



## Assessment of the Effect of Graded Doses Alligator Pepper on Uterus of Female Albino Rats

Uwuigbe Mathew<sup>1\*</sup>, Ohiwerei Wisdom Omogbai<sup>2</sup>, Amina Momodu<sup>3</sup>, Ogbe O Clementina<sup>4</sup>

<sup>1,4</sup> Ambrose Alli University Ekpoma Edo State

<sup>2</sup>Ohilux Global Research and Training Institute, Edo State

<sup>3</sup>Edo University, Iyamho

**Corresponding Author:** Uwuigbe Mathew [Ohiwereiwisdom@gmail.com](mailto:Ohiwereiwisdom@gmail.com)

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### ABSTRACT

The aim of this study is to determine the histological effects of alligator on the uterus of wistar rats, as a result of different doses of alligator administered orally. Fifty (50) adult female wistar rats were use for this study for a period of 28 days (14 days for aclimitization and 14 days for administration). The rats were randomized and divided into five groups (A, B, C, D and E) of ten (10) animals each. the animals were sacrificed two times. All animals were sacrificed by cervical dislocation at least three hours after the last administration of alligator pepper. The results showed the weight before acclimatization, after acclimatization, before sacrifice of control and test subjects in which the Mean±SEM of weight before acclimatization, after acclimatization, before sacrifice were 247±3.00, 226±2.00 and 226±2.00 respectively for control subjects. None of the test groups were found statistically significant ( $p < 0.05$ ) when compared with their respective control. The micrograph result showed that ovary group A in the photomicrograph had a normal histological features. Uterus group B administered with 100mg/kg of aqueous extracts of alligator pepper showed clear carcinoma (neoplastic changes) of the endometry uterus. Uterus group C (200mg of Alligator pepper) and group D (300mg of Alligator pepper) showed hyperplasic cells of the endometry uterus. Uterus group E (400g of Alligator pepper) photomicrograph showed andenomyosis endoutrolysis internal. In conclusion, hyperplasic cells ( a condition suggestive of Hyperplasia) was found to be more common in groups with moderate doses. This study suggests that the oral administration of Alligator pepper at mild or moderate doses could cause severe histopathologic changes in the uterus without having any adverse effect on body weight

## INTRODUCTION

*Aframomum melegueta*, commonly known as alligator pepper, is a species in the ginger family (Zingiberaceae). It is native to West Africa and has both medicinal and nutritional value. The seeds contain active phytochemicals such as flavonoids, tannins, alkaloids, and phenolic compounds, which have been traditionally used for antimicrobial and therapeutic applications. Despite its numerous benefits, concerns have been raised regarding its effects on pregnancy and reproductive health. Studies suggest that alligator pepper could induce histopathological changes in the uterus, influencing pregnancy outcomes. This study aims to evaluate the effects of alligator pepper on the uterine histology of female Wistar rats subjected to different doses.

## LITERATURE REVIEW

*Aframomum melegueta* is a perennial herbaceous plant known for its pungent seeds, which are widely used in traditional medicine. The seeds contain gingerols, shogaols, paradols, and other bioactive compounds with antimicrobial, anti-inflammatory, and antioxidant properties. Studies have demonstrated its efficacy in treating gastrointestinal disorders, infections, and metabolic diseases (Adefegha & Oboh, 2012). The presence of these phytochemicals suggests that alligator pepper may influence hormonal balance and reproductive health. Traditional applications include its use in postpartum recovery, appetite stimulation, and pain relief, but its potential impact on uterine tissues remains largely unexplored.

Ethnobotanical studies indicate that *A. melegueta* is used in various African cultures for its therapeutic benefits, including its role in managing bacterial infections, digestive ailments, and inflammatory conditions (Iwu, 1999). The plant's seeds have been reported to exert uterotonic effects, which could have implications for pregnancy and fertility. Research on its pharmacological effects has also linked it to metabolic benefits, including glucose regulation and lipid metabolism (Mohammed et al., 2014). However, concerns have arisen regarding its potential toxicity and adverse effects on reproductive organs, particularly in prolonged or high-dose consumption.

Given the increasing interest in natural remedies and traditional medicine, understanding the histopathological effects of alligator pepper on the uterus is crucial. This study seeks to bridge the knowledge gap by assessing the impact of *A. melegueta* on uterine histology in Wistar rats under controlled experimental conditions.

## METHODOLOGY

### Study Design

This study utilized an experimental and observational research design involving fifty (50) adult female Wistar rats over 28 days, including 14 days of acclimatization and 14 days of administration.

### Plant Material and Preparation

Samples of alligator pepper were sourced from Ekpoma, Nigeria, and authenticated at the Herbarium of the Botany Department, Ambrose Alli

University. The seeds were air-dried under standard laboratory conditions before preparation.

### **Experimental Animals**

Fifty female Wistar rats (170–250g) were obtained from the College of Medicine, Ambrose Alli University. They were housed in wire mesh cages and fed a standard diet with water ad libitum. The research adhered to international guidelines for laboratory animal use.

### **Experimental Design**

The rats were randomized into five groups (A–E), each containing ten animals:

- Group A (Control): No alligator pepper administration.
- Group B: 100mg/kg aqueous alligator pepper daily.
- Group C: 200mg/kg aqueous alligator pepper daily.
- Group D: 300mg/kg aqueous alligator pepper daily.
- Group E: 400mg/kg aqueous alligator pepper daily.

### **Animal Sacrifice and Tissue Collection**

Animals were sacrificed on days 7 and 14 by cervical dislocation. The uterus was excised, rinsed in cold sucrose solution, blotted dry, and fixed in 10% formal saline for histological examination.

### **Histological Processing and Staining**

The tissues were processed using an automatic tissue processor at Irrua Specialist Teaching Hospital. They were passed through graded alcohol, xylene, and molten paraffin wax before embedding in paraffin blocks. The blocks were cooled at 5°C for 15 minutes, trimmed, and sectioned at 3 $\mu$ m using a rotary microtome. Sections were floated in a 55°C water bath, mounted on frosted slides, dried on a hot plate for 40 minutes, de-waxed, hydrated, and stored for staining. Tissue sections were stained using the Hematoxylin and Eosin (H&E) technique. They were dewaxed in xylene, hydrated through descending alcohol grades, and stained with Harris hematoxylin. After rinsing, differentiation in acid alcohol was performed, followed by bluing in tap water. The sections were counterstained with eosin, dehydrated in ascending alcohol grades, cleared in xylene, and mounted with DPX. Pathological evaluation was conducted using a Swift binocular microscope, and images were captured with a photomicroscope.

### **Data Analysis**

The obtained data were then subjected to statistical analysis using SPSS (version 20). The test groups' values were compared with the values of the control group using ANOVA at 95% level of confidence. Values were represented as mean  $\pm$  standard error of mean (SEM).

## **RESULT**

Table 4.1 presents weight measurements before acclimatization, after acclimatization, and before sacrifice, showing no statistically significant differences ( $p < 0.05$ ) between test and control groups.

Table 4.2 summarizes H&E staining results: Group A had normal uterine histology, Group B (100mg/kg) showed carcinoma, Groups C and D (200mg/kg

and 300mg/kg) exhibited hyperplastic endometrial cells, and Group E (400mg/kg) displayed adenomyosis and endometriosis.

Table 4.1 Comparison of Mean ± S.E of Different Stages of Weight Measurement in Groups and Control Group Using ANOVA.

	Group A (Control)	Test group				F Value	P value
		Group B	Group C	Group D	Group E		
<b>Before Acclimatization</b>	0.247 ±0.003	0.244 ±0.002	0.244 ±0.002	0.241 ±0.002	0.241 ±0.002	1.249	0.3041
<b>After Acclimatization</b>	0.226 ±0.002	0.230 ±0.004	0.234 ±0.003	0.225 ±0.004	0.221 ±0.003	2.410	0.0660
<b>Before Sacrifice</b>	0.2260 ±0.002	0.2300 ±0.004	0.2343 ±0.003	0.2233 ±0.003	0.2213 ±0.004	2.206	0.0873

\* Means statistically significant (p<0.05)

- Group A (Control)
- Group B (100mg/kg of aqueous extracts of alligator pepper)
- Group C (200mg/kg of aqueous extracts of alligator pepper)
- Group D (300mg/ kg of aqueous extracts of alligator pepper)
- Group E (400mg/kg of aqueous extracts of alligator pepper)

Table 4.2 General Tissue Appearance of H and E Staining

	General histologic effect
Control Group A Normal rodent feed	Normal histology
Group B (100mg/kg of aqueous extracts of alligator pepper)	Uterus histology with clear or severe carcinoma
Group C (200mg/kg of aqueous extracts of alligator pepper)	Uterus histology with hyperplastic cells of the endometry uterus
Group D (300mg/kg of aqueous extracts of alligator pepper)	Uterus histology with hyperplastic cells of the endometry uterus
Group E (400mg/kg of aqueous extracts of alligator pepper)	Uterus histology with adenomyosis and endometriosis internal

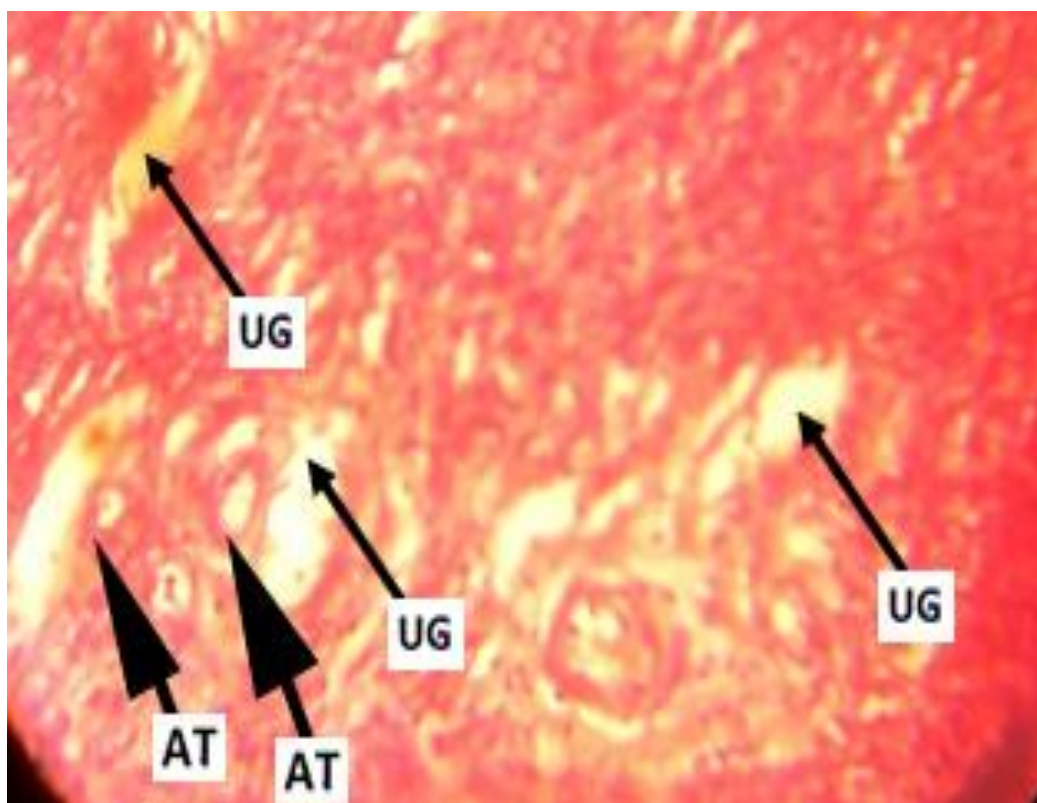


Fig 1- Group A (Control): Photomicrograph of Uterus of Experimental Animal Showing Normal Histology with Numerous Uterine Glands (UG) Lined by Endometrial Cells (Columnar Cells). There are Also Presence of Coiled Arteries (AT) with the Functionalis Layer of the Endometrium. (H and E  $\times 100$ )

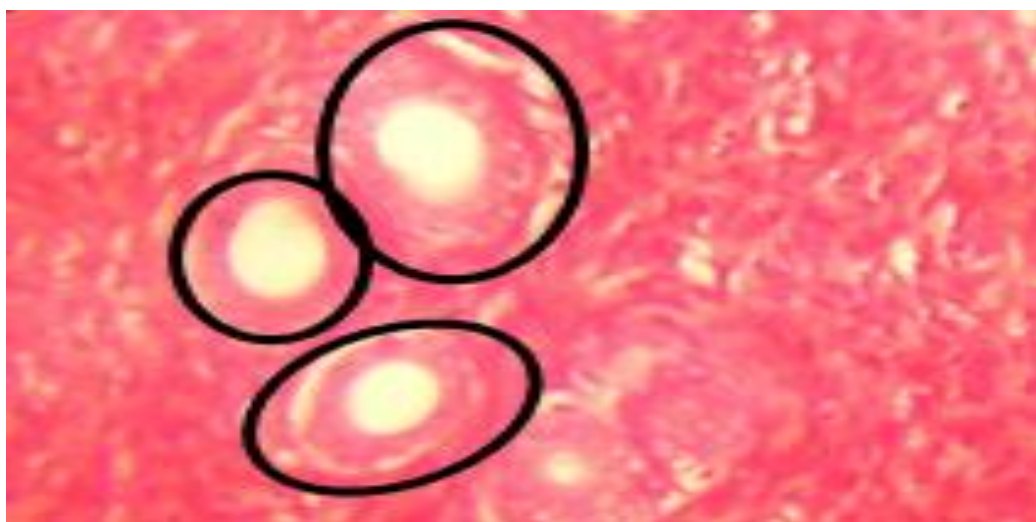


Fig 2- Group B (100mg/Kg of Aqueous Extracts of Alligator Pepper). Photomicrograph of Uterus of Experimental Animal Showing Clear Carcinoma (Neoplastic Changes) of the Endometrium Uterus. The Clear Cell Carcinoma of the Uterus Above Shows Striking Resemblance to Clear Cell Carcinoma of the Ovaries and the Cervix. (H and E  $\times 100$ )

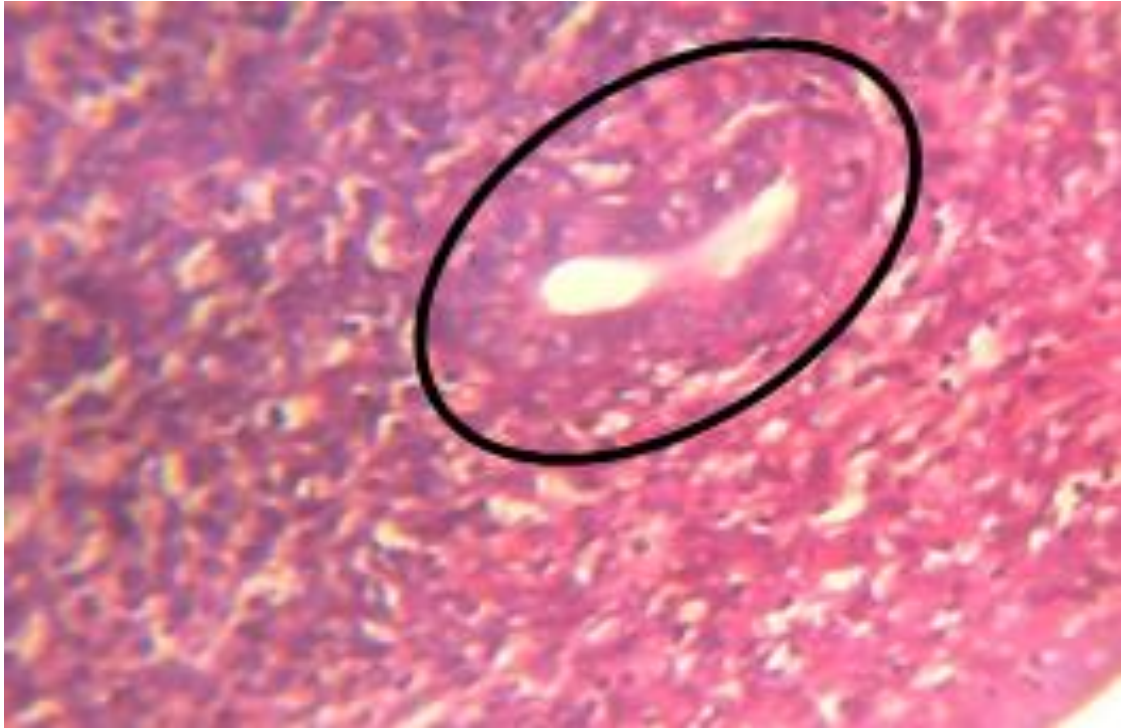


Fig 3- Group C (200mg/Kg of Aqueous Extracts of Alligator Pepper). Photomicrograph of Uterus of Experimental Animal Showing Hyperplastic Cells of the Endometry Uterus. H and E (X100)

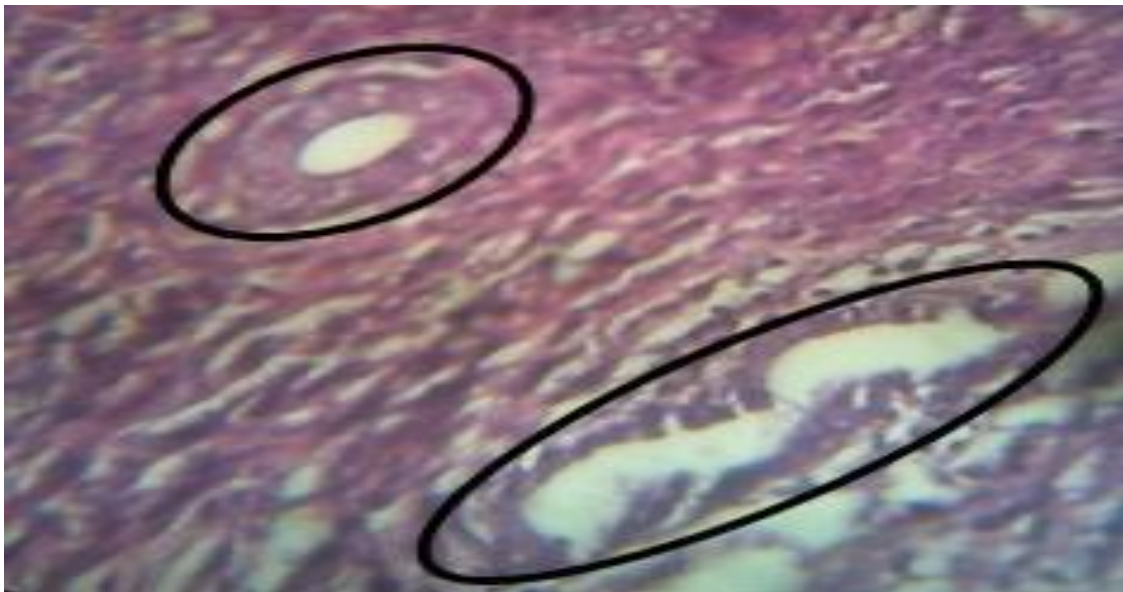


Fig 4- Group D (300mg/Kg of Aqueous Extracts of Alligator Pepper). Photomicrograph of Uterus of Experimental Animal Showing Hyperplastic Cells of the Endometry Uterus. H and E (x100)



Fig 5- Group E (400mg/Kg of Aqueous Extracts of Alligator Pepper).  
Photomicrograph of Uterus of Experimental Animal Showing  
Andenomyosisendoutrolysis Internal. H and E (X100)

## DISCUSSION

The uterus is a hollow muscular organ located in the female pelvis between the bladder and rectum. The uterine cavity opens into the vaginal cavity, and the two make up what is commonly known as the birth canal (Cheong, 2024). Alligator pepper, which is botanically known as *Aframomum melegueta* is a popular herbaceous perennial plant that is originally from West Africa. Alligator pepper contains an essential oil, which is volatile in nature, and is extractable by hydro-distillation from the seeds of *Aframomum melegueta*. Gas chromatography and gas chromatography-mass spectrometry have been used in characterizing 27 compounds, which constitute 98.6% of the essential oil (Ajaiyeoba & Ekundayo, 1999). The aim of this study is to evaluate the effect of Alligator pepper on the Uterus. The result from the body weight of this study was in similarity with that of Carina et al. (2012) as there was no significant alterations in the body weight of animals exposed to aqueous extracts of Alligator pepper (*Zingiberaceae*). The micrograph result showed that ovary group A in the photomicrograph had a normal histological features. Uterus group B administered with 100mg/kg of aqueous extracts of alligator pepper showed clear carcinoma (neoplastic changes) of the endometry uterus. The clear cell carcinoma of the uterus above shows striking resemblance to clear cell carcinoma

of the ovaries and the cervix. The findings of this present study is in similarity with the studies of Lerma et al., (1998) ; Fadare et al., (2006) which concluded that although limited data are available regarding the potential precursor lesions for CCC ( Clear Cell Carcinoma) of the endometrium, the study identified a spectrum of atypical glandular changes (isolated glands or surface epithelium that displayed cytoplasmic clarity and / or eosinophilia with varying degrees of nuclear atypia) with-in the endometrium which might have been the cause of clear cell carcinoma, also suggesting that alligator pepper might have been a causative factor of the cell carcinoma.

Uterus group C (200mg of Alligator pepper) and group D (300mg of Alligator pepper) showed hyperplastic cells of the endometrium. This could have resulted from an overgrowth of the endometrium, the lining of the uterus, due to alligator pepper administration that may progress to or coexist with endometrial cancer. This result was in similarity with that of Ann Pietrangelo, (2018).

Uterus group E (400g of Alligator pepper) photomicrograph showed endometriosis. This could have been due to the alligator pepper causing the inner lining of the uterus (the endometrium) to break through the muscle wall of the uterus (the myometrium). Florence Bryd, (2020) observed a similar findings in its study and suggested that various hormones -- including estrogen, progesterone, prolactin, and follicle stimulating hormone may trigger the condition

## **CONCLUSIONS**

In conclusion, hyperplastic cells (a condition suggestive of Hyperplasia) was found to be more common in groups with moderate doses. This study suggests that the oral administration of Alligator pepper at mild or moderate doses could cause severe histopathologic changes in the uterus without having any adverse effect on body weight.

## **RECOMMENDATIONS**

Based on the histological observations in this study, it may be concluded that ;

- Future studies to further investigate this hypothesis are recommended to properly ascertain the effect of the oral administration of mild and toxic doses of Alligator pepper.
- Considerations should be put in place for extended prophylaxis in patients to prevent such events.

## **FUTURE STUDY**

This study still has limitations so that further research is needed related to the topic of assessment of the effect of graded doses alligator pepper on uterus of female albino rats in order to perfect this study and increase insight for readers.

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