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**Original Research Article**

# Effect of Ethanolic Extract of Breadfruit Seeds on Monosodium Glutamate Induced Uterine Fibroids in Wistar Rats

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**Abstract:** Uterine fibroids (also called leiomyomas) are benign growths made of the uterine smooth muscle. It is a common problem that causes significant health issues in women of reproductive age. Monosodium Glutamate (MSG), a sodium salt of glutamine acid, found in processed foods like instant noodles, canned meats, etc. Breadfruit (Artocarpus altilis) a fruit with numerous benefits, which is rich in nutrients, fiber, anti-inflammatory properties, anti-microbial properties, and rich in anti-oxidants. Forty-eight (48) animals were used for this study. The animals weighed 120 ± 20g. Animals were grouped into eight (8) groups: Control group- received 0.5mls of distilled water orally for 2 weeks; Fibroid Induced group- received 200mg/kg of MSG intraperitoneally for 2 weeks; Breadfruit low dose group- received 500mg/kg of breadfruit extract orally for 2 weeks; Breadfruit high dose group- received 1500mg/kg of breadfruit extract orally for 2 weeks; MSG + Low dose group- received 200mg/kg of MSG intraperitoneally for 2 weeks + 500mg/kg of breadfruit extract orally for 2 weeks; MSG + High dose group- received 200mg/kg of MSG intraperitoneally for 2 weeks +1500mg/kg of breadfruit extract orally for 2 weeks; Low dose + MSG group- received 500mg/kg of breadfruit extract orally for 2 weeks + 200mg/kg of MSG intraperitoneally for 2 weeks; High dose + MSG group- received 1500mg/kg of breadfruit extract orally for 2 weeks +200mg/kg of MSG intraperitoneally for 2 weeks. The animals were sacrificed 24 hours after the last administration using chloroform. Blood samples were collected for hormonal assay. The uterus was harvested and fixed. Data obtained was expressed as mean ± SD, values were considered statistically significant when P≤0.05. From the therapeutic study, the results showed that the extract reduced the estrogen and progesterone level. From the prophylactic study, the results showed that the estrogen level reduced significantly. Statistically significant difference was not observed in progesterone level. Histological section showed simple cuboidal epithelium in the Fibroid induced group, therapeutic low and high groups and prophylactic low and high groups. In the control group the epithelium was simple columnar. The extract may likely reduce the size of the tumor and inhibit its development. It may also inhibit cell proliferation of uterine fibroids in Wistar rats. Histological studies indicated that the ethanolic extract of breadfruit is not cytotoxic.

**Keywords:** Uterine Fibroid, Monosodium Glutamate, Breadfruit seeds, Intraperitoneal.

## INTRODUCTION

Uterine fibroids, known medically as leiomyomas, are the most common benign tumors, affecting the female reproductive system, impacting a significant proportion of women during their reproductive years (Yang *et al.,* 2022). Emerging from the smooth muscle cells of the uterus, these tumors exhibit diversity in size, number, and location within the uterine wall (Williams, and Chennathukuzhi, 2017). While some individuals with fibroids may remain asymptomatic, others may experience pelvic discomfort, excessive menstrual bleeding, and disruptions in reproductive function (Marsh *et al.,* 2018). By the age of 50, an estimated 70% of women will develop one or more uterine fibroid, with approximately 30% experiencing symptoms requiring treatment. Notably, fibroids are more prevalent and tend to develop at an earlier stage in women of African descent (Stewart *et al*., 2017). By age 35, around 60% 0f African- American Women are expected to have fibroid compared to 40% of Caulcasian women of same age. Additional risk factors include age, nulliparity, genetic pre-deposition, early menarchy, caffeine and alcohol consumption, obesity and hypertension (Donnez & Dolmano, 2016). Some risk factors associated with the development of uterine fibroids are modifiable. Fibroids are more common in obese women. Fibroids are dependent on estrogen and progesterone to grow and therefore relevant only during the reproductive years (Parazzini *et al*., 2015).

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Studies show that fibroids tend to grow when hormone levels are higher (like during pregnancy) and shrink when hormone levels are low (like during transition to menopause). Symptoms can include heavy and prolonged periods, bleeding between periods, pressure in the abdomen and pelvic pain (Munro *et al.,* 2018). Some researchers have been trying to employ the anti-inflammatory, and antioxidant properties of the plant extracts to treat hormonal imbalance problems of which uterine fibroid is one of them. Most drug used to reduce the volume or size of uterine fibroids have side effects. Breadfruit extract, known for its antioxidant and anti-inflammatory properties, presents a promising result for the inhibition of the development of uterine fibroids. This study seeks to contribute to the development of supplements for prevention of developing uterine fibroids.

This study seeks to provide the general public with plant extracts that are easily accessible, and are capable of reducing the volume and size of uterine fibroid with little or no effect.

Histologically, they consist of large amounts of extracellular matrix that contain collagen, fibronectin, and proteoglycan. Leiomyoma n the uterus consists of whorled/ fascicular patterns of bland smooth muscle fibers 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 (ECM) in uterine fibroids. The smooth muscle cells in uterine fibroids are elongated with eosinophilic or occasional fibrillar cytoplasm and district cell membrane also they contain fairly large and conspicuous nuclei (Olaranwaju *et al*., 2017)

## MATERIALS & METHOD

Forty-eight (48) albino rats weighing 1600g ± 20g were used for experimentation. The rats were sourced from the Animal House of the Department of Animal and Environmental Biology, Rivers State University. Animals were housed in plastic cages with wire gauze in a well-ventilated animal house with 12 hours of natural light and 12 hours of darkness.

Animals had access to commercial feeds and clean water and libitum. To ensure adaptation to their new environment, a two-week acclimatization period was provided before the commencement of the study.

### Collection and Identification of Plant Material

Breadfruits seeds were obtained from Rivers state University farm. A voucher sample was deposited in the herbarium located in the Department of Plant Science and Biotechnology of the Rivers State University for proper identification. Subsequently, the plant was identified by a botanist and assigned a voucher number: RSU PbH 0130. The remaining samples were washed and air dried for twenty two days. The dried seeds ere pulverized into fine powder using electric blender.

### Ethical Clearance

Ethical clearance was obtained from Research and Ethics Committee of the Faculty of Basic Medical Sciences, Rivers State University, Nkpolu-Oroworukwo, Rivers State.

### Extraction of Breadfruit Seed

The breadfruit was washed and cracked open to remove the seeds. The fine powders of the breadfruit were soaked in 80 % ethanol solvent (ratio 40g of fine powders of the premature plantain fruit: 250 ml of ethanol). In order to achieve maximum extraction, the mixture was agitated periodically. The solution was filtered using Whatman No. I filter paper after 72 hours. The filtrate was concentrated using water bath at a temperature of 42 $^{0}$ C.

**Fig. 1: Diagram of Breadfruit Seeds**

**Animal Grouping / Administration**

**Table 1: Showing the Animal Grouping/Administration**

| Group (No of Animals) | Treatment Schedule | Route of Administration |
|---|---|---|
| Group A | Distilled Water(0.5mls) | Oral |
| Group B | Animals were treated with 200 mg/kg of Monosodium Glutamate (MSG) intraperitoneally for 2 weeks | Intraperitoneal |
| Group C | Animals received Low dose extract (500mg/kgbw) for 2 weeks | Oral |
| Group D | Animals received High dose extract (1500mg/kgbw) for 2 weeks | Oral |
| Group E | Animals received 200 mg /kg of Monosodium Glutamate + low dose extract (500mg/kgbw) for 2 weeks (Therapeutic I) | Oral |
| Group F | Animals received 200mg/kg of Monosodium Glutamate+ high dose extract (1500mg/kgbw) for 2 weeks (Therapeutic II) | Oral |
| Group G | Animals received low dose breadfruit extract (500mg/kgbw) + MSG (200mg/kg) for 2 weeks (Prophylactic I) | Oral |
| Group H | Animals received high dose breadfruit extract (1500mg/kgbw) + MSG (200mg/kg) for 2 weeks (Prophylactic II) | Oral |

**Table 2: Therapeutic Effect of Ethanolic Extract of Breadfruit Seeds on Estrogen Levels**

| | Mean ± SD (pg/ml) | F Value | P Value |
|---|---|---|---|
| Control (NI) | 44.50 ± 3.54 | 72.96 | 0.01 |
| Fibroid Induced (FI) | 78.50 ± 4.95* | | |
| Breadfruit Low (BL) (500mg/kgbw) | 57.50 ± 3.54[a] | | |
| Breadfruit High (BH) (1500mg/kgbw) | 63.00 ± 2.83[a] | | |
| MSG + Extract Low (500mg/kgbw) | 69.00 ± 5.66[a] | | |
| MSG + Extract High (1500mg/kgbw) | 71.50 ± 2.12[a] | | |

**Table 3: Therapeutic Effect of Ethanolic Extract of Breadfruit Seeds on Progesterone Levels**

| | Mean ± SD (ng/ml) | F Value | P Value |
|---|---|---|---|
| Control (NI) | 8.35 ± 0.49 | 79.50 | 0.01 |
| Fibroid Induced (NI) | 23.85 ± 0.49* | | |
| Breadfruit Low (BL) (500mg/kg bw) | 18.45 ± 0.35[a] | | |
| Breadfruit High (BH) (1500mg/kgbw) | 19.40 ± 0.57[a] | | |
| MSG + Extract Low (500mg/kgbw) | 20.65 ± 0.92[a] | | |
| MSG + Extract High (1500mg/kgbw) | 20. 90 ± 0.28[a] | | |

**Table 4: Prophylactic Effect of Breadfruiton Estrogen Levelsin Prophylactic Studies**

| | Mean±SD (pg/ml) | F Value | P Value |
|---|---|---|---|
| Control (NI) | 44.50±3.54 | 47.356 | 0.01 |
| Fibroid Induced (FI) | 78.50±4.95* | | |
| Breadfruit Low 500mg/kgbw | 57.50±3.54a | | |
| Breadfruit High 1500mg/kgbw | 63.00±2.83a | | |
| Extract low + MSG 500mg/kgbw | 84.00±1.41a | | |
| Extract high + MSG 1500mg/kgbw | 87.00±10.61 | | |

**Table 5: Prophylactic Effect of Breadfruit on Progesterone Levels in Prophylactic Studies**

| | Mean ±SD (ng/ml) | F Value | P Value |
|---|---|---|---|
| Control (NI) | 8.35±0.49 | 48.068 | 0.01 |
| Fibroid Induced (FI) | 23.85±0.49* | | |
| Breadfruit Low 500mg/ kgbw | 18.45±0.35a | | |
| Breadfruit High 1500mg/kgbw | 19.40±0.57a | | |
| Extract low+ MSG 500mg/kgbw | 20.45±0.78 | | |
| Extract high+ MSG 1500mg/kgbw | 20.55±2.05 | | |

## RESULTS

The result from the present study showed that the progesterone level in Fibroid induced groups was significantly higher than that of the control group in both the therapeutic and prophylactic study. (Table 3 and Table 5 respectively). In Breadfruit low and high groups, in both the therapeutic and prophylactic studies, Progesterone levels were lower than that of the Fibroid induced groups (Table 3 and Table 5 respectively).

In the therapeutic (MSG + Extract) low and high groups, Estrogen level and Progesterone level was reduced (Table 2 and Table 3 respectively), when compared to the fibroid induced group. In prophylactic (Extract+ MSG) low and high groups, Estrogen increased significantly when compared to fibroid induced and Progesterone level was reduced (Table 4 and Table 5 respectively)

**Histological Study**

The result from the present study showed that In Fibroid induced group (Plate 2), the glands were lined by cuboidal epithelium. The epithelium lining of the control group (Plate 1) is simple columnar epithelium. In Breadfruit low and high groups (Plate 3 & 4), the endometrium was lined by simple columnar epithelium. In the treatment groups (Plate 5 & 6), the endometrium was lined by simple cuboidal epithelium. In the prophylactic groups (Plate 7 & 8), the stroma is normal. The normal endometrium is lined by simple cuboidal epithelium.

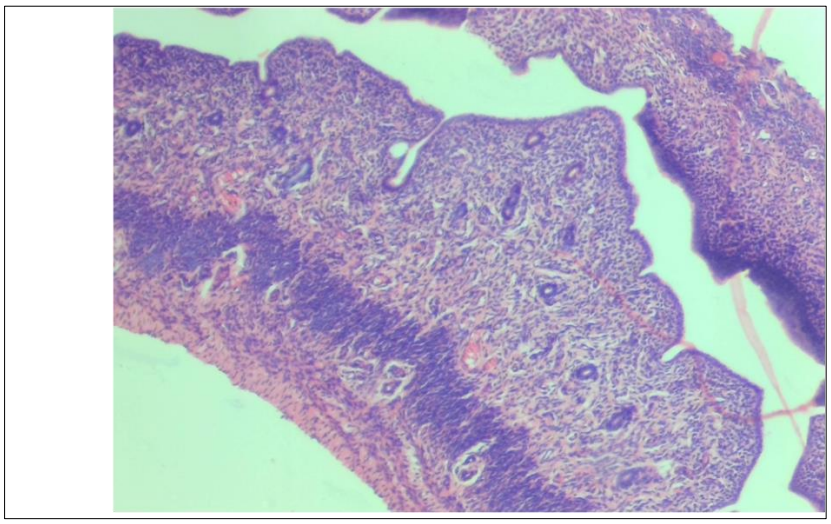

**Plate 1: Photomicrograph section of uterine tissue from animals in the control group (Group A). Section showed proliferative phase of gland with loose and wide endometrial cavity lined by simple columnar epithelium. H&E x 100**

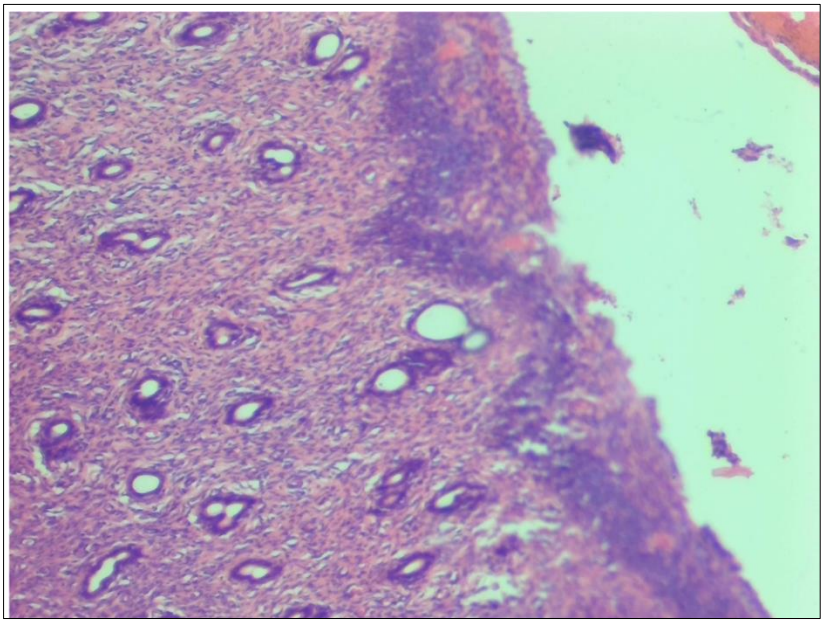

**Plate 2: Photomicrograph section of uterine tissue from animals that received 200mg/kg of MSG (Group B). Section showed normal tubular glands lined by cuboidal epithelium. H&E x 100**

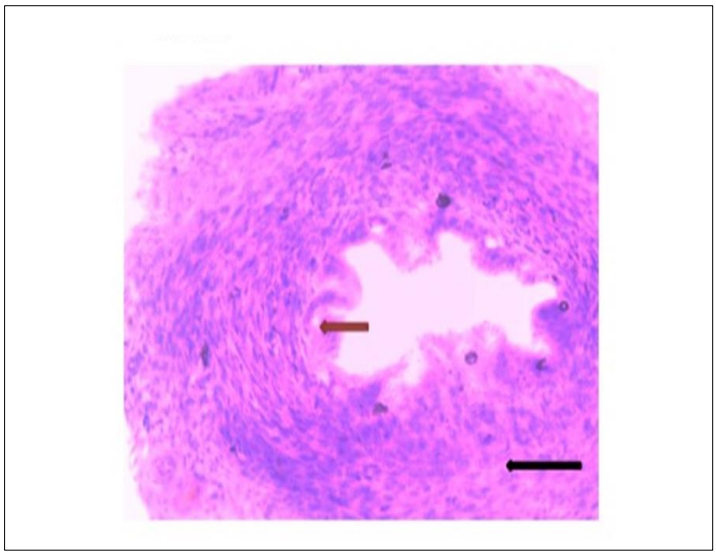

**Plate 3: Photomicrograph section of uterine tissue from animals in low dose breadfruit (500mg/kgbw) group (Group C). Section showed uterine glands in the proliferation on phase (Red arrow). Normal myometrium (black arrow) lined by simple columnar epithelium. H & E X 100**

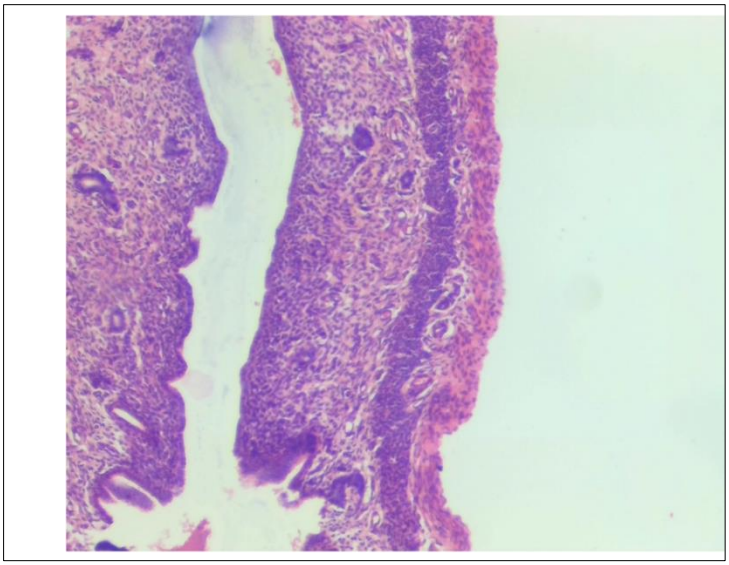

**Plate 4: Photomicrograph section of uterine tissue from animals in high dose breadfruit(1500mg/kgbw) group (Group D). Section showed normal uterus with loose stroma and widened endometrial cavity lined by simple columnar epithelium .H&E x 100.**

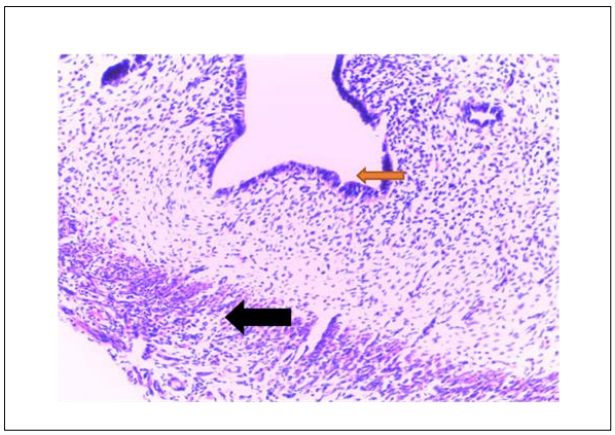

**Plate 5: Photomicrograph section of uterine tissue from animals which received MSG + low dose(500mg/kgbw) breadfruit extract (Group E). Section showed elongated and tubular uterine glands, normal myometrium. H&E x 100**

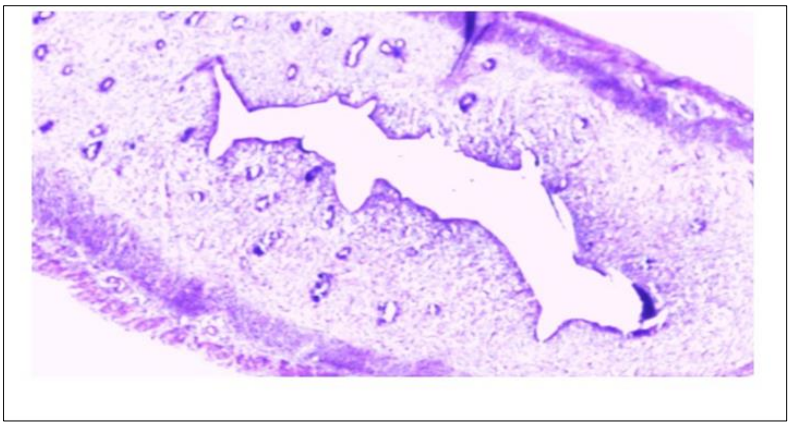

**Plate 6: Photomicrograph section of uterine tissue from animals which received MSG + high dose (1500mg/kgbw) breadfruit extract (Group F). Section showed normal myometrium and uterine glands. H&E x 100**

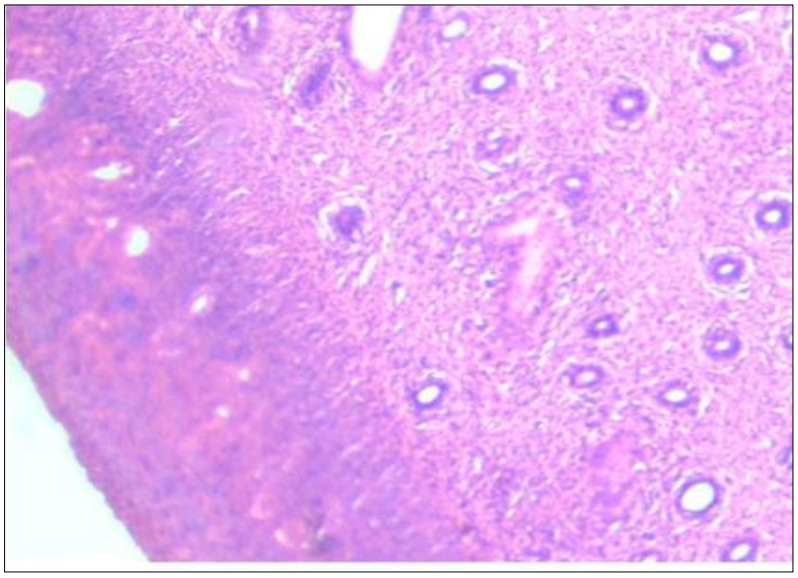

**Plate 7: Photomicrograph section of uterine tissue from animals in low dose breadfruit (500mg/kgbw) + MSG group (Group 5). Section showed normal tubular glands lined by cuboidal epithelial cells. The stroma is normal. H & E ×100**

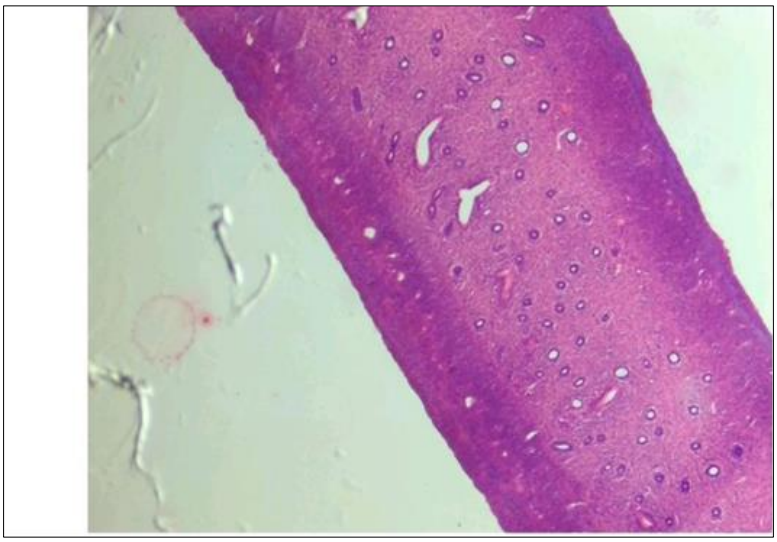

**Plate 8: Photomicrograph section of uterine tissue from animals in high dose breadfruit (1500mg/kgbw)+ MSG group (Group 6). Section showed normal endometrium and myometrium lined by cuboidal epithelium. H & E ×100**

## DISCUSSION

Estrogen and Progesterone have been reported to be important players in the growth and development of uterine fibroids. The serum levels of Estrogen and Progesterone increased in the group treated with Monosodium Glutamate. This implies that Monosodium glutamate increased the concentration of estrogen and progesterone. This agrees with Obochi *et al.,* (2009). They reported that Monosodium glutamate increases estrogen levels by activating aromatase, an enzyme that catalyzes estrogen (estradiol) synthesis by converting testosterone to β-estradiol and subsequent aromatisation of β-estradiol.

Treatment with Breadfruit at high and low doses, reduced the serum level of Estrogen and Progesterone. This implies that Breadfruit reversed the elevated level of Estrogen and Progesterone, thus may have protective effect against the development of uterine fibroids in rats. This agrees with the reports of Olowofalahan *et al*., (2017); Oyebode *et al*., (2019); Olanrewaju *et al*., (2017); Agbadu *et al.,* (2020). They reported that the protective effect of plants was also confirmed by a decrease in serum progesterone levels in the co-treated rats. Estradiol and progesterone are important in the transformation of myometrial cells into leiomyoma cells with progesterone required for the complete development and proliferation of leiomyoma cells.

## CONCLUSION

Ethanol extract of Breadfruit seed was therapeutically effective in reducing Estrogen and Progesterone levels, and prophylactically reduced Estrogen only. This extract may likely reduce the size of the tumor and inhibit its development. It may also inhibit the cell proliferation of uterine fibroids in Wistar rats. Histological studies indicated that the ethanolic extract of breadfruit seed is not cytotoxic.

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