive state of tumor cells to estrogen (Verkauf, 1993), indicating that estrogen receptors have been over expressed in myomas with respect to adjacent myometrium. Therefore, as previously stated, the ability of estrogen to modulate the growth dynamics of uterine fibroid cells occurs by mechanisms involving modulation of cells

proliferation. The growth of fibroid during periods of increased estrogen secretion is primarily due to cellular hypertrophy resulting in increase in intracellular volume (Fisher et al., 1994).

This is in conjunction with works done by Koffuor et al. in 2013 and Obochi et al. in 2009.

The physical and gross morphology of the organ representative in the other groups were also observed. There was presence of what can be referred to as ‘shrinking-in-progress’ of suspected fibroid in the prophylactic, utero-protective and curative I groups (i.e., groups D, E, F); while groups A, C, G showed little or no observable difference in comparison with group A, with no fibroid growth or complete shrinkage of benign tumour as the case may be.

3.4. HISTOLOGICAL ANALYSIS

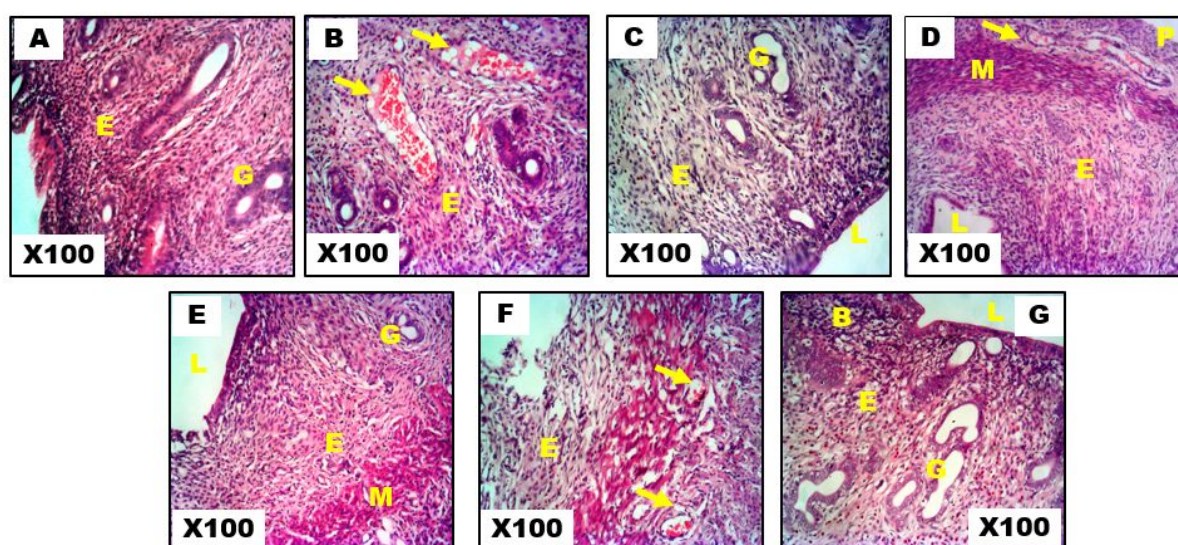


Plate 1: Photomicrographs showing panoramic views of Uterus general morphological presentations in Wistar rats across groups A-G. Hematoxylin and Eosin stain (X100). The Myometrium (M), The Endometrium (E), uterine glands (G), uterine lumen (L), Highly vascularized extracellular matrix (yellow arrows), Whorls of bland smooth muscle cells (black arrow). Group A: Control group Group B: Uterine fibroid Group C: Ginger only Group D: Ginger only → Uterine fibroid Group E: Ginger + MSG Group F: MSG → Ginger (Low dose) Group G: MSG → Ginger high dose

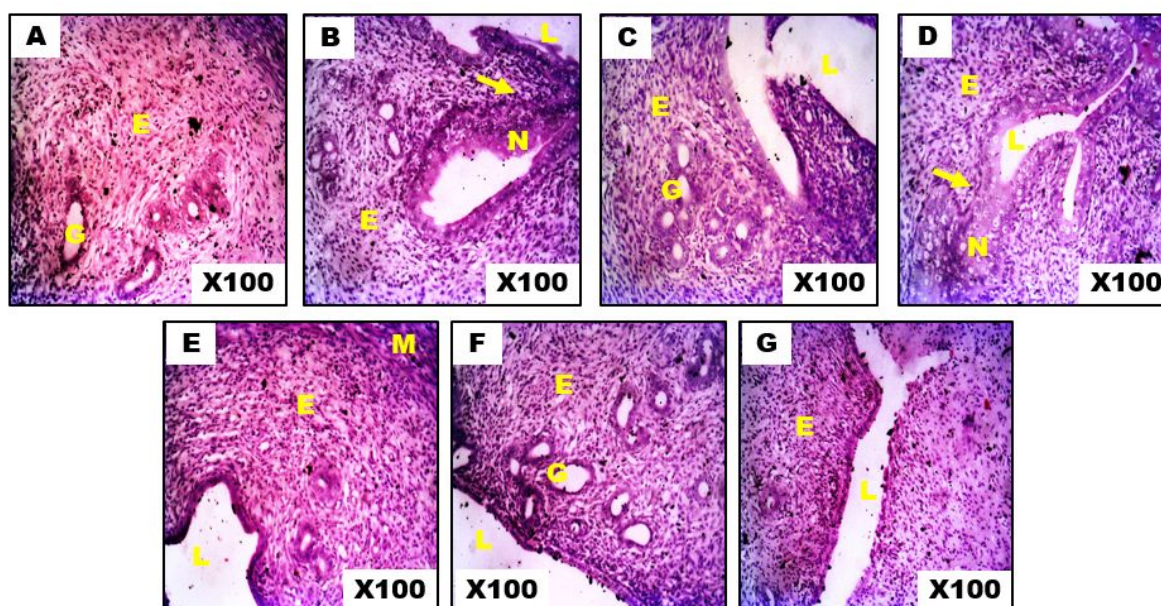


Plate 2: Photomicrographs showing panoramic views uterus general morphological presentations in Wistar rats across groups A-G. Periodic Acid Schiff 'PAS' (X100). The perimetrium (P), Myometrium (M), Endometrium (E), Amyloid deposition in pseudo-capsule vessel (G), uterine Lumen (L), severe endometriosis (yellow arrows), Apoptotic bodies (B), Apoptotic body (N) are well demonstrated. Nuclei: Blue colouration

Group A: Control group

Group B: MSG only

Group C: Ginger only

Group D: Ginger → MSG

Group E: Ginger + MSG

Group F: MSG → Ginger (Low dose)

Group G: MSG → Ginger high dose

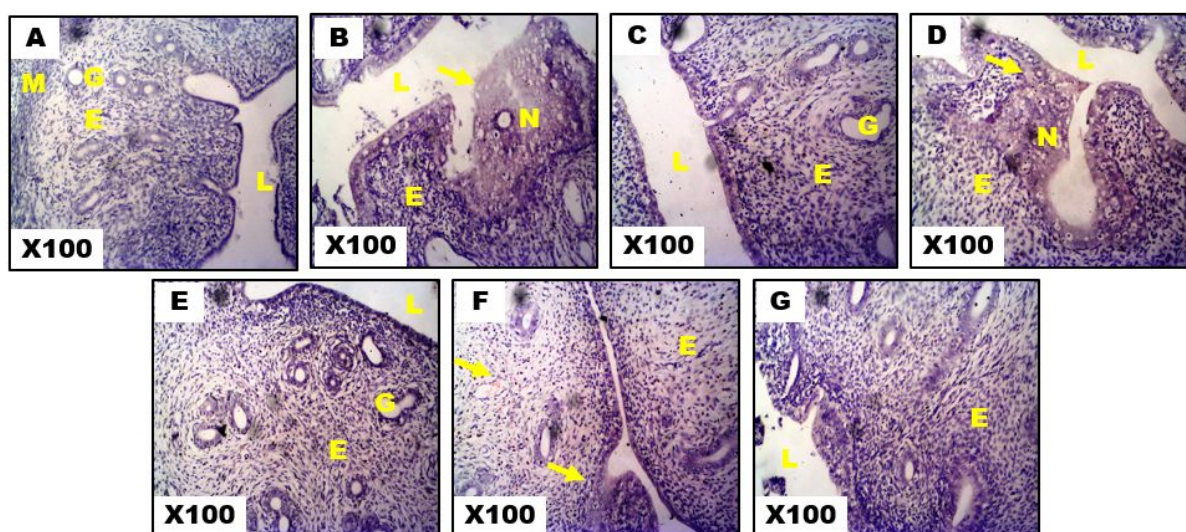


Plate 3: Photomicrographs showing panoramic views uterus general morphological presentations in Wistar rats across groups A-G. Congo Red stain (X100). The perimetrium (P), Myometrium (M), Endometrium (E), uterine glands (G), uterine Lumen (L), severe

endometriosis (yellow arrows), Apoptotic bodies (B), Necrotic bodies (N) and uterine blood vessels are well demonstrated.

Group A: Control group

Group B: MSG only

Group C: Ginger only

Group D: Ginger → MSG

Group E: Ginger + MSG

Group F: MSG → Ginger (Low dose)

Group G: MSG → Ginger high dose

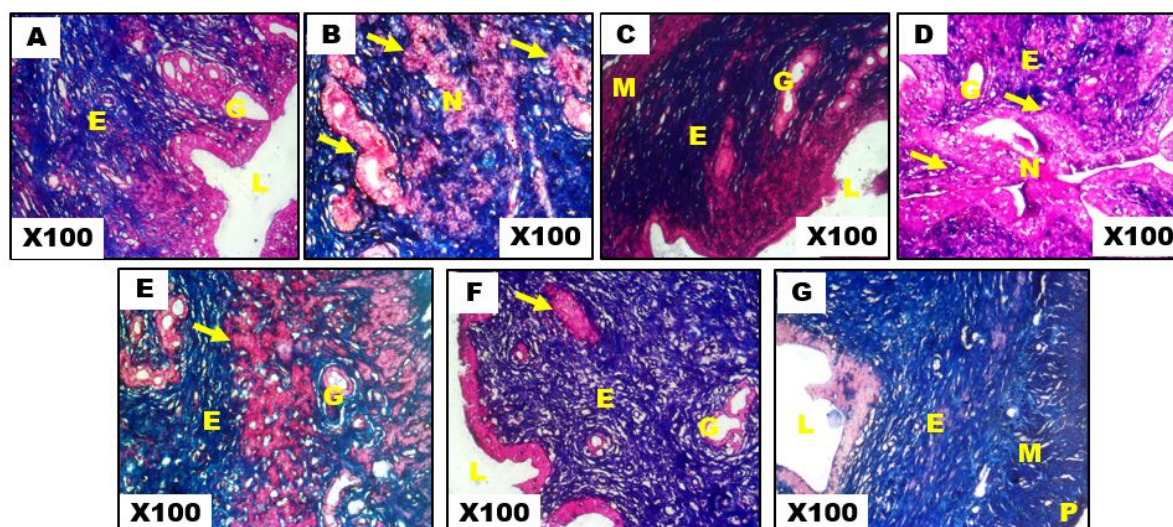


Plate 4: Photomicrographs showing panoramic views uterus general morphological presentations in Wistar rats across groups A-G. Masson Trichrome stain (X100). The perimetrium (P), Myometrium (M), Endometrium (E), uterine glands (G), uterine Lumen (L), severe endometriosis (yellow arrows), Apoptotic bodies (B), Necrotic bodies (N) and uterine blood vessels are well demonstrated.

Group A: Control group

Group B: MSG only

Group C: Ginger only

Group D: Ginger → MSG

Group E: Ginger + MSG

Group F: MSG → Ginger (Low dose)

Group G: MSG → Ginger high dose

Summary report: B, F - severe endometriosis, extracellular matrix accumulation with red blood cells was observed, excessive amyloid and collagen deposition. A, C, E, G- presents normal histology. F- mild histopathological changes were observed

Histologically, the photomicrographs of the uterus and pituitary gland were captured at two different magnification for comparison and better appreciation of cellular components

For the uterus, plate 1A showed the control group at a magnification of X100 stained with H & E dye. The muscular wall and the endometrial lining of the uterus were clearly seen. The glands embedded in stroma are also depicted, the layers of the uterine wall: perimetrium,

myometrium and endometrium are clearly seen. The uterine lumen is also depicted in the photomicrograph.

Plate 1B showed bland/whorled/fascicular patterns of smooth muscle fibres, embedded in a fibrous stroma and separated by well-vascularized connective tissue. Deposition of abundant extracellular matrix was also depicted. Extracellular disorganized matrix is a peculiar characteristic of fibroid growth, mainly consisting of collagen subtypes, fibronectin, and proteoglycans.

Plate 1C showed a normal histo-architecture similar to that of the control group. This could be attributed to the fact that ginger has no adverse effects on the uterus.

Plates 1D, 1E, 1F, 1G showed reversal of the histopathology observed in plate 1B, with some bland smooth muscle fibers depicted, vascularized extracellular matrix seen in plates 1E and 1F

Plate 2A showed normal histology with that amyloid deposition in

Plates stained with PAS and Congo red (i.e., plates 2 and 3 respectively), for group B, showed that fibroblast was proliferated and ECM was overexpressed. Also, amyloid deposited in tissue looked as block mass. PAS and Congo red staining of the tissues in group B showed that all the muscle fiber interstitiums within fibroid entities were negative for amyloid deposition while fibroid capsule and vascular amyloid deposition were positive which suggests that interstitium affected by metabolism of leiomyoma may lead to conformational change of amyloid precursor protein and gradual amyloid deposition. Ginger extract in the subsequent groups seemed to reduce amyloid deposits especially in groups D and G.

Plate 4A showed normal histology with minimal collagen deposition

Plate 4B showed collagen accumulation. This was due to the accumulation of Extracellular matrix of which collagen is a major component of. The collagen production also occurs by tumor myocytes, the interstitium of the tumor fascicle is expanded by abundant blue collagen that separates the red staining fibroid myocytes. No interspersed fibroblasts are noted in the blue collagenous stroma, indicating that the stromal matrix has been produced by the tumor myocytes themselves. Also many of the myocytes of the central portion of the stained section in plate 4B are hypertrophied in size as cellular hypertrophy is a marker for uterine fibroid.

Ginger extracts in groups D and G seemed to nearly-abrogate these effects. Ginger extract, owing to some of its active compounds, down-regulate TGF- β 3 which in turn reduces cell proliferation, hence reducing fibrosis that causes accumulation of collagen.

MMP-2

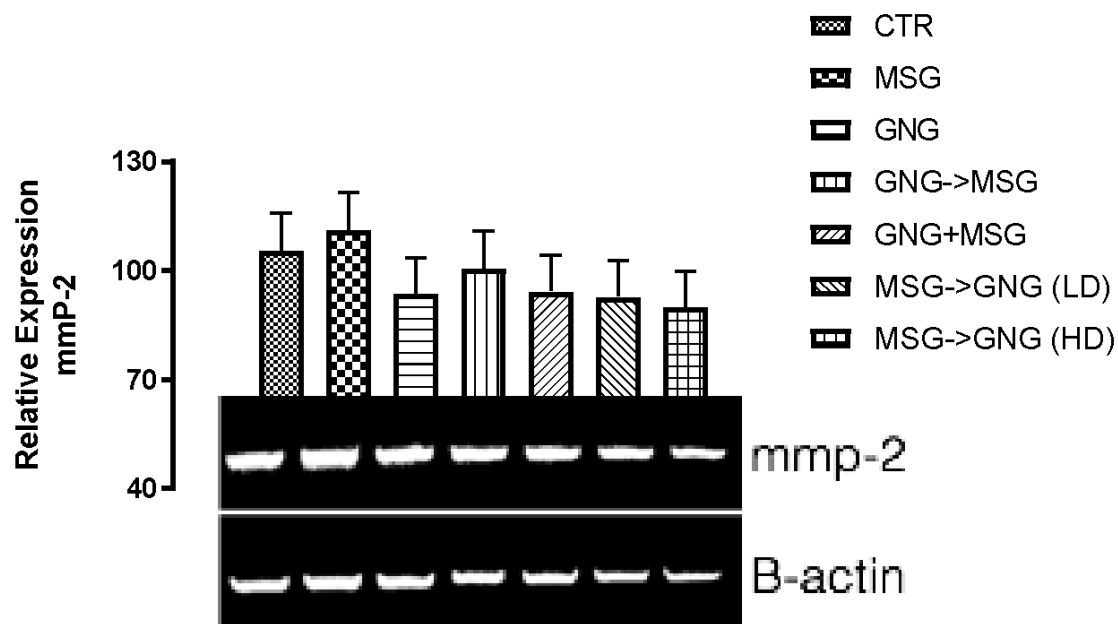


Fig 1.3: Bar graph showing relative expression of MMP-2 across groups through RNA isolation of tissue samples using RNA Snap kit

In conjunction with work done by Bogusiewicz et al (2007), there was increased MMP-2 activity in leiomyomas, hence more MMP-2 was being produced so as to degrade the excess ECM in cases of uterine fibroid, there was a decrease in the groups administered with ginger extract when compared with group B of MSG only.

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