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Full Length Research Paper

Effects of ethanolic extract of *Garcinia kola* on sexual behaviour and sperm parameters in male Wistar rats

N. Ralebona[1], C. R. Sewani-Rusike[1]* and B. N. Nkeh-Chungag[2]

[1]Department of Physiology, Faculty of Health Sciences, Walter Sisulu University, P. Bag X1, Mthatha 5117, South Africa.
[2]Department of Zoology, Faculty of Science, Engineering and Technology, Walter Sisulu University, P. Bag X1, Mthatha 5117, South Africa.

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Erectile dysfunction is a common problem in men. *Garcinia kola* is claimed to possess aphrodisiac effects and as such is used traditionally in the treatment of erectile dysfunction. A 70% ethanolic extract of *G. kola* seeds was prepared and used for treating male Wistar rats (n=8 /group); two doses of *G. kola* (200 and 400 mg/kg body weight) were used for the treatment group, while distilled water was administered to the control group. All the treatments were orally administered daily for 28 days. On day 28, mounting frequency (MF), intromission frequency (IF) and ejaculation frequency (EF) were quantified during sexual behaviour tests. At termination, body and organ weights, gastric ulceration and cauda epididymal sperm counts were determined. Serum was collected for determination of testosterone levels. Both doses (200 and 400 mg/kg) showed marked aphrodisiac activity with significantly increased sexual behaviour parameters compared to controls. However lower dose of *G. kola* was more effective than the higher dose. Testosterone levels were higher in both treatment groups compared to controls. Sperm counts were similar to controls however testes weights were higher in *G. kola* treated rats compared to controls. Thus these results show that *G. kola* enhances sexual activity in normal male rats.

Key words: *Garcinia kola*, ethanolic extract, sexual behaviour, sperm count, testosterone, aphrodisiac.

INTRODUCTION

Sexual health is an important component of an individual's quality of life and well being (WHO, 2002). Sexual dysfunction can, therefore be a very distressing condition for men. It can erode the male essence (NIH, 1992; Monga, 1999). One form of sexual dysfunction is erectile dysfunction.

Erectile dysfunction (ED) is defined as the difficulty in achieving or maintaining an erection sufficient for sexual activity or penetration at least 50% of the time for the last 6 months (Laumann et al., 1999). Unfortunately, it is a problem often neglected by the health practitioners as they strive to deal with life threatening complications of disease. Successful treatment of ED may improve not only sexual relationships, but also the overall quality of life. The prevalence of ED in South African blacks and mixed race males has been reported to be as high as 77% (De Klerk et al., 2003). The etiological factors which contribute toward ED are multifactorial including aging, diabetes mellitus, neurogenic factors, and iatrogenic factors (Post et al., 2009). Available commercial treatments for ED include, but are not limited to, selective phosphodiesterase inhibitors, for example, sildenafil, tadanafil and vardenafil and other oral medications used to mediate and sustain an erection via enhancing the effect of nitric oxide (Lipschultz and Kim, 1999; Rendell et al, 1999). However, due to the prohibitive cost of these and other treatment methods for ED, patients in developing countries use the traditional approach. Traditional herbs have been used to improve sexual performance or to treat ED. Such plants as *Massularia acuminata* (Yakubu et al., 2008), *Tribulus alatus* (El-Tantawy et al., 2007), *Montanoa tomentosa* (Carro-Juarez et al., 2004); *Ferula hermoni* (Colman-

*Corresponding author: E-mail: crusike@wsu.ac.za. Tel: +2747502 2773. Fax: +27475022758.

Saizarbitoria et al., 2006) and *Myristica fragrans* Houtt (Tajuddin et al., 2003), have been shown to possess aphrodisiac activity. There are claims that the seeds of *G. kola* (Family: Guttiferae, sub-family: Clusoideae), a plant cultivated in West and Central Africa and exported worldwide, possesses aphrodisiac activity. Commonly known as bitter kola, this nut is served as refreshment and also used medicinally for the treatment of abdominal pain, cough, laryngitis, liver disease and erectile problems (Irvine, 1961; Odebunmi et al., 2009). The aim of this study was to investigate the aphrodisiac effect of the seed of *G. kola* in mature male rats.

METHODS

Preparation *G. kola* seed extract

The *G. kola* seeds were bought at a local market in Cameroon and were authenticated at the herbarium of the Department of Botany, University of Cameroon where a voucher specimen already exists. Extract was prepared using the method of Adaramoye (2010) with modifications, using 70% ethanol as solvent. The seeds were peeled, cut into small pieces and then crushed using a household blender. The paste was dried and then extracted twice with 70% ethanol at room temperature with continuous agitation. Ethanol was removed using rotary evaporator (Buchi RE111) under reduced pressure at 60°C. Water was removed by freeze drier (Modulyo Edwards) for 12 h. From approximately 1200 g of *G. kola* seeds, 564 g of solid matter was obtained giving a yield of 47%. The extract powder was stored at -70°C until used for bioassays. The dried down extract was reconstituted in distilled water for animal oral treatments.

Animals and treatment groups

Fourteen week old male and female albino rats of Wistar strain weighing 250 to 300 g and 175 to 200 g, respectively, were used for the study. All animals were housed in polypropylene cages and maintained in a 12:12 h light: dark cycle, at a temperature of 24 to 28°C, with water and solid pellet food (Epol-SA) *ad libitum*. Ethical care and handling of experimental animals was observed at all times and the study was approved by the Walter Sisulu University ethics committee (clearance number 0023/009). The male rats were divided randomly into three groups, each consisting of 8 rats. The groups were: Control group receiving 2 ml of distilled water; Low dose group receiving 200 mg/kg body weight of *G. kola* seed extract; High dose group receiving 400 mg/kg body weight of *G. kola* seed extract. All groups were treated orally daily for 28 days.

Sexual behaviour test

These were carried out as described by Yakubu et al. (2007). To familiarise the animals to the behaviour testing environment, animals were brought to the laboratory and exposed to dim light at the stipulated time of testing daily for 6 days before the experiment. Female rats were only allowed to mate when they were in oestrus. Females were brought into oestrus chemically by giving a single dose of 500 µg/animal oestradiol benzoate (Sigma) 48 h prior to pairing and then injecting progesterone subcutaneously at a dose of 5 mg/kg, 4 h before the experiment. Female receptiveness was confirmed by exposing them to males that were not part of this study, prior to the study. The most receptive females were selected for the study. The sexual behaviour was carried out on day 28 after commencement of the treatment of male rats. Sexual behaviour monitoring was conducted in a darkened room 2 h after the day's treatment dose. In this test, female rats were introduced into the cages of the male animal with 1 female to 1 male ratio. The observation for mating behaviour was started immediately after introduction of the female and parameters were recorded as they occurred for 20 min. The parameters of male sexual behaviour that were monitored were pre-coital behaviour (qualitative observation of chasing, nosing, sniffing and genital grooming); mount frequency (MF = number of mounts without intromission from the time of introduction of the female); intromission frequency (IF= number of intromissions from the time of introduction of the female) and ejaculation frequency (EF= number of ejaculations from the time of introduction of the female). Animals exhibiting low activity (no intromission after 10 min) were excluded from the final result.

Termination of animals

Two days after behaviour testing, rats were weighed and deeply anaesthetized with sodium pentobarbital (65 mg/kg IP). Blood was collected by cardiac puncture into plain tubes and serum collected by centrifugation (3000 ×g for 10 min) and stored at -70°C until biochemical tests.

Organ weights

At termination of the experiment, organs were dissected out, fat cleaned off and the organs weighed. Vital organs weighed were liver, heart, kidney, and spleen to monitor the toxicity of *G. kola*. Reproductive organs weighed were prostate, seminal vesicles, epididymis and testes to assess the effects of *G. kola* extract on reproductive organs. All organ weights were reported as % of body weight.

Gastric ulceration

The stomach was incised along the greater curvature and washed carefully with distilled water. Mucosa of both control and extract treatments were evaluated for ulceration and any visible hemorrhagic changes.

Sperm count

Cauda epididymis was separated and used for sperm count using the method of Robb et al. (1978). Briefly, the organ was placed in a Petri dish with 10 ml of warmed buffered normal saline, pH 7.2. Cauda epididymis was minced using a pair of scissors and the Petri dish placed at 37°C to allow sperm to swim into the warmed normal saline to form a suspension. A tissue-free aliquot was loaded onto the Neubauer haemocytometer (Deep 1/10 mm, Labart, Germany). Five squares were counted in triplicate per sample. Sperm count was reported as millions of sperm/ml.

Testosterone assay

Serum collected at termination was used for assaying for total testosterone. Testosterone was measured using a commercial ELISA kit (IBL) which is based on competitive binding of testosterone on immobilised antibody. Horse radish peroxidase was used for colour development and absorbance at 420 nm measured on a plate reader (Multiskan EX). Values are reported as ng/ml of serum.

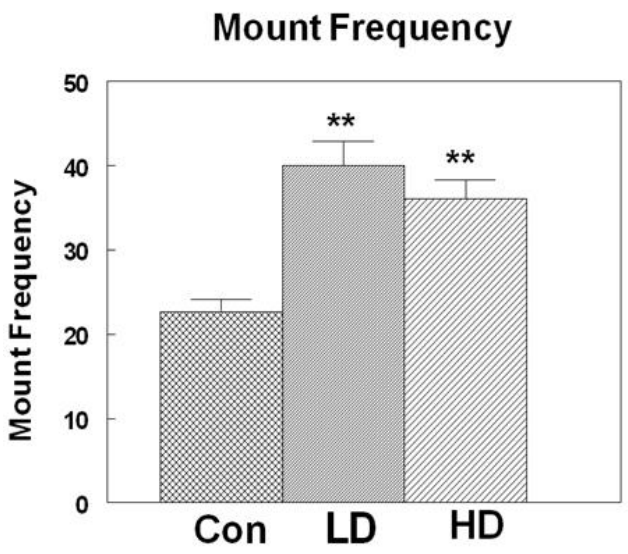

Figure 1. Effect of 28 day treatment with 200 mg/kg (LD) or 400 mg/kg (HD) *G. kola* on mount frequency during a 20 min sexual behaviour observation. Con = Controls. Values are mean ± SEM. **, p<0.01 compared to controls. n=8 for all treatment groups.

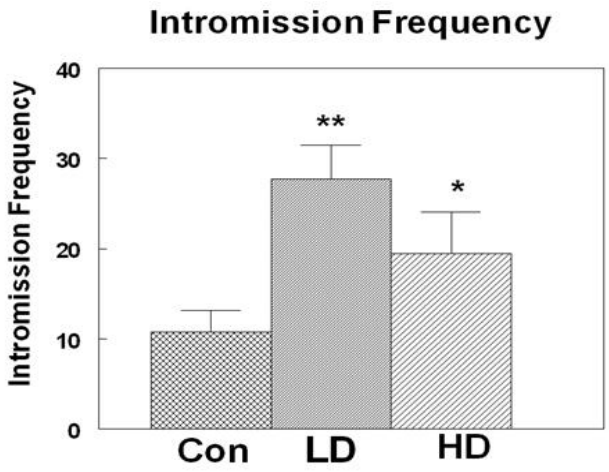

Figure 2. Effect of 28 day treatment with 200 mg/kg (LD) or 400 mg/kg (HD) *G. kola* on intromission frequency during a 20 min sexual behaviour observation. Con = Controls. Values are mean ± SEM. ** P<0.01; *P<0.05 compared to controls. n=8 for all treatment groups..

Acute toxicity studies

Acute toxicity was determined in healthy adult albino mice of either sex as previously described (Asare et al., 2011) Two groups of 5 mice/group received single oral dose of *G. kola* extract at 1200 and 2400 mg/kg body weight. The animals were observed continuously for 1 h, then hourly for the next 4 h, intermittently over the next 48 h and at least once a day for two weeks. Physical manifestations of toxicity such as writhing, gasping, salivation, hyperactivity, drowsiness and death were looked for during animal observations.

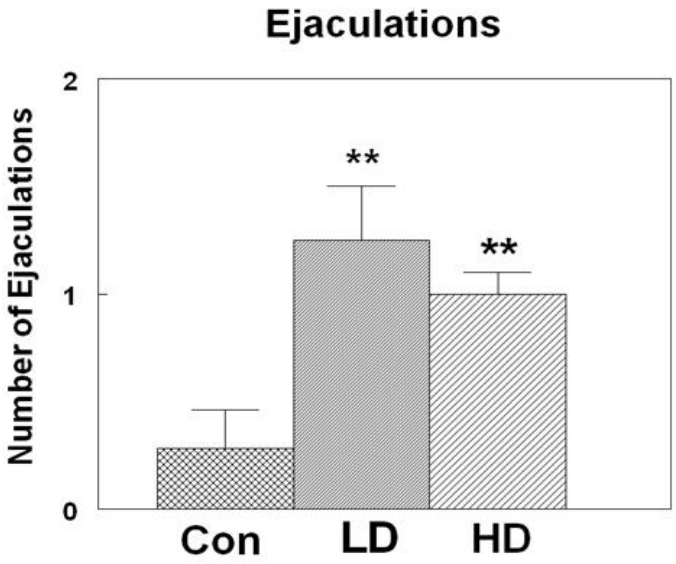

Figure 3. Effect of 28 day treatment with 200 mg/kg (LD) or 400 mg/kg (HD) *G. kola* on number of ejaculations during a 20 min sexual behaviour observation. Con = control. Values are mean ± SEM. **, p<0.01 compared to controls. n=8 for all treatment groups.

Statistical analysis

Results were expressed as mean ± standard error of mean (SEM). Statistical analysis was done using GraphPad InStat Version 3 and data was compared using the non paired ANOVA. Values were considered significantly different when p<0.05.

RESULTS

Effect of *G. kola* treatment on sexual behaviour

Qualitative observation of pre-coital sexual behaviour (chasing, nosing, sniffing and genital grooming) was increased in *G. kola* treated rats at both doses compared to the control group. *G. kola* treated animals were more aggressive and persistent in chasing compared to the untreated rats. *G. kola* treatment at both low and high doses produced a highly significant (p<0.01) increase in the number of mounts compared to controls (Figure 1). There was a highly significant (p<0.01) and a significant increase (p<0.05) in number of intromissions in the rats treated with low dose and high dose *G. kola* respectively (Figure 2). The low dose (200 mg/kg) was therefore more effective in eliciting intromissions than the high dose (400 mg/kg) of *G. kola*. There was a highly significant increase (p<0.01) in the number of ejaculations for both low and high dose *G. kola* treated rats compared to controls (Figure 3). Overall, both doses elicited an increase in sexual behaviour compared to controls.

Body, organ weights and sperm counts

At termination, body weights were equal between

Table 1. The effect of treatment with *G. kola* ethanolic extract on vital organs weights in grams.

Treatment group	Body weights (g) and vital organs weights (% of body weight)				
	Body	Heart	Liver	Kidney	Spleen
Control	340±7.5	0.29±.01	2.818±0.11	0.672±0.01	0.192±0.01
200 mg/kg *G. kola*	366±18	0.28±.0.04	2.958±0.14	0.648±0.22	0.193±0.01
400 mg/kg *G. kola*	347±22	0.296±0.01	2.848±0.08	0.643±0.02	0.198±0.01

All values are presented as Mean ±SEM.

Table 2. The effect of treatment with *G. kola* ethanolic extract on reproductive organ weights in grams.

Treatment group	Reproductive organ weights (% of body weight) and sperm count (x10^6)				
	Testes	Prostate	Epididymis	Seminal vesicles	Sperm count
Control	0.979±0.02	0.165±0.007	0.313±0.021	0.785±0.122	1.98±0.4
200 mg/kg *G.kola*	1.346±0.029*	0.162±0.011	0.341±0.01	0.802±0.106	2.21±0.13
400 mg/kg *G.kola*	1.385±0.023*	0.173±0.184	0.358±0.013	0.798±0.125	2.32±0.32

All values are presented as Mean ± SEM. *, p<0.05.

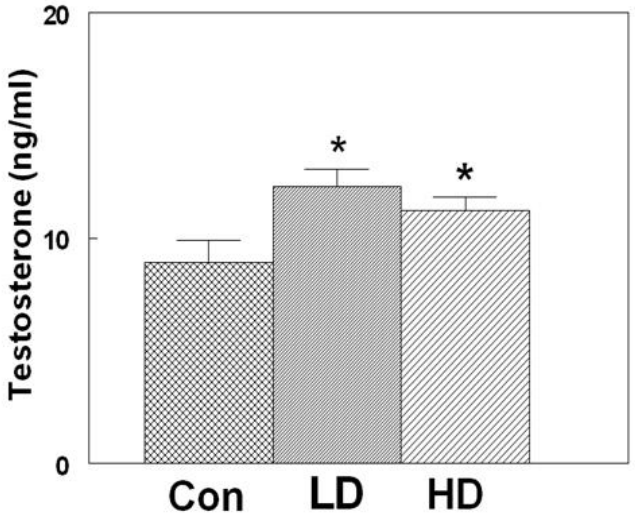

Figure 4. Effect of 28 day treatment with 200 mg/kg (LD) or 400 mg/kg (HD) *G. kola* on serum testosterone levels. Con = controls. Values are mean ± SEM. * P<0.05 compared to controls. n=8 for all treatment groups.

treatment groups and the control group. The vital organ weights (heart, spleen, liver and kidneys; Table 1) and reproductive organ weights (prostate, seminal vesicle and epididymis) showed no significant difference (p>0.05) compared to the control group (Table 2). However, testes weights for both low and high dose treatment groups showed a significantly increased weight (p<0.05) compared to controls after 28 days of treatment. Despite the increase in testicular weights, there was no observed increase in sperm count in *G. kola* treated rats, at both doses, compared to control (Table 2).

Testosterone

There was a significant increase (p<0.05) in the total testosterone levels in both *G. kola* treated groups compared to controls (Figure 4).

Acute toxicity and ulcerogenicity studies

Toxicity studies carried out using oral doses of 1200 and 2400 mg/kg *G. kola* extract did not cause any obvious signs of toxicity as assessed by behavioural changes in the experimental mice. Mice were observed to be active and healthy after 2 weeks of observation. There were no haemorrhages nor ulcers observed in the gastric mucosa of both low and high dose treated rats after a daily treatment period of 28 days.

DISCUSSION

The present study sought to investigate the effects of oral administration of ethanolic extract of *G. kola* on male sexual behaviour, testosterone levels and sperm parameters. The present study confirmed that *G. kola* possesses sexual enhancing effects on male rats as evidenced by the increased mounting (MF) and intromission (IF) frequencies with increased number of subsequent ejaculations over the 20 min observation period. These parameters are considered to be a measure of both libido and potency (Bitran and Hull, 1987; Meisel and Sachs, 1994) and indicative of

improved sexual arousal and performance (Abdulwaheb et al., 2007). Thus our results show that *G. kola* treatment increases both libido and potency in normal rats.

Drugs that enhance sexual function may act via an increase in circulating testosterone levels, the male sex hormone responsible, among other functions, in enhancing sexual function via central and peripheral effects (Mills et al., 1996). Indeed testosterone supplementation has been shown to improve libido and intensifying orgasm and ejaculation (Fabbri, 2001). In this study, *G. kola* treatment resulted in increased testosterone levels, which may account for the enhanced sexual activity. The mechanisms for increased total testosterone levels may be via central influences to increase gonadotropins or locally via increase in the number of Leydig cells or their sensitivity to luteinising hormone (LH). Further studies to elucidate mechanisms are required.

There was a significant increase in testicular weights with no change in the weights of accessory reproductive organs and epididymal sperm count. Increase in spermatogenesis is usually accompanied by increased testicular weight since the bulk of testicular weight is made up of seminiferous tubules that house spermatids and spermatozoa (Oyewopo et al., 2011; Kenjale et al., 2008; Chauhan et al., 2007). Despite the increased testosterone levels there was maintenance of accessory organ weights. Normal accessory organ structure and function is maintained by circulating androgens (Mooradian et al., 1987). The actual duration of increase in the levels of testosterone was not determined in our study; therefore, the accessory organs may have not experienced the increased testosterone long enough for weight change. Threshold levels of required testosterone might be different for the different accessory organ functions (Chauhan et al., 2007). Furthermore, despite the increased testicular weight, *G. kola* had no effect on epididymal sperm count after 28 days of treatment. One complete spermatogenic cycle takes 58 days in the rat (Franca et al., 1998). Since our study was less than 58 days, spermatogonia exposed to *G. kola* treatment had not been deposited into the epididymis for observation. A longer study is therefore necessary to make meaningful observations on the effects of treatment on epididymal sperm counts.

The body and vital organ weights were not altered after treatment with *G. kola* extract. Monitoring of organ weights gives information on general wellbeing of the animal (Tsai et al., 2003). These results show that *G. kola* treatment over 28 days did not affect organ weights, since they remained similar to those of the controls. Furthermore, acute toxicity and ulcerogenicity studies confirm nontoxic effects of *G. kola*.

In conclusion, *G. kola* treatment at two doses showed enhanced libido and potency in male rats after treatment for 28 days. Furthermore, *G. kola* treatment enhances testosterone secretion and increases testicular weights.

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