



## METHANOLIC EXTRACTS OF THE FRUIT OF *ABELMOSCHUS ESCULENTUS* (OKRO) CAUSES INCREASE IN THE SERUM CONCENTRATION OF SOME REPRODUCTIVE HORMONES AND DECREASES TOTAL SPERM COUNT IN MALE ALBINO WISTAR RATS

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**ABSTRACT**

This study was aimed at determining the effect of the methanolic extract of *abelmoschus esculentus*(okro) on the serum concentration of key male reproductive hormones and total sperm count in male albino wistar rats. We also tried to ascertain the reproductive safety of the fruit in males using animal models. In this study, twenty five male albino wistar rats weighing 180-220g were grouped into five of five rats each, after two weeks of acclimatization. *Group1 served as the control and were given water and normal rat chaw while groups 2, 3,*

*4 and 5 served as the test groups and were orally administered with (250, 500, 750 and 1000)mg/kg of okro extract respectively. After six weeks of extract administration, the rats were anaesthetized using chloroform and 5ml of blood was collected from each rat through cardiac puncture. The blood was centrifuged and the serum was used for hormonal assay of testosterone (T), luteinizing hormone (LH) and follicle stimulating hormone (FSH). Scrotal incision was done on the rats and semen was collected from their caudal epididymis for the analysis of total sperm count. The result of the study showed a significant ( $p < 0.05$ ) increase in the serum level of T, LH and FSH with a surprising significant ( $p < 0.05$ ) decrease in total sperm count in the test groups when compared with the control group. The result of this study depicts that okro may not be very good for males who are still producing children especially*

*those that have previously had infertility problems. We therefore advise males of this category to consume okro meals with caution.*

**KEY WORDS:** *Abelmoschus Esculentus*, Testosterone, Infertility, Sperm, Hormones.

## 1.0 INTRODUCTION

*Abelmoschus esculentus* (L. *moench*) (okro) is a flowering plant in the mallow family whose fruit is widely eaten around the world. It is called so many names by so many people. For instance, it is called lady's finger or gumbo in many English speaking countries. In the United States, it is called okra, in the Caribbeans it is known as okro. In Asia, the Chinese call it qui-kui and the Indians call it bende kayi. In Europe, it is called quiabo by the Portuguese; guigambo by the Spanish and gombo by the French and the Dutch. This plant has a disputed origin, as the Supporters of South Asian origins point to the presence of its proposed parents in that region; supporters of west Africa origins point to the greater diversity of okro in the region while the Egyptians and moors of the 12<sup>th</sup> and 13<sup>th</sup> century used the Arabic word 'bamia' for the plant, suggesting it had come from the east (Ethiopia). However, the word 'okro' may have originated from Nigeria, West Africa and it is cognate with 'okwuru' in the Igbo language spoken in Nigeria.<sup>[1]</sup>

On the account of its nutritional and chemical composition, Okro is often associated with Creole food and has a wide range of nutrients and chemicals. It is very rich in vitamin C and follate, with good levels of magnesium<sup>[2]</sup>, manganese, calcium, potassium and small but useful amount of thiamine, riboflvine, niacin and vitamin E.<sup>[3]</sup> A measure of low levels of carotenoids which decrease with all kinds of processing and phenolic content of 108.80mg/100g has also been reported.<sup>[4]</sup> Due to the presence of so many vitamins and minerals in okra, it provides a very good antioxidant activity.<sup>[2]</sup> Furthermore, green-yellow oil is pressed from okra seed. This oil has a pleasant taste and odour and is high in unsaturated fats like oleic and linoleic acids.<sup>[5]</sup> A 1920 study revealed that a sample of okra contained 15% of oil; while in 2009, a study found out that okra oil is suitable for use as a biofuel.<sup>[6]</sup>

Medicinally, the crude extract of okra has been shown by different studies to have different pharmacological effects on the different organs and systems of the body. Studies have reported that okro possess an impressive antioxidant activities.<sup>[7, 8, 9]</sup> In a study that investigated the antioxidant activity of okro by the PPPH and hydroxyl radical scavenging assays, it was reported that okro showed an increased antioxidant activity.<sup>[10]</sup> On cholesterol

lowering activity, an in vitro study into the bile-binding capability of okro, beets asparagus, eggplant, turnipet, green beans, carrot and cauliflower; it was found that okro was more significantly effective than the other vegetables.<sup>[11]</sup> This was reasoned to be because of the high viscous fibre content (0.39g/100g) which binds with bile acid and lower cholesterol.

On anti-inflammatory activity, the products of 5-lipoxygenase that mediates allergy and inflammatory responses like thrombosis, atherosclerosis and asthma can be inhibited by okra extract.<sup>[12]</sup> Also a study has reported that okra contains rhamnogalacturonans which increases kerocyte proliferation in vitro thereby potentially aiding in skin healing and rejuvenation; hence okra can be used as skin therapy.<sup>[13]</sup>

On ulcer, okra is traditionally used in Asia to treat gastric problems on account of its mucilaginous content.<sup>[14]</sup> Also, an investigation on the effect of the various okra extract on the bacteria 'H. pylori' which causes chronic gastritis, gastric and duodenal ulcer using an adhesion model based on sections of the gastric mucosa revealed that pretreatment of the bacteria with preparation of fresh okra juice inhibited the adhesion of the bacteria almost completely. Freeze-drying and reconstitution of the juice reduced its effectiveness; the study therefore concluded that rhamnogalacturonans with considerable amount of glucuronic acid identified as the major active components of okra with certain glycosylated proteins together with glycoproteins and highly acidic sugar compounds formed a three dimensional complex structure that interacts with the surface adhesion molecules of the H. pylori to inhibit ulcer.<sup>[14]</sup> Furthermore, 100mg/kg and 200mg/kg of okra has been shown to effectively lower serum blood glucose level in streptozotocin (60mg/kg IP) induced diabetic rats.<sup>[15]</sup> Similarly, a significant reduction in the blood glucose level of alloxan induced diabetic rats treated with aqueous and ethanol extract of okra at 300mg/kg for just 7 days has been reported.<sup>[16, 17]</sup>

Other pharmacological effect of okra include; anti-cancer effect<sup>[18]</sup> and anti-proliferative and proapoptotic effects.<sup>[19]</sup> Whether or not okra affects the male reproductive system or male fertility has been a matter of speculation. However, the extraction of the oil 'gossypol'<sup>[20]</sup> which has been established as having an irreversible deleterious effect on male reproduction<sup>[21]</sup> from okra seed raises a serious question. Gossypol is richly found in cotton seed<sup>[22]</sup> and cotton belongs to the same family of plant with okra; hence, there is the possibility of having some quantities of gossypol in okra which can possibly cause infertility in males. However, there has been reports of decreases in the mean weight of the testes of

Sprague Dawley rats treated with aqueous fruit extract of *Abelmoschue esculentus* (okra fruit) which did not reverse after a 28 days recovery period<sup>[23]</sup> Another study reported that the methanolic extract of the fruit of okro negatively affects the testes and sperm parameters.<sup>[24]</sup> Nothing has however been reported on the male reproductive hormones. This therefore necessitates the present study.

## 2.0 MATERIALS AND METHOD

### 2.1 PREPARATION OF PLANT MATERIAL

Fresh fruits of *Abelmoschus esculentus* (okra) were purchased from mile three market in Port Harcourt Nigeria and were identified in the department of Plant Science and Biotechnology. The specimen were documented and preserved at the university of Port Harcourt herbarium with the reference number **UPH/PSB/016**.

The fruits were washed, air dried and 3kg of it ground to powdered form using manual grinding machine. 500gms of the powdered fruit was then soaked in 99% methanol at 60-70<sup>0</sup>c for 36 hours in continues extraction using soxhlet apparatus. The resultant extract was filtered and concentrated under reduced pressure at 40% using rotary evaporator. The dark-brown jelly yield was then stored in an air-tight bottle and preserved in a refrigerator at -4<sup>0</sup>c, pending usage. The stock solution was prepared by dissolving 10gms of the extract in 100ml of distilled water. The volume of the stock given to each animal was calculated using the modified formula below.

$$\text{Vol. (ml)} = \text{dose of extract (mg.kg}^{-1}\text{)} \times \text{weight of the rat (kg)} / \text{conc. of stock (mg/ml)}$$

### 2.2 PROCUREMENT AND PREPARATION OF EXPERIMENTAL ANIMALS

Twenty five (25) adult male albino wistar rats weighing 200-260gms were acquired and housed in a clean wooden cage at the animal house of the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Nigeria. Standard room temperature of 25-30<sup>0</sup>c, humidity of 60-70% were maintained and the rats were allowed natural light-dark cycle with free access to rat chow and normal tap water at libitum. The rats were allowed to acclimatize under this condition for three weeks (14 days).

International guidelines for the care and use of laboratory animals in biomedical research as recommended by the Canadian council of animal care<sup>[25]</sup>, and the recommendation of the

guiding principles in the care and use of animals for research were adhered to, in the course of this study.

### 2.3 EXPERIMENTAL DESIGN

At the end of acclimatization, the rats were weighed and divided into 5 groups of 5 rats each according to their weights. Group 1 served as the control group and was administered with 1ml of water orally while groups 2, 3, 4 and 5 received oral administration of okro extract at 250mg/kg, 500mg/kg, 750mg/kg and 1000mg/kg respectively and served as the test groups. Six weeks (42 days) following treatment, the rats were reweighed anaesthetized and 5ml of blood sample was collected from each rat through cardiac puncture. The blood samples were temporarily stored in a plain centrifuge tube and labeled. After some time, the plain centrifuge tubes containing the blood samples were centrifuged at 3000rev/min for 15 minutes using a centrifuging machine with model No.800D Ocean made<sup>+</sup>, England; then the sera was separated from the cells and stored in sample bottles labeled and frozen at 4<sup>o</sup>c before being used for hormonal assays.

Seamen was collected from the rats by scrotal incision to expose the testes with the epididymis and vas deference, seamen was then gently squeezed out from the tail of the epididymis through the vas deference into a Petri dish and following following WHO outline as contained in WHO protocol MB-50<sup>[26]</sup>, total sperm count was determined.

The stored sera were analyzed for testosterone, follicle stimulating hormone (FSH) and luteinizing hormones (LH) at the Department of Chemical pathology, University of Port Harcourt teaching hospital, Port Harcourt Nigeria following established the outlines<sup>[27]</sup>: with the hormonal kit supplied by Monobind Inc. Lake Forest, CA 92630. USA with product code: 3725-300; for testosterone test system and Biocheck, Inc 323 Vintage Park Dr. Foster city, CA 94404; for FSH and LH. The principle for testosterone assay is based on the competitive enzyme immunoassay type 7 while FSH and LH assays share the same principle which is based on a solid phase enzyme-linked immunosorbent assay (ELISA). This system makes use of mouse monoclonal anti- $\alpha$ -hormone antibody for solid phase (microwells) immobilization and another mouse monoclonal anti- $\beta$ -hormone antibody in the antibody-enzyme conjugate solution.

At the end of the study, data were collected and analyzed using the software “statistical package for social sciences (SPSS; version 17.0 IBM)”. One way analysis of variance

(ANOVA) was used to analyze the means and significant differences were determined. Comparisons between the groups were made using post Hoc, Turkey HSD and Dunnett's test. Differences at the probability level  $p \leq 0.05$  (95% confidence interval) were taken to be statistically significant. All results were presented in tables and expressed as mean plus/minus standard error of mean ( $M \pm S.E.M$ ).

### 3.0 RESULT AND DISCUSSIONS

**Table 1: Showing changes in total sperm count and serum concentration of reproductive hormones in male albino Wistar rats following six weeks treatment with methanolic extract of the fruit of *Abelmoschus esculentus*.**

	Group1 (control) N=5	Group2 (250mg/kg) N=5	Group3 (500mg/kg) N=5	Group4 (750mg/kg) N=5	Group5 (1000mg/kg) N=5
Total Sperm count(million/ml)	72.80 $\pm$ 1.59	18.20 $\pm$ 0.92*	33.00 $\pm$ 4.06*	28.00 $\pm$ 4.39*	42.80 $\pm$ 0.86*
Testosterone (ng/ml)	0.90 $\pm$ 0.03	2.34 $\pm$ 0.05	2.30 $\pm$ 0.88	3.22 $\pm$ 0.41*	2.34 $\pm$ 0.03
FSH(ng/ml)	1.82 $\pm$ 0.09	2.50 $\pm$ 0.11*	2.72 $\pm$ 0.06*	2.36 $\pm$ 0.07*	2.24 $\pm$ 0.07*
LH(ng/ml)	1.20 $\pm$ 0.05	1.88 $\pm$ 0.05*	1.80 $\pm$ 0.05*	1.64 $\pm$ 0.08*	1.54 $\pm$ 0.03*

All values are expressed as Mean  $\pm$  S.E.M. \*=statistically significant compared to the control at  $p \leq 0.05$ , N= sample size

### 3.1 DISCUSSIONS

Total sperm count was seen to have significantly decreased when compared the test groups with the control. This could be as a result of an earlier report of the possible destructive effect of the extract on the testicular tissues which in turn reduces the testicular weight and hence, spermatogenesis.<sup>[23]</sup>

On the reproductive hormones, it was surprising to observe that there were significant increases in the serum concentration of the assayed reproductive hormones (testosterone, LH and FSH) in the test groups when compared with the control group. The cause of this is not very clear but could be due to the damaging effect of the extract on the sertoli cells which is known to secrete inhibinB. InhibinB acts on the pituicytes to cause the down-regulation of the secretion of male sex hormones. The disruption in the secretion of inhibinB could cause the pituitary gland to become hyper-active and result in the increase in serum concentration of LH, FSH and testosterone seen in this study. In a 1995 and 2004 study in infertile male humans, there were reports of increases in serum concentration of male sex hormones (LH, FSH and testosterone) and reduction in total sperm<sup>[28,29]</sup> which is in concordance with the present report.

The result of this study also suggests that the destructive effect of the extract on the sertoli cells could trigger autocrine and paracrine secretion from nearby cells which may inturn have a trigerring effect on the gonadotropes of the pituitary gland to produce more LH and FSH, which will inturn cause an increase in the secretion of testosterone from the leydig cells of the testes.

#### 4.0 CONCLUSION AND RECOMMENDATIONS

The ever increasing decline in the quantity and quality of semen<sup>[30]</sup> could be a combination of many factors; one of which could be the use of some substances as food and drugs. One of such substances investigated in this study is okro. From our study, we observed that the methanolic extract of the fruit of okro significantly ( $p<0.05$ ) decreased total sperm count while at the same time significantly ( $p<0.05$ ) increasing the serum concentration of testosterone, LH and FSH in male albino wistar rats. From the observation, we therefore conclude that consistent consumption of the fruit of okro in meals could impair fertility in males and we recommend further mechanistic studies on the fruit to establish its male reproductive safety.

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#### REFERENCES

1. Carney, Judith and Richard Nicholas Rosomoff (2009). In the Shadow of Slavery: Africa's botanical legacy in the Atlantic World. University of California Press. Berkeley, CA.
2. Savello P.A., Martin F.W. and Hill J.M (1980). Nutritional composition of okra seed meal, *Journal of Agric food chemistry*, 28(6): 1163-1166.
3. Athar N, Taylor G, McLaughlin J, Skinner J (2004). *FOODfiles 2004*.
4. El-Malak G.A.A., (2007). Evaluation of free radical scavenging by natural antioxidants in some fresh, blanched, cold and frozen storage of vegetables. *Annals of Agricultural Science (Cairo)*, 52(2): 367-374.



5. Mays, D.A., W. Buchanan, B.N. Bradford, and P.M. Giordano (1990). "Fuel production potential of several agricultural crops". *Advances in new crops.*, 260–263.
6. Farooq, Anwar; Umer Rashid; Muhammad Ashraf; Muhammad Nadeem (March 2010). "Okra (*Hibiscus esculentus*) seed oil for biodiesel production". *Applied Energy*, 87(3): 779–785.
7. Yang R.Y, Tsou SCS, Lee T.C, Wu W.J, Hanson P.M, Kuo G, Engle L.M, Lai P.Y (2006). Distribution of 127 edible plant species for antioxidant activities by two assays. *Journal of the Science of Food and Agriculture*, 86(14): 2395-2403.
8. Adelakun O.E, Oyelade O.J, Ade-Omowaye B.I.O, Adeyemi I.A, Venter M.V.D. (2009). Chemical composition and the antioxidative properties of Nigerian Okro seed (*Abelmoschus esculentus* Moench) flour, 47(6): 1123-1126.
9. Adetuji F.O, (2008). Antioxidant Degradation in Six Indigenous Okra *Abelmoschus esculentus* (L) Moench Varieties During Storage in Nigeria, *Journal of Food Technology*, 6(5): 227-230.
10. Sunilson J.A.J., Jayaraj P., Mohan M.S., Kumari A.A.G., Varatharajan R. (2008). Antioxidant and hepatoprotective effect of the roots of *Hibiscus esculentus* Linn. *International Journal of Green Pharmacy.*, 2(4): 200–203.
11. Kahlon TS, Chapman MH, Smith GE 2007a. In vitro binding of bile acids by okra, beets, asparagus, eggplant, turnips, green beans, carrots, and cauliflower. *Food Chemistry*, 103(2): 676-680.
12. Sekiya K (1997). Inhibition of cyclooxygenase and lipoxygenase by foods. *Bulletin of the Shikoku National Agricultural Experiment Station*, 61: 31-37.
13. Deters A.M, Lengsfeld C, Hensel A (2005). Oligo- and polysaccharides exhibit a structure dependent bioactivity on human keratinocytes in vitro. *Journal of Ethnopharmacology*, 102(3): 391-399.
14. Lengsfeld C, Titgemeyer F, Faller G, Hensel A (2004). Glycosylated compounds from okra inhibit adhesion of *Helicobacter pylori* to human gastric mucosa. *Journal of Agricultural and Food Chemistry*, 52(6): 1495-1503.
15. Sabitha V., Ramachandra S., Naveen K.R., and Panneerselvam (2011). Antidiabetic and Antihyperlipidemic potential of *Abelmoschus esculentus*(L) Moench. in streptozotocin-induced diabetic rats. *Journal of pharmacy and bioallied sciences.*, 3(3): 397-402.
16. Dibyajyoti Saha, Bindu Jain, Vibhor K. Jain. (2011). phytochemical evaluation and characterization of hypoglycemic activity of various extracts of *abelmoschus esculentus* linn. Fruit. *International Journal of Pharmacy and Pharmaceutical Sciences.*, 3(2): 34



17. Zhenzhong X. and Hondjun S. (2010). Effects of okra capsule combined with valsartan in Treatment of Early Diabetic Nephropathy with Microalbuminuria, *Modern Journal of Integrated Traditional Chinese and Western Medicine*, 3.
18. Dan R.D. and Gu C, (2010). Inhibition Effect of Okra Polysaccharides on Proliferation of Human Cancer Cell Lines. *Food science.*, 2010; 21.
19. Vayssade M., Sengkhampan N., Verhof R., Delaigue C., Oundiam O., Vingneron P., Voragen A.G.J., Schols H.A. and Nagel M.D, (2010). Antiproliferative and Proapoptotic Actions of Okra Pectin on B16F10 Melanoma Cells, *Phytotherapy Research*, 24(7): 982-989.
20. Martin F.W., Telek L., Roberte R., Santago A.G.(1979). Protein, oil and gossypol contents of a vegetable curd made from okra seeds, *Journal of food science*, 44(5): 1517-1519.
21. Burgos, M.; Ito, S.; Segal, J. S.; Tran, T. P. (1997). "Effect of Gossypol on Ultrastructure of Spisula Sperm". *Biological Bulletin.*, 193: 228–229.
22. Coutinho E.M. (2002). "Gossypol: a contraceptive for men". *Journals of Contraception.*, 65(4): 259–63.
23. Olatunji-Bello I.I., Ijiwole, T. Fawobajo.O.(2009). Evaluation of The Deleterious Effects of Aqueous Fruit Extract of *Abelmoschus Esculentus* (Okro Fruit) on Some Male Reproductive Parameters in Sprague Dawley Rats; *Journal of Phytology*, 1-6
24. Nwoke Kyrian Uchenna, Konyefon Nweze Godswill, Ifedi Izuchukwu Charles (2014). Effects of the Methanolic Extract of *Abeloschus esculentus* (L) Moench (Okro) Fruit on the testes and sperm characteristics of Male Albino Wistar Rats. *International Journal of Advanced Biological and Biomedical Research*. Res, 2(10): 2686-2690.
25. Canadian Council of Animal Care (CCAC), (1985). Guide to the handling and use of experimental animals. *Ottawa: ont; 2 United States NIH publications*, 85-230: 45-47.
26. W.H.O. (2010). Laboratory manual for the examination and processing of human semen 5th ed. WHO Press World Health Organization, 20 Avenue Appia, 1211 Geneva27, Switzerland., 7-113.
27. Uotila M, Rouslahti E, Engvali E.J. (1981). Two-site sandwich enzyme immunoassay with monoclonal antibodies to humans. *African Journal of immunological methods.*, 42. 11-15.
28. Sulthan, C., Craste-de-paulet, B., Audran, F., Iqbal, Y. and Ville, C. (1985) Hormonal evaluation in male infertility. *Annals of Biological and Clinical Sciences Paris.*, 43(1): 63-66.

29. Ramesh S. Babu, Sadhani M.D., Swarna M., Padmavathi P., and Reddy P.P (2004). Evaluation of FSH, LH and Testosterone levels in different subgroups of infertile males. *Indian journal of clinical biochemistry.*, 19(1): 45-49.
30. Carlsen, E., Giwercman, A., Keiding, N., Skakkeback, N. E. (1992). Evidence for decreasing quality of semen during past 50 years. *Biomedical Journal*, 12: 609- 613.