THE HISTOLOGICAL EFFECTS OF ALLIGATOR ON THE UTERUS OF WISTAR RATS

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ABSTRACT
Aframomum melegueta (Alligator pepper), a member of the Zingiberaceae family, is a widely used spice with potential antimicrobial and therapeutic properties. The seeds of this plant are rich in phenolic compounds, which have been traditionally utilized in treating infections. This study aimed to evaluate the histopathological effects of aqueous extracts of *Aframomum melegueta* on the uterus of female Wistar rats. Fifty rats were divided into five groups (A-E), with Group A serving as the control and Groups B-E receiving increasing doses of aqueous *A. melegueta* extract (100, 200, 300, and 400 mg/kg, respectively) over a 14-day period. The rats' body weights were measured before and after treatment, with no significant weight changes observed across the groups. Histological analysis of uterine tissues showed normal architecture in the control group (Group A), while varying degrees of uterine abnormalities were observed in the treatment groups. Group B (100 mg/kg) exhibited clear cell carcinoma in the endometrium, while Groups C (200 mg/kg) and D (300 mg/kg) showed endometrial hyperplasia. Group E (400 mg/kg) displayed findings consistent with adenomyosis. These results suggest that moderate to high doses of *A. melegueta* may induce severe histopathological changes in the uterus, including neoplastic and hyperplastic alterations, without affecting body weight. Further research is needed to elucidate the mechanisms underlying these effects and to assess the long-term safety of *Aframomum melegueta* consumption.

Keywords: *Aframomum melegueta* (alligator pepper), uterine histopathology, hyperplasia, carcinoma, Wistar rats, herbal medicine, antimicrobial.

INTRODUCTION
Aframomum melegueta, a perennial plant from the Zingiberaceae family (ginger family), is closely related to cardamom. Its seeds, known for their sharp, pungent flavor with hints of black pepper and citrus, are commonly used as a spice. This plant is variously referred to as grains of paradise, melegueta pepper, alligator pepper, Guinea grains, ossame, or fom wisa. The term "Guinea pepper" is sometimes used for *Aframomum melegueta*, though it is more accurately attributed to *Xylopia aethiopica* (grains of Selim) (Ajaiyeoba & Ekundayo, 1999).

Indigenous to West Africa, historically known as the "Pepper Coast" or "Grain Coast" due to its spice trade, Aframomum melegueta is also an important agricultural product in Ethiopia's Basketo district (Basketo special woreda). This tropical plant flourishes in rainforests and regions such as Ghana, Cameroon, Sierra Leone, Liberia, Nigeria, Togo, and Côte d'Ivoire.. Its seeds contain aromatic ketones, giving them their characteristic sharp, peppery flavor (Galal, 1996; Tackie et al., 1975). Known for both medicinal and nutritional benefits, Aframomum melegueta has been used

traditionally for treating infections, and its bioactive compounds show promise for developing local antimicrobial treatments (Oyegade, 1999).

The seed of Aframomum melegueta contains phenolic compounds, marking it as a natural antimicrobial resource. Phenols and phenolic compounds are widely recognized for their disinfectant properties and serve as a benchmark for evaluating other bactericidal agents (Okwu, 2001). Aframomum melegueta seed extracts, which possess strong antiseptic and bactericidal effects, have been applied in wound care and infection prevention (Okwu, 2004).

Traditional medicine, often referred to as herbal or natural medicine, has a long history globally (Hazan and Atta, 2005). Since ancient times, humans have relied on plants to address infectious diseases. Long before microbial science, plants with healing properties were widely acknowledged for their therapeutic benefits (Rios and Recio, 2005). Medicinal plants are defined by their ability to contain therapeutic substances in their tissues, which are often used directly or as bases for drug synthesis. Numerous plants have a long history in traditional medicine owing to their antimicrobial properties (Sofowora, 1993), the therapeutic benefits of these plants stem from their bioactive compounds, mainly secondary metabolites like alkaloids, flavonoids, tannins, and phenolic compounds, which exert significant physiological effects on both humans and animals (Edeoga *et al*., 2005).

2.0 MATERIALS AND METHODS

2.1 Study Design

The study involved the use of both experimental and observational study design. 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)

2.2 Plant Material/Preparation

The sample of alligator pepper were obtained from market square Ekpoma Edo State, Nigeria and was botanically identified and verified at the Department of Botany Herbarium in Ambrose Alli University, Ekpoma. It was air-dried under standard laboratory conditions following the protocol of Adekomi, (2010)

2.3 Experimental Animals

Fifty (50) female Wistar rats, weighing between 170-250g, were acquired from the laboratory animal house at the College of Medicine, Ambrose Alli University, Ekpoma, Edo State.

2.4 Experimental Design

The rats were randomly assigned to five groups (A, B, C, D, and E), with ten animals in each group. They were housed in closed glass chambers, each with a volume of approximately 0.1m³ (37cm x 54cm x 30cm), featuring a 2cm opening at the top, following the protocol outlined by Onarlioglu *et al*. (1999).

Group A (Control rats):Where not administered with alligator pepper
Group B: Administered with 100mg/kg aqueous alligator pepper once per day (9.00am)
Group C: Administered with 200mg/kg aqueous alligator pepper once per day (9.00am)
Group D: Administered with 300mg/kg aqueous alligator pepper once per day (9.00am)
Group E: Administered with 400mg/kg aqueous alligator pepper once per day (9.00am)

2.5 Animal Sacrifice

The animals were sacrificed on two separate occasions. The first group from each category was sacrificed on the seventh day, while the second group from each category was sacrificed on the fourteenth day.

All animals were sacrificed by cervical dislocation at least three hours after the last administration of alligator pepper. They were laid down on the dissection board in a supine position and their anterior thoraco-abdominal cavities were carefully dissected in the midline to expose the organs of interest (uterus). The uterus were rinsed with a cold sucrose solution, carefully blotting it dry using filter paper before transferring into 10% formol saline to fix for at least 72 hours before further histological protocol and analysis was performed. Body weights were also measured at baseline and before animals were sacrificed.

2.6 Histological Protocol

The histological analysis including the Staining procedure, Microscopy and photomicroscopy was done using standard laboratory procedure.

2.7 Data Analysis

The collected data were analyzed statistically using SPSS (version 20) and micrographs represented scientifically.

RESULTS

3.0: WEIGHT BEFORE ACCLIMATIZATION, AFTER ACCLIMATIZATION AND BEFORE SACRIFICE OF CONTROL AND TEST SUBJECTS.

Table 1 presents the weights of control and test subjects before acclimatization, after acclimatization, and prior to sacrifice. For the control group, the mean ± SEM weights were 247±3.00 g before acclimatization, 226±2.00 g after acclimatization, and 226±2.00 g before sacrifice. No significant differences ($p<0.05$) were observed between the test groups and their respective controls.

Table 2 shows the general tissue appearance of H & E staining. In which Group A shows a normal histology. Group B (100mg/kg of aquesous extracts of alligator pepper) with uterus histology with clear or severe carcinoma. Group C, which received 200 mg/kg of aqueous extract of alligator pepper, exhibited uterine histology showing hyperplastic cells in the endometrial lining. Group D, treated with 300 mg/kg of the same extract, also displayed hyperplasia in the endometrial cells. In Group E, which was administered 400 mg/kg of the aqueous extract, uterine histology revealed signs of adenomyosis and endometrial changes associated with internal endometriosis.

Table 1: Mean changes in stages of weight measurement In all groups

	Group A (Control)	Test group				F Value	P value
		Group B	Group C	Group D	Group E		
Before Acclimatization	0.247 ±0.003	0.244 ±0.002	0.244 ±0.002	0.241 ±0.002	0.241 ±0.002	1.249	0.3041
After Acclimatization	0.226 ±0.002	0.230 ±0.004	0.234 ±0.003	0.225 ±0.004	0.221 ±0.003	2.410	0.0660
Before Sacrifice	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)**

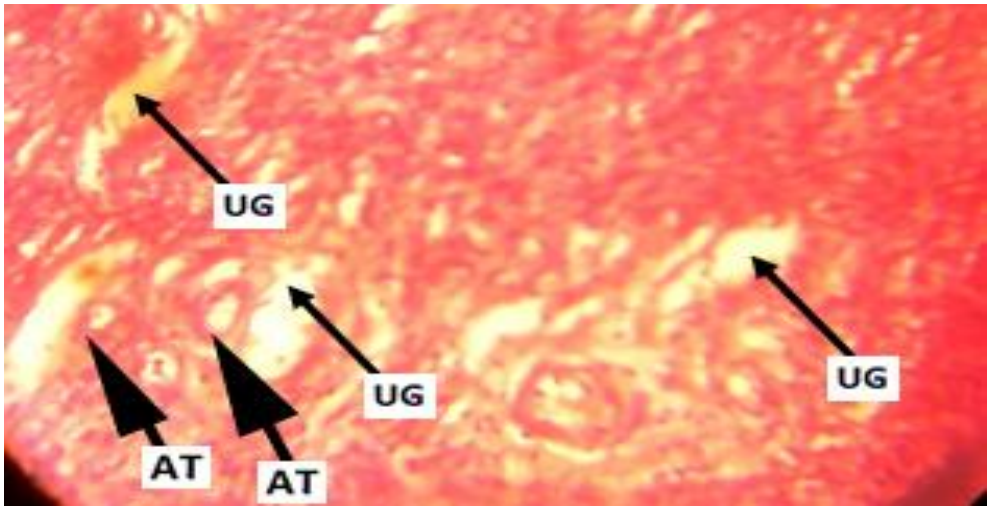

Fig 1- Group A (Control): Photomicrograph of uterus of experimental animal showing normal histology with numerous uterine glands (UG) lined by endometrial cells (coloumal cells). There are also presence of coiled arteries (AT) with the functionalis layer of the endomentrum. (H and E ×100)

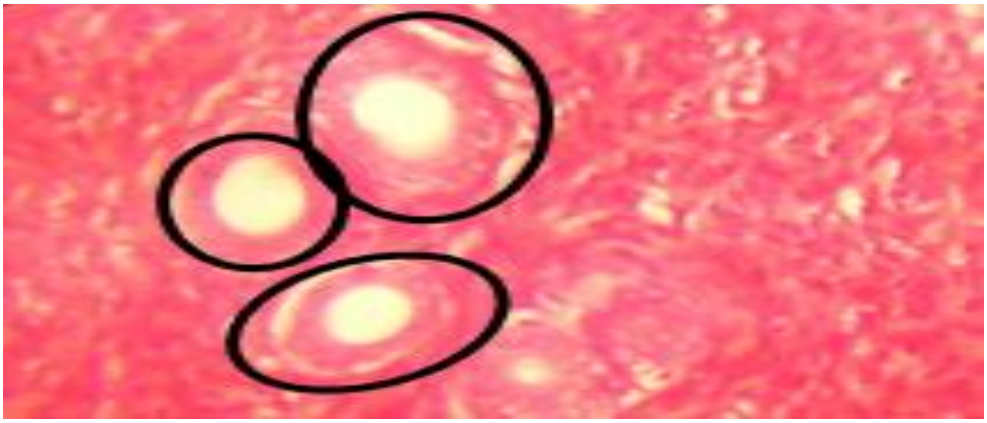

Fig 2- Group B (100mg/kg of aquesous extracts of alligator pepper): Photomicrograph of uterus of experimental animal showing clear carcinoma (neoplastic changes) of the endomentry uterus. The

clear cell carcinoma of the uterus above shows striking resemblance to clear cell carcinoma of the ovaries and the cervix. (H and E ×100)

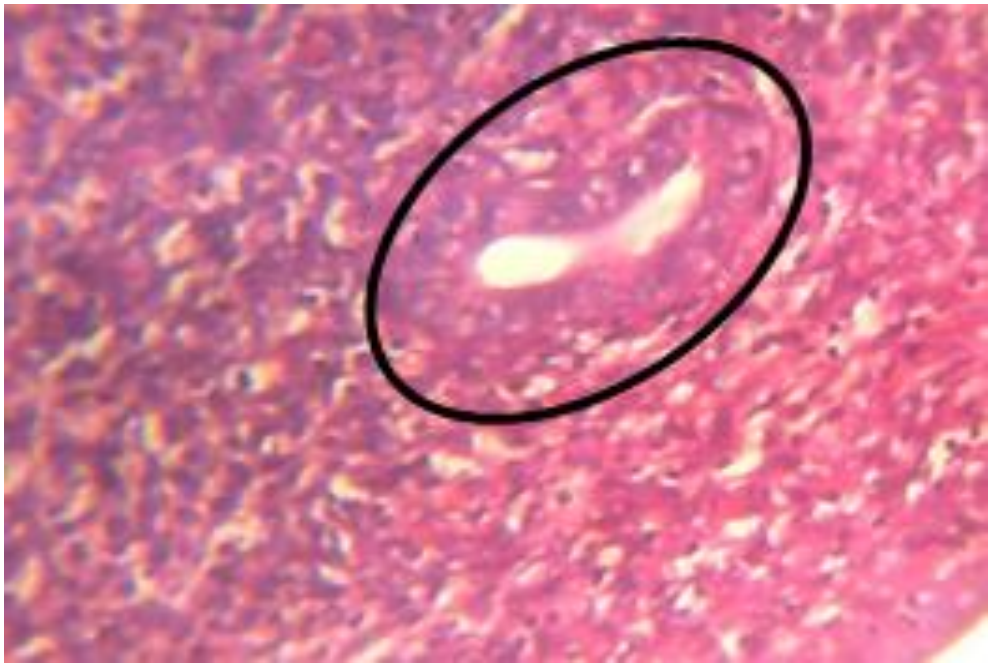

Fig 3- Group C (200mg/kg of aqueous extracts of alligator pepper): Photomicrograph of uterus of experimental animal showing hyperplasic cells of the endomentry uterus. H and E (x100)

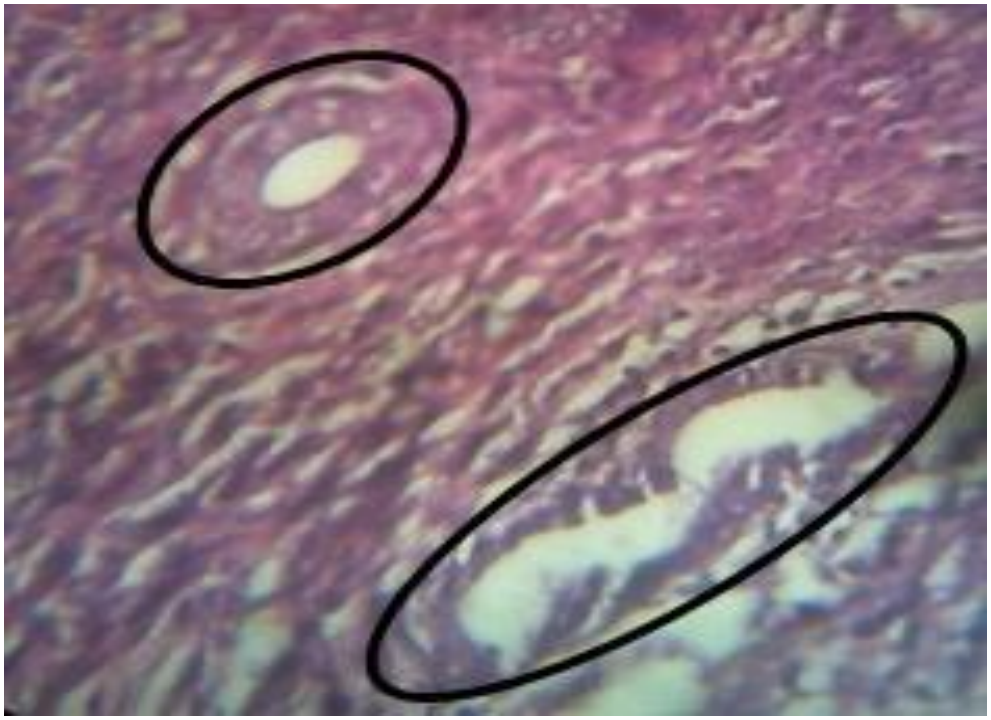

Fig 4- Group D (300mg/kg of aqueous extracts of alligator pepper): Photomicrograph of uterus of experimental animal showing hyperplasic cells of the endomentry uterus. H and E (x100)

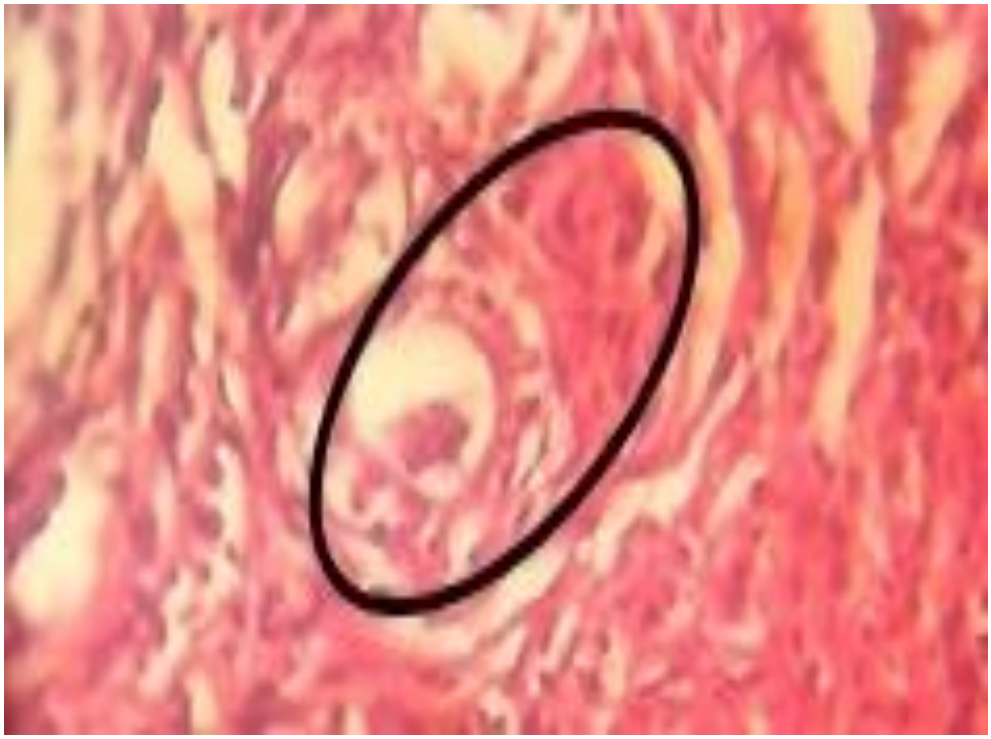

Fig 5- Group E (400mg/kg of aqueous extracts of alligator pepper): Photomicrograph of uterus of experimental animal showing andenomyosisendoutrolysis internal. H and E (x100)

DISCUSSION

The uterus is a hollow, muscular organ located in the female pelvis between the bladder and the rectum. It is connected to the vaginal canal, collectively forming the birth passage (Kara Rogers, 2019). *Aframomum melegueta*, commonly known as alligator pepper, is a well-known perennial plant native to West Africa. The seeds of *Aframomum melegueta* contain a volatile essential oil, which is typically extracted through hydro-distillation. Gas chromatography and gas chromatography-mass spectrometry analyses have identified 27 compounds, making up 98.6% of the oil's composition (Ajaiyeoba & Ekundayo, 1999). This study seeks to evaluate the effects of alligator pepper on the uterus. In line with the observations of Carina et al. (2012), no significant changes were noted in the body weight of animals treated with aqueous extracts of alligator pepper (Zingiberaceae). Histological examination revealed normal ovarian tissue in group A. However, group B, which was given 100 mg/kg of aqueous alligator pepper extract, showed evidence of clear cell carcinoma (neoplastic changes) within the endometrium. The carcinoma observed in the uterus was similar to the clear cell carcinoma seen in the ovaries and cervix. These findings are consistent with reports by Lerma et al. (1998) and Fadare et al. (2006), which, while offering limited data on precursor lesions for endometrial clear cell carcinoma (CCC), identified atypical glandular changes—such as isolated glands, surface epithelium with cytoplasmic clarity, eosinophilia, and nuclear abnormalities—within the endometrium. This suggests that these changes might play a role in the development of clear cell carcinoma, with evidence indicating that alligator pepper could be a potential contributing factor. In group C (200 mg/kg of alligator pepper) and group D (300 mg/kg of alligator pepper), signs of endometrial hyperplasia were observed, indicating an abnormal proliferation of cells in the uterine lining. This overgrowth of the endometrium, likely triggered by the administration of alligator pepper, has the potential to develop into or coexist with endometrial carcinoma. These results are consistent with findings reported by Ann Pietrangelo (2018). Photomicrographs from uterus group E (400 mg

of alligator pepper) revealed evidence of adenomyosis and internal endometriosis, suggesting that the alligator pepper may have caused the endometrium to penetrate the uterine muscle wall (myometrium). A similar phenomenon was noted by Florence Bryd (2020), who proposed that various hormones, including estrogen, progesterone, prolactin, and follicle-stimulating hormone, might trigger such conditions.

CONCLUSION.

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.

RECOMMENDATIONS.

From the histological findings in this study, the following conclusions can be drawn:

- Further research is needed to thoroughly explore this hypothesis and to better understand the effects of administering both mild and toxic doses of alligator pepper orally.
- Measures should be considered for extended preventive care in patients to avoid the occurrence of such events.

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