

Evaluation of Hydromethanolic Seed Coat Extract of *Garcinia kola* on Fertility Profile of Male Wistar Rats

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Abstract: *The traditional application of Garcinia Kola in the treatment of many ailments is common practice in Nigeria and the African sub region. Although, the pulp part of Garcinia kola seed has been scientifically evaluated, the seed coat may not have been investigated for any biological effects. The present study was then carried out to investigate the effects of the seed coat of Garcinia kola on the fertility indices of male wistar rats. Male wistar rats were randomly assigned into three (3) groups of eight (8) rats each. Group one (1) served as control and received distilled water. Group two (2) and group three (3) received 100mg/kg and 200mg/kg of the hydromethanol (20%:80%) seed coat extracts respectively. Hormonal and sperm quality studies lasted for 30 and 58 days respectively. Thereafter, recovery study was done. Results obtained showed that the extract caused significant reductions in the serum levels of Luteinizing Hormone, Follicle stimulating hormone and testosterone as well as a significant reduction in the sperm count. However, these were completely reversed upon cessation of extract administration. This study proved that, the seed coat of Garcinia kola possesses anti fertility effects which are reversible in male wistar rats.*

Keywords: Garcinia kola, seed coat, hydromethanol, antifertility, reversible

1. Introduction

Garcinia kola (GK), also called bitter kola has been described as a wonder plant because, every part of the plant is considered to have some medicinal importance[1]. GK tree is medium sized and grows up to 12 meters in West and Central Africa where it is mainly cultivated and distributed[2], often in moist forest [3,4]. The seeds of GK are smooth and elliptical in shape and consist of a yellow pulp and brown coat[5]. They are also bitter and possesses an astringent taste[6]. In Nigeria and some other West African countries, the seeds are commonly consumed for medicinal purposes and also presented as refreshment in traditional and social gatherings[7,8]. The folkloric application of GK in treatment of many ailments is deep rooted in Nigeria and the African sub region. This includes its use in the treatment of liver disorders, hepatitis, jaundice[9], diarrhea[10], as well as cough and erectile dysfunction[11-13], amongst others. Extracts of the plant has also been reported to have hypolipidaemic[14] and antimicrobial effects[15]. Some consumers of GK seed discard the seed coat before consuming the pulp. Many previous researchers worked on the pulp of GK and reported on its effects on the histological integrity of the testis[16]; its aphrodisiac activity[17,18], and as a possible hop substitute in beer making[19-21]. Although, the presence of certain bioactive compounds have been discovered in the seed coats of almond, peanuts(*Arachis hypogea*), lotus seeds(*Nelumbo nucifera*), African yambean(*Sphenostylis stenocarpa*)[22-24] and GK seed and hulls[5]; literature is scarce about the biological effects of the seed coat of GK.

Fertility in males is sensitively hinged on normal levels of Luteinizing hormone (LH), Follicle stimulating hormone (FSH) and testosterone(TET); which are employed as very important complementary assessment of male fertility profiling[25-28], and they quantitatively affect the

production of spermatozoa[29]. Sperm count is a very useful test for spermatogenesis and it is highly correlated with fertility[30]. In view of the apparent lack of scientific information on the biological effects of the seed coat of GK seed, the present study was carried out with the objective to explore the effects of the seed coat of GK on male fertility.

2. Materials and Methods

2.1 Preparation of Plant extract

The seeds of GK were procured in a local market and identified in the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria.

The outer coat of the seeds were removed, dried and blended to fine powder.

The crude extraction was carried out with hydromethanol (1:4) at 60 – 70°C using the soxhlet apparatus.

The solution was filtered after 24 hours and the filtrate concentrated under reduced pressure of 60°C to a semi solid form using the rotary evaporator. The net yield was weighed and the extract preserved in a refrigerator at 4°C.

To prepare the stock solution, the extract was reconstituted to obtain 100mg/ml and 200mg/ml of solution for animal oral treatments.

2.2 Animal models

Ethical approval for this study was obtained from the ethics committee in the College of Health Sciences, University of Port Harcourt, Nigeria.

Adult male rats were randomly selected from the animal house of the Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria. They were housed in clean cages and maintained in relatively constant environmental conditions with proper ventilation and 12 hour light and 12 hour dark cycle. They had access to food and water *ad libitum*. Further, the procedures involving the animal models conformed to the guiding principles in the care and the use of animals by the American Physiological society [31].

2.3 Experimental design

This study was designed to investigate the effects of the seed coat of GK. In the first phase of this study, male wistar rats were divided into three (3) groups of eight rats each for hormonal and sperm quality studies which lasted for 30 and 58 days respectively. 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 seed coat respectively. In the second (recovery) phase, the rats received same dose of extract for 30 and 58 days for hormonal and sperm quality studies respectively; then allowed to stay without treatment for equal duration before sacrifice. The extracts were administered as single oral doses per day using animal feeding hypothermic syringes. Eight (8) rats from each group were sacrificed under chloroform anaesthesia on day 31 and 59 after 24hours of last administered dose and also on day 61 and day 117 after recovery period.

2.4 Collection of blood

Blood samples were collected through cardiac puncture into dry test tubes and allowed to stand for about 15 minutes to clot. It was further centrifuged at 3000 rev/min for 10-15 minutes using a table centrifuge machine. The sera was separated using a pasteur pipette into sterile sample tubes and stored at -4°C until used.

2.5 Semen collection/ Sperm quality analysis

Semen was collected by making a small incision at the inguinal region to reach the caudal epididymis where an incision of about 1mm was made. Semen was gently squeezed through the vas deferens. The epididymal sperm count was obtained by the method of cytometry using the improved Neubauer cytometer and was expressed as million/ml [32,33]. The procedures employed in the determination of all sperm parameters have been documented [34].

2.6 Hormone assay

The assay for LH, FSH and TET, was done in accordance with established principles [35]; using appropriate hormonal kit. The assay for testosterone depended on competitive binding of the hormone on immobilized antibody. The procedure involved in assay of these hormones was based on a solid phase enzyme linked immunosorbent assay (ELISA). This system incorporates the mouse monoclonal anti - α - hormone antibody for solid phase (microwells) immobilization and another mouse monoclonal anti- β -

hormone antibody in the antibody enzyme conjugate solution.

2.6 Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 20.0 was used for the statistical analysis of data. This involved the use of analysis of variance (ANOVA). Results were expressed as Mean \pm SEM and regarded as significant at $p < 0.05$.

3. Result

3.1 Result presentation

The results of the study are presented in tables 1 to 4 and figure I.

Table 1: Effect of the seed coat of *Garcinia kola* on some hormones

Groups	Hormones		
	LH (IU/L)	FSH (IU/L)	TET (ng/ml)
Group 1 (Control)	6.49 \pm 0.48	6.01 \pm 0.47	0.88 \pm 0.09
Group 2 (100mg/kg)	5.05 \pm 0.41*	4.71 \pm 0.39	0.81 \pm 0.06
Group 3 (200mg/kg)	4.58 \pm 0.40 [‡]	3.55 \pm 0.24 [‡]	0.55 \pm 0.07*

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 the seed coat 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 1.64	65.63 \pm 1.99	64.38 \pm 1.48
Group 3 (200mg/kg)	67.50 \pm 2.11	66.25 \pm 1.83	63.13 \pm 1.88

Table 3: Effect of the seed coat of *Garcinia kola* on tissue/organ weights

Groups	tissue/organ weights (g)	
	TESTIS	EPIDYDIMIS
Group 1 (Control)	0.96 \pm 0.07	0.24 \pm 0.06
Group 2 (100mg/kg)	1.06 \pm 0.05	0.23 \pm 0.04
Group 3 (200mg/kg)	1.03 \pm 0.05	0.24 \pm 0.04

Table 4: Level of various hormones and sperm count following recovery period

Groups	Hormones/Sperm count			
	LH (IU/L)	FSH (IU/L)	TET (ng/ml)	Sperm count($\times 10^6$ /ml)
Group 1 (Control)	5.99 \pm 0.30	6.05 \pm 0.13	2.04 \pm 0.07	65.00 \pm 2.04
Group 2 (100mg/kg)	5.83 \pm 0.36	5.90 \pm 0.21	1.95 \pm 0.07	62.25 \pm 1.28
Group 3 (200mg/kg)	5.79 \pm 0.18	5.59 \pm 0.22	1.90 \pm 0.05	60.50 \pm 2.78

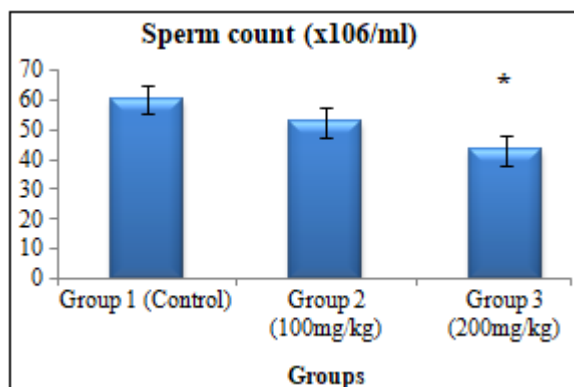


Figure 1: Effect of the seed coat of *Garcinia kola* on Sperm count

3.2 Result analysis

Hydromethanolic extracts of the seed coat of GK were administered for a period of 30 and 58 days for hormonal and sperm quality studies, as low dose of 100mg/kg bw (group 2) and higher dose of 200 mg/kg bw (group 3).

Table 1 showed that LH level reduced significantly at $p < 0.05$ for the low dose of 100mg/kg and $p < 0.01$ for the higher dose of 200mg/kg. The reductions occurred in a dose dependent manner when compared with control. The level of serum FSH and TET also reduced significantly at ($p < 0.01$) and ($p < 0.05$) at the higher dose of 200mg/kg respectively.

In table 2, the effects of the extracts on some sperm parameters were highlighted. The parameters includes the viable sperm cells, normal morphology and actively motile cells. The difference when compared with controls were not found to be significant ($p < 0.05$). Also, there were no significant ($p < 0.05$) differences found in the weights of the testis and epididymis (table 3) in comparison with control.

Table 4 show the level of LH, FSH, TET and sperm count following a recovery period of 30 days. The differences between the control and test groups were not statistically significant.

Figure 1 illustrates dose dependent reductions in sperm count. This was found to be significant ($p < 0.05$) at the higher dose (200mg/kg) of the extract.

4. Discussion

This study investigated the effect of the hydromethanolic extract of the seed coat of GK on various physiological indices of fertility in male wistar rats.

The proper assessment and management of male related fertility problems and on the other hand, the development of safe male contraceptive which will also be reversible, no less requires a thorough understanding of the hormonal regulation of spermatogenesis [36].

FSH and the LH are glycoprotein hormones secreted by the anterior pituitary gland in response to hypothalamic gonadotropin releasing hormone (GnRH), and they act directly on the testes where they stimulate somatic cell

function in support of spermatogenesis [37]. These two hormones also regulate the secretion of TET by the testis which is important in the initiation and maintenance of spermatogenesis [38]. These regulations occur via their actions on specific receptors expressed on various cells. In, males, the expression of the FSH receptor (FSH – R) is limited to the testicular sertoli cells [39], whereas the LH receptor (LH – R) are expressed primarily in the leydig cells even though, receptor staining is also observed in spermatogenic cells [40,41].

In this study, the seed coat extract of GK caused significant reductions in the serum levels/concentrations of the pituitary gonadotropins. While the low and higher doses caused reductions in LH, only the higher dose of 200mg/kg significantly reduced the FSH. The implication thus, is that the plant extract may have acted directly on the anterior pituitary gland to inhibit synthesis of the gonadotropins. The significantly reduced TET level observed with the higher dose of the extract may be due to inhibitions to the release of pituitary gonadotropins which manifested as lower concentrations of these hormones in the blood. These changes led to a diminished gonadal stimulation to the secretion of TET in an intricate hormonal interplay which exist at the hypothalamo-hypophyseal-gonadal axis. Also, the extract may have disrupted the function of the luteinizing hormone releasing hormone receptor (LH RH receptor) which resulted in reduced LH release.

The epididymal sperm parameters showed that the extract did not affect the percentages of viable sperm cells and cells with normal morphology as well as sperm motility.

The actively motile sperm cells were evaluated and reported in this study since the sluggishly motile or immotile sperm cells appear to be physiologically ineffective, as they are very much unlikely to penetrate the cervical mucus to fertilize the ova [42,43]. However, the extract caused a significant reduction in the sperm count. The significant reductions in the secretion of the FSH and LH which caused a decrease in the testosterone output in this study may be responsible for the reduction in spermatogenesis leading to a reduced sperm count. In another study, a decline in testosterone secretion was stated as the reason for an observed impairment of spermatogenesis [44].

Meanwhile, the sperm count has been reported to be a very important test of spermatogenesis which is directly associated with fertility [32].

The testicular and epididymal weights were not affected in spite of the fact that the plant extract reduced spermatogenesis.

Plant based contraceptives inhibits male fertility following administration of the natural substances by causing a decrease in the density of spermatozoa [45].

But the reversibility of a depressed or inhibited fertility remains the cardinal feature of an ideal plant based male contraceptive.

In this study, the reduced hormone secretion (FSH, LH and TET) and the sperm count were completely reversed upon cessation of extract administration.

5. Conclusion

The seed coat of GK possesses anti fertility effects which are reversible in male wistar rats. The reversibility of these effects show that the extract could be a potential male contraceptive.

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