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Effect Of Ethanolic Extract From The Leaf Of *Gnetum Africanum* On Endocrine Function In Female Rats

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Abstract
Activity of ethanolic extract of the leaf *Gnetum africanum* impacted on endocrine functions in female rats was investigated. The plant is extensively consumed as vegetable by most people of Niger Delta region in Nigeria. The ethanolic extract of the plant was soxhlet extracted followed by standard procedure. 40 female rats weighing between 120 and 150 g were divided into 4 group. Group 1, 2, 3 received orally 10, 200 and 700 mg/kg/d, ethanolic extract of the leaf of *Gnetum africanum* respectively; and group 4 received 2 ml normal Saline as control for 3 days. On 4th day after treatment rats of all groups were sacrificed and blood samples were collected through cardiac puncture into centrifugal tubes and allowed to clot. Serum-samples were centrifugally separated and collected into reagent bottles for use in hormonal assays. Results showed that treatment with various doses of ethanolic extract caused dose dependent decrease in serum levels of Follicle Stimulating Hormone (FSH), Progesterone and increase in Luteinizing Hormones (LH), Estrogen (Es) and no alteration in prolactine serum level. These observations allow the suggestion that reduction in serum levels of FSH and progesterone might be due to the negative feedback mechanism with respect to increased serum levels of estrogen.

Keywords: Ethanolic extract, *Gnetum africanum*, endocrine function, female rats and hormonal assays.

1. Introduction

Afang plant, identified as *Gnetum africanum* belonging to the family of *Gnetacea* is one of the superior vegetables to most people in Niger Delta region of Nigeria [Udoh, 2007]. It is been used since ancient time by most Nigerians for preparation of soupy meals for very important visitors and during traditional ceremony by cultures in most states of the Niger Delta region of Nigeria. The genus is cultivated in farms of most Nigeria people as a cash crop. For decades, the popular use of afang vegetable in preparation of meals has dramatically increased.
The plant vegetable is native to Africa of origin of west and central Africa. The phenotypic diversity within the *Gnetucea* is significant. Some species of *Gnetum africanum* plants have short stems that are completely hidden by the leaves of other wild plants. Other species have long sterms being arborescent, shrubby, sprawling and climbing plants. Arborescent species can reach 30 m height and may be branched or unbranched on their supportive tree-plants.

Gnetum africanum which is native to Africa is locally called afang by the people of Akwa Ibom and Cross River States of Nigeria, and has been employed in the country's herbal medicine as a diagnostic tool for measles in children. Certainly, the leaves of this plant have been consumed extensively as vegetable in diets.
However, the common assumption that *Gnetum africanum* is naturally harmless may be misleading. As a cash crop the plant-vegetable contributes to the poverty alleviation of some less privileged Nigerians serving as an income earning [Besong et al, 2001]. Many Nigerians develop strong appetite for afang-soup because of its nutritional values. This leads to high rate of consumption of the plant vegetable. The intensed consumption of *Gnetum africanum* leaves in diets probably the cause of stomach protrusion in some women of the Niger Delta Region of Nigeria. However, women who were addicted to *Gnetum africanum* meals often complained of menstrual cycle irregularity and heavy menstrual flow exceeding a normal period of 5 days [Udoh, 2007].

The acceptability and optimal utilization of the leaves of *Gnetum africanum* as vegetable in Nigerian diets have given us inclination to carry out investigation into its effect on female reproductive hormones.

2. Material and Method

2.1. *Collection of plant material*

The leaves of *Gnetum africanum* plant were harvested from thick bushes in Calabar, Cross River State, Nigeria. The leaves of the plant were collected in the months of March and April, 2010. It was identified as *Gnetum africanum* by Dr. 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 allowed to dry overnight at room temperature of 28±1°C. The leaves were further subjected to oven dry (Behrmanning Iroy, N.Y.) at the temperature of 40°C for 8h. The dried leaves of the plant were sliced off into bits and ground into powder using an electrical blender (Behrmanning Iroy, N. Y).

The powder sample (100 g) of the leaf of *Gnetum africanum* was wrapped in a wattman filter paper, size 40 and placed in 500 ml Soxhlet extractor and the alkaloid extract was extracted following a modified method of Udoh, (2007). 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 evaporated *in vacou* at a reduced temperature of 40°C. About 40 g ethanolic solid extract was partitioned in equal volumes (100 ml) of both distilled water and chloroform for 24 h for proper separation into two phases. The two phases were separated into chloroform and water fractions, respectively, using separating funnel. Either chloroform or water fractions was evaporated into solid form. The solid extract of the water fraction was tested to contain alkaloids with negligible quantity of tannins.

2.3 Treatment

40 adult female rats weighing between 120 – 150 g were obtained from Animal House Unit of the Department of Pharmacology, University of Calabar, Nigeria. The rats were allowed to acclimatize in the Laboratory for a period of 7 days. The rates were housed in standard pelleted diet and water *ad libitum*. An ambient temperature was 28±1°C. The rats were divided into 4 groups of 5 rats per group. Groups 1, 2 and 3 received alkaloids extract of *Gnetum africanum* 10, 200 and 700 mg/kg/d, respectively. While group 4 received normal saline as control, for 3 days. After treatment, rats of all groups were sacrificed.

2.4. Serum Sample

Blood samples were collected through cardiac puncture from rats of all groups into centrifugal tubes. The blood samples were allowed to clot for 30 minutes and the serum-samples were separated by centrifugation. The Seri separated were aspirated into blood sample bottles for use in the experiment.

2.5 Hormonal assays

The methods of microwell (sigma, USA) enzyme linked immunoassay (ELISA) were employed to estimate the serum levels of estrogen, progesterone, follicle stimulating hormone (FSH) and prolactine (PRL). These methods required calibration curves for determination of the corresponding concentration of the test samples.

2.6. Uterine Weight Metric

Seventy-five immature female rats weighing about 90± g were divided into 15 groups of 5 female rats per group. Ethanolic extract was administered to 9 groups of rats; groups 1 – 3 received 10 mg/kg/d; groups 4 – 6 received 200 mg/kg/d and groups 7 – 9 received 700 mg/kg/d; while groups 10 - 12 received 17^{β} estradiol, 1 mg/kg/d as standard and 13 -15 received normal saline 0.5 ml per rat per day as control, for 3, 14 and 30 days respectively. On day one post treatments rats of all the groups were sacrificed and their uteri were isolated, placed on size 40 wattman filter papers to dry. The isolated dried uterine muscles were weighed using an electric balance (Ohaus, USA).

2.7 Statistical Analysis

Data obtained from this work were analyzed statistically using ANOVA followed by a post test (Turkey-Kramer multiple Comparison test). Differences between means were considered significant at 1% and 5% level of significance, $P < 0.01$ and 0.05.

3. Results and Discussion

3.1. Results

Ethanolic extract of the leaf of *Gnetum africanum* (10, 200 and 700 mg/kg/d) administered to both male and female adult rats for 3 days induced increase serum levels of Luteinizing hormone in them. Ironically, the same treatment caused a significant decrease ($P< 0.01$) in serum level of Follicle Stimulating Hormone (FSH) of female rats and significant increase ($P< 0.01$) in that of the male rats when compared to control (Table1).

However, treatment of rats with three graded doses of the ethanolic extract of *Gnetum africanum* caused a dose dependent increase in the serum levels of estrogen ($P< 0.01$), table 1. Whereas, treatment with dose levels of 10 and 700 mg/kg/d of *Gnetum africanum* for 3 days exhibited a significant decrease ($P< 0.05$) in the serum level of progesterone, while rats treated with the extract (200 mg/kg/d) for 3 days showed a significant increase ($P< 0.05$) in the serum level of progesterone (Table 1).

Actually, progesterone was not detected in the serum of male rats.

In another study, uterine metric system revealed that the alkaloids extract administration to female rats induced dose related increase in uterine muscle weight. These increases were similar to the increase uterine weight induced by 17β- estrogen (Table 2). The induced increase in uterine weights of rats treated with extract were significantly different ($P< 0.1$) compared to control.

3.2 Discussion

The results of this investigation show that repeated consumption or oral administration of *Gnetum africanum* leaf extract influences the physiological activities of the reproductive hormones. The extracts pretreatment caused estrogen-progesterone hormonal imbalance, decrease in serum concentration of the Follicle Stimulating Hormone (FSH) as well as an increase in Luteinizing Hormone (LH). Estrogens and progesterone are among the most important hormones for implantation of the blastocyst and pregnancy maintenance in humans and other species [Winter et al, 1998; Udoh et al, 2004; Srirajaskanthan et al, 2009; Lyakhovich et al, 2010].

Oral administration of ethanolic extract of *Gnetum africanum*, daily for 3 or 30 days caused increase in the serum concentrations of estrogen and Luteinising Hormone (LH). The increased serum levels of estrogen and Luteinising Hormone (LH) were dose dependent. This observation is similar to the effect of *Garcinia kola* on female reproductive hormones [Braide et al, 2004]. A number of investigators showed that estrogen and progesterone inhibited ovarian function [Goldsieher et al, 1974; Spellacy et al, 1980; Stadel, 1981]. Increase in serum level of estrogen also exerts a negative influence on fertility in women [Braide et al, 2004]. It is also known that LH stimulates ovulation growth of corpus Luteum and progesterone release [Miller et al, 2002; O' Rahilly, 1973]. Therefore, LH acts to augment progesterone secretion by granulose cells, which stimulates FSH release at midcycle [Bowman and Rand, 1980]. Ironically, ethanolic extract administration induces reduction in serum FSH level. The reduction in the serum level of FSH may be due to increased serum levels of estrogen observed in the study. It has been revealed that ingestion of the ethanolic extract of the leaves of *Gnetum africanum* caused a dose related increase in uterine muscle weights. This increase could be as the result of increase level of serum estrogen. The study therefore reveals that the ethanolic extract of *Gnetum africanum* possesses oestrogenic activity with inhibitory release of FSH by the anterior pituitary. The oestrogenic action of the extract could bring about negative feedback effect of oestrogen on FSH production in a manner similar to the observations by Okokon et al (2011), Jones, (1981); Cousins et al, (1980) and Spellacy et al, (1979).

4. Conclusion

In Conclusion, this finding suggests that the ethanolic extract of the leaf of *Gnetum africanum* is a phytoestrogen and could be pharmaceutically formulated as a female contraceptives.

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Table 1: **Effect of Ethanolic extract of leaves of *Gnetum africanum*, repeated administration on the serum levels of Follicle Stimulating Hormone (FSH), Lutenizing Hormone (LH), estrogen (es), progesterone (Pro) and Prolactin (PRL) in female Rats.**

Treatment	Dosage (mg/kg)	Duration of Treatment (Days)	n	Serum Level of Hormone				
				FSH (µ/l)	LH (µ/l)	Es (pg/ml)	Pro (Pg/ml)	PRL (µ/l)
Control	0.85% NaCl	3	5	14.7 ± 0.1	2.4 ± 0.1	8± 0.05	8± 0.1	12.8± 2
alk	10	3	5	1.0 ± 0.1*	2.6 ± 0.1	10.0 ± 0.05*	4.6 ± 0.5*	ND
alk	200	3	5	1.3 ± 0.1*	3.8 ± 0.05	11.7 ± 0.1*	10.0 ± 0.05**	ND
alk	700	3	5	1.2 ± 0.05*	2.9 ± 0.05	13.2 ± 0.05*	5.0 ± 0.05*	ND

*P< 0.01 and **P< 0.0.05

Key:

ND = Not Detected

**P = Significantly different from Control

n = Number of rats used.

Table 2: **Effect of Ethanolic extract of leaves of *Gnetum africanum*, repeated administration on the uterine muscle weight (uw) of female Rats.**

Duration of Treatment (days)	Wt of Rats (g)	Control (0.85% NaCl)		Standard; 17β estradiol (1mg/kg)		Effect of Ethanolic extract treatment (mg/kg/d) on the uterine muscle weight of female rats					
						10		200		700	
		uw	Ruw	uw	Ruw	Uw	Ruw	Uw	Ruw	uw	Ruw
3	90	0.6 ± 0.1	0.7	2.5± 0.1*	2.8	2.8 ± 0.05**	3.1	3.0 ± 0.05	3.3	2.6 ± 0.01	2.9
14	90	1.1 ± 0.1	1.2	2.7± 0.1*	3.0	2.0 ± 0.05**	3.3	3.4 ± 0.05	3.8	2.8 ± 0.05	3.1
30	90	1.3 ± 0.1	1.4	2.4 ± 0.05**	2.7	3.5 ± 0.1**	3.9	3.1 ± 0.1	3.4	2.7 ± 0.1	3.0

*P < 0.01; **P 0.05 and n=5

Key:

uw = Uterine weight

Ruw = Relative uterine weight

*P, **P = Significantly different from Control

Wt =weight

Mg/kg/d= milligram per kilogram body weight per day.

n = 5= Five rats per group.

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