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An evaluation of sodium fluoride toxicity and the ameliorative potential of extract of *Phyllantus amarus* on reproductive hormones and lipid profile of adult male albino rats

CE Achikanu *, II Ujah and MO Ezenwali

Department of Applied Biochemistry, Faculty of Applied Natural Sciences, Enugu State University of Science and Technology, Enugu, Enugu State, Nigeria.

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Abstract

This study investigated the toxicity of sodium fluoride on male reproductive hormones and the ameliorative effects of extract of *Phyllantus amarus* on male reproductive hormones of adult albino rats. The study consisted of six equal groups of five albino rats that were administered with sodium fluoride and the *Phyllantus amarus* over a period of two weeks. The result indicates a possible toxicity of sodium fluoride on hormone and lipid profile and the probable capacity of the extract of *Phyllantus amarus* to ameliorate this toxicity.

Keywords: Toxicity; Sodium fluoride; Reproductive; Hormones; *Phyllantus amarus*

1. Introduction

Plants are medicinal and contain bioactive phytochemicals or bionutrients [1]. Phytochemicals have an important function in preventing long term diseases like cancer, diabetes and coronary heart disease. These phytochemicals acts as dietary fibre, antioxidants, anticancer, detoxifying agents, immunity-potentiating agents and neuropharmacological agents [2].

Phyllantus amarus (Euphorbiaceae) is a widely spread small erect, tropical perenial herbal shrub whose stem has a green capsule, and grows up to 10-60 cm high depending on the environmental factors [3]. It exhibits anti-viral, anti-inflammantory and hepatoprotective, radioactive and antioxidant activities have been documented [4]. It grows well in India, China, Phillippines, Cuba, Guam, and from Sierra Leone, Nigeria, Equitorial Guinea, and everywhere in tropical regions [5]. It is variously known for as gale of wind, stonebreaker, carry me seed, seed on the leaf and pick-a-back.

Traditionally *Phyllanthus amarus* herb has found its traditional usefulness in several health problems such as diarrhoea, dysentery, dropsy, jaundice, intermittent fevers, urinogenital disorders, scabies and wounds [6]. *P. Amarus*is used in the treatment pain, gonorrhea, diabetes and chronic dysentery, itchiness, wounds, bruises, scabies, ulcers and sores. It has effect in excretory system; thus, it is used in the treatment of kidney/gallstones, kidney urinary bladder disturbances, and prostate problems [7]. *Phyllantu samarus* efficacious in the treatment of gastro-intestinal like dyspepsia, colic, diarrhea, constipation and dysentery [3]. The young shoots of plant are administered in the form of an infusion for the treatment of chronic dysentery. Fresh leaf, whole plant, flower, stem juice and root extract has been applied in the treatment of numerous diseases and has effects on the lipid profile [8].The plant has also reported to increase testosterone level, a male fertility hormone [9].

*Corresponding author: CE Achikanu; E-mail: cosmasachikanu@gmail.com
Department of Applied Biochemistry, Faculty of Applied Natural Sciences, Enugu State University of Science and Technology, Enugu, Enugu State, Nigeria.

Infertility is becoming increasingly a societal problem, and cause of many divorce in marriage and resultant effect of so many unresolved social problems and/or relationships [10]. Men are also to be blamed in so many cases where there is lack of procreation in the family [11]. A lot of factors have been suggested to be responsible for the infertility in male [12]. The ultimate consequence of infertility may be divorce, injuries especially on the part of the women and bruised relationship [13]. The solution to this our societal problem can be found in plants around us with little or no cost.

Plants have been employed in traditional medicine for the prevention and treatment of various diseases. Sodium fluoride available in various products has been shown to cause toxicity in various biochemical parameters. This research was thus carried out to investigate the toxicity of sodium fluoride on reproductive hormones and lipid profile, and the ameliorative effects of extract of *Phyllanthus amarus* on the reproductive hormones and lipid profile of adult Male Albino Wistar rats.

2. Methodology

2.1. Animals

Male albino rats were obtained from Nnamdi Azikiwe University, Awka. These animals were housed in steel cages within the laboratory of Applied Biochemistry of Enugu State University of Science and Technology. They were maintained, given standard feed and clean drinking water *ad lbitum.* The animals were acclimatized for a period of one week prior to the experiment. All experiments were in compliance with the National Institute of Health Guide for care and use of laboratory animals.

2.2. Collection and preparation of plant materials

*Phyllantusamarus*leaves were collected from Agbani, in Nkanu of Enugu State and identified by Prof. Nwamba of Applied Biology, Enugu State University of Science and Technology. The plucked leaves were air dried for seven days and then were ground into powder using manual grinding machine.

2.3. Extraction of samples

A quantity, 800 g of the ground sample was weighed using analytical weighing balance and soaked in 4000 ml of petroleum ether, it was shaken vigorously and left inside the bottle for 24 hours.The ratio of mixing the sample and solvent is 100 g: 500 ml. The percentage yield of the extract is calculated by the formula:

$$\% \text{ yield } = \frac{\text{Weight of concentrate (in grams)}}{\text{Weight of grounded sample (in grams)}} \times \frac{100}{1}$$

The mixture was decanted and filtered into a beaker using separating funnel and a filter paper. The weight of the residue was taken after it is dried andthe extract was concentrated.

2.4. Feeding of rat

The weights of the rats were taken immediately they were purchased and were subjected to one week acclimatization. During this period of acclimatization, the rats were all fed with the grower's mash mash feed. The feeding method was *adlibitum*.

2.5. Experimental design

Forty-eight sexually mature male adult albino rats were obtained from the animal house of Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. These animals were housed in steel cages within the laboratory of Applied Biochemistry of Enugu State University of Science and Technology. They were maintained, given standard feed and clean drinking water *ad libitum.* The animals were acclimatized for a period of one week prior to the experiment. After acclimatization, the rats were grouped in seven equal groups of five rats each as follow:

Group I: received daily oral dose of normal feed (positive control)

- Group II: received daily oral of NaF (10 mg/kg) (negative control)
- Group III: received daily oral dose of NaF (10 mg/kg) + extract (200 mg/kg)
- Group IV: received daily oral dose of NaF (10 mg/kg) + extract (300 mg/kg)
- Group V: received daily oral dose of NaF (10 mg/kg) + extract (800 mg/kg)
- Group VI: received daily oral dose of NaF (10 mg/kg) + Viagra (2 mg)

2.6. Sacrifice and sample collections

The rats were at the time of first weighted and then anaesthetized with chloroform and cardiac puncture as performed to obtain blood samples for FSH, LH and testosterone analysis. Then cervical dislocation was carried out. The testes were collected, cleared from the surrounding fat and weighed using analytical weighing balance, body weights were assessed relative to animal testes weight.

2.7. Determination of hormonal profile

Blood was collected into sample bottles through rectobulba plexus in the eye into non-heparinised sample bottles to obtain serum for the determination of hormonal profile.

2.8. Test procedure for testosterone

Sufficient wells for calibrators, control and the test samples were properly placed in duplicate. 50 µl of each calibrator, control and test samples was added to the corresponding labelled wells in duplicate followed by addition of 100 µl of conjugate working solution. The solution was properly mixed and incubated on a plate shaker (approximately 200 rpm) for 1hr at room temperature. Each well was washed properly with 300µl of diluted washed buffer and the plate firmly tapped against absorbent paper to ensure that it was dry. A volume, 150 µl of tetramethylbenzidine (TMB) substrate was added to each well and incubated for 15 minutes at room temperature before the addition of 50 µl stop solution. The absorbance was read at 450 nm within 20 minutes after addition of the stop solution using spectrophotometer (model: Labtech: Advanced microprocessor uv-vis spectrophotometer single beam- 295).

2.9. Leutanizing hormone (LH) and follicle stimulating hormone (FSH) assay

In the assay of LH and FSH, 50 ml of standard or test sample was measured into appropriate well. A volume, 100 ml of enzyme conjugate reagent was added into the well. This was gently mixed for 10 seconds and incubated at room temperature for 45 minutes. The incubated mixture was removed by flicking the plate contents into the well and washed 5 times with water. A volume, 100 ml of tetramethyl was added to the incubated mixture at room temperature and allowed to react for 20 minutes. The reaction was stopped by addition of 100ml of stop solution to the well and readings were taken at 450 nm within 15 minutes. Concentration of the test (A) was calculated as follows:

$$A = \frac{[\text{Absorbance of test}]x\ [\text{Concentration of standard}]}{\text{Absorbance of standard}}$$

3. Lipid Profile

3.1. Determination of cholesterol

Three test tubes were labelled blank, standard and sample respectively. Into the blank were added 10 µl of distilled water and 10 µl of standard to the labelled test tubes. Sample serum (10 µl) was added to the appropriately labelled test tube. Reagent (1 ml) was added to the three sets of the tubes, mixed and incubated at 37 C for 5 minutes. The absorbance of the sample (A sample) was measured against the reagent blank within 60 minutes at 500 nm.

Conc. Of cholesterol in sample = (ΔA sample/ ΔA standard *Conc. Of standard in mg/dL).

3.2. Determination of high density lipoprotein (HDL)

High density lipoprotein Cholesterol (HDL) level was determined using Randox kit.

The procedure involved two steps.

3.2.1. Precipitation step

The serum sample (0.3 ml) was pipetted into labelled centrifuge tubes. A drop of the precipitant solution or reagent was added to each of the centrifuge tubes. The contents in the various tubes were thoroughly mixed and allowed to stand for 15 minutes at room temperature (20 -25 C), then centrifuged at 2000 rpm.

3.2.2. Determination of cholesterol concentration in the supernatant

Three test tubes were labelled blank, standard and sample respectively. Into the blank were added 10 µl of distilled water and 10 µl of standard to the labelled test tubes. Sample serum (10 µl) was added to the appropriately labelled test

tube. Reagent (1 ml) was added to the three sets of the tubes, mixed and incubated at 37 C for 5 minutes. The absorbance of the sample (A sample) was measured against the reagent blank within 60 minutes at 500 nm.

Conc. of HDL-Cholesterol in sample = (ΔA sample/ ΔA standard *Conc. of standard in mg/dL).

3.3. Determination of low density lipoprotein (LDL) cholesterol concentration

The procedure involved two steps.

3.3.1. Precipitation step

The serum sample (0.2 ml) was pipetted into labelled centrifuge tubes. A quantity (0.2 ml) of the precipitant solution or reagent was added to each of the centrifuge tubes. The contents in the various tubes were thoroughly mixed and allowed to stand for 15 minutes at room temperature (20 -25 C), then centrifuged at 4000 rpm for 15 minutes.

3.3.2. Determination of cholesterol concentration in the supernatant

Three test tubes were labelled blank, standard and sample respectively. Into the blank were added 20 µl of distilled water and 20 µl of standard to the labelled test tubes. Sample serum (20 µl) was added to the appropriately labelled test tube. Reagent (1 ml) was added to the three sets of the tubes, mixed and incubated at 37 C for 5 minutes. The absorbance of the sample (A sample) was measured against the reagent blank within 60 minutes at 500 nm.

Conc. of Cholesterol in the supernatant = (ΔA sample/ ΔA standard *Conc. Of standard in mg/dL)

The LDL Cholesterol concentration in the sample is calculated as follows:

LDL Cholesterol =Total Cholesterol – Cholesterol in Supernatant

3.4. Determination of triacylglycerol concentration

Triacylglycerol concentration was determined using the method of Albers *etal*., (1978).

One hundred microliter (100 µl) of the reagent-1 was pipette into the reagent blank tube, standard tube and the sample tubes. In the standard test tube was added 10 µl of the standard (CAL) while 10 µl of the sample was pipette into the sample tube mixed thoroughly and incubated for 10 minutes at 20 – 25 C. Absorbance of the sample and the standard were measured against the reagent blank within 60 minutes at 546 nm.

Triacylglycerol Concentration = (ΔA sample/ ΔA standard *Conc. Of standard in mg/dL)

4. Results

The hormone levels were reduced in group II which were treated with sodium fluoride except for testosterone. The levels were restored particularly at higher concentrations in groups IV and V. The Viagra (group VI) also produced a restoration effect.

Table 1 Effects of *Phyllantus amarus* leaf extraction some hormones of male albino rats.

Groups/Hormones	Follicle stimulating hormone (FSH)	Luteinizing hormone (LH)	Testosterone
Group I (control)	14	16	14
Group II (Neg. control)	13	14	15
Group III	9	12	15
Group IV	13	15	15
Group V	17	16	16
Group VI	14	15	16

The lipid profile levels were reduced in group II except for cholesterol levels. There was again a restoration effects in the lipid profile particularly in groups IV, V and VI.

Table 2 Effects of *Phyllantus amarus* leaf extraction lipid profile of male albino rats.

Groups/Parameter	T. Cholesterol	LDL	HDL	VLDL	Triglyceride
Group I	2.8	1.3	0.8	0.7	0.7
Group II	2.6	1.2	0.6	0.3	0.7
Group III	2.5	1.4	0.7	0.4	0.5
Group IV	3.0	1.4	0.8	0.8	0.6
Group V	2.9	1.4	0.9	0.7	0.7
Group VI	2.8	1.3	0.8	0.7	0.6

5. Discussion

Plant based medication has been man's ultimate therapeutic agent over the years and is still in the frontline for improving human health [14].

Admiration of sodium fluoride reduced the levels of follicle stimulating hormone and luteinizing hormone, but the level of the testosterone remained unaffected. According to [15] observed that fluoride treatment brought about a significant decrease in the weight of testis, epididymis, and ventral prostate. The sperm motility and density were significantly reduced and marked reduction in the number of primary spermatocytes, secondary, spermatocyte, and spermatids. The levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), progesterone (P) and estradiol (E2) affected. The concentration of reproductive hormones was significantly lower in the three NaF-treated groups, and the endometrium was damaged and the maturation of follicles was inhibited. In addition, the total number of follicles of all types was significantly lower in the NaF-treated groups [16]. Sodium fluoride has also been noted to affect thyroid hormones such asT_3, T_4 and TSH hormones [17].

The administration of the *Phyllantus amarus* leaf extract had an effect on all the hormones which was dose-dependent with group V which had the highest dose producing the highest effect. This result agrees with work of [18] who observed that the methanol extracts of *Phyllanthus amarus* (800 mg/kg) caused an insignificant change in the levels of Luteinizing (LH) and Follicle stimulating (FSH) hormone. [19] in the study of the effect of *Phyllanthus amarus* on some reproductive indices of male albino rats observed reduction in weight of testes and epididymes, sperm motility, sperm viability, sperm count and sperm head abnormalities in male rats treated with *Phyllanthus amarus* when compared to the control.

Sodium fluoride also decreased the level of lipid parameters examined. [20], observed a concentration of sodium fluoride for the high-density lipoprotein (HDL)-cholesterol level, it showed lower results. An amelioration effect was observed upon the administration of the extract of *P. Amarus*. This agrees with the work of [8] on Effect of *Phyllanthus amarus* leaf extract on these rum lipid profile of alloxan-induced diabetic albino wistar rats.

Phyllantus amarus contains important bioactive component [21] whose effect can singly or in concert improve the biosynthetic processes involved in hormonal production. This study shows the effects of *phyllanthus amarus* leaf extract on the hormonal parameters of albino rats. The increase in the testosterone may act as a libido enhancer of fertility agent as claimed by traditional medicine practitioners. This is so because optimum level of testosterone is required for normal sex drive in adult male and an increase in the level of testosterone can lead to an increase in the spermatozoa [22] and hence an increase in male fertility [22].

The phytochemicals found present in the leaf of *P. amarus* include: flavonoids, tannins, saponins, alkaloids, terpenoids, steroids and cardiac glycosides. Flavonoids present in this plant has been shown to possess many pharmacological properties such as: anti-oxidant activities, anti-inflammatory activities, anti- cancer activities and anti- microbial effects hence, flavonoids may have a contributory effect to its fertility properties and other pharmacological effects the plant possesses [23]. Favonoids as an anti-oxidant, has a rejuvenating effect on cells or tissues, it is anti-aging hence can contribute substantially on the fertility effect of this plant. Alkaloids and tannins may also contribute to the plant's effects as antimalarial, anti-diarrhea and analgesic agents. Elevated serum levels of reproductive hormone stimulate spermatogenesis processes through testosterone production. Therefore, it could be that concentration of

phytochemicals in *Phyllantus amarus* at a moderate concentration is sufficient enough to stimulate the secretion of GnRH from the hypothalamus, thereby, resulted to higher serum levels of testosterone as observed in this study.

6. Conclusion

Sodium fluoride caused toxicity in the in the sex hormones and lipid profile. The administration of leaf extract of *Phyllantus amarus* to albino rats at different (increasing) doses and time (increasing) duration resulted in increases in serum concentrations of male sex hormones and lipid profile showing a possible ameliorating effect of the extract of the leaf.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

Statement of ethical approval

All the experiments were conducted in accordance with Animal Care Guide Line of Nigeria. There are no human subjects in this research and informed consent is not applicable.

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