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# EVALUATION OF THE HYDROETHANOLIC LEAF EXTRACTS OF *SOLANUM AETHIOPICUM* (GARDEN EGG) ON THE CONCENTRATION OF REPRODUCTIVE HORMONES AND HISTOLOGY OF THE TESTIS OF MALE WISTAR RATS

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## ABSTRACT
*Solanum aethiopicum* forms part of Nigerian foods and indigenous medicines and it is commonly consumed. This study evaluated the effects of the hydroethanolic leaf extract of *solanum aethiopicum* on the concentration of reproductive hormones and histology of the testis of male Wistar rats. 40 adult male rats with average weight of 245g were used for the study. The rats were divided into four groups of 10 rats each. Group 1 served as control and received only distilled water while groups 2, 3 and 4 served as the experimental groups and received 100mg/kg, 200mg/kg and 300mg/kg of *Solanum aethiopicum* leaf extract respectively for 30 days, after which half of the rats in each group were sacrificed while the rest were allowed for another 30days without the administration, to evaluate the effects of discontinued administration of the extract. The effects of the extract on the serum levels of Testosterone, Follicle-stimulating Hormone, Luteinizing Hormone and Histology of the testis were evaluated. The results showed a significant increase in the concentration of all the reproductive hormones in the test groups in a dose dependent manner, which reversed significantly upon discontinued administration. The histology of the testis in the extract treated groups showed improved spermatogenic features, when compared with the control. It was therefore concluded that the hydroethanolic leaf extract of *S. aethiopicum* can boost the production of the reproductive hormones and preserve the histology of the testis and its continuous intake may improve fertility in male Wistar rats.

**KEYWORDS:** Sperm, Solanum, Hormones, Reproductive, Administration and Extract.

## INTRODUCTION
Plants are vital sources of modern medicines and also serve as natural sources of fertility boosting and anti-fertility substances.[1] Infertility and subfertility have been a major medical and social preoccupation since the dawn of human existence and women have always been the ones used to identify fertility. For ages now, couples have been more concerned with conception and its difficulties.[2] Infertility refers to an inability to conceive after having regular unprotected sex. Medical evidence shows that both genders have same rates of infertility. Yet African traditions view infertility as always the women's fault. Male infertility can present as oligospermia, azoospermia, erectile dysfunction, poor libido, psychological challenges, poor hormonal levels, poor sperm quality (low sperm count, abnormal morphology, and sluggish sperm cells) due to high level of oxidative stress and a lot more.

Although there are proven treatment plans (fertility booster or aphrodisiacs) for male infertility, they are either very expensive like hormonal therapies and medications or highly invasive like surgeries.[3] In order to tackle this disorder, one of the less expensive and non-invasive methods is the use of medicinal plants or herbs. The world health organization (WHO) estimated that 80 percent population of some countries presently uses herbs for some aspect of primary health care. Approximately 25 percent of modern drugs in the United States are gotten from plants.[4, 5, 6, 7] Medicinal plants have been extensively used to improve or regulate fertility in males. The beneficial effects of medicinal plants on male fertility include increased sexual desire (libido),[8] [8, 9, 10] hormone stimulatory activity (GnRH LH, FSH and testosterone),[12, 13] increased semen volume, increased sperm count and increased viability and motility of spermatozoa. The interest in using herbal medicine in spite of the presence of synthetic treatments may be accounted for by the belief that natural aphrodisiacs or fertility boosters have less or no side effects, nutritional value and are cheaper.[3] These medicinal plants are leafy vegetables or fruits we could

easily find around us and incorporate into our daily diets. Most of them contain flavonoids, saponins, tannins, alkaloids and many other constituents known to have fertility boosting effects.[14]

The *Solanum aethiopicum* (garden egg) is a common traditional vegetable in tropical Africa, grown mainly for its leaves and fruits. The fruits and leaves of *S. aethiopicum* are mostly eaten or used in its fresh state. It could also be steamed, juiced, pickled, boiled or used in preparing stews with other vegetables or meats, while its leaves are mostly used in soups and also in combination with other vegetables or juiced. The leaves of this garden egg are characterized by its small roundish nature, green in color and have a bitter-sweet taste. The leaves of *Solanum aethiopicum* are eaten as a leaf vegetable and are actually more nutritious than the fruit and just like other vegetables contains essential nutrients such as vitamins and minerals in a good quantity which play an important role in human health improvement.

This study is therefore aimed at establishing the impact of the consumption of *Solanium aethiopicum* leaf on male fertility, through the evaluation of its effect on the serum concentration of the gonadotropic hormones (FSH and LH), Testosterone and the histology of the Testes. The effects of discontinuing the consumption of the leaf on the serum level of the above reproductive hormones were also evaluated.

## MATERIALS AND METHODS

A total of 40 male Wistar rats with average weight of 245g were used for this study. The animals which were purchased from the animal house of Department of Human Physiology, University of Port Harcourt, were housed in an environment of normal room temperature separately. They were fed with standard finisher feeds (Top feed, Nigeria) and water for 14 days to allow for acclimatization before the experimental processes began. *Solanum aethiopicum* leaves used for this study was bought from a Mile 3 Market in Port Harcourt, Rivers state and sent to the Plant Science and Biotechnology Department of the University of Port Harcourt for identification and authentication, with Herbarium Number (UPH/P/185). The leaves were cleaned properly before drying under the sun until they were well dried. The dried leaves were ground into powder and 3 kg of the powder was put into a maceration jar. 6000 mL of 70% ethanol (hydro-ethanol) was added to the plant sample in the maceration jar. The mixture was allowed to stay for a period of three days (72 hours), during which it was continuously agitated thrice daily so as to give room for the sample to absorb the solvent. After the period, the substance was then filtered with a white handkerchief into another maceration jar and re-filtered with Whitman filter paper which was folded into four portions and placed in a glass funnel so as to get a clear filtrate. The filtrate containing the extract was then poured into an evaporating dish and dried in a water bath at a temperature of $45^0$C and a constant observation until it dried into paste.

The study was divided into two phases.

- **Phase 1** -30days administration of the extract
- **Phase 2-** 30days of discontinuation of administration of extract.

The 40 male rats used for this study were divided into four (4) groups with each group containing ten (10) rats as shown in the table below.

**Table 1: Experimental Design of the Study.**

| S/N | GROUP NAME | SUBSTANCE ADMINISTERED |
|---|---|---|
| 1 | **Group 1(Control)** | Distilled water |
| 2 | **Group 2** | 100mg/kg BW hydro-ethanol extract of leaves of *S. aethiopicum* |
| 3 | **Group 3** | 200mg/kg BW hydro-ethanol extract of leaves of *S. aethiopicum* |
| 4 | **Group 4** | 300mg/kg BW hydro-ethanol extract of leaves of *S. aethiopicum* |

After 30 days of oral administration (using an oral gavage method), half of the male Wistar rats from each group by random selection were sacrificed under light chloroform anesthesia. The blood samples were collected by jugular puncture using syringes into universal bottles and taken to the laboratory for analysis of the reproductive hormones (Follicle Stimulating Hormone, Luteinizing Hormone and Testosterone) while the remaining half was sacrificed from each of the test groups after 30days discontinuation using the same method of sample collection.

The testes of all the rats were harvested through abdominal incision and fixed in 10% formalin and processed by the usual method for molten paraffin embedding at the Anatomy Department in University of Port Harcourt, Rivers State, Nigeria. Section of 4-20µm thickness by microtome was taken and stained with haematoxylin and eosin stain for histopathological examination through light microscope by the usual method described by other researchers.[15]

Ethical approval was obtained from the research Ethics committee of the University of Port Harcourt (UPH/CEREMAD/REC/MM74/015). The statistical analysis was done using the standard package for social sciences (SPSS version 20.0). The results were analysed using the one-way analysis of variance (ANOVA) with a significant difference at $p<0.05$. LSD and turkeys' multiple comparison were used to test for significant differences between the groups. The results were presented as mean ± standard error of mean.

## RESULTS

**Table 2: Effects of *Solanum aethiopicum* leaf extract on Reproductive Hormones.**

| Groups | FSH (miu/ml) | LH (miu/ml) | TESTOSTERONE (miu/ml) |
|---|---|---|---|
| **1 (control)** | 0.21±0.01 | 0.46±0.09 | 2.02±0.08 |
| **2 (100mg/kg)** | 0.28±0.02 | 0.55±0.09[a] | 2.46±0.29 a |
| **3 (200mg/kg)** | 0.38±0.12[a] | 0.75±0.12[a] | 2.60±0.06 [a] |
| **4 (300mg/kg)** | 0.68±0.15[a] | 1.06±0.11 [a] | 3.19±0.07 [a] |

**Data are expressed as Mean ± SEM of 5 rats, [a] represents significant differences relative to the control for test groups 2,3 and 4 at p<0.05.**

**Table 3: Effects of discontinuation of administration of *Solanum aethiopicum* leaf extract on Reproductive Hormones.**

| Groups | FSH (miu/ml) | LH (miu/ml) | Testosterone (miu/ml) |
|---|---|---|---|
| 1 (control) | 0.23±0.02 | 0.48±0.10 | 2.10±0.14 |
| 2 (100mg/kg) | 0.25±0.13 | 0.37±0.01[b] | 0.65±0.05[b] |
| 3 (200mg/kg) | 0.30±0.06[c] | 0.58±0.26[c] | 0.98±0.04[c] |
| 4 (300mg/kg) | 0.46±0.02[d] | 0.69±0.15[d] | 1.81±0.79[d] |

***b -mean significant differences of test group 2 (between 30 days administration and after 30 days of discontinuation of administration).***
***c -mean significant differences of test group 3 (between 30 days administration and after 30 days of discontinuation of administration).***
***d -mean significant differences of test group 4 (between 30 days administration and after 30 days of discontinuation of administration).***

**Table 4: Comparison of the concentration of Reproductive Hormones following discontinuation of the administration of *Solanum aethiopicum* leaf extract.**

| Groups | FSH (miu/ml) | | LH (miu/ml) | | TESTOSTERONE (miu/ml) | |
|---|---|---|---|---|---|---|
| | Test group | Reversal group | Test group | Reversal group | Test group | Reversal group |
| 2 | 0.28±0.02 | 0.25±0.13 | 0.55±0.09 | 0.37±0.01 | 2.46±0.29 | 0.65±0.05 |
| % Difference | -10.7 | | -32.7[b] | | -73.6[b] | |
| 3 | 0.38±0.12 | 0.30±0.06[c] | 0.75±0.12 | 0.58±0.26 | 2.60±0.06 | 0.98±0.04 |
| % Difference | -21.1[c] | | -22.7[c] | | -62.3[c] | |
| 4 | 0.68±0.15 | 0.46±0.02[d] | 1.06±0.11 | 0.69±0.15 | 3.19±0.07 | 1.81±0.79 |
| % Difference | -32.4[d] | | -34.9 [d] | | -43.3[d] | |

**The negative (-) sign shows the reduction that occurred after discontinuation of the administration of *S. aethiopicum* leaf extract in wistar rats within a particular group.**

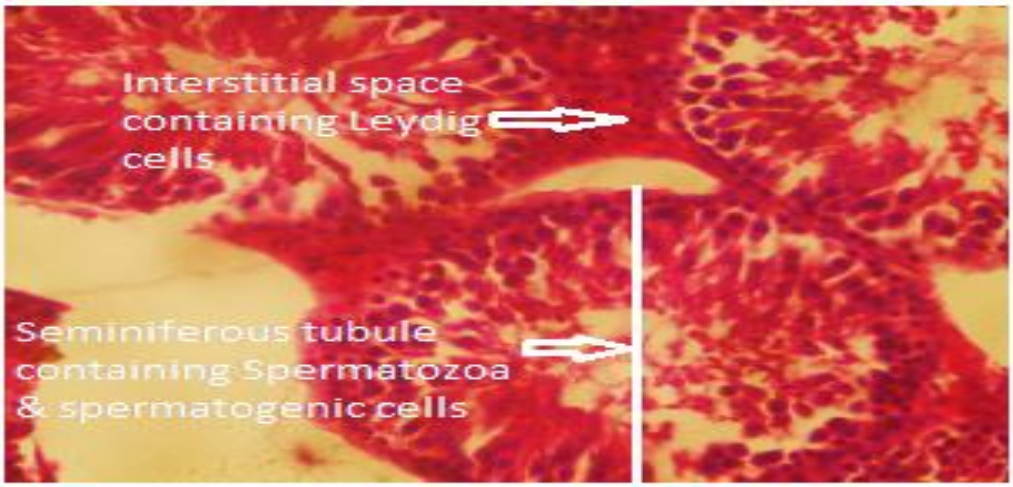


**Plate 1: Photomicrograph of a section of the Testis of rat (control group) after 30 days of administration of the extract. It reveals a histologically normal Testis, showing interstitial spaces containing Leydig cells and seminiferous tubules containing spermatozoa and spermatogenic cells. Stain: haematoxylin and eosin; Magnification: x 100.**


**Plate 2: Photomicrograph of a section of the Testis of rat (100mg/kg group) after 30 days of administration of the extract. It reveals a histologically normal Testis, showing interstitial spaces containing Leydig cells and seminiferous tubules containing spermatozoa and spermatogenic cells. Stain: haematoxylin and eosin; Magnification: x 100.**

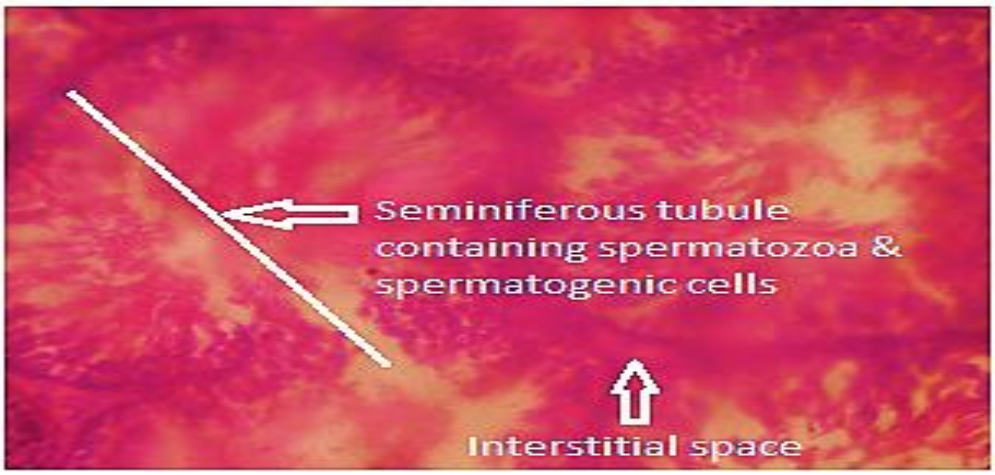


**Plate 3: Photomicrograph of a section of the Testis of rat (200mg/kg group) after 30 days of administration of the extract. It reveals a histologically normal Testis, showing interstitial spaces containing Leydig cells and seminiferous tubules containing spermatozoa and spermatogenic cells. Stain: haematoxylin and eosin; Magnification: x 100.**

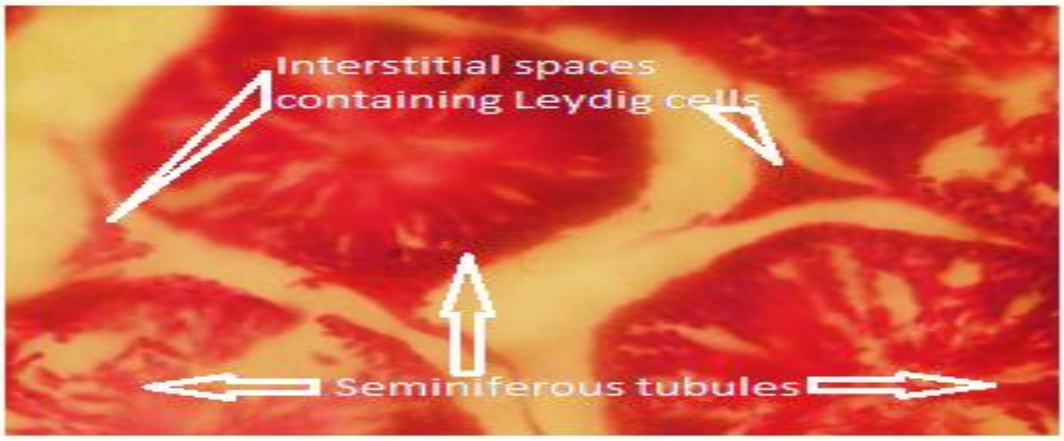


**Plate 4: Photomicrograph of a section of the Testis of rat (300mg/kg group) after 30 days of administration of the extract. It reveals a histologically normal Testis, showing interstitial spaces containing Leydig cells and seminiferous tubules containing spermatozoa and spermatogenic cells. Stain: haematoxylin and eosin; Magnification: x 100.**

## DISCUSSION OF FINDINGS

### Effect of administration of Hydro-ethanolic leaf extract of *S. aethiopicum* on Reproductive Hormones

The study revealed that 30 days' administration of 100 mg/kg, 200 mg/kg and 300mg/kg of *S. aethiopicum* leaf extract significantly increased the serum levels of Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and Testosterone (TET) in a dose-dependent manner as shown in table 2. This suggests that the extract had pro-fertility effects by increasing the serum levels of the reproductive hormones in a dose dependent manner. The increase caused by the extract could be attributed to the presence of saponins which help in improving testosterone levels and flavonoid which improves androgen levels.[16] Male fertility in mammals is regulated by the two adenohypophyseal gonadotropic hormones: Luteinizing hormone (LH) and Follicle stimulating hormone (FSH). Follicle stimulating hormone stimulates the production of spermatogonia to spermatocytes and also maintains the spermatogenic process. Follicle stimulating hormone and Luteinizing hormone are necessary for meiosis and development of spermatids.[17] Testosterone is the main male gonadal hormone produced by the interstitial cells of Leydig the testis. It is the major hormonal marker of androgenecity, in addition to LH and FSH. It is required for the growth, development and maintenance of male reproductive organs.[18] In association with FSH, testosterone acts on the seminiferous tubules to initiate and maintain spermatogenesis.[19]

The significant increase in the serum concentration of FSH therefore suggests that the extract has a stimulatory effect on the hypothalamic-pituitary axis. The pro-gonadotropic effect by the extract is an indication that the extract may enhance the normal functioning of the Sertoli cells which will increase sperm cell maturation. The significant increase in the concentrations of the gonadotropic hormones in all the test groups suggests a direct effect on the anterior pituitary hormongenesis.

These findings agree with the studies on the fertility effects of other plant extract (Allium sativum) by other researchers[14] who reported that saponins, flavonoids, alkaloids and tannins present in plant extracts have the capability of increasing the body's natural Testosterone, LH and FSH levels. According to another group of researchers,[16] LH which is released by the pituitary gland helps to maintain testosterone levels. This therefore means that the increase in Luteinizing hormone level may be responsible for the increase in testosterone concentration which enhances male fertility.

### Effect of the discontinuation of administration of the extract on reproductive hormones with respect to their experimental counterparts and to the control group

Upon discontinuation of the administration of the extract, there was a decline or reversal in the serum levels of FSH, LH and testosterone in a dose dependent manner (tables 3 and 4). The effect of the extract on the reproductive hormones declined (progressive decrease in the serum concentration of the hormones) faster in the group that received 100mg/kg than those that received 200mg/kg and 300mg/kg body weight. However, the decline in the serum concentration of the group that received 300mg was found to be slower than their corresponding experimental groups (groups 2 and 3). This pattern of reversal/decline in the serum concentrations of the reproductive hormones suggests that their elimination rates from the body are both dose and time dependent. This is true because those with lower doses were eliminated faster than those which received a higher dose. The reversal pattern in the serum concentrations of LH and Testosterone supports earlier reports that Luteinizing Hormone being released by the pituitary gland helps to maintain testosterone levels, such that as the serum concentration of LH increases, the serum concentration of Testosterone also increases and vice versa.[16] This result therefore suggests that to achieve the desired effect of the extract on male reproductive hormones, the extract has to be consumed in a reasonably large quantity.

### Effect of administration of Hydro-ethanolic leaf extract of *S. aethiopicum* on the Histology of the Testes

The histology of the testes in all the experimental groups after 30 days of administration of the extract revealed a normal histology, when compared with the control group. The extract improved spermatogenesis as seen in the histology of the testes. The treated animals had more spermatogenic cells and the Leydig cells were more with clear interstitial spaces (Plates 1-4). This suggests that the leaf extract of *S. aethiopicum* may have a protective effect on the testis thereby promoting fertility. This protective effect of the extract on the testis may be attributed to the presence of flavonoid in the leaf extract which has been found to possess cyto-protective activities. Flavonoids have also be found to be effective in the prevention of lesion and in the scavenging of free radicals, generated by the natural and experimental stress that may damage testicular cell structure.[20, 21]

## CONCLUSION

The study showed that the hydroethanolic leaf extract of *Solanum aethiopicum* caused a significant dose dependent increase in the serum concentration of reproductive hormones such as Follicle stimulating hormones, luteinizing hormone and Testosterone. It is also found to be capable of protecting or preserving the cytoarchitecture of the testis. There was however a significant reversal of the effect of the leaf extracts on the reproductive hormones when the administration was discontinued for 30 days. These results show that the continous intake or consumption of the leaf of *solanum aethiopicum* (garden egg) may increase the concentration of reproduction hormones in male wistar rats and boost their reproductive potential.

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