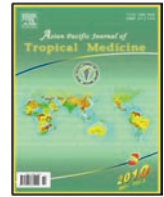


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## Document heading

Effects of aqueous extracts of *Irvingia gabonensis* seeds on the hormonal parameters of male guinea pigsObianime Acubowyedia Wolfe<sup>1</sup>, Uche Fidelia Ijeoma<sup>2\*</sup><sup>1</sup> Department of Pharmacology, University of Port Harcourt Nigeria<sup>2</sup> Department of Pharmacognosy and Phytotherapy, University of Port Harcourt Nigeria

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## ABSTRACT

**Objective:** To investigate the effects of the aqueous extracts of *Irvingia gabonensis* (Irvingiaceae) seeds on the hormonal parameters of male guinea pigs. **Methods:** The hormonal effects of *Irvingia gabonensis* on hormonal parameters of male guinea pigs were investigated and compared with that of proviron using enzyme immuno assay method, which was done by reaction of antibody with serum testosterone and testosterone label, magnetic solid phase separation and colour development step. The phytochemical screening of *Irvingia gabonensis* seeds was also carried out using standard procedures. **Results:** The aqueous extract of the *Irvingia gabonensis* seeds (50–400 mg/kg) caused a statistically significance increase ( $P < 0.05$  ANOVA) of testosterone in male guinea pigs, from  $(2.70 \pm 0.26)$  ng/mL to  $(3.10 \pm 0.42)$  ng/mL on the 7th day and to  $(3.30 \pm 0.48)$  ng/mL on the 28th day of the administration of the extracts. The highest increase was  $(3.30 \pm 0.48)$  ng/mL, being obtained after 28 days of treatment. These effects were similar to that of proviron, which was  $(2.80 \pm 0.28)$  ng/mL and  $(3.00 \pm 0.41)$  ng/mL on the 7th and 28th day of treatment respectively. The phytochemical screening of *Irvingia gabonensis* seeds revealed the presence of flavonoids, tannin, alkaloids, carbohydrate, volatile oils, terpenoids, saponins and cardiac glycosides. **Conclusions:** This study supports the claims on the use of the seeds of *Irvingia gabonensis* by traditional medicine practitioners as a fertility agent. However, further studies need to be done to isolate and characterize the active principle(s) responsible for this activity in this plant.

## 1. Introduction

*Irvingia gabonensis* (family of Irvingiaceae) is a large tree with dense compact crown of evergreen large leaves with edible seeds and sweet edible fruit pulp. It is found in rainforests, widely distributed in tropical West Africa and represented by two varieties: the fruit with sweet edible scanty fibrous pulp, fluted or cylindrical holes and fruits with bitter in edible very fibrous pulp and buttressed whole<sup>[1, 2]</sup>. *Irvingia gabonensis* tree usually reach maturity and begin flowering at 10–15 years old.

*Irvingia gabonensis* is commonly known as African Mango, Dikanut, or bush Mango. The seeds are known as Ogbuno in Ibo, Apon in Yoruba Nigeria. The paste from the kernel is known as Dika bread in Gabon and Etima in Cameroon<sup>[3, 4]</sup>.

*Irvingia gabonensis* is very useful to man. The bark of *Irvingia gabonensis* is used for diarrhea or dysentery, the fruit is rich in vitamin C and is consumed as a desert

fruit throughout Western and Central Africa<sup>[5]</sup>. The pulp is used for making jelly, jam and juices<sup>[6]</sup>. The seeds are used as thickener for soup, stew or as additive for flavoring<sup>[5, 7]</sup>. Traditionally the *Irvingia gabonensis* bark is given to women to shorten their breast feeding period. It is also used for colic and dysentery<sup>[6]</sup>, for hernias, yellow fever and as anti-poison<sup>[3]</sup>. It also has antimicrobial properties; the decoction of the bark is used for treating scabies, toothache and skin diseases<sup>[6]</sup>. *Irvingia gabonensis* has hypoglycemic effect hence its' used as anti-diabetic agent<sup>[8]</sup>.

*Irvingia gabonensis* is used by traditional medicine practitioners to increase fertility in men but there is no scientific based report on this claim. This study therefore, seeks to establish for the first time scientific information on the use of this plant as a fertility agent.

## 2. Materials and methods

## 2.1. Plant materials

The seeds of *Irvingia gabonensis* were collected in June 2008 from Bayelsa state, Nigeria. The plant was

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authenticated by H. D. Onyehusim, a taxonomist at Botany Herbarium of University of Port Harcourt Nigeria, where voucher specimen was deposited. All the chemicals used were of analytical grade.

## 2.2. Preparation and extraction of plant sample

The plant seeds were dried under the oven at temperature of 28 °C for 2 hours. The dried seeds were ground with hammer mill and the fine powdered were extracted using Soxhlet apparatus. The yields of the extract were obtained after removal of solvent. The extracts were stored in the refrigerator for subsequent reconstitution and use.

## 2.3. Animals

Adult male guinea pigs of average weight 300–600 g were obtained from the animal house of University of Port Harcourt. They were housed in a cage of five animals per cage and were allowed to acclimatize with the new environment for 10 days. The animals were properly fed on elephant grass throughout the experimental period.

## 2.4. Phytochemical screening

Chemical tests were carried out on the extracts and on the powdered specimens using standard procedures to identify the constituents [9, 10] by characteristic colour changes as described by Sofowara *et al* [11, 12].

## 2.5. Hormonal assay

The animals from different groups were given diethylether anesthesia and dissected. The blood samples were respectively collected in lithium heparinized tubes.

## 2.6. 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. 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. 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 100 mL of stop solution to the well and readings were taken at 450 nm within 15 minutes.

Concentration of the test was calculated as follows:

Concentration of test

$$= \frac{\text{Absorbance of test} \times \text{Concentration of standard (U/L)}}{\text{Absorbance of standard}}$$

## 2.7. Testosterone enzyme immunoassay

This was carried out in three stages namely: reaction of antibody with serum testosterone and testosterone label; magnetic solid phase separation step and colour

development step.

In the reaction of antiserum with serum testosterone and testosterone label, 50  $\mu$  L of test blood sample was pipetted into different tubes. The testosterone blocking reagent, diluted testosterone label and testosterone antiserum (100  $\mu$  L) were added to the test tube, covered, and mixed with vortex mixer.

## 2.8. Magnetic separation reagent reaction

100  $\mu$  L of testosterone separation reagent was added to different test tubes, covered and mixed with vortex mixer. The tubes were incubated in water bath at 37 °C for 30 minutes. The assay tubes were removed from water bath and placed on a magnetic base. The rack of tubes was kept upright in magnetic separation for 5 minutes after which the supernatant liquid from all the tubes were decanted. Then the tubes were changed from upright position and removed from the magnetic base.

## 2.9. Washing step

50  $\mu$  L of dilute testosterone enzyme immune assay (EIA) wash buffer was added to different tubes and mixed with vortex mixer. The rack of tubes was placed on a magnetic base. The tubes were kept upright in the magnetic separation for 5 minutes. The supernatant liquid were decanted from all the tubes and the separator was returned to an upright position. The rack of tubes was removed from the magnetic base. The whole process was repeated. This process is essential to remove all unbound components.

## 2.10. Colour development step

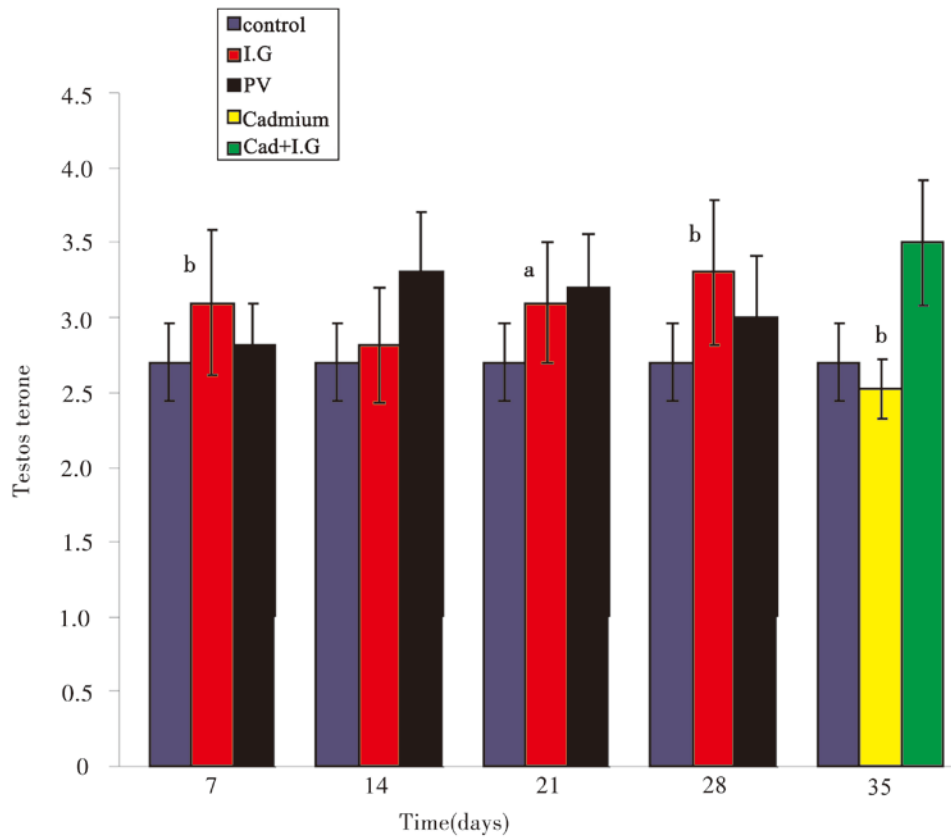
500  $\mu$  L of substrate solution was added to different test tubes, covered and mixed with vortex mixer. The tubes were transferred to 37 °C water bath and incubated for 6 minutes. The tubes were removed from the bath and 1 mL of EIA stop buffer was added to the different tubes and mixed with vortex mixer. The rack of tubes was placed onto a magnetic base and then tubes were kept upright in magnetic separation for 10 minutes. The absorbance of the test sample and standard were recorded with spectrophotometer and compared with the blank.

## 2.11. Statistical analysis

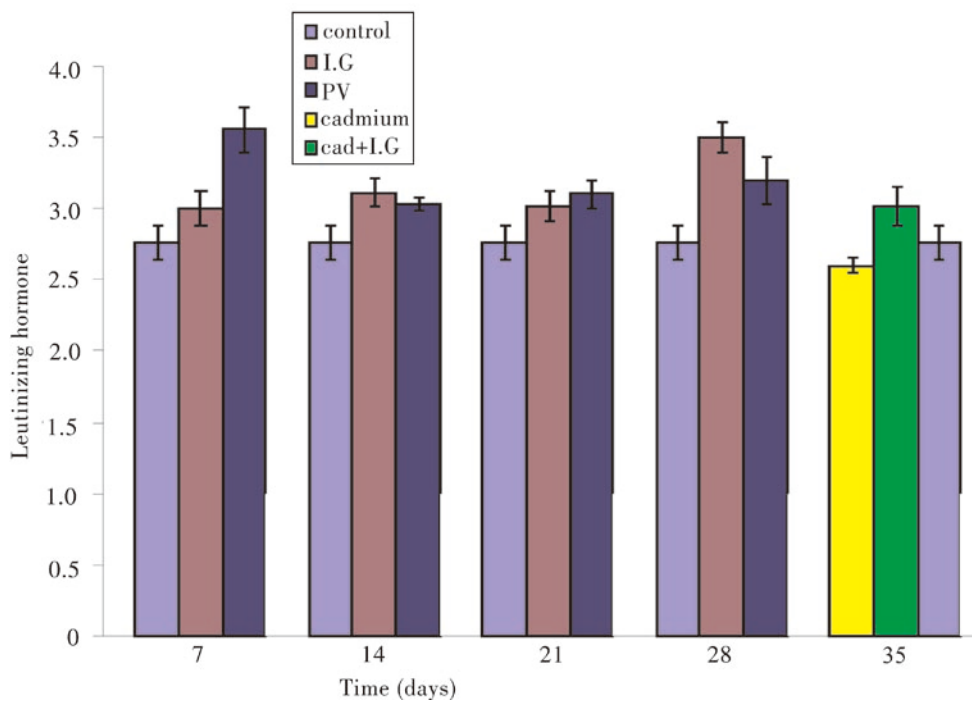
Data were expressed as mean  $\pm$  standard error of mean (SEM) of five observations. Statistical analysis of data was performed using analysis of variance (ANOVA). Results were subjected to Graph Pad prism 5 demo (software) analyses, the differences between mean accepted as significant at  $P < 0.05$  (ANOVA).

## 3. Results

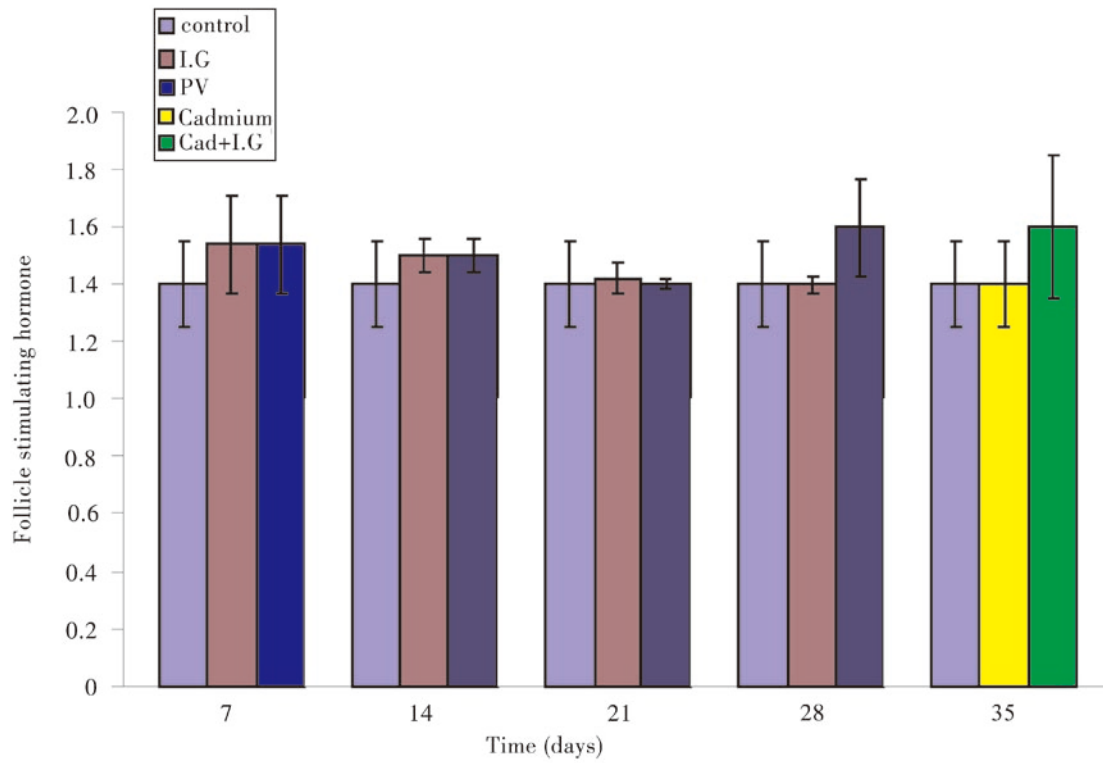
In time-dependent study, an optimum effect ( $3.30 \pm 0.60$ ) ng/mL, that is, an increase in testosterone level of male guinea pigs was obtained on the 7th and 28th day of post exposure period with 200 mg/kg of *Irvingia gabonensis* (Figure 1). This effect was statistically significant at  $P < 0.001$  (ANOVA) and was very comparable to the observed effects



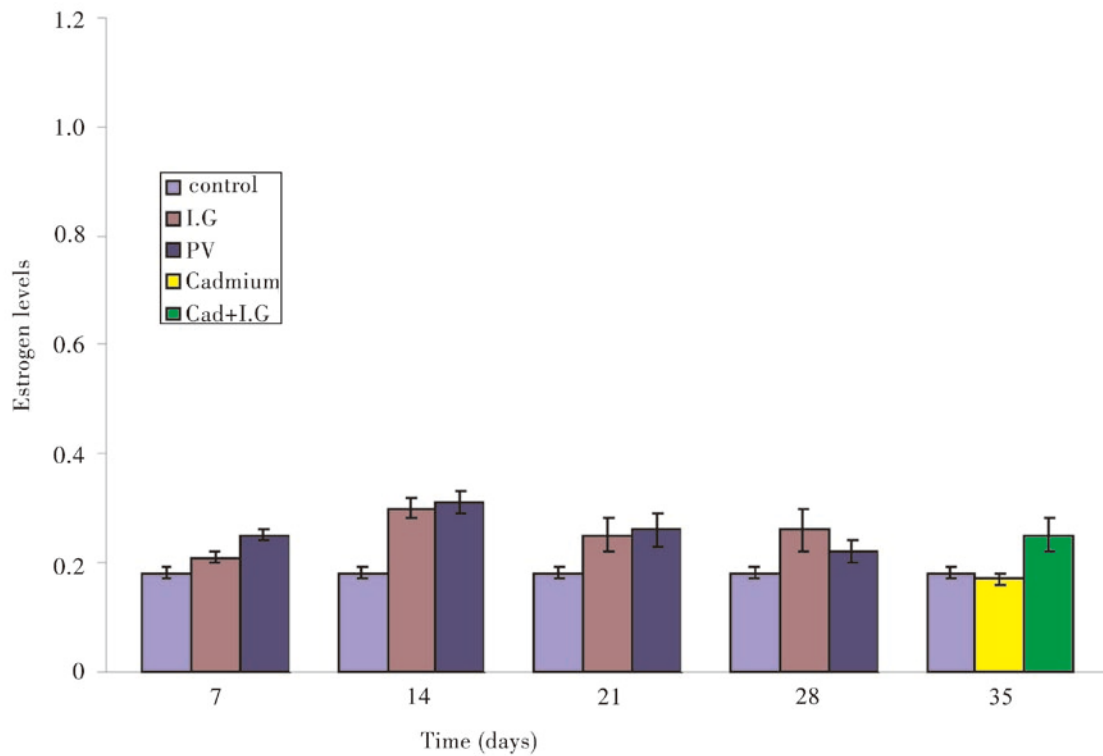
**Figure 1.** Time-dependent effect of *Irvingia gabonensis* on the testosterone of male guinea pigs. <sup>a</sup>represents significant values at  $P < 0.05$ , <sup>b</sup>represents significant values at  $P < 0.001$  (ANOVA); I.G: *Irvingia gabonensis* seed extracts, cad: cadmium, PV: Proviron.



**Figure 2.** The time-dependent effect of *Irvingia gabonensis* on leutinizing hormone of male guinea pigs. I.G: *Irvingia gabonensis* seed extracts, cad: cadmium, PV: proviron.



**Figure 3.** The comparative effect of *Irvingia gabonensis* and proviron on FSH of male guinea pigs. I.G: *Irvingia gabonensis* seed extracts, cad: cadmium, PV: proviron.



**Figure 4.** The effect of *Irvingia gabonensis* on the estrogen level of male guinea pigs. I.G: *Irvingia gabonensis* seed extracts, cad: cadmium, PV: proviron.

of proviron (12.5 mg/kg), which was  $(2.80 \pm 0.28)$  ng/mL and  $(3.00 \pm 0.41)$  ng/mL on the 7th and 28th day of treatment respectively. However, proviron exhibited the highest effect on the 14 and 21 day which persisted with slight decrease on the 28 days treatment. While *Irvingia gabonensis* exhibited optimum effects on the 7 and 28 days treatment (Figure 1). Furthermore, in a pathological study, using cadmium as a known reproductive toxicant (0.05 mg/kg), *Irvingia gabonensis* (400 mg/kg) caused significant increases in testosterone level  $(3.50 \pm 0.42)$  ng/mL. This was previously decreased to  $(2.50 \pm 0.20)$  ng/mL by pretreatment with cadmium ( $P < 0.001$  ANOVA) (Figure 1).

Furthermore, the aqueous extracts of *Irvingia gabonensis* (50–400 mg/kg) caused slight but not statistically significant increase on the levels of follicle stimulating hormone (FSH), and leutinizing hormone (LH) levels from  $(2.8 \pm 0.12)$  m/u/L and  $(1.40 \pm 0.15)$  m/μ/L to  $(3.10 \pm 0.05)$  m/μ/L and  $(1.50 \pm 0.06)$  m/μ/L respectively (Figure 2, 3). The estrogen level was also insignificantly increased from  $(0.18 \pm 0.01)$  nmol/L to  $(0.30 \pm 0.02)$  nmol/L (Figure 4). This was also comparable to the observed effects of proviron on above hormones of male guinea pigs.

The phytochemical screening of *Irvingia gabonensis* seeds revealed the presence of flavonoids, tannin, alkaloids, carbohydrate, volatile oils, terpenoids, saponins, cardiac glycosides and volatile oils, no resins was found.

#### 4. Discussion

This study shows the effects of aqueous extract of the seeds of *Irvingia gabonensis* on the hormonal parameters of male guinea pigs. The results show that *Irvingia gabonensis* seeds extract causes an increase in the level of testosterone but has an insignificant effect on the levels of follicle stimulating hormone (FSH), estrogen and leutinizing hormone (LH). The increase in the level of testosterone was found to be statistically significant ( $P < 0.05$  or  $0.001$ , ANOVA). The increase in the testosterone may be responsible for the effect of this plant as a libido enhancer or fertility agent, as claimed by traditional medicine practitioners. 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<sup>[6]</sup> and hence an increase in male fertility<sup>[6]</sup>.

The phytochemicals found in the seeds of *Irvingia gabonensis* include: flavonoids, tannins, saponins, alkaloids, terpenoids, volatile oils, 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 this plant<sup>[13–16]</sup>. Flavonoids as an anti-oxidant, has a rejuvenating effects 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 ant malarial, anti-diarrhea and analgesic agents<sup>[6, 14]</sup>.

This study therefore, supports the claims on the folkloric use of the seeds of this plant to improve libido and

reproductive function in men. However, further study needs to be done to isolate, identify and characterize the active principle present in the seeds of this plant.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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