

Evaluation of Pharmacodynamic Effects of Ethanolic Extract of the Leaves of *Gnetum africanum* on Uterine and Ovarian Tissues Morphology of Rats.

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Abstract

Dynamic effect of Ethanolic extract of medicinal plant, *Gnetum africanum* repeated oral administration on uterine and ovarian tissues histology of rats was investigated. Three tolerated doses of the extract 10, 200 and 700 mg/kg obtained from acute toxicity test were administered orally to three respective groups of female rats daily for 3 days. On the 4th day, rats of all the groups were sacrificed and their uteri and ovaries were carefully isolated and fixed in 10% formal-saline, for 28 days. The fixed tissues were sectioned and histologically processed and examined under light microscope. Photomicrographs of the tissue sections revealed uterine endometrial hyperplasia with formation of several tiny cysts due to glandular dilatation and formation of ovarian fibromas, cytological atypia and stroma. The findings allow the conclusion that excessive oral administration of the medicinal plant, *Gnetum africanum*, a popular Nigerian medicinal plant, could impact a negative influence on the female reproductive physiology.

Keywords: Ethanolic Extract, leaves of *Gnetum africanum*, Ovarian tissues, Uterus, female rats.

1. Introduction

The plant, *Gnetum africanum* (family, Gnetaceae), is a popular medicinal plant in Nigerian. It is commonly called Afang or Ogazi in the Niger Delta Region of Nigeria. The leaves of the plant are prepared in most Nigerian diets as vegetable.

In Niger Delta Region of Nigeria the plant, *Gnetum africanum* is used for preparation of a popular Nigerian diet (Afang-soup) mixed with another vegetable, *Talinum triangulare* (water leaf) as additive (Akpanabiatu, et al, 1998).

Most women of this region are additive to the Nigerian native diet, afang soup. It has been reported that, plants possess a diversity of biological activities capable of modifying normal functions of human body system (Ekanem and Udoh, 2010). Based on this fact, it is speculated that short or long term repeated consumption of the plant as vegetable, could have a dynamic impact on the uterine and ovarian muscles morphology.

In most Nigerian restaurants, afang soup is the most popular vegetable soup. Despite its role in Nigeria traditional medicine, it is considered to be an important source of protein, fatty acids, essential amino acids and mineral element (Baker, 1972). Increasing demand for afang soup would lead to abuse of the plant by most Nigerians.

Phytochemical studies of the crude extract of *Gnetum africanum* earlier carried out showed that the plant contains in greater quantity, alkaloids and fats. Our preliminary investigation into the effect of the Ethanolic extract of the leaf of *Gnetum africanum* on endocrine function in female rats revealed, elevated serum levels of oestrogen (submitted for publication). The elevated blood concentration of oestrogen following the extract treatment allowed the speculation that the plant vegetable, *Gnetum africanum* is phytoestrogenic.

Based on the above facts about the biological activity of *Gnetum africanum*, it becomes necessary to carry out investigation into the effect of the extract on the morphology of uterus and ovary of rats.

2. Materials and Methods

2.1 Collection of Plant Material:

The leaves of the plant, *Gnetum africanum*, were fetched from the tropical forest of Calabar, Cross River State of Nigeria, around the months of March and April, 2011. The plant was identified as *Gnetum africanum* by Professor Ani Nkang of the Department of Botany, University of Calabar, Nigeria. The voucher specimen was preserved in the Herbarium of the Department of Pharmacology, University of Calabar, Nigeria.

2.2 Preparation of Ethanolic Extract:

The leaves of Gnetum africanum were washed clean in tap water and allow to air-dry overnight at room temperature of $28\pm 1^{\circ}\text{C}$. The dried leaves of Gnetum africanum were sliced off into bits and ground into power using an electrical Blender (Behrmaning Troy, N. Y).

The power sample (100 g) of the leaf of Gnetum africanum was wrapped in a Wattman filter paper, size 40 and placed in IL Soxhlet extractor (M & G Scientific company, England) and the Ethanolic extract was prepared following a modified method of Udoh (1995). The ground leaf sample of Gnetum africanum was first extracted in absolute petroleum ether for 8 h to remove fats. The fat-free plant sample residues left in the Soxhlet chamber were re-extracted in absolute ethanol for 72 h. The Ethanolic liquid extract was evaporated using a Rotary Evaporator (Astell Hearson, England) in vacuo at a reduced temperature of 40°C into solid extract. The solid extract was chemically screened to contain alkaloids with negligible quantity of tannins.

2.3 Treatment:

About 50 adult female rats of 30 weeks old, weighing between 100 and 120 g were obtained from Animal House Unit, Department of Pharmacology, University of Calabar, Nigeria. The rats were allowed to acclimatize in the laboratory for a period of 7 days. The rats were housed in standard cages and allowed free access to standard pelleted diet and water ad libitum. An ambient temperature was $28\pm 1^{\circ}\text{C}$.

Three tolerated doses (10, 200 and 700 mg/kg) of the Ethanolic extract of Gnetum africanum were selected from the results of acute toxicity test. Rats were divided into 5 groups of 5 rats per group. Group 1 received normal saline (control), group 2 received 17β -estradiol (1mg/kg) as standard, groups 3, 4 and 5 received the estimated tolerated doses of the Extract 10, 200 and 700 mg/kg for 3 days respectively. After treatment, rats of all groups were sacrificed. Their uteri and ovaries were carefully isolated and fixed in 10% formal-Saline for 28 days.

2.4 Histopathology:

The fixed tissue sections were processed for histopathological examination. The tissue sections were washed in tapwater for 30 min, later dehydrated in graded concentration of ethanol, then passes through two changes of equal volumes of chloroform; xylene mixture and cleared in two changes of pure xylene. The sections were impregnated in two changes of molten paraffin wax at 60°C to remove clearing agents, and embedded in the molten paraffin enblocked in a mould. The blocks were allowed to solidify. Solid blocks of tissues in paraffin wax were sectioned to the required thickness of $15\mu\text{m}$, using microtome (BehrManning Troy, N.Y). The embedded specimens were cut into thin paraffin ribbons and smeared on the slide and stained with haematoxylin (Sigma, U.S.A) and eosin (Sigma, USA) following a standard staining procedure. The prepared slides were examined under light microscope (Olympus, Japan).

3. Result

3.1 Uterine Muscle Morphology

Ethanolic extract of the leaf of Gnetum africanum orally administered at doses of 10, 200 and 700 mg/kg to female rats, daily for three days caused endometrial hyperplasia, endometrial lining to become thickened due to proliferation of the endometrial glandular tissue with the formation of numerous tiny cysts (fig 1c, d and e) compared to control (fig 1a) and standard (fig b). The microscopic examination of the histology of uterus also showed features which indicated possible failure of normal growth regulation of endometrium.

Highest dose (700 mg/kg) of Ethanolic extract of Gnetum africanum caused severe endometrial hyperplasia with proliferation of the endometrial glands, glandular epithelial cells atypia, cytoplasmic pleomorphism and enlarged uterine cavity (fig 1e).

3.2 Ovarian Morphology

Ethanolic extract of the leaf of Gnetum africanum (10, 200 and 700 mg/kg) administered to female rats orally for 3 days induced formation of fibromas in the granulosa and thecal cells of the ovarian stroma (fig 2b, c and d) compared with control (fig 2a) and standard (fig 2e). The photomicrographs showed some mucinous tumours which is an evidence of cytological atypia (fig 2b, c, d and e). Dose of 700 mg/kg/d administered for 3 days caused formation of marked fibromas, thecal cells of the ovarian stroma, papiliferous ingrowths and columnar epithelium filled with cystic cavity (fig 2d).

4. Discussion

Observations of this experiment reveal that repeated administration of the Ethanolic extract of the plant, Gnetum africanum cause some pathological effects on uterine and ovarian muscles morphology in rats. Pharmacodynamic activity of the plant extract is similar to that of 17β -estradiol (Rang and Dale 2009; Sumino et al. 2005; Nakata, 2011).

Repeated oral administration of the plant extract causes uterine endometrial hyperplasia and localized areas of polypoid hyperplasia forming endometrial polyps which contain cristically dilated endometrial glands. Examination of the photomicrographs of the uterine muscle sections reveals thickening of endometrial lining as a result of proliferation of the endometrial glandular tissue with the formation of numerous tin and scattered cysts, resulting from the dilatation of endometrial glands. These effects are similar to that of excessive oestrogen secretion (Sumino, et al, 2005; Schmid, 2008; Srirajaskanthan, et al, 2009; Lyakhovich and Gasche, 2010). This finding may suggest that, the plant extract of Gnetum africanum possesses some estrogenic effect on the uterine muscle or causes excess release of oestrogen into the blood circulation which in turn induces pathological changes in the uterine muscle endometrium.

Further study of the activity of plant extract of Gnetum africanum ingestion in rat's ovary reveals formation of fibromas from granulosa cells and cells of the ovarian stroma. The photomicrographs of ovarian tissues further reveal mucinous cystic tumours, seen as benign mucinous cystadenoma, mucinous cystadenocarcinoma and mucinous cyst of borderline malignancy. These observations are similar to the report that ovarian tissue produces oestrogenic hormones that cause uterine endometrial hyperplasia (Udoh, et al, 1996; Udoh and Udoh, 2005; Nakata, 2011).

However, oestrogenic influence of the plant extract of Gnetum africanum might interact with ovarian follicles to release excess oestrogen which could interfere with female reproductive efficiency. The findings allow the suggestion that excessive oral administration of the plant, Gnetum africanum, could result in dysfunctional female reproductive system.

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Legend

Figure 1:

- a. The Photomicrograph of the cross section of uterus from a normal rat. Note, normal Uterine muscle morphology (arrows). Magnification, X400, H & E stain.

- b. Photomicrograph of the cross section of uterine muscle of rat pretreated daily for 3 days with 17 β -estradiol (standard). Note, proliferation of the endometrial hyperplasia (arrows A), with proliferation of the endometrial glands (arrows B), glandular epithelial cells atypia (arrows C), Cytoplasmic pleomorphism (arrows D), and enlarged uterine cavity (arrow E). Magnification, X400, H & E stain.
- c. Photomicrograph of the cross section of uterine muscle of rat pretreated daily for 3 days with the Ethanolic extract of Gnetum africanum, (10mg/kg). Note, proliferation of the endometrial glandular tissue (arrow A), formation of numerous tin cyst (arrows B), dilation of endometrial glands (arrows C), and Uterine cavity enlargement, (arrow D). Magnification, X400, H & E stain.
- d. Photomicrograph of the cross section of uterine muscle of rat pretreated daily for 3 days with the Ethanolic extract of Gnetum africanum, (200mg/kg). Note, proliferation of the endometrial glandular tissue (arrows A), formation of numerous tin cyst, (arrows B), dilation of endometrial glands, (arrows C), and uterine cavity enlargement, (arrow D). Magnification, X400, H & E stain.
- e. Photomicrograph of the cross section of uterine muscle of rat pretreated daily for 3 days with the Ethanolic extract of Gnetum africanum, (700mg/kg). Note, endometrial hyperplasia (arrow A), with proliferation of the endometrial glands (arrow B), glandular epithelial cells atypia (arrow C), cytoplasmic pleomorphism (arrow D), and enlarged uterine cavity (arrow E). Magnification, X400, H & E stain.

Figure 2:

- a. Photomicrograph of the cross section of ovary of control rat. Note normal ovarian cell morphology. Magnification, X400, H & E stain.
- b. Photomicrograph of the cross section of ovarian tissues from rat pretreated daily for 3 days with Ethanolic extract of Gnetum africanum, (10mg/kg). Note, formation of fibromas (arrow A), formation of thecal cells of the ovarian stroma (arrow B), and ovarian cell atypia (arrow C). Magnification, X400, H & E stain.
- c. Photomicrograph of the cross section of ovarian tissues from rats pretreated daily for 3 days with Ethanolic extract of Gnetum africanum, (200mg/kg). Note, formation of fibromas (arrow A), formation of thecal cells of the ovarian stroma (arrow B), and ovarian cell atypia (arrow C). Magnification, X400, H & E stain.
- d. Photomicrograph of the cross section of ovarian tissues from rat pretreated daily for 3 days with Ethanolic extract of Gnetum africanum, (700mg/kg). Note, formation of fibromas (arrow A), formation of thecal cells of the ovarian stroma (arrow B), papiliferous ingrowth (arrow C) and columnar epithelium filled with the cystic cavity (arrow D). Magnification, X400, H & E stain.
- e. Photomicrograph of the cross section of ovarian tissues from rat pretreated daily for 3 days with 17 β -estradiol (1mg/kg), as standard. Note, formation of fibromas (arrow A), formation of thecal cells of the ovarian stroma (arrow B), and papiliferous ingrowths (arrow C) and columnar epithelium filled with the cystic cavity (arrow D). Magnification, X400, H & E stain.
- f.



Fig 1a (Control)

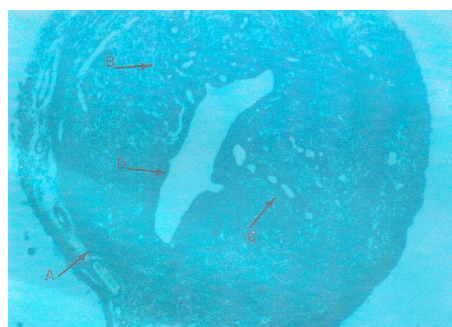


Fig 1b (17 β -estradiol: Standard)



Fig 1c (Ethanolic Extract: 10mg/kg)

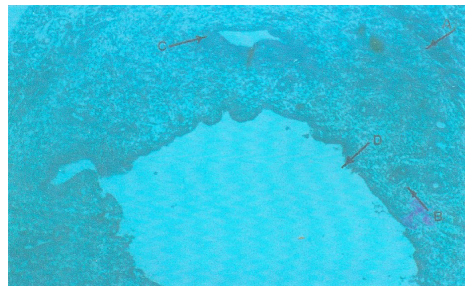


Fig 1d (Ethanolic Extract 200mg/kg)



Fig 1e (Ethanolic Extract 700mg/kg)

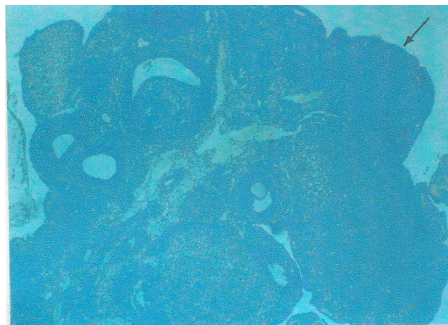


Fig 2a (Control)

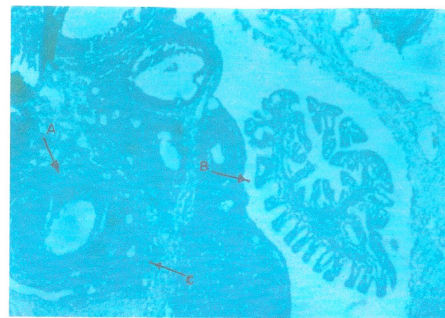


Fig 2b (Ethanolic Extract: 10mg/kg)

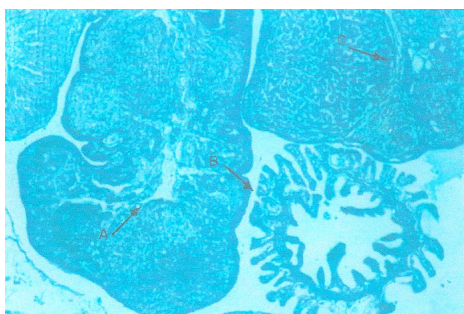


Fig 2c (Ethanolic Extract: 200mg/kg)

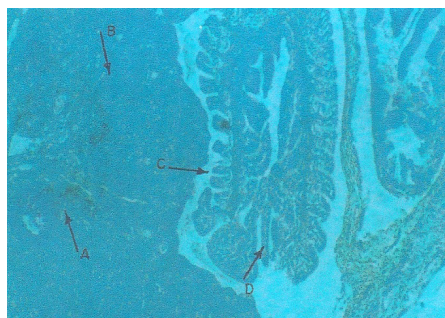


Fig 2d (Ethanolic Extract: 700mg/kg)

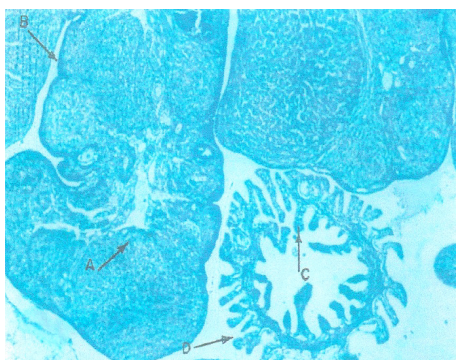


Fig 2e (17β-estradiol: Standard)

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