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Original Research Article

Effects of Ethanolic Extract of Cocoa Seeds on Monosodium Glutamate Induced Uterine Fibroids in Wistar Rats

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Abstract: Fibroid is a reproductive health problem which affects females of reproductive age in all parts of the world. Various health implications associated with fibroid which ranges from miscarriages, infertility etc. The aim of the study is to investigate the effects of ethanolic extract of cocoa seeds on monosodium glutamate induced uterine fibroids in wistar rats. Forty eight (48) animals were used for the study. Animals were grouped into six(6) groups (control, fibroid induced, cocoa low, cocoa high, low dose+ MSG, high dose+ MSG group, MSG + high dose group and MSG + low dose) of 6 animals each. Extracts were administered for 14days, Control received 0.5 ml of distilled water; Fibroid induced received 200mg/kg bw of MSG; cocoa low group received 500mg/kg bw; cocoa high group received 1500mg/kg bw; cocoa low group +MSG received 500mg/kg bw+200mg/kg bw of MSG.; cocoa high group +MSG received 1500mg/kg bw + 200mg/kg bw of MSG, MSG + low dose group MSG was administered for 2 weeks, followed by 500mg/kg body weight of cocoa for 2 weeks. MSG + high dose group MSG was administered for weeks, followed by 1500mg/kg body weight of cocoa for 2 weeks. 24 hours after last administration animals were sacrificed. Blood samples were collected for hormonal assays. The uterus was harvested and fixed. Data obtained was expressed at Mean ± Standard deviation, values were considered statistically significant when $p \leq 0.05$. The results showed that ethanolic extract of cocoa seeds reduced the level of progesterone in the prophylactic groups when compared to the fibroid induced group. Results from the therapeutic studies showed that ethanolic extract of Cocoa seed reduced the level of progesterone only. It had no effect on the estrogen level of Wistar rats. The plant extract may arrest tumor progression and may be effective in prevention of MSG induced uterine fibroids in rats.

Keywords: Leiomyoma, Monosodium glutamate (MSG), Cocoa extract, Estrogen, Progesterone.

INTRODUCTION

Uterine fibroids, also known as leiomyomas or myomas, are benign smooth muscle tumors that develop within the uterus and represent the most common gynecological tumors among women of reproductive age. These tumors may occur in different anatomical locations within the uterus, including subserosal, intramural, and submucosal regions, and their clinical manifestations largely depend on their size and location. While some fibroids remain asymptomatic, others may lead to significant health complications such as heavy menstrual bleeding, pelvic pain, infertility, and recurrent pregnancy loss (Brosens *et al*., 2006; Wallach *et al*., 2004). The prevalence of uterine fibroids is estimated to range between 20% and 40% among women in their reproductive years, with a higher incidence reported among women of African descent. The etiology of uterine fibroids is multifactorial, involving hormonal, genetic, environmental, and lifestyle factors. Estrogen and progesterone are recognized as key regulators in fibroid development and growth. Studies have shown that fibroid tissues often exhibit higher concentrations of estrogen compared to adjacent normal myometrium, and they possess the ability to locally synthesize estrogen through aromatase activity, thereby promoting cellular proliferation (Wallach *et al*., 2004).

Monosodium glutamate (MSG), a widely used food additive known for enhancing flavor, has been implicated in various adverse health effects, particularly when consumed excessively. MSG is the sodium salt of glutamic acid and is commonly present in processed foods. Experimental studies have demonstrated that MSG can induce oxidative stress, disrupt hormonal balance, and cause tissue damage in several organs,

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including reproductive organs. In animal models, MSG exposure has been associated with increased levels of estrogen and other biochemical alterations that may contribute to the development of uterine fibroids (Ataseven *et al*., 2016; Zanfirescu *et al*., 2019). Oxidative stress plays a significant role in the pathogenesis of uterine fibroids. It occurs due to an imbalance between reactive oxygen species (ROS) and antioxidant defenses, leading to cellular damage and abnormal tissue growth. MSG-induced oxidative stress has been shown to promote lipid peroxidation, reduce antioxidant enzyme activity, and alter cellular homeostasis, thereby contributing to reproductive toxicity and fibroid formation (Sharma, 2015; Lushchak & Storey, 2021). Cocoa (Theobroma cacao) is a natural plant product widely consumed in various forms, particularly as cocoa powder and chocolate. It is rich in bioactive compounds such as flavonoids, polyphenols, and essential minerals, which possess potent antioxidant and anti-inflammatory properties. Cocoa has been reported to reduce oxidative stress by inhibiting inflammatory pathways and enhancing antioxidant defense mechanisms. These properties suggest that cocoa may have therapeutic potential in preventing or mitigating diseases associated with oxidative stress, including uterine fibroids.

Given the increasing prevalence of uterine fibroids and the limitations associated with current treatment options, there is a growing interest in exploring natural plant-based interventions. The potential protective effect of cocoa extract against MSG-induced uterine fibroids may be attributed to its antioxidant and anti-proliferative properties. Therefore, this study aims to investigate the prophylactic effects of ethanolic extract of cocoa seeds on monosodium glutamate-induced uterine fibroids in Wistar rats, with particular emphasis on hormonal regulation and oxidative stress modulation.

MATERIALS AND METHODS

Animal Procurement /Acclimatization

Forty eight (48) albino rats weighing 160+_20g were used for this experiment. These rats were purchased from the department of Animal and environmental biology, Rivers State University. Animals were housed in a plastic cage with wire gauze in a well ventilated house, within 12h of light and 12h of darkness.

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

Collection and Identification of Plant Material

Cocoa 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 0132. 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 the Research &Ethics committee of the Faculty Basic Medical Sciences, Rivers state University, Nkpolu-Oroworuko, Rivers state.

Extraction of Plant Material (Cocoa)

The cocoa was washed and cleaned to remove any dirt or debris on the surface. Following this, it was washed, air dried, cracked open to get the seeds. The cocoa was washed and cracked open to remove the seeds. The fine powders of the cocoa 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.

Determination of LD50

LD50 is the amount of substance that will cause mortality in half of the test population. Eniola *et al*., (2015) reported the LD50 value of cocoa seed as 5000mg/kg. 10% of the LD50 value was used as low dose and 30% as high dose.

Animal Grouping /Administration

Treatment Protocols

Group/Number of Animals	Treatment/Schedule	Rationale
Group A (6)	Placebo of Water	Control group
Group B (6)	Animals will be treated orally with 200mg/1kg MSG only, for 14 consecutive days, intraperitoneally	(Negative control group) To introduce Uterine fibroid
Group C (6)	Animals receiving low dose of cocoa extract only, for 14 consecutive days	(Positive control group) (I) A placebo for cocoa extract
Group D (6)	High dose cocoa for 14 days	Positive control (I)
Group E (6)	Low dose Extract for 14days + MSG interaperetoneally for 154days .	(Prophylactic 2) To prevent the onset of uterine fibroid
Group F (6)	Animal receiving 200mg/kg cocoa for 14days, oral administration 200mg/kg MSG for 14days.	Prophylactic group to prevent the onset of uterine fibroid

Group/Number of Animals	Treatment/Schedule	Rationale
Group G	200mg/kg of MSG for 14 days + high dose extract 1500mg/kg for 14 days (Therapeutic)	Oral
Group H	200mg/kg of MSG for 14 days + low dose extract 500mg/kg for 14 days (Therapeutic)	Oral

Duration of Studies

The study was conducted in the period of 28days and Animals were sacrificed 24 hours after the last administration.

Induction of Uterine Fibroid Using MSG

Uterine fibroid was used using the method described by oluwofolahan *et al*., 2018. Uterine fibroid will be induced by administering 200mg/kg of MSG daily for 15days intraperitoneally.

Histological Methods

i. **Fixation:** The organs were fixed with 10% formalin.
ii. **Dehydration:** Graded percentage of alcohol (ethanol) was used to dehydrate the tissues. These include 50%, 70%, 95% and absolute 1, 2 & 3.
iii. **Clearing:** The tissues were cleared in xylene 1, 2 and 3 and each lasted for one hour. iv. Impregnation/Infiltration: The tissues were impregnated in 60° molten paraffin wax 1 and 2 in the laboratory oven and each lasted for one hour.
iv. **Embedding:** Tissues were embedded in 70 molten paraffin wax using tissue cassettes and metal molds and allowed to block (solidify) immediately in the freezer.
v. **Sectioning:** Tissues were sectioned with a microtome using two microns. The ribbons will be spread on glass slides with 20% ethanol.

The ribbons floated in warm water (about 30°-40°) in a water bath.

Sectioned ribbons were picked later with properly labelled glass slides and placed on a Hot plate under temperature of 60°.

vi. **Dewaxing:** Sectioned tissue slides were dewaxed under 80° temperature in the laboratory oven.
vii. **Staining:** Dewaxed tissues slides were sent to water i.e., passing the dewaxed tissues from xylene to absolute ethanol to 95% to 70% to 50% and finally to water and allowed to bluing. Tissues slides will be stained in Cole hematoxylin for 10 minutes, rinsed in water and differentiated with 1% acid alcohol.

Then it was stained in 1% eosin for five minutes and rinsed in absolute ethanol. ix. Mounting: Slides were rinsed in xylene, mounted with Dibutylphthalate Polystyrene Xylene (DPX) mountant and then cover slipped.

Statistical Analysis

Data were expressed as mean + SEM. The mean difference between the tested groups and the control was tested using a One-Way ANOVA. Values were considered statistically significant when $p \leq 0.02$ and $p \leq 0.01$The significant difference was assessed using the LSD Posthoc test.

RESULTS

Results are represented in Tables and Plates.

Table 1: Effects of cocoa on estrogen level in the prophylactic studies

Groups	Mesn±SD	F. Value	P value
Control (NI)	44.50 ±3.54	32.404	0.02
Fibroid Induced(FI)	78.50±4.95		
Cocoa Low(500kg/bw)	71.50±9.19		
Cocoa High(1500kg/bw)	75.00±2.83		
Lowdose extract (500kg/bw)+ MSG	87.00±7.07		
High dose extract(1500kg/bw) + MSG	91.50±.6.36		

Table 2: Effects of cocoa on progesterone level in the prophylactic studies

Groups	Mean±SD	F- Value	p value
Control (NI)	8.35±0.49	59.231	0.01
Fibroid induced(FI)	23.85±0.49		
Cocoa Low(500kg/bw)	15.85±1.20		
Cocoa High (1500kg/bw)	13.15±2.33		
Low dose extract 500kg/bw+ MSG	14.25±3.89		
High dose extract 1500kg/bw +MSG	13±2.05		

Table 3: Effect of ethanolic extract of Cocoa seeds on Estrogen Level in therapeutic study

Groups	Mean± SD	F-Value	P Value
Control (NI)	44.50 ± 3.54	54.124	0.05
Fibroid induced (FI)	78.50 ± 4.95*		
Cocoa low (CL)	71.50 ± 9.19[a]		
Cocoa high (CH)	75.00 ± 2.83		
MSG + low dose extract	95.5 ± 3.54[a]		
MSG + high dose extract	98.5 ± 0.71[a]		

Values expressed as mean + SD
*= significantly different from control at P ≤ 0.05
a = significantly different from fibroid induced at P ≤ 0.05
Pg/ml- picogram per milliliter

Table 4: Effect of ethanolic extract of Cocoa seeds on progesterone level in therapeutic studies

Groups	Mean ± SD	F-Value	P value
Control (NI)	8.35 ± 0.49	87.8	0.05
Fibroid induced (FI)	23.85 ± 0.49*		
Cocoa low (CL)	15.85 ± 1.20[a]		
Cocoa high (CH)	13.15 ± 2.33[a]		
MSG + low dose extract	18.00 ± 1.27[a]		
MSG + high dose extract	16.00 ± 1.41[a]		

Values expressed as mean ‡ SD
*= significantly different from control at P ≤ 0.05
a = significantly different from fibroid induced at P ≤0
Ng/ml nanogram per milliliter

Histological Slide Results

Histological slides of animals in control group (group A), showed proliferation phase of glands with loose and wide endometrial cavity lined by columnar epithelium. H&Ex 100.

Animals that received 200mg/kg of MSG (group B) showed normal tubular glands lined by cuboidal epithelium. H&E x 100. Animals in low dose (500 mg/kgbw) Cocoa seeds group (group C) showed normal myometrium, swollen glands lined by columnar epithelium. H&E x 100. Animals in high dose (1500mg/kgbw) Cocoa seeds group (group D) showed columnar epithelium, normal endometrial glands and actively proliferating myometrium. H&Ex 100.

Animals treated with MSG + low dose cocoa extract showed uterine gland lined by simple cuboidal epithelium. Animals treated with AMSG + high dose extract showed straight tubular gland lined by cuboidal cell. The stroma is normal.

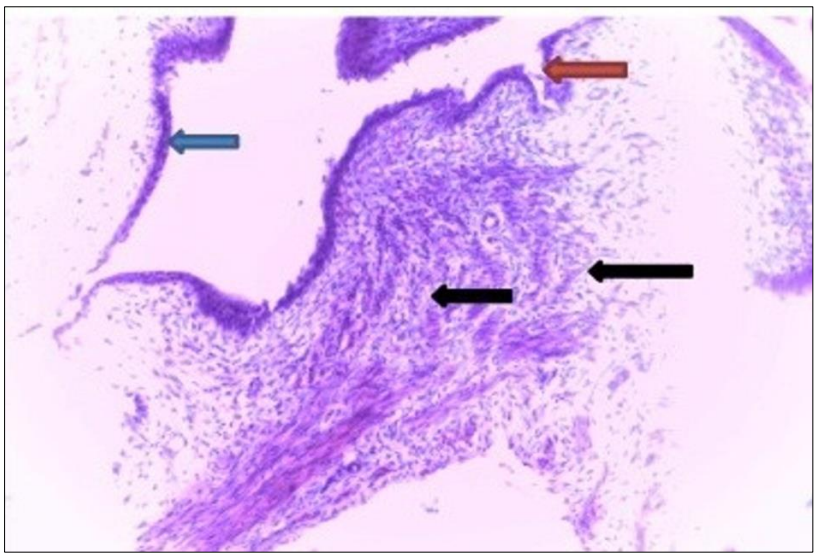
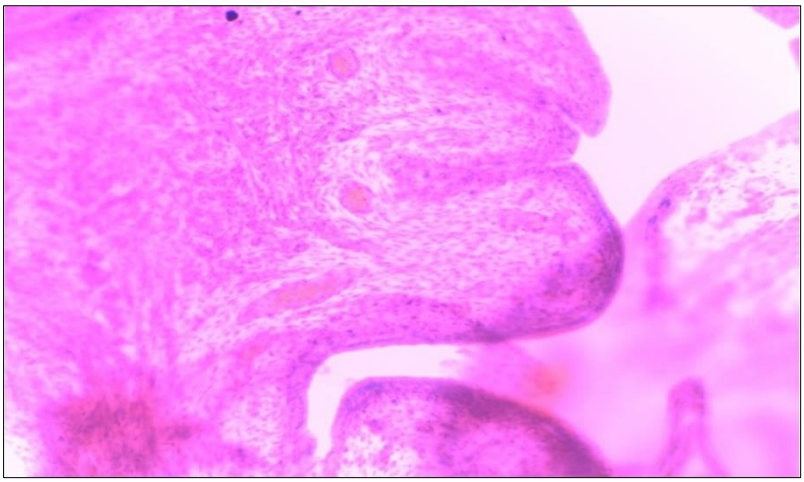


Plate 1: Photomicro graph section of uterine tissue from animals in the control group. Section showed proliferation phase of gland with loose & wide endometrial cavity lined by simple columnar epithelium. H&E × 100



Pate 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 × 100

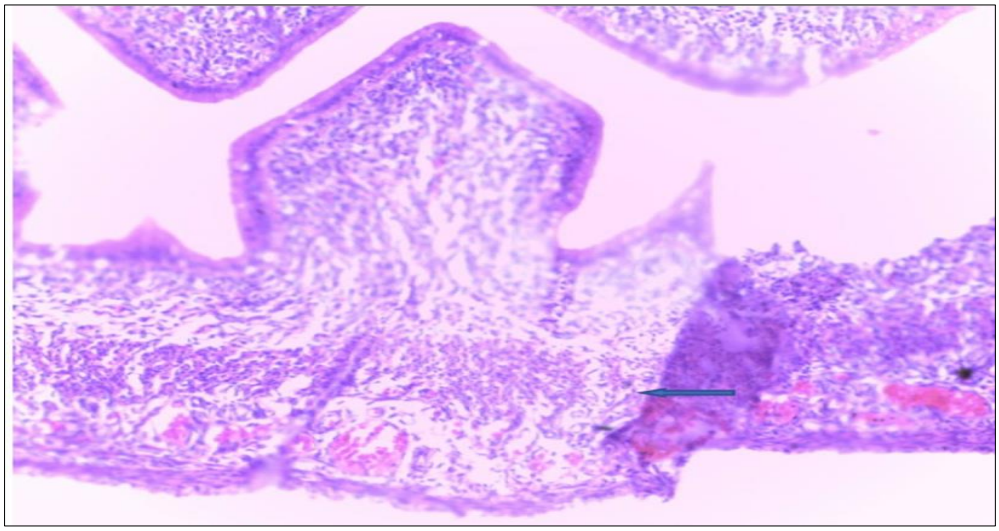


Plate 3: Photomicro graph section of uterine tissue from animals in low dose (500mg/kgbw) cocoa seeds group. Section showed normal myometrium, swollen glands lined by simple columnar epithelium. H&E × 100

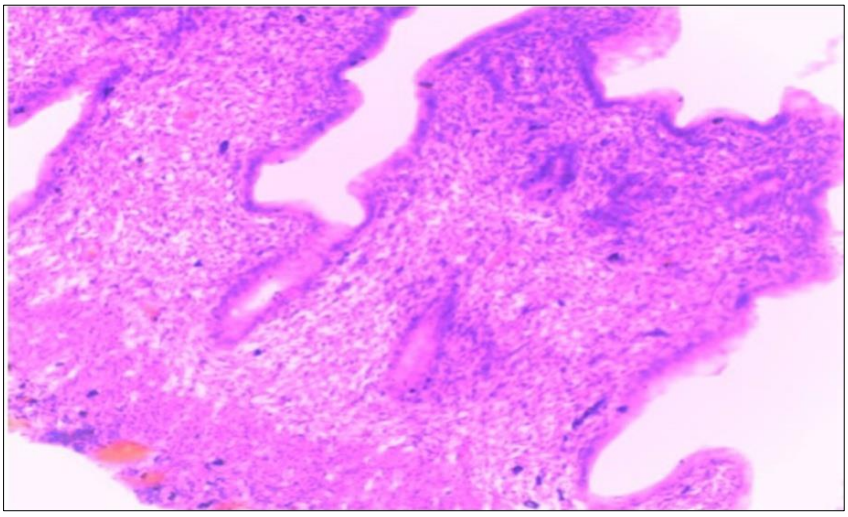


Plate 4: Photomicro graph section of uterine tissue from animals in high dose (1500mg/kgbw) cocoa seeds group. Section showed columnar epithelium, and normal endometrial glands actively proliferating myometrium. H&E × 100

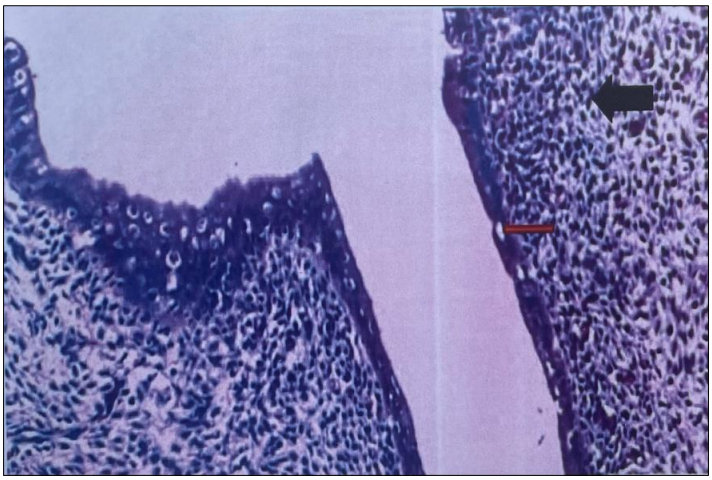


Plate 5: Photomicrograph section of uterine tissue from animals treated with MSG + low dose (500 mg/kgbw) cocoa extract. Section showed uterine gland lined by simple cuboidal ep ithe lium

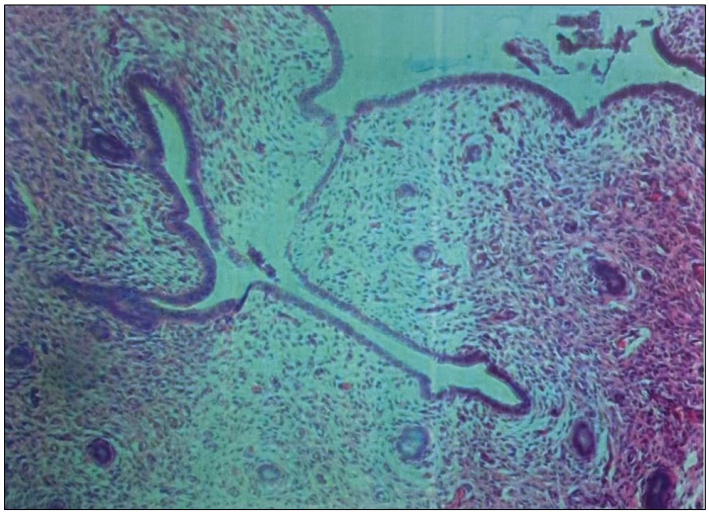


Plate 6: Photomicrograph section of uterine tissue from animals treated with MSG + high dose (1500mg/kgbw) extract. Section showed straight tubular gland lined by cuboidal cells. The stroma is normal.

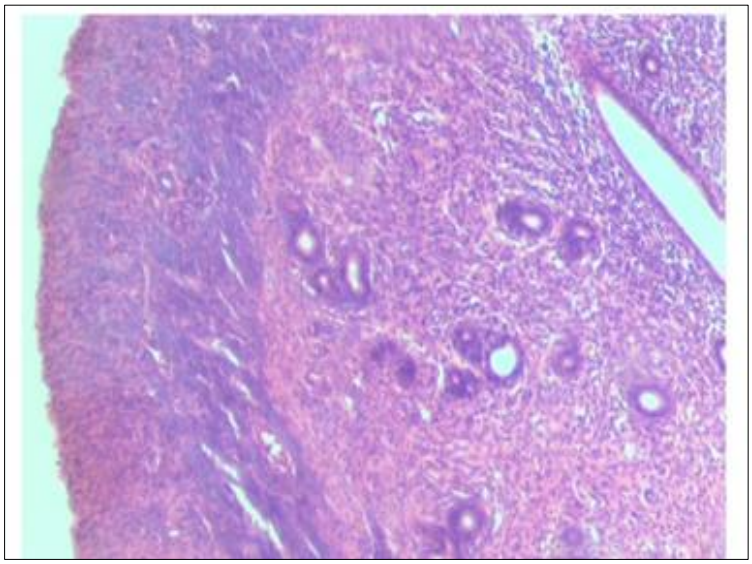


Plate 7: Photomicrograph section of uterine tissues from animals in low dose (500kg/bw) + MSG. Section showed normal myometruim and endometrium

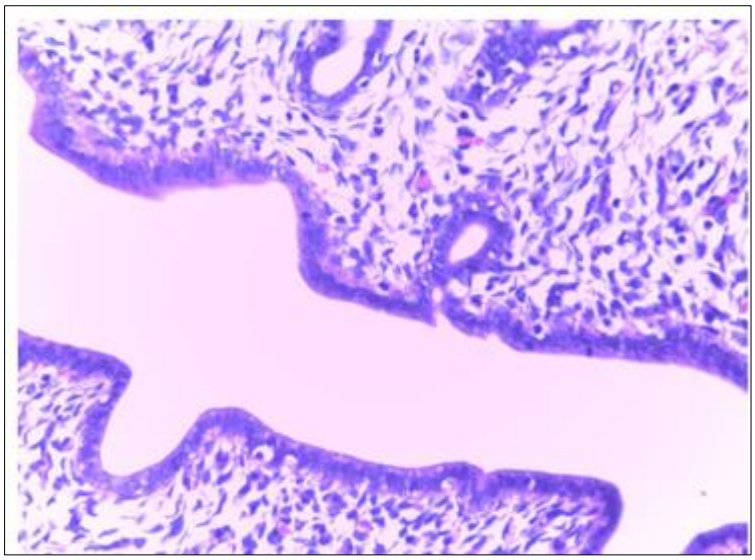


Plate 8: Photomicrograph sections of uterine tissues from animals in high dose(1500kg/bw)+ MSG. Section showed vacuoles in the endometrium and edematous stroma

DISCUSSION

Studies have demonstrated that ovarian hormones are important molecular indicators linked to the development and proliferation of uterine fibroids. The development of uterine fibroids has been attributed mainly to estrogen, but progesterone also has a significant impact (Yashunsky, 2021).

Administration of 200mg/kg of MSG caused significant increase in the serum level of estrogen and progesterone seen in fibroid induced groups. This implies that MSG activated aromatase. This agrees with Agbadua *et al*., (2020) who reported that MSG activated the enzyme aromatase which catalyses the synthesis of estrogen thereby increasing estrogen level.

In the prophylactic group, treatment with ethanolic extract of cocoa seeds reduced the elevated level of progesterone which was elevated by MSG. This implies that cocoa seeds may likely inhibit the proliferation of leiomyoma cells in rats. This agrees with Reis *et al*., (2015), who reported that progesterone is required for the complete development and proliferation of uterine fibroids.

In the therapeutic group, treatment with ethanolic extract of cocoa seeds increased estrogen but reduced progesterone level. The plant extract reversed the elevated progesterone caused by monosodium glutamate. This implies that ethanolic extract of cocoa may inhibit cell proliferation (Foidart *et al*., 1998).

CONCLUSION

Ethanolic extract of Cocoa seed reduces the level of progesterone, it has no effect on estrogen level. The plant extract may arrest tumor progression.

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