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Preliminary investigation of oral lethal dose of monosodium glutamate that would induce leiomyoma in Wistar rats

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ABSTRACT

Uterine leiomyoma or fibroid is prevalent among black women of reproductive age. Known as an oestrogen factor disease, food flavouring agents like monosodium glutamate has been implicated in inducing fibroid. Monosodium glutamate (MSG) is a popular food flavouring agent and there is no regulation in its usage and consumption in Nigeria. This work investigated the lethal dose of MSG that could induce fibroid in adult female Wistar rats and effects of this chemical on uterus and liver of female albino Wistar rats. Twelve (12) adult female albino Wistar rats weighing between 150g – 250g were used for the work to determine the dose of monosodium glutamate (MSG) that would induce leiomyoma in rats. Animals were given unlimited access to food (poultry growers mash) and water and were housed in cages divided into six (6) different groups of two (2) rats each and allowed two weeks for acclimatization. Group one (1) rats received 100mg/kgbw, group two (2) was administered with 500mg/kgbw, group three (3) was administered with 800mg/kgbw, group four (4) rats were given 1200mg/kgbw while group five (5) rats received a 1500mg/kgbw of MSG, respectively. Group six (6) did not receive MSG and thus served as the negative control. The MSG was administered to the rats by oral gavage method. At the end of the administration, oestrogenic effect of the monosodium glutamate (MSG) was determined following the estimation of serum oestradiol levels of rats and 750mg/kgbw of MSG was chosen as the dose that could induce leiomyoma.

Keywords: Leiomyoma, fibroids, monosodium glutamate, Wistar rats, histology

INTRODUCTION

We live in a world in which man-made chemicals have become part of our everyday life. Some of these chemicals affect the endocrine system, interfering with the developmental processes of humans, pets, and wildlife species. A key concern is that human and wildlife health depends on the ability to reproduce and develop and this is not possible without a healthy endocrine system. As many endocrine-related diseases and disorders are on the increase, the World Health Organization (WHO) with the United Nations Environment Programme (UNEP) reported that close to 800 chemicals are known to be capable of interfering with hormone receptors, synthesis or conversion (WHO/UNEP, 2013). However,

only a small fraction of these chemicals known as endocrine disruptors have been investigated in tests capable of identifying overt endocrine effects in intact organisms (Bergman *et al.*, 2012).

Uterine leiomyoma or fibroids (Day Baird *et al.*, 2003), afflicts more than 70% of reproductive-aged black women (Eze-Steven, 2019). Hormonal factors play high roles in its development and progression (Kuffor *et al.*, 2013).

Oestrogen has been implicated in various uterine pathologies including leiomyoma (Donnez and Dolmans, 2016). The increased level of oestrogen is the most common cause of fibroid and painful menstruation (Bernard *et*

al., 2002) and the primary promoter of uterine leiomyoma growth (Martinez *et al.*, 2010).

Monosodium glutamate (MSG), a flavouring agent, is believed to be an oestrogenic agent that can induce uterine fibroid in laboratory rats by eliciting an increase in the levels of serum oestradiol (Zia *et al.*, 2014). This food flavouring agent is popular among Nigerians and is sold in Nigerian markets as Ajinomoto®.

Statement of the problem

There is a heavy intake of food seasonings, particularly MSG, among Africans. This intake has resulted to predisposing African women to development of fibroid (Stewart *et al.*, 2013) with high resultant impact in their reproductive life (Ekine *et al.*, 2015). This study seeks to understand the amount of MSG that would be deleterious to uterine walls while using laboratory rats as models.

Research Objectives

The objectives of this study are:

- (i) To establish the initiation of leiomyoma (uterine fibroid) in Wistar rats following the ingestion of monosodium glutamate (MSG).
- (ii) To assess the effect of MSG on fibroid induced albino Wistar rats' uterus, kidney, and liver cells.

Hormones

Hormones regulate human and animal cells. They modify biological processes and are therefore essential for reproduction, survival and biological performance (Wuttke *et al.*, 2010). Meanwhile, biological and cellular functions of these hormones are disrupted by chemicals (EDCs). These EDCs are often taken up in diet (like the MSG) or drinks (example bisphenol A) (Ehrlich *et al.*, 2014). They can leak from polycarbonate plastics, bottles, and cans use to line food and water containers (Liao and Kannan 2011) or through inhalation of contaminated gases (e.g. cigarette smoke or dust) (Braun *et al.*, 2011; He *et al.*, 2009). Endocrine disruptors (EDs) with oestrogenic action have direct effects on genes where they cause DNA damage by promoting malignant differentiation of affected cells (Bolton and Thatcher, 2008).

Other endocrine disruptors include polychlorinated biphenyls (PCBs), dioxins, plastics, pesticides [dichlorodiphenyltrichloroethane (DDT), methoxychlor] and pharmaceutical agents [diethylstilbestrol (DES)]. Natural chemicals including phytoestrogens like genistein and

coumestrol and found in human and animal foods, can also act as endocrine disruptors (Dickerson and Gore, 2007). Results have shown that early puberty in girls, while contributed to by many factors including nutrition, stress, and ethnicity, may in part be due to exposures to oestrogenic EDCs (Mouritsen *et al.*, 2010; Biro *et al.*, 2013). Such oestrogenic compounds are also associated with uterine fibroids, ovarian dysfunction, and subfertility in humans and in animal models (Jefferson *et al.*, 2012).

Oestrogen has been implicated in the prevalence of uterine fibroid (De Vivo *et al.*, 2011). Factors that influence the overall exposure to oestrogen affect the incidence of developing uterine fibroids, consequently, exercise which decreases oestrogen concentration, slows its development (Cook and Walker, 2004). Oestrogen is carcinogenic according to animal studies (Cavalieri and Rogan, 2014) and the International Agency for Research on Cancer (IARC, 2007). It promotes cell proliferation, which may increase the likelihood of spontaneous mutations caused by errors during DNA replication. Once these mutations are present within the genome, continued replication would create clones of the mutations or perhaps more mutations. If these errors are uncorrected (either through repair or apoptosis) they can lead to a carcinogenic phenotype.

Oestrogenic hormones exert their actions when bound to oestrogen receptors (ERs). Endocrine disruptors bind to these ERs altering the functions of normal oestrogen. And because EDCs are not natural hormones, a single EDC may have the ability to affect multiple hormonal signalling pathways. Thus, it is quite likely that one type of EDC can disrupt two, three, or more endocrine functions, with widespread consequences on the biological processes that are controlled by those vulnerable endocrine glands.

Uterine Leiomyoma

Leiomyomas are benign tumours (Eze-Steven, 2019) composed of disordered smooth muscle cells (Bowden *et al.*, 2009) resulting in excessive uterine bleeding, anaemia, defective implantation of an embryo, recurrent pregnancy loss, preterm labour, obstruction of labour, pelvic discomfort, and may mask malignant tumours (Catherino *et al.*, 2011).

Risk Factors of Leiomyoma

Diet is another factor with a link to the development of uterine fibroid. There is a correlation between the consumption of beef, and other red meat, with the development of fibroid while a high intake of green vegetables has a protective effect (He *et al.*, 2013). Meanwhile, some plants like soy and flax which are rich in phytoestrogens are known to prevent the development of fibroid. Phytoestrogens are diphenolic compounds that become converted into oestrogenic substances in the gastrointestinal tract (Ginsburg and Prelevic, 2000). These compounds act as "natural" selective oestrogen receptor (ER) modulators (SERMs, such as tamoxifen) (Nikov *et al.*, 2000).

Oestrogen Metabolism

Oestrogen is a steroid hormone mainly produced by the ovaries prior to menopause. Its regulation, biosynthesis, and secretion are controlled by gonadotropins (Goldfien, 2001). Circulating oestrogens in the body are primarily bound to sex hormone binding globulin and albumen. The unbound oestrogens enter target-tissue cells and induce biological activity (Goldfien, 2001). Oestrogen gains entrance into cells through passive diffusion. Once inside the cell, it binds nuclear oestrogen receptors (ER). Two forms of the oestrogen receptor are ER α and ER β and these differ in their distribution, binding affinity, and biological function (Yasar *et al.*, 2017). The ratio of expression of the two receptor types determines the biological response to oestrogen. The ER is primarily an intranuclear binding protein (Hye-Rim *et al.*, 2012), but it can shuttle between the nucleus and cytoplasmic compartments (Maruvada *et al.*, 2003). Once oestrogen binds to the ER, the ER changes its conformation, allowing it to activate the transcription of oestrogen-responsive genes (Hye-Rim *et al.*, 2012). Overall, oestrogen receptor levels are higher in leiomyomata tissue than in homologous myometrium (Yasar *et al.*, 2017).

Monosodium Glutamate

Monosodium glutamate (MSG) is a salt of glutamate and used as a flavour enhancer in foods (Wijayasekara and Wansapala, 2017). It produces a flavour that cannot be provided by other foods – a taste described, in Japanese, as umami, which is translated to “savoury” (Husarova and Ostatnikova, 2013). 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 (Kurihara, 2009).

Monosodium glutamate has been proved to be toxic for both humans and experimental animals (Tawfik and Al-Badr, 2012). Its prolonged consumption causes a number of toxic effects, referred to as the Chinese Restaurant Syndrome in humans (Jinap and Hajeb, 2010). Results from animal studies have reported a decrease in the red blood cells count, haemoglobin concentration, and packed cell volume (PCV) of animals given monosodium glutamate (Al-Mousawi, 2017).

Materials and Methods

The study was carried out at the Uterine Leiomyoma and Nutrigenomics Research Laboratory at the Enugu State University of Science and Technology (ESUT), Agbani, Enugu State Nigeria.

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 the animal house of the Faculty of Veterinary Sciences, University of Nigeria, Nsukka (UNN).

Preparation of Monosodium Glutamate (MSG) Solution

The respective solutions of the MSG given to the various animals were prepared following 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).

Determination of Monosodium glutamate (MSG) Lethal Dose

Twelve (12) adult female albino Wistar rats weighing between 150g – 250g were used to determine the lethal dose of monosodium glutamate (MSG) that would induce leiomyoma in rats and lasted for twenty-eight (28) days. Animals were given unlimited access to food (poultry growers mash) and water. These animals, housed in cages were divided into six (6) different groups of two (2) rats each and allowed two (2) weeks for acclimatization. Group one (1) rats received 100mg/kgbw, group two (2) was administered with 500mg/kgbw, group three (3) was

administered with 800mg/kgbw, group four (4) rats were given 1200mg/kgbw while group five (5) rats received a 1500mg/kgbw of MSG, respectively. Group six (6) did not receive MSG and thus served as the negative control. The MSG was administered to the rats by oral gavage method (Wheatley, 2002). At the end of twenty-eight (28) days administration, the oestrogenic effect of the monosodium glutamate (MSG) was investigated by the estimation of serum oestradiol levels of rats and 750mg/kgbw of MSG was chosen as the dose that could induce leiomyoma.

Animal Sacrifice and Samples Collection

After the MSG administration, test animals were anaesthetized according to the method described by Close *et al.*, (1997) and their organs eviscerated. Histology investigations were performed on rats eviscerated organs to determine fibroid induction. The eviscerated rat’s uteri were harvested and trimmed of extraneous materials and preserved in 10% formalin and used for the histological studies.

Assay Method

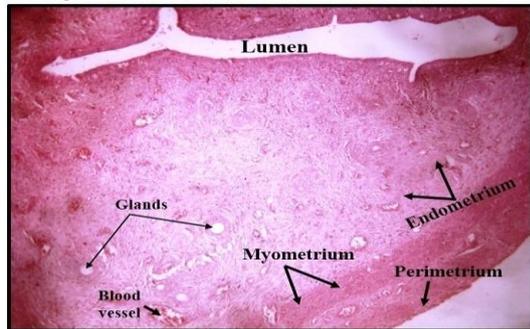


Plate 1: Uterus section photomicrograph from Group 6 rat (Negative Control) showing normal histoarchitecture of the tissue. The perimetrium, myometrium, endometrium bearing the glands, and blood vessels, appear normal. (Stain: H&E; Mag: -x100)

Histopathology Technique

Histopathology procedures were carried out on rat’s uteri using the method described by Slaoui and Fiette, (2011).

Results

Table 1: Oestradiol levels of rats given various doses of monosodium glutamate (MSG) per kilogram body weight

Dose (mg/kg)	Animal Group	Absorbance	Concentration (pg/ml)
100	1	2.955	0.6
500	2	2.963	1.2
800	3	2.951	1.4
1200	4	2.978	1.5
1500	5	NA	NA

Key: NA = Not Available.

Histopathology Investigations



Plate 2: Uterus section photomicrograph from another Group 6 rat (Negative Control) 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 3: Uterus section photomicrograph from Group 5 rat after ingestion of 1500mg/kgbw of MSG. There is evidence of hyperplasia of the luminal epithelium (LE), infiltration of inflammatory cells (arrows) and degeneration of endometrial glands (dG). (Stain: H&E; Mag: - x100)

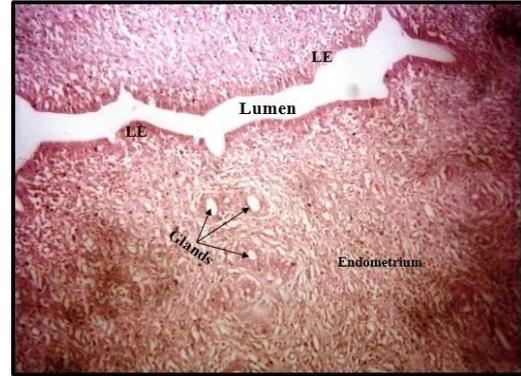


Plate 5: Uterus section photomicrograph from Group 1 rat treated with 1000mg/kgbw of MSG. Mild cellular infiltration is observed within the endometrium. However, the endometrial glands and luminal epithelium (LE) appear fairly intact. (Stain: H&E; Mag: - x200)

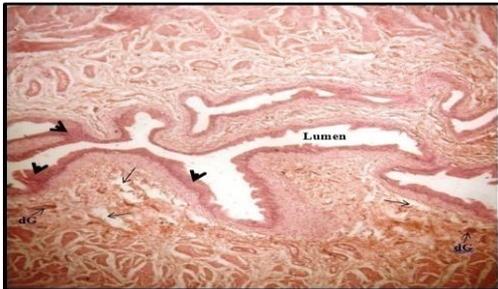


Plate 4: Uterus section photomicrograph from Group 1 rat ingested with 100mg/kgbw of MSG. Histoarchitectural alteration (arrows) is observed at the apical areas of the endometrium extending to the luminal epithelia, and some of the glands appear to be degenerating (dG). Mild hyperplasia (arrow heads) is also noted in the luminal epithelium. (Stain: H&E; Mag: - x100)

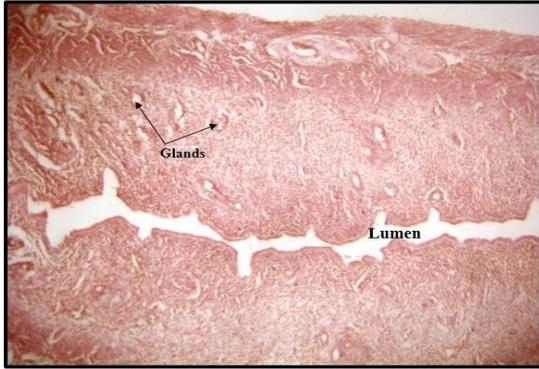


Plate 6: Uterus section photomicrograph from Group 2 rat ingested with 500mg/kgbw of MSG. The tissue parenchyma shows no obvious tissue architectural alteration. (Stain: H&E; Mag: - x100)

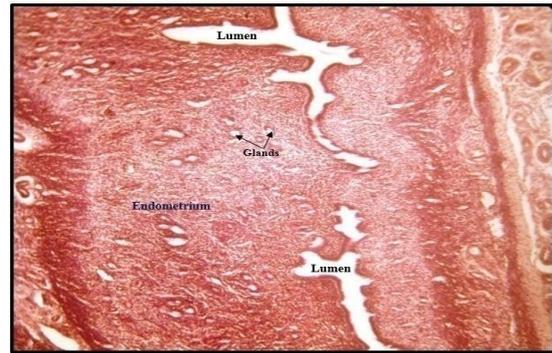


Plate 7: Uterus section photomicrograph from Group 2 rat ingested with 500mg/kgbw of MSG. The histomorphology of the organ shows no observable changes. (Stain: H&E; Mag: - x100)

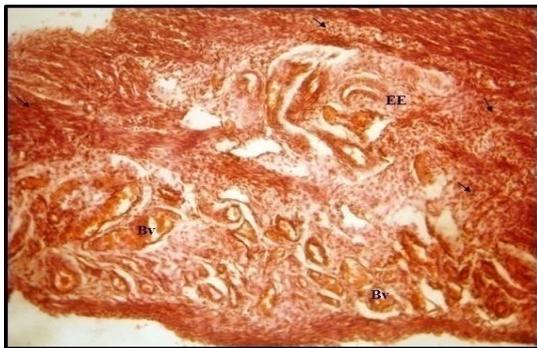


Plate 8: Uterus section photomicrograph from Group 3 rat ingested with 800mg/kgbw of MSG showing markedly degenerated endometrial epithelium (EE) and inflammatory cellular infiltration (arrows). Most blood vessels (Bv) at the basal region appear congested. (Stain: H&E; Mag: - x100)

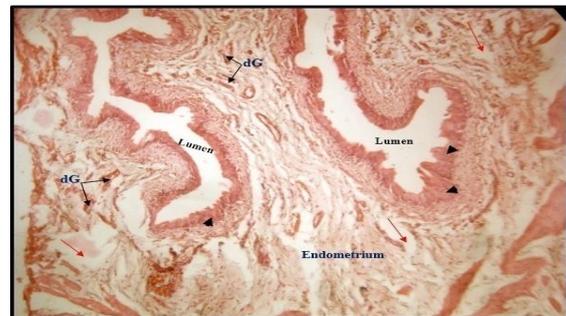


Plate 9: Uterus section photomicrograph from Group 4 rat ingested with 1200mg/kgbw of MSG. The intervening stroma of the endometrium appears degenerated (red arrows), and degenerating glands (dG) are also noted. Mild hyperplasia of the luminal epithelium is also noted (arrow heads). (Stain: H&E; Mag: - x100)

Discussion

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 gave a hepatotoxic and nephrodestructive properties. This could be due to its oxidizing effect on rat's uterine walls. These oxidative damages support the findings indicating the hepatotoxic effects of MSG (Kazmi *et al.*, 2017).

Histopathology investigations revealed morphological damages (Plates 3, 4, 5, 6, 8 and 9) in rat's uterus when compared to the negative control group (Plates 1). The

ingestion of monosodium glutamate (MSG) at 750mg/kgbw has a deleterious effect on the uteri as seen in Plates 3, 4, 5, 6, 8, and 9.

Conclusion

The work indicates that oral intake of monosodium glutamate (MSG) is potentially harmful to the uterine walls of Wistar albino rats when given at a dose beyond 700mg/kg body weight.

Recommendation

It is recommended that this result, when extrapolated to humans, will serve as template

for the regulated ingestion of MSG or its addition to foods as a seasoning agent.

Contribution to Knowledge

1. Monosodium glutamate is an oxidizing agent and its usage as food additive should be discouraged.

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