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Effect of Ethanolic Extract of Garcinia Kola Seed on Some Reproductive Parameters of Male Wister Rats

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ABSTRACT: *The ethanolic extract of Garcinia kola seed was investigated for its activity on selected haematological parameters of male wistar rats. Thirty (30) male wistar rats weighing 150.42 ± 3.98 were divided into three groups (I, II and III) comprising ten animals each. Animals in group I (control) received 0.5 ml of distilled water while those in groups II and III were administered 100 and 200 mg/kg body weight of the extract respectively once daily. After 3 weeks of extract administration, 5 rats from each group were sacrificed. Same was done after 6 weeks. Preliminary phytochemical screening of ethanolic extract of Garcinia kola seed revealed the presence of alkaloids, saponins, tannins, carbohydrate, steroids and flavonoids. The lethal dose (LD_{50}) of the extract was found to be safe up to 5000 mg/kg body weight. Results of this study revealed that the extract has no significant ($p > 0.05$) change on body weight. However the weight of testes decreased significantly ($p < 0.05$) in groups II and III after 3 weeks of administration. The change in weight was not significant ($p > 0.05$) at 6 weeks of administration. The weight of the epdididymis increased significantly ($p < 0.05$) in group III after 6 weeks of administration. Serum testosterone levels increased significantly ($p < 0.05$) in group III after 3 weeks and groups II and III after 6 weeks of administration. The administration of the extract caused a significant ($p < 0.05$) increase in sperm count in groups II and III at 3 weeks and group III at 6 weeks. Sperm motility increased significantly ($p < 0.05$) whereas percentage of abnormal sperms decreased significantly ($p < 0.05$) in groups II and III at 3 and 6 weeks of treatment. This study has shown that administration of 100mg/kg and 200mg/kg of G kola ethanolic extract for 6 weeks enhances sperm characteristics in wistar rats.*

KEYWORDS: *Garcinia kola, Reproductive Parameters, Ethanolic Extract, Enhancing Effects.*

I. INTRODUCTION

The environment of man is endowed with plants and fruits which have been found to be of great nutritional and health importance to man and animal. Such plants and fruits constitute sources of spices in food, stimulants and some micronutrients while others may have aphrodisiac properties. Over the years, some of these plant materials have been extensively investigated and their properties documented. (Adeyeye and Ayejuyo, 1994 ;Alliet al., 2000). Quite a number of plant materials have antimicrobial properties with tremendous therapeutic potentials; one of such being garcinia kola.(Cowan, 1999; Chatterjee, 2000).

In the last few years, herbal medicine has gained a lot of relevance and popularity both in developing and developed countries because of their natural origin and less side effects (Grover et al., 2002). Herbal products cause few adverse effects but have beneficial pharmacological and therapeutic uses in a number of illnesses, where they have been examined for their capacity to reduce symptoms and improve quality of life (Weber et al., 1999).

Garcinia kola is a dicotyledonous plant distributed throughout West and Central Africa. It is found in Sierra Leone, Ghana, Nigeria, Benin, Cameroon, Senegal, Gabon and Congo. (Adedejiet al.,2006). Garcinia kola grows well in the coastal rainforest of the Southwestern and Southeastern Nigeria. It is used as food and herbal medicine and produces reddish, yellowish or orange colour fruits containing 2 to 4 seeds (Adesanyaet al., 2007). The seed is elliptical or oval in shape, is hard and has a slightly aromatic odour (Maziet al., 2013).The plant is commonly called “bitter kola” or “male kola” because of its bitter taste, or for its claimed aphrodisiac activity, respectively. The stem bark is used as a purgative among the natives of Eastern Nigeria and the latex is externally applied to fresh wounds to prevent sepsis, assisting in wound healing(Ukoet al., 2001).

It plays prominent and significant roles in the life and culture of the people especially among the Yoruba and Ibo tribes in Southern Nigeria; as it is used in entertaining guests and for special occasions.

Garcinia kola had been identified to contain properties that are of biochemical and physiological interest such as antibacterial, antioxidative, antihepatotoxic, and hypoglycemic. (Farombiet al., 2002 ;Okunjiet al., 2007).

The seeds are used to cure cough, dysentery, or chest colds in herbal medicine (Chairungsrilerdet al., 1996). This plant has been referred to as a 'wonder plant' because every part of it has been found to be of medicinal importance. It is used in folklore remedies for the treatment of ailments such as liver disorders, hepatitis, diarrhoea, laryngitis, bronchitis, and gonorrhea (Iwu, 1993). This study present the effects of garcinia kola extract on some reproductive parameters in male wister rats.

II. MATERIALS AND METHODS

MATERIALS

Plant Materials and Authentication

Garcinia kola seeds which were purchased from Karu market, in the Federal Capital Territory, Abuja and were authenticated at Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria with voucher specimen number (F.H.I. 10847).

Experimental Animals

Male Wistar rats (Rattusnorvegicus) weighing 150.42 ± 3.98 g were obtained from the Animal House of Federal College of Animal Husbandry, Kuru, Jos, Plateau State, Nigeria.

Other Reagents

All other chemicals and reagents used which were of analytical grade were products of sigma Aldrich Ltd., Buchs, Canada and are prepared in volumetric flask using glass wares with distilled water except otherwise stated.

III. METHODS

Preparation of Ethanolic Extract of Garcinia kola Seed

Dried seeds of Garcinia kola were peeled to remove the testa. This was cut into smaller sizes and thereafter pulverized in a blender (PHILIPS, Model HR-1724, Brazil) to obtain smooth powder. A known weight (200 g) of the powder was extracted in 1000 ml of ethanol for 72 hours at room temperature. The extract was filtered with Whatman No. 1 filter paper (Maidstone, UK) and the resulting filtrate concentrated in a Rotary Evaporator. The mixture was further transferred into steam bath where it was evaporated to give the required brownish-black residue. This was then reconstituted in distilled water to give the required doses (100 and 200 mg/kg body weight) used in the study.

Phytochemical Screening

Preliminary phytochemical screening to detect the presence of alkaloids, saponins, tannins, flavonoids, steroids, anthraquinones, cardiac glycosides and carbohydrate were carried out by adopting the procedures described by Harborne (1973), Walls et al., (1996), Odebiyi and Sofowora (1978), Awe and Sodipo (2001), El-Olemyet al., (1994), Mainasaraet al., (2012), Sofowora (1993) and Trease and Evans (1983) respectively.

Acute Toxicity Study (LD_{50})

The method described by Lorke (1983) was adopted to determine lethal dose (LD_{50}). The experiment was carried out in two stages:

Stage I: Here, nine rats were involved. They were divided into 3 groups of 3 rats each. The animals in group A received ethanolic extract of Garcinia kola seed at a dose of 10 mg/kg body weight. Group B received 100 mg/kg body weight. Group C received 1000 mg/kg body weight. The animals were observed/monitored for 24 hours. The number of deaths in each group was noted.

Stage II: This stage was carried out based on the results of the first stage. Here, another three groups of one rat each was used. The animal in Group A received 1000 mg/kg body weight of the extract. The animal in Groups B and C received 3000 and 5000 mg/kg body weight of the extract respectively. The animals were monitored for another 24 hours during which the number of deaths or abnormal reaction/behaviour was noted.

Animal Grouping and Extract Administration

A total of thirty male albino rats, housed in clean aluminum cages contained in well ventilated standard housing conditions (temperature: 28-31^{0}C; photoperiod: 12 hours; humidity: 50-55%) was used for the study. The animals were allowed free access to rat pellets (Premier Feed Mill Co. Ltd., Ibadan, Nigeria) and tap water ad libitum. The cages were also cleaned on daily basis. The animals were acclimatized for two weeks before the commencement of the experiment. The thirty (30) male Wistar rats weighing 150.42 ± 3.98 were completely randomized into three groups (I, II and III) comprising ten animals each. Animals in group I (control) received 0.5 ml of distilled water while those in groups II and III were administered 100 and 200 mg/kg body weight of

the extract respectively once daily. After 3 weeks of extract administration, 5 rats from each group were sacrificed. Same number was sacrificed after 6 weeks. Extract administration was done daily using polystyrene. This research was carried out in Physiology Department of Bingham University, according to the rules in Nigeria (Revised Helsinki Declaration, 2008) governing the care and use of laboratory animals as acceptable internationally.

Organ Weights

When the animals are sacrificed, the epididymis and testes were dissected out, fat cleaned off and the organs weighed to assess the effects of G. kola extract on these reproductive organs.

Sperm Count Determination

Methods of Selmanogluet al (2009) were used to analyse sperm count. The caudal epididymis was dissected and minced in 1ml of normal saline. The suspension was then fixed in formal saline and spermatozoa counted using the Neuberhematocytometer chamber and light microscope.

Sperm Motility

The method of Biswaset al (2002) was used with a minor modification. The content of the vas deferens was collected with aid of syringe and needle and a drop was placed on a clean slide (37ºC) and covered with cover slip. The motility was determined by visual estimation (using microscope at a magnification of 400X) of the proportion of spermatozoa moving forward (motile) and those that didn't move were considered non-motile.

Sperm Morphology

A drop of stained sperm suspension (which was prepared for sperm count) was smeared on a glass slide, air dried for a maximum of 3 minutes and visualized microscopically at a magnification of 400X. For each rat, the sperms were screened and the percentage of total abnormality of heads (such as microcephalus, detached head, flattened head, double head) and/or tails (such as coiled tail, bent tail and double tail) was determined.

Statistical Analysis

The grouped data obtained were statistically evaluated and significance of the various treatments were tested using t test in SPSS version 20. All the results were expressed as mean ± SD from 5 rats in each group at 3 and 6 weeks. P values of ≤ 0.05 were considered statistically significant.

IV. RESULTS

Preliminary phytochemical screening of the ethanolic extract of Garcinia kola seed revealed the presence of alkaloids, saponins, tannins, carbohydrate, steroids and flavonoids. Anthraquinones and cardiac glycosides were not detected (Table 1).

Table 1: Phytochemical constituents of ethanolic extract of Garcinia kola seed

Constituent	Inference
Alkaloids	++
Flavonoid	+++
Saponins	++
Tannins	++
Carbohydrate	++
Anthraquinones	-
Steroids	+
Cardiac glycoside	-

Key:

- \- Absent
- \+ Present
- ++ Significantly present
- +++ Abundantly present

For acute toxicity study, all the graded doses of the ethanolic extract of Garcinia kola seed administered to the animals showed no signs of toxicity and no deaths were recorded. Therefore, the LD_{50} of ethanolic extract of Garcinia kola seed was found to be safe up to 5000 mg/kg body weight.

Effect of Treatment with Ethanol Extract of Garcinia kola on whole Animal Weight

After 3 weeks of administration of the extract, animal weight increased from 151.40±14.21 (control) to 156.60±15.58 and 164.20±21.01 in groups II and III respectively. This increase was however statistically not significant (p= 0.596 and 0.292 respectively) (Table 2).

After 6 weeks of administration, animal weight decreased from 214.20±35.87 to 189.20±9.04 in group II while there was an increase from 214.20±35.87 to 215.60±37.07 in group III. Both the decrease and increase observed in both groups were not significant with p values of 0.169 and 0.953 respectively (Table 2).

Effect of Treatment with Ethanol Extract of Garcinia kola on Testicular Weight

After 3 weeks of administration, the ethanol extract of G kola significantly decrease the weight of testes from 1.38±0.11 (control) to 1.17±0.13 and to 1.06±0.16 in groups II and III respectively (p=0.023 and 0.006 respectively) (Table 2)

After 6 weeks of administration, there was increase in the weight of the testes from 1.15±0.16 (control) to 1.23±0.18 in group II which was not significant (p=0.512). There was however decrease in testicular weight to 1.12±0.26 in group III which was also not statistically significant (p=0.736) (Table 2)

Effect of Treatment with Ethanol Extract ofGarcinia kola on Weight ofthe Epididymis

After 3 weeks of administration of the extract, the weight of the epididymis increased from 0.16±002 (control) to 0.17±0.04 and 0.18±0.04 in groups II and III which was statistically insignificant (p=0.597 and 0.432 respectively) (Table 2).

After 6 weeks of administration of the extract, the weight of the epididymis increased from 0.15±0.01 (control) to 016±0.02 in group II which was statistically insignificant (p=0.447). However there was statistically significant increase to 0.18± 0.01 in group III when compared with the control group (p=0.002) (Table 2).

Table 2: Effect of Ethanol Extract of Garcinia kola on whole Animal and Reproductive Organs Weight after Six Weeks of Oral Administratiion

Parameters	3 weeks			6 weeks		
	Treatment			Treatment		
	Control	100mg	200mg	control	100mg	200mg
Animal weight (g)	151.40±14.21 [a]	156.60±15.58 [a]	164.20±21.01 [a]	214.20±35.87 [a]	189.20±9.04 [a]	215.60±37.07 [a]
Weight of testis (g)	1.38±0.11[a]	1.17±0.13[b]	1.06±0.16[b]	1.15±0.16[a]	1.23±0.18[a]	1.12±0.26[a]
Epididymis weight (g)	0.16±0.02[a]	0.17±0.04[a]	0.18±0.04[a]	0.15±0.01[a]	0.16±0.02[a]	0.18±0.01[b]

Means with different superscripts (a, b) along the rows are statistically significant when compared with the control group

Effect of Treatment with Ethanol Extract of Garciniakola on Serum Testosterone

After 3 weeks of administration of the extract, the serum testosterone decreased from 3.90±0.29 (control) to 3.75±0.26 in group II while there was an increase from 3.90±0.29 (control) to 4.53±0.22 in group III. The decrease in group II and increase in group III were statistically insignificant and significant with p values of 0.103 and 0.004 respectively (Figure 1).

After 6 weeks of administration, the serum testosterone increased from 2.88±0.51 (control) to 3.98±0.15 and 4.05±0.13 in groups II and III respectively. These changes were statistically significant with p values of 0.043 and 0.034 respectively (Figure 1).

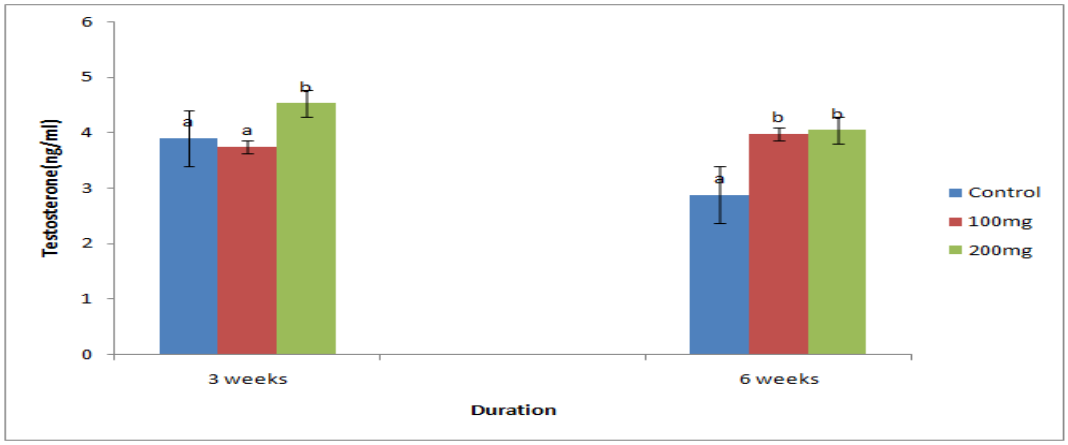

Figure 1: Effect of Ethanol Extract of Garcinia kola on Serum Testosterone Concentration after 3 and 6 Weeks

Effect of Treatment with Ethanol Extract of Garciniakola on SpermCount

After 3 weeks of administration of the extract, there was statistically significant increase in sperm count from 18.40±1.45 (control) to 22.76±3.72 and to 31.18±2.01 in groups II and III with p values of 0.040 and 0.001 respectively (Table 3).

After 6 weeks of administration, there was also an increase in sperm count from 18.54±2.65 (control) to 18.56±4.63 in group II which was statistically insignificant (p=0.994) whereas there was a statistically significant increase in sperm count from 18.54±2.65 (control) to 27.58±4.60 in group III (p=0.005) (Table 3).

Effect of Treatment with Ethanol Extract of Garcinia kola on SpermMotility

After 3 weeks of administration of the extract, there was significant increase in sperm motility from 95.18±1.04 (control) to 97.00±1.00 in group II and to 97.70±0.50 in group III with p values of 0.023 and 0.001 respectively (Table 3).

After 6 weeks administration of the extract, there was statistically significant increase in sperm motility from 83.80±1.79 (control) to 89.00±3.16 and to 89.60±4.39 in groups II and III with p values of 0.013 and 0.026 respectively(Table 3).

Effect of Treatment with Ethanol Extract of Garcinia kola on Sperm Abnormality

After 3 weeks of administration of the extract, sperm abnormalities significantly decreased from 5.88±1.26 (control) to 2.90±0.99 and to 3.00±1.05 in groups II and III with p values of 0.008 and 0.040 respectively (Table 3).

After 6 weeks administration of the extract, there was also significant decrease in sperm abnormalities from 6.20±1.48 (control) to 3.00±1.0 and to 3.00±1.58 in groups II and III with p values of 0.004 and 0.011 respectively(Table 3).

Effect of Treatment with Ethanol Extract of Garcinia kola on SpermHead Abnormality

After 3 weeks administration of the extract, sperm head abnormalities significantly decreased from 7.70±1.01 (control) to 2.02±0.84 in group II and to 1.54±0.67 in group III with p values of 0.001 in both groups (Table 3).After 6 weeks of administration, the sperm head abnormalities also significantly decreased from 3.50±0.91 (control) to 1.92±0.42 and to 1.42±0.87 in groups II and III with p values of 0.008 and 0.006 respectively(Table 3).

Effect of Treatment with Ethanol Extract of Garcinia kola on SpermTail Abnormality

After 3 weeks administration of the extract, sperm tail abnormalities significantly decreased from 9.24±1.67 (control) to 1.10±0.46 in group II and to 1.12±0.63 in group III with p values of 0.001 in both groups (Table 3). After 6 weeks of administration, the sperm tail abnormalities also significantly decreased from 3.10±0.93 (control) to 1.68±0.40 and to 1.38±0.70 in groups II and III with p values of 0.014 and 0.011 respectively (Table 3).

Table 3: Effect of Ethanol Extract of Garcinia kola on Sperm Parameters after Six Weeks of Oral Administration

	3 weeks			**6 weeks**		
	Treatment			**Treatment**		
Parameters	**Control**	**100mg**	**200mg**	**control**	**100mg**	**200mg**
Sperm count (millions/ml)	18.40±1.45[a]	22.76±3.72[b]	31.18±2.01[b]	18.54±26.54[a]	18.56±50.74[a]	27.58±46.27[b]
Motile (%)	95.18±1.04[a]	97.00±1.00[b]	97.70±0.50[b]	83.80±4.60[a]	89.00±3.16[b]	89.60±4.39[b]
Abnormal(%)	5.68±0.26[a]	2.90±0.99[b]	3.00±1.08[b]	6.20±1.48[a]	3.00±1.00[b]	3.00±1.58[b]
H/abnormal (%)	7.70±1.01[a]	2.02±0.84[b]	1.54±0.67[b]	3.50±0.91[a]	1.92±0.42[b]	1.42±0.87[b]
T/abnormal (%)	9.24±1.62[a]	1.10±0.46[b]	1.12±0.63[b]	3.10±0.93[a]	1.68±0.40[b]	1.38±0.70[b]

Means with different superscripts (a, b) along the rows are statistically significant when compared with the control group

V. DISCUSSION

The use of plants in traditional medical practice has a long drawn history and remains the mainstay of primary health care in most of the third world countries. Traditional medicine is used by about 60% of the world's population in both developing and developed countries (Mythilypriyaet al., 2007).Garcinia kola has been reported to possess numerous therapeutic potentials, along with phytochemical components (Ukoet al., 2001). These phytochemicals have been shown to have a broad spectrum of pharmacological activity, such as antibacterial, antioxidative, antihepatotoxic and hypoglycemic (Okunjiet al., 2007).

There was a statistically insignificant (p>0.05) increase body weight of the treated animals in both groups after 3 weeks and in the high dose group after 6 weeks with an insignificant (p>0.05) decrease in body weight after 6 weeks in the low dose group. This shows that G kola extract has an insignificant effect on the

body weight of the treated animals after 6 weeks. Body weight is an indication of an animal wellbeing. The acute toxicity study also shows that 50% ethanol extract of G kola has no toxic effect on the treated animals. This finding is consistent with that of Ralebonaet al (2012) who also noted no significant effect of ethanol extract of G kola on body weight of treated animals after 28 days of oral administration. Agbaiet al (2013) however reported a significant decrease in body weight of treated animals after 16 weeks administration of 300mg/kg of methanolic extract of G kola. Reports indicates that kolaviron in G kola exerts a hypocholesterolaemic effect on the body (Agbaiet al., 2013).

The results of this study showed a significant dose dependent decrease in testicular weight in groups II and III after 3 weeks of administration and an insignificant decrease in testicular weight in group III after 6 weeks of administration of the extract. Agbaiet al (2013) also noted a dose dependent decrease in testicular weight following 100/kg, 200mg/kg and 300mg/kg administration of ethanolic extract of G kola for 6 weeks. However, Ralebonaet al (2012) reported that 200mg/kg and 400mg/kg of ethanolic extract of G kola caused a significant increase in testicular weight with no significant change in the weights of accessory reproductive organs. A dose dependent increase in weight of the epdididymis was seen in this study. This was however not significant in the low dose group at 3 weeks and 6 weeks respectively but significant in the high dose group at 6 weeks of administration. This was consistent with the finding of Agbaiet al (2013) and is most likely due to the effect of increase in serum testosterone levels and increase in sperm count seen in this study.

There was a significant dose dependent increase in serum testosterone levels in all the treated groups when compared with the controls both at 3 weeks and 6 weeks of administration. The mechanism for the increase in serum testosterone levels may be via the central influences to increase gonadotropins or locally via increase in number of leydig cells or their sensitivity to luteinizing hormone.Akpantahet al., (2003) has found that G.kola increases the peripheral testosterone level. This ability of G.kola to increase peripheral testosterone has been attributed to anti-oxidant compounds present in them (Oluyemiet al., 2007; Akpantahet al., 2003).

A dose dependent increase in sperm count was noted in all the treated groups when compared with the controls which were significant in both groups at 3 weeks and high dose group at 6 weeks. Adesanyaet al (2007) and Iwuji and Herbert (2012) also reported a dose dependent increase in sperm count following oral administration of 100mg/kg and 200mg/kg of ethanolic extract of G kola for 6 weeks. Increase in spermiogenesis is facilitated by antioxidants and increase in serum testosterone levels which are properties of biflavonoids and xanthones found in G kola (Oluyemiet al., 2007 and Akpantahet al., 2003).

The significant increase in sperm motility in all the treated groups when compared with the controls in this study was consistent to the results obtained by Iwuji and Herbert (2012) who reported increase in sperm motility of rabbit bucks fed diets containing G kola seed meal. This was however inconsistent with the results obtained by Adesanyaet al (2007) who reported a decrease in sperm motility of adult wistar rats. They postulated that the decrease in sperm motility could be due to the rapid development of the spermatozoa which may need a moderate but progressive development for them to have excellent motility.

There was significant decrease in the percentage of the abnormal sperms in all the treated groups when compared with the controls in this study both at 3 weeks and 6 weeks of treatment. This was consistent with the findings of Iwuji and Herbert (2012) who demonstrated significant decrease in abnormal sperms in all the treated groups. Decrease in the abnormalities of the head and tail in all the treated groups when compared with the controls was noted in this study. This is likely due to the presence of antioxidants such as kolaviron in the G kola extract. Antioxidants are known to protect spermatogenesis in animals exposed to toxicants (Attehsahlinet al., 2006).

VI. CONCLUSION

This study concludes that administration of 100mg/kg and 200mg/kg of G kola ethanolic extract for 6 weeks caused increase in the weight of the accessory reproductive organs, serum testosterone level and sperm count. It also caused increase in sperm motility and reduction in the percentage of sperm abnormalities in wister rats.

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