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Effects of the Aqueous Extract of the *Abelmoschus esculentus* (Linn. Moench) Fruit on the Male Reproductive Parameters during Infertility

Fatoba, T. A.
Department of Animal Production and Health,
Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria
(Formerly Ondo State University of Science and Technology)
E-mail: fathom434@yahoo.com

Nwanja, N.
Department of Biological Sciences,
Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria
(Formerly Ondo State University of Science and Technology)

ABSTRACT

With the help of male albino Wistar rats, this study sought to ascertain the effects of the aqueous extract of Abelmoschus esculentus (okra) on the male reproductive parameters as well as the fruit's safety for use during infertility in male animals. Twenty male albino Wistar rats, weighing between 150 and 180g, were divided into groups of four by five after two weeks of acclimatization for this study. Groups 2, 3 and 4 served as the test groups and were given (250, 500, 750) mg/kg of okra extract orally, whereas Group 1 served as the control group and was given water and standard rat chaw. The rats were put to sleep with chloroform after receiving the extract for four weeks, and 5 ml of blood was drawn from each rat's heart. The serum from the centrifuged blood was used to measure the hormone testosterone (T) and glucose levels. Rats' caudal epididymis semen was collected for the purpose of semen analysis after scrotal incision. The study's findings indicated that when compared to the control group, the tested animals had a significantly higher serum level of T and a marginally higher total sperm count, both of which were statistically significant ($p<0.05$) but lower insignificant ($p>0.05$) for glucose. Contrary to reports from other researchers who saw male infertility issues, the findings of this study suggested that okra may be safe. Therefore, humans and male animals should cultivate the habits of consuming cooked okra meals.

Keywords: *Abelmoschus esculentus, Infertility, Hormones, Semen, Testosterone,*

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INTRODUCTION

Abelmoschus esculentus (Linn. Moench) commonly called okra is a flowering plant belong to family Malvaceae whose fruit is generally acceptable as condiment throughout the world. The species is a perennial that frequently reaches a height of 2 meters (6.6 feet), is coarse, erect, branched, and more or less hairy. It is frequently grown as an annual in temperate climates. Long-petioled, heart-shaped, orbicular or orbicular-ovate leaves with 3- to 5-lobed margins are about 25 centimeters long or less. Petioles are at least as long as the blade; solitary, axillary flowers with a large, yellow corolla that is deep purple inside at the base. A fruit with rows of rounded, kidney-shaped seeds is elongated, 10 to 25 centimeters long, 1.5 to 3 centimeters in diameter, tapering to a blunt point, and containing the fruit.

Okra is frequently associated with various foods due to its chemical and nutritional makeup, which includes a wide variety of nutrients and chemicals with a high viscous fiber content (0.39g/100g), which binds to bile acid and lowers cholesterol (Kahlon *et al.,* 2007). Okra extract, skin therapy (Deters *et al.,* 2005), vitamin C, and folate, along with adequate levels of magnesium (Savello et al., 1980; Yang *et al.,* 2006; Adelakun *et al.,* 2009; Adetuyi, 2008; Sunilson *et al.,* 2008; Kahlon *et al.,* 2007), manganese, calcium, potassium, and a small but useful amount of selenium (Athar *et al.,* 2004).

With all types of processing, some secondary metabolites such as carotenoids decrease, and phenolic content of 108.80mg/100g has also been reported (El-Malak, 2007). Additionally, okra seed is used to extract green-yellow oil, and various studies have shown that okra crude extract has a variety of pharmacological effects on the body's various organs and systems. Okra is commonly used in Asia to treat gastric issues, including ulcers, due to its mucilaginous content (Lengsfeld *et al.,* 2004), which also helps to lower serum blood glucose levels (Sabitha *et al.,* 2011; Dibyajyoti *et al.,* 2011; Zhenzhong and Hondjun, 2010).

Okra also has an anti-cancer effect, as well as anti-proliferative and proapoptotic effects (Dan and Gu, 2010) (Vayssade *et al.,* 2010). It has been debated whether or not okra has an impact on male fertility or the male reproductive system. The extraction of the oil "gossypol" (Martin *et al.,* 1979) from okra seeds, which has been proven to have a permanent negative impact on male reproduction (Burgos, 1997), raises a serious issue. Given that okra and cotton are both members of the same plant family and that gossypol is abundant in cotton seed, there is a chance that some gossypol in okra may contribute to male infertility.

Gossypol is a naturally occurring phenolic substance produced by pigment glands in cotton stems, leaves, seeds, and flower buds of the cotton plant (genus Gossypium).

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Gossypol has been investigated as a potential male contraceptive for decades due to its potent antispermatogenic effects. It is a phenolic aldehyde that permeates cells and inhibits several dehydrogenase enzymes (Qian and Wang 1984; Dodou, 2005). However, the efficacy and reversibility of infertility brought on by gossypol have long been debatable and are still being researched by numerous organizations (Coutinho *et al.,* 2000; Yang *et al.,* 2004). There have been numerous reports on the effects of gossypol on the testis, including changes in Sertoli cell secretion and the arrest of germ cell meiosis (Zhuang *et al*., 1983; Ojha *et al.,* 2008).

Abelmoschus esculentus (okra fruit) aqueous extract has been reported to cause Sprague Dawley rats to lose weight on average in their testicles, and these losses persisted after a 28-day recovery period (Olatunji-Bello *et al.,* 2009). The testes and sperm parameters are adversely impacted by the methanolic extract of the fruit of the okra, according to another study (Nwoke *et al.,* 2014). On the okra as a condiment on male reproductive, however, there has been no information reported. Therefore, this study sought to ascertain the effects of the aqueous extract of *Abelmoschus esculentus* (okra) on the male reproductive parameters as well as the fruit's safety for use during infertility.

MATERIAL AND METHOD

Assessment of Plant Materials

In Okitipupa, Ondo State, Nigeria, the fresh fruits of *Abelmoschus esculentus* (okra) were purchased at the market's main entrance. The fruits were washed, air dried and 10kg subjected to autoclave, later ground to powdered form using mortar and pestle, then kept pending usage.

Procurement of Experimental Animals

At the Department of Biological Sciences, Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria, twenty (20) adult male albino rats weighing 150-180g were purchased, kept in a clean wooden cage at standard room temperature, and given free access to food and ordinary tap water whenever they needed it. The rats were given two weeks to adjust to this environment (14 days). The Canadian Council on Animal Care's recommendations for international guidelines for the care and use of laboratory animals in biomedical research as well as recommended guiding principles in the care and use of animals for research were

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followed throughout the course of this study. Four groups of animals were formed, one of which served as the control group. Each group of animals was sedated before their semen was examined at the conclusion of the experimental period. Its testes and epididymis were used for the analysis of the semen. Through cardiac puncture, blood samples were taken from the animals and placed in sterile bottles for hormonal analysis.

Laboratory Design

The rats were weighed at the conclusion of acclimatization and divided into 4 groups of 5 rats each based on weight. Group I served as the control group and received untreated feed, whereas groups II, III, and IV, which served as the test groups, received okra samples at doses of 250 mg/kg, 500 mg/kg, and 750 mg/kg, respectively. The rats were reweighed 30 days after treatment, anesthetized, and 5 ml of blood were drawn from each rat through a cardiac puncture. The blood samples were labeled and temporarily stored in a standard centrifuge tube. After some time, using a centrifuge with model number 800D Ocean made+, England, the plain centrifuge tubes containing the blood samples were centrifuged at 3000 rev/min for 15 minutes. The sera were then separated from the cells and stored in sample bottles with labels and frozen at $4^{o}C$ before being used for hormonal assays. Semen was removed from the rats by making a scrotal incision that exposed the testes, epididymis, and vas deference. Semen was then carefully squeezed into a Petri dish through the vas deference, and the total sperm count was calculated in accordance with WHO guidelines found in WHO protocol MB-50. At the Specialist State Hospital, Okitipupa, the Department of Chemical Pathology examined the stored sera for testosterone and glucose.

Analytical Statistics

Data were gathered and analyzed using the Statistical Package for Social Sciences (SPSS; version 17.0 IBM). The means were examined using one-way analysis of variance (ANOVA), and significant differences were found. Dunnet's test, Turkey HSD, and post Hoc were used to compare the groups. Differences were considered statistically significant if they were at the probability level of 0.05 (95 percent confidence interval). All findings were tabulated and expressed as mean +/- standard error of mean (M + S.E.).

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RESULTS AND DISCUSSION

Table 1: Effect of *Abelmoschus esculentus* (Linn. Moench) fruit on Spermiogramic parameter of male albino rat

Parameters	T1	T2	T3	T4	S.E
Colour	White	White	White	White	-
Mass activity	1	2	2	2	-
Mass motility	62	64	66	65	-
Sperm concentration $x10^{7}$/ml	6.7^{b}	7.1^{a}	6.9^{ab}	7.2^{a}	0.0217
Dead sperm	30	28	29	28	-
Abnormality					
Primary	6	4	4	4	-
Secondary	3	2	3	3	-
Tertiary	4	2	3	2	-
Testosterone (ng/ml)	1.56^{b}	2.36^{a}	2.46^{a}	2.76^{a}	0..2631
Glucose (mg/dl)	87,34	82.77	83.56	81.43	3.1073

Values in the same row with various superscripts are significant (a, b) ($p< 0.05$)

Effect of *Abelmoschus esculentus* (Linn. Moench) fruit on Spermiogramic parameter of albino rat is shown on Table 1. Semen colour of the treated group and the control appeared white but treated rats exhibited higher mass activity, motility, sperm concentration and live sperm than the control but a reduced abnormality. Sperm concentration values differ statistically ($p< 0.05$). The treated group has fewer abnormalities (primary, secondary, and tertiary) than the control group. The values were statistically indifferent ($p> 0.05$) when compared.

The results of some researchers who reported an increase with the methanolic crude extract of the plant extract were supported by the increased semen characteristics in the treated group, but the concentration of semen decreased. This could be explained by a potential destructive effect of the extract on the testicular tissues, which lowers testicular weight and, in turn, inhibits spermatogenesis (Olatunji-Bello *et al.,* 2009 and Nwoke *et al.,* 2015). However, inability of the treated animals to suffer from the extract could be adduced to the ability of heat which might ameliorate the gossypol to non-toxic products. The values of mass activity, motility and concentration agreed with the report of (Oyeyemi *et al*., 2001). Testosterone values are higher in the treated groups than the control (Fig 1) but a reduction in the values of glucose was observed in the treated (Fig 2).

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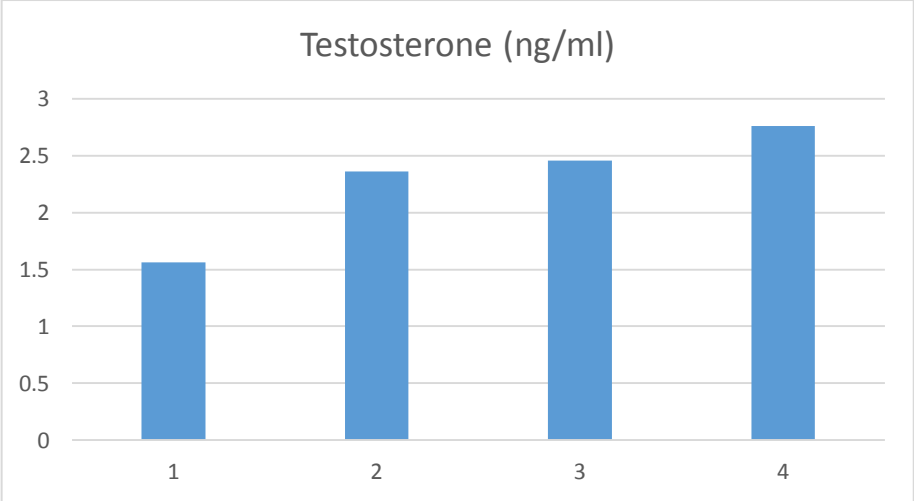
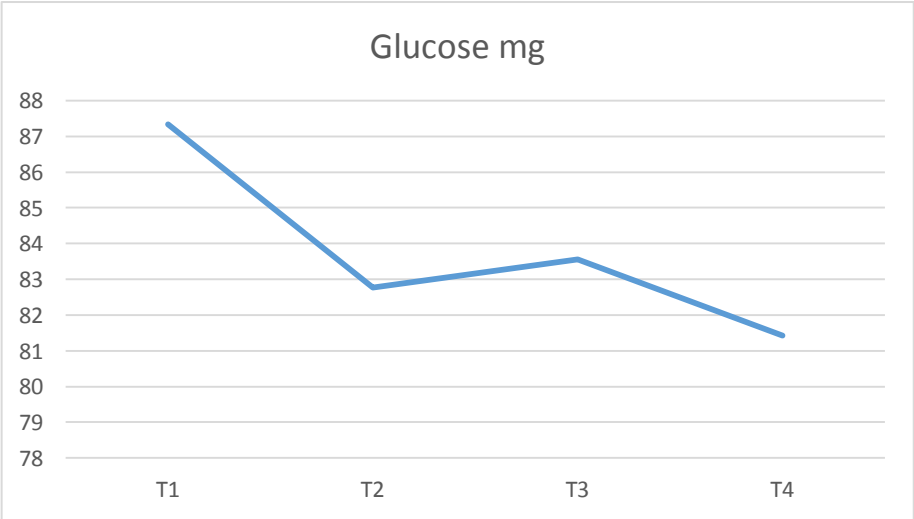


Treatments

The study's findings are consistent with a report by Javellonar *et al.* (1998) that the level of these active ingredients in the body, as well as each person's mode and level of sensitivity, determines their mechanism of action. Additionally, each person's rate of body accumulation from their extremely low food level varies, making it even more difficult to predict when and how they will affect the body.



Treatments

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The result was similar to some workers who reported that the crude extract of okra produced astounding pharmacological effects on the body's various organs and systems and also posed an impressive antioxidant activity (Yang *et al.,* 1006; Adelakun *et al.,* 2009). Although the active ingredient gossypol, which inhibits sperm production (spermatogenesis) and motility by blocking a number of crucial enzymes for energy metabolism in sperm and sperm-producing cells, may have been lessened by heat, which increased protein binding and converted more free - gossypol (toxic form) to the bound form (non-toxic form), the result of this study is supported by this theory. The significant increased effect of the extract on the hormonal testosterone and reduction in glucose levels is in line with other workers (Olatunji-Bello *et al.,* 2009; Zhenzhong and Hondjun, 2010; Dibyajyoti *et al.,* 2011 and Nwoke *et al.,* 2015). It's interesting to note that, when compared to the control group, the test groups' serum concentrations of the measured reproductive hormones (testosterone) significantly increased. The relationship between testosterone, metabolic syndrome (MetS), type 2-diabetes (T2D) and insulin resistance (IR), which has been linked with the development of cardiovascular disease and erectile dysfunction, is suggested to be that testosterone reduces body fat and thus plays an important role in regulating insulin, glucose, and fat metabolism (ED). Because visceral fat acts as an endocrine organ, androgen deficiency is linked to IR, T2D, MetS, and increased visceral fat deposition, which in turn promotes endothelial dysfunction and vascular disease by producing inflammatory cytokines (Traish *et al.,* 2009)

Table 2: Effect of *Abelmoschus esculentus* (Linn. Moench) fruit on white albino rat's organs

ORGAN	Control (0)	T1	T2	7.5	S.E
Heart	0.50	0.60	0.53	0.60	0.0547
Kidney	1.10	1.12	1.15	1.15	0.0596
Liver	5.50	6.20	6.32	6.28	0.0252
Lung	1.80	1.90	2.03	2.00	0.0633
Testis	2.00	2.30	2.34	2.31	0.0633
Pancreas	0.46	0.53	0.56	0.55	0.07416
Intestine	16.20^{b}	14.60^{a}	14.33^{ab}	14.20^{b}	0.2394

Values in the same row with various superscripts are significant (a, b) ($p < 0.05$)
S.E = Standard error

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The effects of Abelmoschus esculentus (Linn. Moench) fruit on the visceral organs of albino rats is depicted in Table 2. The findings demonstrated that the treated animals had heavier organ weights than the untreated animals. When compared to the control group, animals in the 750mg/kgBW group had the highest body weight. With the exception of the intestines, the values of the heart, kidney, liver, lung, testis, and pancreas are higher than the control and are statistically indifferent ($p>0.05$) when compared. The plant's high levels of dietary fiber, carbohydrates, minerals, and vitamins (A, C, and E), which aid in digestion, lower caloric intake, have the ability to limit nitric oxide (antioxidant) buildup in the blood and lipid (fat) and lower the risks of cancer, may be responsible for the improved morphological characteristics (Yang *et al.,* 2006; Adelakun *et al.,* 2009). Due to its chemical components, it contains a unique combination of and a strong anti-diabetic source (Zhenzhong and Hondjun 2010; Sabitha *et al.,* 2011). The outcome shows that gossypol has been denatured by heat, making it safe to consume the okra fruit. Gossypol could affect protein and mineral utilization by lowering palatability, digestibility, or metabolism, and may even exert a toxic effect resulting in liver damage (Martin *et al.,* 1979).

CONCLUSION

With the help of male albino Wistar rats, this study sought to ascertain the effects of the aqueous extract of *Abelmoschus esculentus (okra)* on the male reproductive parameters as well as the fruit's safety for use during infertility in male animals. Data were gathered and analyzed using the Statistical Package for Social Sciences (SPSS; version 17.0 IBM). The means were examined using one-way analysis of variance (ANOVA), and significant differences were found. Dunnet's test, Turkey HSD, and post Hoc were used to compare the groups. Differences were considered statistically significant if they were at the probability level of 0.05 (95 percent confidence interval). All findings were tabulated and expressed as mean +/- standard error of mean (M + S.E.). Semen colour of the treated group and the control appeared white but treated rats exhibited higher mass activity, motility, sperm concentration and live sperm than the control but a reduced abnormality. The findings indicated that when compared to the control group, the tested animals had a significantly higher serum level of T and a marginally higher total sperm count, both of which were statistically significant ($p<0.05$) but lower insignificant ($p>0.05$) for glucose. Contrary to reports from other researchers who saw male infertility issues, the findings suggested that okra may be safe. Therefore, humans and male animals should cultivate the habits of consuming cooked okra meals.

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