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Histopathological Investigations Of *Curcuma longa* (Turmeric) and *Zingiber officinale* (Ginger) On Rats With Monosodium Glutamate-Induced Leiomyoma

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Abstract

Uterine leiomyoma also known as fibroid is a medical problem of the female reproductive tract and prevalent among black women of child-bearing age. Monosodium glutamate (MSG), a popular food seasoning agent is an oestrogen disruptor but its intake has not been linked to fibroid. Fibroid has no known chemotherapy and hysterectomy leaves huge financial burden with side effects. It is necessary to determine its safer management method. This work investigated the effects of aqueous extracts of *Curcuma longa* (turmeric) and *Zingiber officinale* (ginger) on uterus and kidney sections of rats with monosodium glutamate-induced leiomyoma. Twenty-eight rats were used. They were divided into four groups of seven rats each and acclimatized. Fibroid was induced on three groups after daily ingestion of 750mg/kg body weight of MSG for 28 days. Negative control had no fibroid. Positive control had fibroid but remained untreated. Groups III and IV also had fibroid. Groups III and IV were continuously ingested with the same dose of MSG for the next 28 days with daily oral treatment using 250mg/kg body weight aqueous extracts of turmeric and ginger, respectively. Histological examinations were performed on the 2nd and 4th weeks, respectively, on two rats from each group for the next 28 days. Results showed nephrotoxic effects of MSG with endometrial degeneration. Group III rats gave mild histological textures of their tissues compared with Group IV rats. MSG ingestion is nephrotoxic but the use of turmeric aqueous extract alleviated this effect and could be used in fibroid of management.

Keywords: Leiomyoma, monosodium glutamate (MSG), *Curcuma longa* (turmeric), *Zingiber officinale* (ginger), uterus, kidneys.

INTRODUCTION

Use of man-made chemicals, which have become part of our everyday lives, is threatening human health. Some of these chemicals affect the endocrine system, interfering with human developmental processes (WHO/UNEP, 2013). Endocrine disruptors are exogenous compounds with the potential to disrupt normal oestrogenic functions (Fujisawa and Castellot, 2014). Some examples are monosodium glutamate (MSG) and polychlorinated biphenyls (PCB) (Hunter et al. 2000). Sharma et al. (2013) proposed a link between MSG consumption and uterine fibroid but this has not been scientifically established.

Uterine leiomyoma popularly known as uterine fibroids are the most prevalent medical problem of the female reproductive tract (Taylor et al. 2015). It afflicts more than 70% of reproductive-aged women (Levy et al. 2012) and black women have the highest prevalence of developing it than women of other races (Taylor et al. 2015).

Monosodium glutamate (MSG) has been shown to induce uterine fibroid in laboratory rats (Zia et al. 2014). In Nigeria, it is a popular food seasoning sold as Ajinomoto® and is in use as a bleaching agent for the removal of stains from clothes (Olugbenga et al. 2014).

Medicinal plants, have contributed immensely to the management and treatment of diseases. *Zingiber officinale* Roscoe (Ginger) has been found useful in pregnancy-related morning sickness (Shakya, 2015). *Curcuma longa* Linn (Turmeric) has anti-inflammatory and fertility properties (Labban, 2014). Despite these, their use in the management of uterine fibroid has not been documented.

This study aims to evaluate the option of

treating uterine fibroid with the use of locally available bulbs like turmeric and ginger while the objective is to assess the effects of the bulbs on fibroid induced albino Wistar rats' uterine and kidney cells.

MATERIALS AND METHODS

Plant Material and extraction:

This work was carried out at Biochemistry Department of Enugu State University of Science and Technology (ESUT), Agbani, Enugu State and Histopathology Laboratory of ESUT Teaching Hospital, Enugu, Enugu State.

Two plant materials used were ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*). The rhizomes were harvested young from a farm in Awgu LGA of Enugu State around 4.00pm and replanted in the Botanical Garden of the Faculty of Applied Natural Sciences, Enugu State University of Science and Technology. Both rhizomes were nurtured to maturity and later used for the experimental analysis. These rhizomes were identified by Professor J. C. Okafor, a Taxonomist with the Applied Biology and Biotechnology Department, Enugu State University of Science and Technology, Enugu State.

Extracts from these rhizomes were prepared according to the Soxhlet method described by Redfern et al. (2014).

Animal Studies

Experimental animals used for this research study were apparently healthy adult female Wistar albino rats of about 6 to 8 weeks old with average weight of 150 to 250g. Rats were confirmed as adults following the method described by Lenschow et al. (2017). All the rats were obtained from Faculty of Veterinary Sciences, University of Nigeria, Nsukka (UNN).

Preparation of Monosodium Glutamate (MSG) Solution

The solutions of the MSG given to the animals were prepared folowing the dissolution of a calculated volume of MSG in a warm water (MSG is sparingly soluble in cold water/water at room temperature but readily soluble in hot water).

Experimental Design

Leiomyoma induction and treatment

Twenty-eight (28) adult female albino Wistar rats were used in this study. Animals were acclimatized for two (2) weeks and divided into four (4) groups of seven (7) rats each. All animals were fed orally according to the methods described by Wheatley, (2002).

The negative control group received feed and water only. The positive control group received feed, water and 750mg/kgbw of MSG daily for twenty-eight (28) days. Group III rats (MSG+Tur) received feed, water and 750mg/kgbw of MSG daily for twenty-eight (28) days and later received food, water, 750mg/kgbw of MSG and 250mg/kgbw of aqueous extract of turmeric daily for another twenty-eight (28) days.

Group IV rats (MSG+Gin) had food, water and 750mg/kg/bw of MSG daily for first twenty-eight (28) days. In the second twenty-eight (28) days, they were treated with oral ingestion of 250mg/kgbw of aqueous extract of ginger (*Zingiber officinale*) while receiving food, water and 750mg/kgbw of MSG.

Leiomyoma was induced in rats in the positive control, Groups III,and IV following the initial administration of 750mg/kgbw of MSG daily for twenty-eight (28) days. Groups III and IV rats continued to receive MSG with the different extracts in the second twenty-eight (28) days as specified above according to the method described by Cheng et al. (2011).

Histopathology procedures carried out on the tissues was according to the method described by Slaoui and Fiette, (2011). Sections were stained according to Hematoxylin and Eosin (H&E) technique for general tissue structure. After staining the sections, the slide was mounted with DPX (Diphenyl Phthalate Xylene). Care was taken to avoid air bubbles while mounting the slide. The sections were examined after staining using Olympus Binocular Microscope with in-built lighting system. The sections were then photographed, by a professional histopathologist, using a AmScope Microscope Digital Camera (Model Mu500) attached to an eyepiece of the microscope.

RESULT AND DISCUSSION

Photomicrographs of tissues of negative control female albino Wistar rat compared with the positive control.

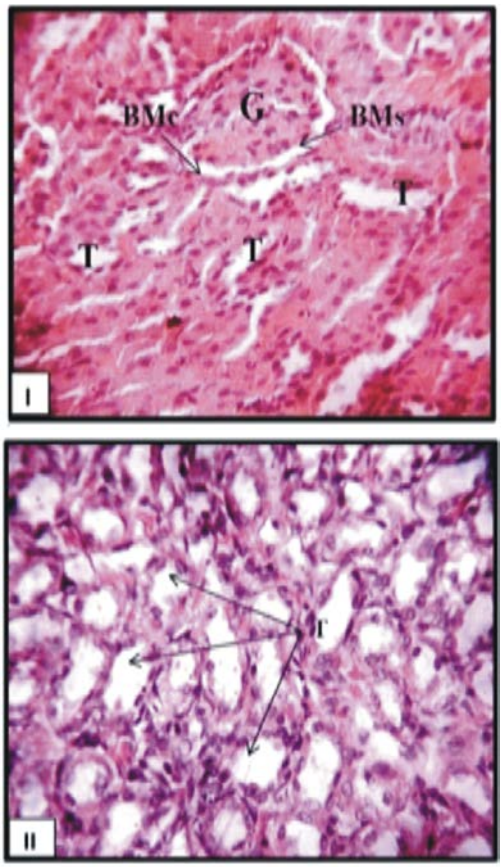

Plate 1: Kidney section photomicrographs from negative control rat showing normal histoarchitecture of the cortex (I) and medullary (II) regions. The glomerulus (G), cortical and medullary tubules (T), Bowman's capsule (BMc) and space (BMs) appear normal. (Stain: H&E; Mag: I&II-x400)

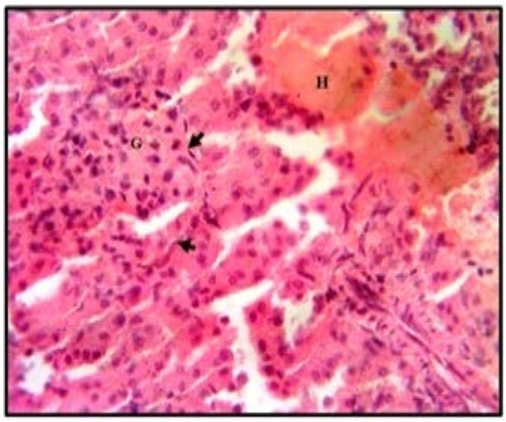

Plate 2: Kidney section photomicrograph from positive control rat after MSG intoxication without treatment. Adhesion of glomerulus (G) to Bowman's capsule (arrow heads) and evidence of haemorrhage (H) are noted. (Stain: H&E; Mag: - x400)

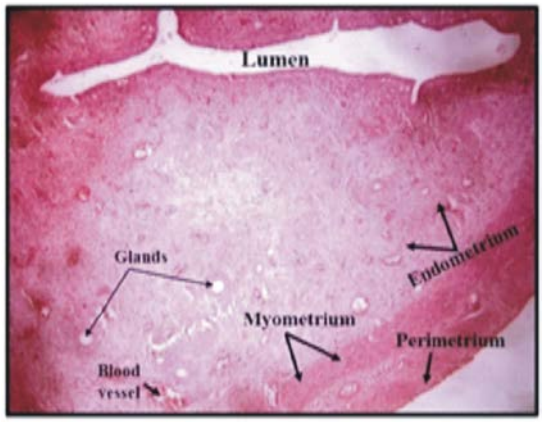

Plate 3: Uterus section photomicrograph from negative control rat showing normal histoarchitecture of the tissue. The perimetrium, myometrium, endometrium bearing the glands, and blood vessels, appear normal. (Stain: H&E; Mag: -x100)

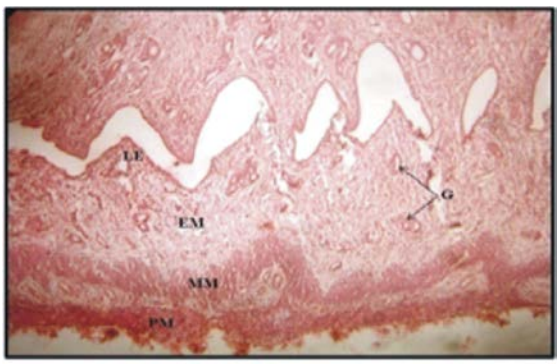

Plate 4: Uterus section photomicrograph from the same negative control rat showing evidence of a moderately preserved histomorphology. The luminal epithelium (LE), endometrium (EM) bearing the glands (G), myometrium (MM) and perimetrium (PM) show no observable abnormality.
(Stain: H&E; Mag: - x100)

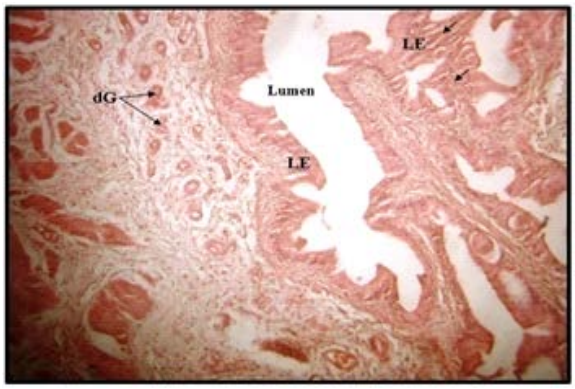

Plate 5: Uterus section photomicrograph from positive control rat shows evidence of hyperplasia of the luminal epithelium (LE), infiltration of inflammatory cells (arrows) and degeneration of endometrial glands (dG). (Stain: H&E; Mag: - x100).

Photomicrographs of tissues of Group IV female albino Wistar rats treated with aqueous extract of Ginger (*Zingiber officinale*) rhizome.

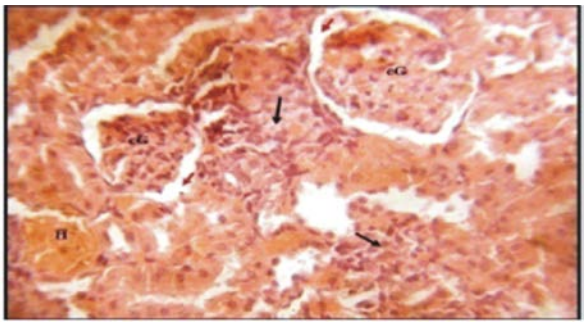

Plate 6: Kidney section photomicrograph from rat treated with 250mg/kgbw of *Ginger* (*Zingiber officinale*) for 2 weeks following MSG intoxication. Collapse of some glomeruli (cG) is noted with resultant increase in the bowman's capsular space (red arrow). Infiltrations of inflammatory cells are observed within some degenerating tubules (black arrows). Evidence of haemorrhage (H) is also noted. (Stain: H&E; Mag: - x400)

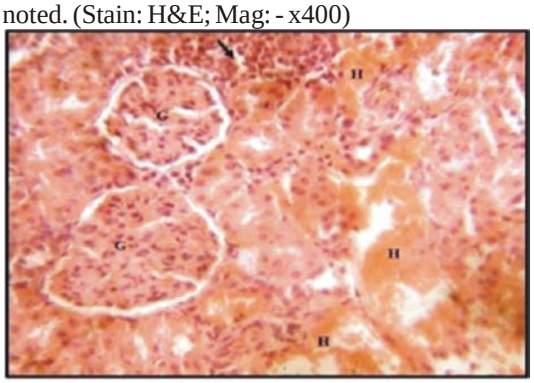

Plate 7: Kidney section photomicrograph from rat treated with 250mg/kgbw of *Ginger* (*Zingiber officinale*) for 4 weeks following MSG intoxication. Most glomeruli (G) appear intact. Mild cellular infiltration is observed within few degenerating tubules (arrow). Haemorrhage (H) is remarkably noted. (Stain: H&E; Mag: - x400)

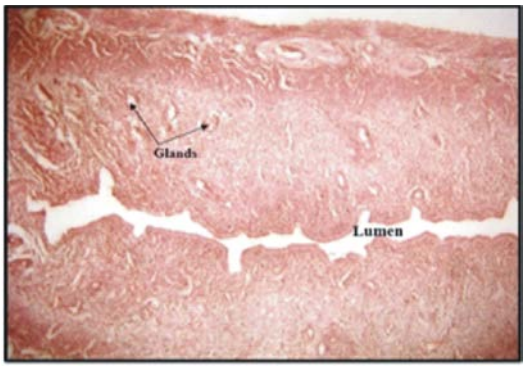

Plate 8: Uterus section photomicrograph from rat treated with 250mg/kgbw of ginger (*Zingiber officinale*) for two weeks following MSG intoxication. The tissue parenchyma shows no obvious tissue architectural alteration. (Stain: H&E; Mag: - x100)

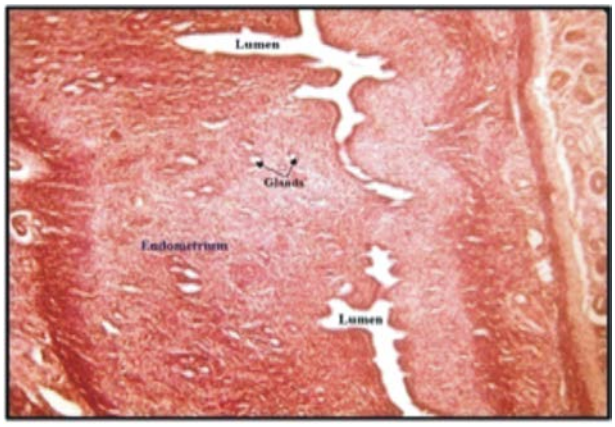

Plate 9: Uterus section photomicrograph from rat treated with 250mg/kgbw of ginger (*Zingiber officinale*) for four weeks following MSG intoxication. The histomorphology of the organ shows no observable changes. (Stain: H&E; Mag: - x100)

Photomicrographs of tissues of Group III female albino Wistar rats treated with aqueous extract of Turmeric (*Curcuma longa*) rhizome.

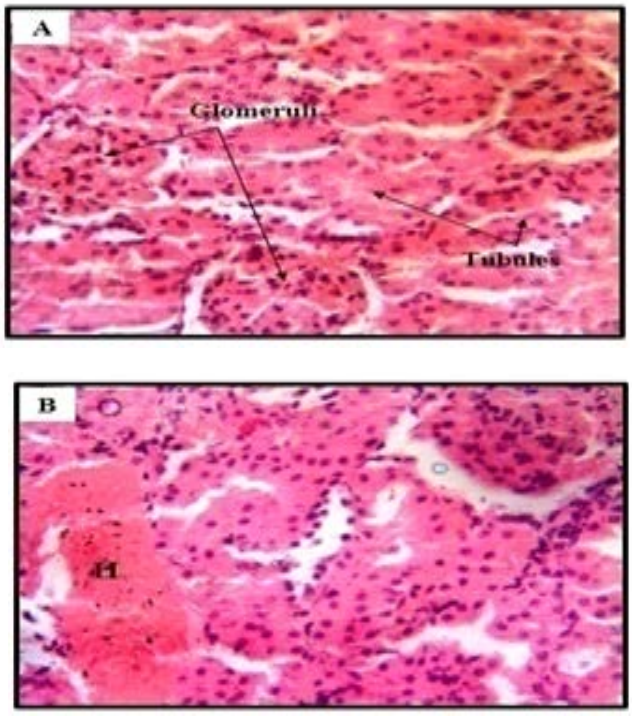

Plate 10: Kidney section (cortical region) photomicrographs from rat treated with 250mg/kgbw of Turmeric for two weeks following MSG intoxication. Some portions of the section appear fairly intact (A) whereas evidence of haemorrhage (H) is seen in few areas. The central vein and surrounding hepatocytes appear intact. (Stain: H&E; Mag: - x100)

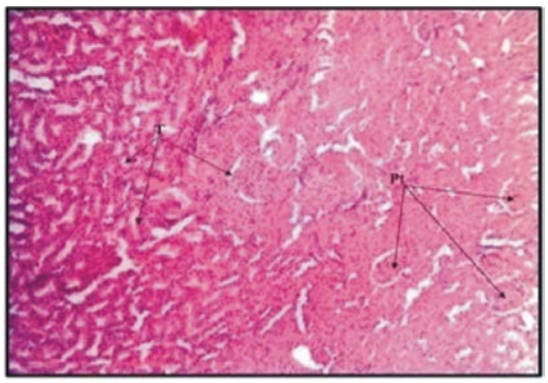

Plate 11: Kidney section photomicrograph of the cortico-medullary region from rat treated with 250mg/kgbw of Turmeric for four weeks following MSG intoxication. The glomeruli (G) and tubules (T) shown appear normal. (Stain: H&E; Mag: - x100)

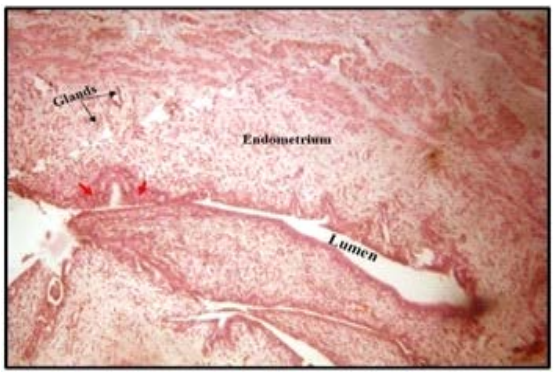

Plate 12: Uterus section photomicrograph from rat treated with 250mg/kgbw of turmeric for two weeks following MSG intoxication showing moderately preserved tissue morphology. The luminal epithelium show mild presence of cellular infiltrates. However, the endometrium bearing the glands show no obvious histopathological alteration (Stain: H&E; Mag: - x100)

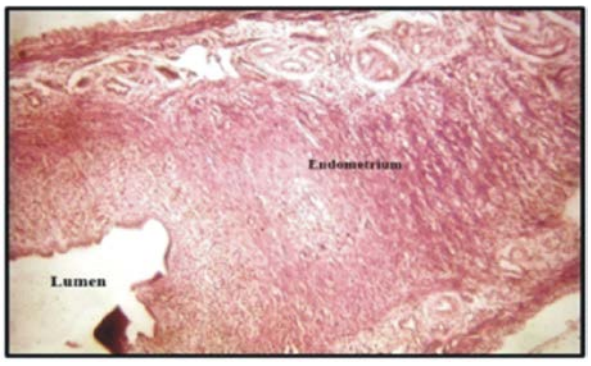

Plate 13: Uterus section photomicrograph from rat treated with 250mg/kgbw of turmeric for four weeks following MSG intoxication showing moderately preserved tissue morphology. The luminal epithelium and layers of the uterine tissue show no obvious histopathological alteration (Stain: H&E; Mag: - x100)

The lethal dose of MSG in humans is 1500mg per 100g (Freeman, 2006) compared to the effective dose (ED) of 750mg/kgbw in Wistar rats from this study. At this dose, MSG ingestion is toxic. This could be due to its oxidizing effect on internal organs and tissues.

Photomicrographs of kidney sections of negative control rat show normal histoarchitecture of the kidney cortex (Plate 1) and uterine sections of the same rat (Plates 3 and 4) show normal perimetrium, myometrium, and endometrium with no observable abnormality. The uterine section of the positive control rat (Plate 5) shows evidence of hyperplasia of the uterine epithelium unlike the negative control rat (Plates 3 and 4). Evidences of tissue degeneration seen in Plates 2 and 5 are indications of the oxidative powers of MSG. Kazmi et al. (2017) reported that MSG is toxic to hepatocytes; however this study shows it also causes degeneration of kidney cells and uterine walls (Plates 2 and 5).

Two weeks of MSG ingestion and treatment with *Z. officinale*, show higher evidences of inflammatory cellular infiltrations (Plate 6) (Tawfik and Al-Badr, 2012) compared to those treated with *C. longa* extracts (Plate 12). Also, at the fourth week following treatment with *Z. officinale*, which shows some histomorphological alterations (Plate 7), there were no observed histopathological alterations in rats treated with *C. longa* extracts (Plate 13). The kidney cells (Plates 6 and 7) were not spared since evidence of haemorrhage was noted at two and four weeks. These infiltrations are in conformity with the findings that MSG ingestion is the cause of oxidative stress which affects ovaries (Mustafa et al. 2015) and also kidneys. Though ginger extract preserved the endometrium at two (Plate 8) and four (Plate 9) weeks; this extract show a less protective effect than *C. longa* on rat's tissues.

At two (2) weeks, the uterine epithelium of Group III rat shows mild cellular infiltrates (Plate 12) compared to four week at which no histopathological alteration was observed (Plate 13). It indicates that treatment with aqueous extract of *C. longa* preserved the myometrium from oxidative damages as reported in rats in the positive control (Plate 5). Similar results are seen

in the kidney cells (Plates 10 and 11) where the surrounding hepatocytes appear normal.

Histopathology investigations revealed morphological damages (Plates 2 and 5) in examined tissues for each rat group compared to the negative control group (Plates 1, 3, and 4). And continuous use of these rhizomes at 250mg/kgbw, significantly reduced the oxidizing damages done to the uterine walls and kidney cells (Plates 6 to 13). Effect of turmeric (*Curcuma longa*) aqueous extract on these tissues (Plates 10 to 13) shows a better improvement when compared with that of the ginger extract, histologically (Plates 6 to 9). This could be due to the presence of curcumin a major component of turmeric. Curcumin has a hepatoprotective property (Labban, 2014), which is due to its ability to decrease the formation of proinflammatory cytokines, on tissues (Park et al. 2000).

CONCLUSION

Aqueous extracts of ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) can independently protect the uterine and nephritic cells of female rats induced with leiomyoma. However, at 250mg/kgbw, aqueous extract of *Curcuma longa* can offer a greater protection to the uterine myometrium of Wistar rats than *Zingiber officinale*.

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