

Effect of the hydromethanolic Extract of the Pulp of *Garcinia kola* Seed on Fertility Indices of Male Wistar Rats

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Abstract: The incidence of infertility is on the increase, globally. However, the largest burden exist in Africa where it is still posing a major challenge in the reproductive health of couples. Male related factors also play a significant role in the incidence of infertility among couples. This study was done to investigate the effects of the pulp of *Garcinia kola* seed on fertility of male wistar rats. Male wistar rats were divided into three (3) groups of eight (8) rats each. Group one (1) which served as control received distilled water. Group two (2) and group three (3) were treated with 100mg/kg bw and 200mg/kg bw of the hydromethanolic (1:4) extract of the pulp of *Garcinia kola* seed respectively. This design was used in each case, for the sperm quality and hormonal studies which lasted for 58 and 60 days respectively. The results obtained showed that the extract caused significant increase in the serum levels of luteinizing hormone, testosterone, testicular weight as well as the percentage of actively motile cells and sperm count. This study proved that, the pulp of *Garcinia kola* possesses fertility enhancing effects in male wistar rats.

Keywords: Pulp, *Garcinia kola*, hydromethanolic, wistar rats, fertility

1. Introduction

The inability of couples to reproduce has increased to the extent that it has become a social and public health concern globally [1, 2]. It is estimated that approximately 5–8% of couples suffer from infertility at some point in their reproductive lives and it is considered to be a major problem associated with reproductive health in sub-saharan Africa [3]. Available evidence shows that, the highest burden of infertility is concentrated in Africa where 10.1% of couples on the average were reported to experience infertility, with as high as 32% in some countries and ethnic groups [3]. Indeed in Africa and some other developing countries, the inability to bear children is a tragedy for many couples. The failure to bear offsprings could be due to a problem of the male. Male factor infertility is indeed, very common and even on the increase worldwide, constituting a source of global concern [4, 5, 6]. Male Infertility may result from the failure of the pituitary gland to secrete the gonadotrophin hormones [Follicle stimulating hormone (FSH) and Luteinizing hormone (LH)] which could lead to a disruption of testicular function. These hormones, in addition to Testosterone (TET) are important hormones that influence male fertility and reproduction [7]. Furthermore, decreased levels of TET and FSH were reportedly implicated as cause for reduced spermatogenic activities, infertility and reproductive toxicity [8-9]. There are various treatment options for male infertility. However, in Africa and many developing countries, there is still a high dependence on plant sources as medicine in treating the sick [10]. Some medicinal plant extracts including *Titonia diversifolia* [11] have been investigated and found to improve male fertility. *Garcinia kola* [GK] plant which is largely distributed throughout West and Central Africa, Asia and Europe [12-14], is highly valued for its medicinal attributes [15-16]. In African traditional medicine, the nut-

like seed of the plant which consist of a yellow pulp and brown coat is said to be of immense therapeutic benefits [5, 17-18] and therefore, applied in treatment of various ailments like diabetes [19] and guinea worm infestations [20]. Although, studies have been carried out to investigate the effects of *Garcinia kola* on male fertility indices, the findings are at variance. *Garcinia kola* was reported to cause significant increase in gonadotrophins and non significant changes in TET and sperm count [21]; whereas, in a similar study, it caused non significant change in the gonadotropins but a significant increase in the level of TET and sperm count [22]. The studies were carried out using the pulp of *Garcinia kola* seed. The objective of this study was to investigate the effects of the pulp of *Garcinia kola* seed on the physiological indices of male fertility.

2. Materials and Methods

2.1. Animal models

Male wistar rats which weighed between 120g-150g at the beginning of experiment, were sourced from the animal house of Faculty of Basic Medical Sciences, University of Port Harcourt. The rats used in the study were randomly selected and allowed two (2) weeks to acclimatize. They were given free access to feeds (Top feeds Nigeria Limited) and water while housed in clean cages. They were exposed to standard conditions and temperature of 25°C – 30°C and 12h light and 12 h dark schedules. The cages were cleaned and their beddings, water and feeds were changed on daily basis throughout the period of the experiment.

Generally, the study was carried out in conformity with the recommendations in the care and use of laboratory animals by the American Physiological Society [23]

Volume 7 Issue 3, March 2018

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2.2. Preparation of plant materials

Garcinia kola seeds were procured from local vendors in Rivers State, Nigeria and subsequently identified at the herbarium of the Plant Science and Biotechnology department of the University of Port Harcourt, Nigeria.

The seed coat was removed and the pulp was dried and blended to fine powder. Hydromethanol (1:4) was used for extraction at 60-70°C with the Soxhlet apparatus. The extract solution was filtered after 24h and the filtrate was concentrated under reduced pressure of 60°C to a semi-solid form using the rotary evaporator. The extract was now weighed and preserved in refrigerator until used. The extract was later dissolved to obtain 100mg/ml and 200mg/ml of solution which was used for oral treatment of the rats.

2.3. Experimental design

This study was carried out to investigate the effects of the pulp of *Garcinia kola* seed. In this study, male wistar rats were randomly assigned into three (3) groups containing eight (8) rats each for sperm quality and hormonal studies. Group one (1) which served as control received distilled water. Group two (2) and group three (3) were treated with 100mg/kg bw and 200mg/kg bw of the hydromethanol extract of the pulp of *Garcinia kola* seed respectively. The treatments for the sperm quality and hormonal studies lasted for 58 and 60 days respectively. The administration of extracts was done once daily with specialized hypothermic syringes used in animal feeding. The animals were sacrificed under anaesthesia induced with chloroform on days 59 and 61, 24hours after administration of last dose.

2.4. Collection of blood

Blood samples were collected through cardiac puncture into sample tubes and centrifuged at 3000 rev/min for 10-15 minutes to obtain serum which were stored in the refrigerator and later used for hormone analysis.

2.5. Collection of semen / sperm analysis

The caudal epididymis was accessed through a small cut made at the inguinal region. Again, an incision of about 1mm was made on the caudal epididymis to enable the collection of semen. Semen was gently squeezed through the vas deferens. The epididymal sperm count was determined through the method of cytometry with the use of the improved Neubauer cytometer. The sperm count was expressed as million/ml [24, 25]. Sperm parameters were analysed through methods that has been documented [26].

2.6. Hormone assay

The assay for hormones (LH, FSH and TET), was done in accordance with established methods [27] ; using appropriate hormonal kit. The competitive binding of the hormone on immobilized antibody formed the basis for the assay of testosterone. The procedure involved in assay of

all these hormones was based on a solid phase enzyme linked immunosorbent assay (ELISA). In this system, the mouse monoclonal anti - α - hormone antibody for solid phase (microwells) immobilization was incorporated with another mouse monoclonal anti- β -hormone antibody in solution of the antibody enzyme conjugate.

2.7. Statistical analysis

Statistical analysis of data was done using statistical package for social sciences (SPSS) version 20. The results were expressed as mean \pm SEM. The difference between the mean(s) was determined by one way analysis of variance (ANOVA). In all statistical tests, a value of $p < 0.05$ was considered significant.

3. Result

3.1 Result presentation:

The results of this study are presented in tables 1 to 3 and figure 1.

Table 1: Effect of hydromethanolic extract of the pulp of *Garcinia kola* on some hormones

Groups	Serum hormones		
	LH FSH TET		
Group 1 (Control)	6.29 \pm 0.64	6.29 \pm 0.51	2.01 \pm 0.10
Group 2 (100mg/kg)	7.20 \pm 0.53	6.75 \pm 0.53	2.05 \pm 0.09
Group 3 (200mg/kg)	7.76 \pm 0.76*	7.68 \pm 0.40	2.44 \pm 0.18 [‡]

Values expressed as Mean \pm SEM. n=8. Significant at [[‡](P<0.01)] and [* (P<0.05)] when compared with control group.

Table 2: Effect of hydromethanolic extract of the pulp of *Garcinia kola* on some sperm parameters

Groups	Sperm parameters		
	Viable sperm cells (%)	Normal morphology (%)	Actively motile (%)
Group 1 (Control)	68.13 \pm 2.10	68.75 \pm 3.10	63.75 \pm 2.80
Group 2 (100mg/kg)	67.50 \pm 2.11	70.00 \pm 2.31	66.88 \pm 1.32
Group 3 (200mg/kg)	69.38 \pm 2.20	68.75 \pm 1.57	71.88 \pm 2.10*

Values expressed as Mean \pm SEM. n=8. Significant at * (P<0.05) when compared with control group.

Table 3: Effect of hydromethanolic extract of the pulp of *Garcinia kola* on tissue/organ weights

Groups	Tissue/organ weights (g)	
	Testis	Epididymis
Group 1 (Control)	1.03 \pm 0.10	0.25 \pm 0.05
Group 2 (100mg/kg)	1.06 \pm 0.06	0.28 \pm 0.05
Group 3 (200mg/kg)	1.28 \pm 0.05 [‡]	0.31 \pm 0.06

Values expressed as Mean \pm SEM. n=8. Significant at [‡](P<0.01) when compared with control group.

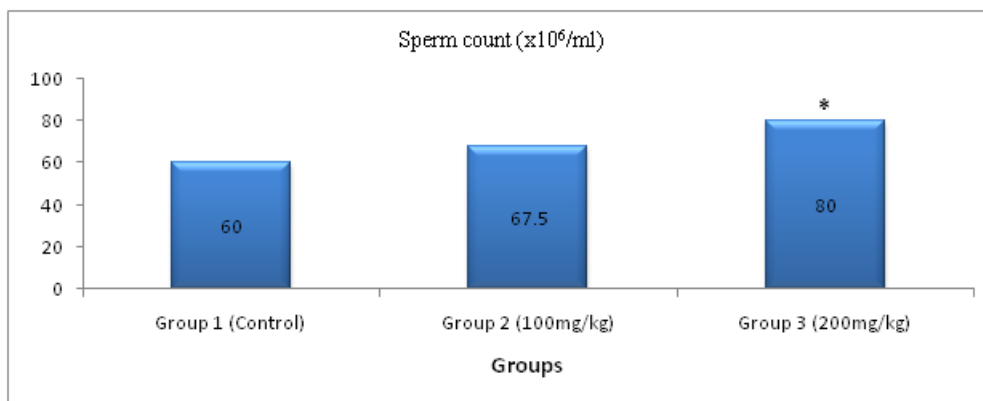


Figure 1: Effect of hydromethanolic extract of the pulp of *Garcinia kola* on sperm count

3.2 Result analysis

The extract was administered for 60 days duration, as low dose of 100mg/kg bw to group 2 and higher dose of 200mg/kg bw to group 3. Group 1 served as control as presented in the result.

In table 1, the LH level increased significantly ($p < 0.05$) while the FSH level was only marginally increased in group 3. The increase occurred in a dose dependent manner in comparison to control.

The level of serum TET also increased significantly ($p < 0.01$) at the higher dose of the extract.

Table 2 highlights the effects of the extract on some sperm parameters. The parameters shown are the viable sperm cells, sperm cells with normal morphology and actively motile cells. The percentage viable cells and percentage cells with normal morphology were not significantly ($p < 0.05$) altered when the test groups were compared to control.

However, the percentage of actively motile cells were significantly increased ($p < 0.05$) in group 3 when compared to control.

As shown in table 3, the weight of the testis was significantly ($p < 0.01$) increased in group 3 where as, the weight of the epididymis was not significantly altered ($p < 0.05$) when compared to control.

Figure 1 illustrates the changes in sperm count in the different groups. A significant increase ($p < 0.05$) occurred in sperm count in group 3.

4. Discussion

This study was done to evaluate the effects of a low dose (100mg/kg) and a higher dose (200mg/kg) bw of the hydromethanolic extract of the pulp of *Garcinia kola* on various physiological indices of fertility in male wistar rats.

An array of messenger hormones which act through the endocrine, paracrine and autocrine pathways maintain proper functioning of the mammalian testis. The primary

messengers are the FSH and LH which are called gonadotrophins, as well as the androgens.

The gonadotrophins are tasked with the maintenance of the proper functioning of somatic cells of the testis [28]. The testicular somatic cells include the interstitial cells of Leydig, whose primary function is the production of TET [29]; the myoid cell which lines the seminiferous tubules providing it with physical support and enabling its contractile motion [30]; and the sertoli cells which essentially provide physical and nutritional support for spermatogenesis because it lies in close proximity with proliferating and differentiating germ cells [31]. The actions of these hormones which influences male fertility are usually targeted at any of these somatic cell types. Furthermore, the receptors for the hormones are expressed on the cells.

In males, the FSH receptor (FSH-R) is expressed on the testicular sertoli cells [32], and the LH receptors (LH-R) are found primarily in the Leydig cells, although receptor staining is also observed in spermatogenic cells [33-34].

In the present study, the gonadotrophins (LH & FSH) secreted by the anterior pituitary gland were found to be elevated following the administration of the pulp extract of *Garcinia kola*. The increase in LH was found to be significant ($P < 0.05$) with the higher dose when compared to control. The plant extract may have acted directly on the anterior pituitary gland to cause increased secretions of these hormones; which was evidenced by the significantly elevated LH and marginal rise in FSH in blood. The TET level was found to be significantly increased with the higher dose of the extract. The increased TET level may be due to the significantly increased release of LH which then acted on the receptors expressed on the leydig cells. The stimulation of these receptors may have led to an increased secretion of testosterone and the promotion of spermatogenesis. TET regulates the process of germ cell differentiation [28]. The increased gonadal stimulation of testosterone release exemplifies the complex order of hormone interplay in the hypothalamus-pituitary-gonadal axis in males.

The epididymal sperm parameters showed that the extract did not significantly alter the percentages of viable spermatozoa and sperm cells with normal morphology.

But the higher dose of the extract caused a significant increase in the actively motile cells when they were all compared to control; which agrees with the findings in a similar study [35].

The extract also caused a significant increase in the sperm count. This was observed with the higher dose of the extract when it was compared to control. The significant increase in the secretion of the LH that consequently caused an increase in TET secretion in this study may be responsible for the increased spermatogenesis leading to increased sperm count. Indeed, the amount of spermatozoa in the seminiferous tubules, which is an indication of the degree of spermatogenesis in quantitatively maintained by the TET and FSH [36].

Also, motile spermatozoa in adequate concentrations and devoid of abnormalities are highly correlated with fertility [37].

On the other hand, decreased numbers of spermatozoa, reduced motility and/or morphologically distorted spermatozoa are the leading causes of disturbed fertility or infertility in animals [38].

The weight of the testis was also increased significantly with the higher dose of the extract. But the weight of the epididymis was not significantly altered. Increases in testicular weight usually accompany an increase in spermatogenesis. This is because the seminiferous tubules which contain the spermatids and spermatozoa accounts for large part of the testicular weight [39-40].

The observed changes in the cascade of hormones and its impact on testicular and spermatogenic activities of the higher dose (200mg/kg) of the pulp extract of *Garcinia kola* improved fertility and reproductive parameters in male wistar rats. The findings in the present study are in agreement with the findings in a similar study [41] but, disagree with reported findings in another related study [42]. The variation in the findings with the latter study may be due to different methods of extraction. The result obtained in this study showed that the pulp of *Garcinia kola* increased physiological indices of fertility in male wistar rats.

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