

Dose dependent effect of *Gacinia kola* ethanolic extract on sperm profile in fasting induced male Wistar rats

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Received 23rd September 2025; Accepted 29th October 2025

ABSTRACT: This research determines the effects of starvation-induced stress and *G. kola* ethanolic extracts on sperm indices using male rats. Thirty (30) male rats that weigh 110-130g were purchased from the faculty of BMS and acclimatised for 2 weeks. They were separated into GP1 (control), GP2 15 hours normal diet and water with 9 hours fasting, GP3 6 hours normal diet and water with 18 hours fasting and GP4, 5 and 6 were treated at 25, 50 and 100 mg/kg *G. kola* ethanolic extract daily for 4 weeks. Sperm count was calculated using the hemocytometer method. The findings from this study show a significant decrease in sperm count among the group treated with *G. kola* ethanolic extract at different doses compared with the control. There was an increase in progressive sperm motility at 50 and 100 mg doses compared with the control. Moreover, a significant increase in non-progressive sperm motility was observed among treated groups with *G. kola* ethanolic extract compared with the control. However, there was a non-significant change in immotile sperm count at different concentrations with *G. kola* extract. Regarding fasting hours on sperm count, there were no significant changes compared with the control group. In conclusion, the study reveals that ethanolic extracts of *G. kola* possibly contain certain phytochemical constituents that induced deleterious effects in male sperm profile on prolonged consumption.

Keywords: Extract, *G. kola*, hormones, rats, sperm.

INTRODUCTION

Worldwide, between 10 and 20 per cent of couples are unable to conceive (Eisenberg *et al.*, 2023). When sexual activity without the use of a contraceptive does not result in pregnancy for more than a year, infertility can be diagnosed (Practice Committee of the American Society for Reproductive Medicine, 2015; May *et al.*, 2025). The male partner is responsible for the infertility of the couple in about half of the cases. Interventional options primarily target women, despite the fact that this fact is widely recognised (Akpantah *et al.*, 2011; Winters and Walsh, 2014; Kimmins *et al.*, 2023). According to WHO guidelines, male subfertility can have a variety of causes and show up

as compromised sperm quality, which is assessed using particular criteria like ejaculation volume, sperm motility, and total sperm count (WHO, 2010). Even in fertile men, sperm quality is highly variable and greatly influenced by lifestyle (Gollenberg *et al.*, 2010; Luk and Loke, 2015; Köhn *et al.*, 2021; Chen *et al.*, 2023).

Adesuyi *et al.* (2012) delved into an investigation titled “Nutritional and Phytochemical Screening of *Garcinia kola*” (Figure 1). Mineral content revealed a high calcium level (2200 ppm), Sodium (852 ppm) and Potassium (968). *G. kola* contains vital minerals despite its anti-nutrient contents. Findings show a high level of Saponin (2.5%),

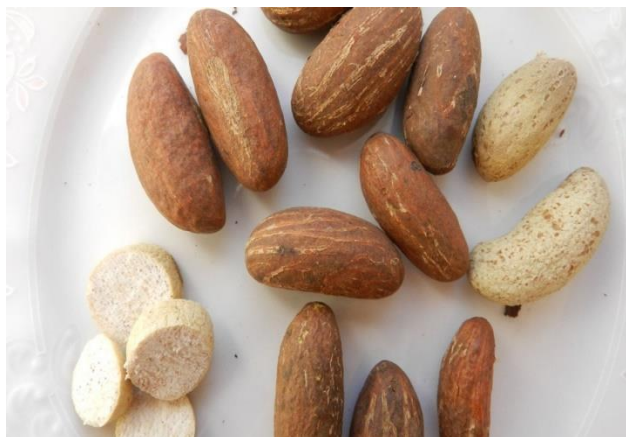


Figure 1. *Garcinia kola* seeds.

Cardiac Glycosides (3.421%) and Flavonoids (2.041%). With the considerable level of Cardiac glycosides, *G. kola* can be used as an active and anti-inflammatory component of drugs derived from plants. *G. kola* is a remarkable plant that belongs to the Clusiaceae family (Einser, 1990; Ikeuba *et al.*, 2013).

The maturation of germ cells via spermatogenesis is presumably an aspect of reproduction in males that is often evaluated in complicated experiments. A fascinating process of cellular differentiation called spermatogenesis, leading to the output of millions of spermatozoa per day, involves the organised expression of specified genes and specific production of gene product at each process step, coupled with the progressive communication linking developing gene cells (Jégou, 1993; Huang *et al.*, 2005; Essader *et al.*, 2005; Rolland *et al.*, 2007). More clinical and experimental research is being done on medical fasting and prolonged fasting are becoming more and more well-liked as non-pharmacological preventative and therapeutic approaches (Longo and Mattson, 2014; Li *et al.*, 2017; Cheng *et al.*, 2017; Brandhorst and Longo, 2019). Sperm quality is negatively impacted by a metabolic state that is pre-diabetic (Ferreira *et al.*, 2015), and it has been discovered that fasting may improve insulin sensitivity (Stange *et al.*, 2013). Spermatogenesis is known to be directly dependent on testosterone levels, and a small sample among obese and non-obese men has previously reported possible benefits of two days of food starvation on the pituitary-testicular axis (Michalsen *et al.*, 2019).

Important roles are played by nutrition, genetics, and the environment in the physiology of reproduction. Reproductive dysfunctions have been linked to diets high in fats and sugars (Plotan *et al.*, 2013). Individual composition is greatly influenced by the circadian timing system, which is typified by cycles of body temperature, sleep-wake patterns, and feeding habits (Dibner *et al.*, 2010; Pendergast *et al.*, 2016).

However, due to limited information from literature on the

effects of ketogenic diets and *G. kola* seed on starvation and stress, this study is therefore aimed at investigating the effects of starvation-induced stress and *G. kola* seed extracts on semen analysis using male Wistar rats as research models. Although it has been reported by Mingoti *et al.* (2003) that stress decreased sperm production, but does not interfere with the general fertility using male rats. Hence, knowledge regarding starvation-induced stress on reproduction may still be essential. The effects when starvation is induced are yet to be known, as a certain diet itself is a type of stress on the body system.

METHODOLOGY

Experimental design

The experimental design consisted of six groups of animals subjected to different feeding and treatment conditions. Group 1 (Control) received a normal diet (ND) and water *ad libitum* for 24 hours daily with no starvation. Group 2 was provided with a normal diet and water for 15 hours daily, followed by 9 hours of starvation. Group 3 received a normal diet and water for 6 hours daily and was subjected to 18 hours of starvation. Group 4 was administered 25 mg/kg of *Garcinia kola* extract, while Group 5 received 50 mg/kg of the extract. Group 6 was treated with 100 mg/kg of *Garcinia kola* extract.

Sample collection

Thirty male w-rats were purchased from the departmental animal house, while the *G. kolas* were purchased from the Benin boundary market for this study.

Sperm analysis

Sperm analysis was conducted in accordance with the guidelines of the World Health Organisation (WHO, 1999) and MB-50 (1983). The evaluation focused on key sperm parameters, including sperm count and sperm motility. The assessment of motility was further categorised into three distinct classes: progressive motility, representing sperm cells with active forward movement; non-progressive motility, indicating sperm cells that move but fail to advance effectively; and immotile sperm cells, which exhibit no movement. These parameters were analysed to determine the overall quality and functionality of the sperm samples.

Statistical analysis

Data obtained from the experiment were statistically analysed using the Statistical Package for the Social Sciences (SPSS) software, version 24.0. All results were expressed as the Mean $\pm$ Standard Error of the Mean

Table 1. Comparative analysis of sperm mean values in Wistar rats fed with normal diet.

Parameters	Control	9 Hours Starvation	18 Hours Starvation	p-value
Sperm count (cells/mm ³)	556.3 ± 19.37	521.8 ± 3.35	550.5 ± 13.05	0.32
Progressive motility	62.75 ± 0.85	66.50 ± 1.32	65.50 ± 0.64	0.04
Non-Progressive motility	12.00 ± 1.08	11.50 ± 0.86	12.25 ± 1.03	0.07
Immotile sperm	25.50 ± 0.86	21.50 ± 0.95	22.25 ± 1.31	0.03

p < 0.05 indicates significant difference.

(SEM) to indicate the degree of variability and reliability of the data. Differences between groups were evaluated, and statistical significance was determined at a probability level of $p < 0.05$, implying that any observed variation with a p -value below this threshold was considered statistically significant.

RESULTS

The comparative analysis of sperm parameters in Wistar rats subjected to varying feeding and treatment conditions revealed notable differences among the experimental groups. Table 1 presents the sperm mean values for rats maintained on a normal diet and exposed to different durations of starvation. The sperm count showed no significant variation among the control (556.3 ± 19.37 cells/mm³), 9-hour starvation (521.8 ± 3.35 cells/mm³), and 18-hour starvation groups (550.5 ± 13.05 cells/mm³) ($p = 0.32$). Progressive motility, however, exhibited a significant increase in the 9-hour starvation group ($66.50 \pm 1.32\%$) compared with the control ($62.75 \pm 0.85\%$) ($p = 0.04$), while non-progressive motility did not differ significantly across groups ($p = 0.07$). Conversely, immotile sperm cells were significantly reduced in the 9-hour starvation group ($21.50 \pm 0.95\%$) relative to the control ($25.50 \pm 0.86\%$) ($p = 0.03$). These results indicate that moderate starvation improved sperm motility without adversely affecting sperm count.

The administration of *Garcinia kola* ethanolic extract demonstrated a concentration-dependent effect on sperm characteristics (Figures 2 to 5). Sperm count significantly decreased at all tested concentrations (25, 50, and 100 mg/kg) compared with the control group ($p < 0.05$). Progressive motility was also significantly reduced at 50 mg/kg and 100 mg/kg doses, whereas no significant difference was observed at 25 mg/kg. In contrast, non-progressive motility significantly increased at all extract concentrations compared with control; however, a marked reduction occurred at 100 mg/kg when compared with 50 mg/kg ($p < 0.05$). Immotile sperm cells showed no significant variation across the different concentrations of *G. kola* extract.

Furthermore, evaluation of the effect of starvation on sperm count among rats fed a normal diet (Figure 6) showed no significant difference between the 9-hour and 18-hour starvation groups relative to the control.

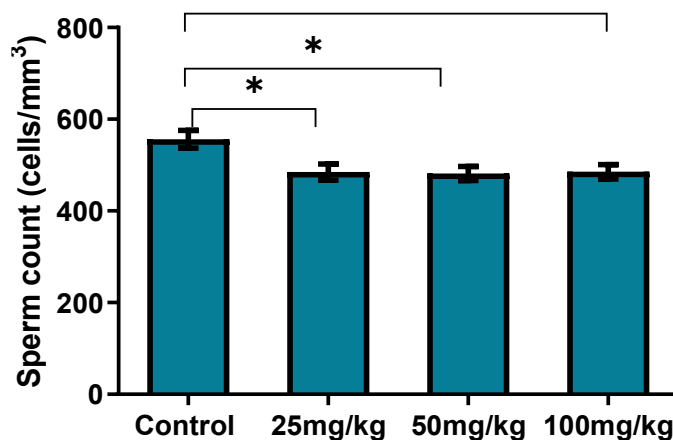


Figure 2. *G. kola* ethanolic extracts Effect on Sperm Count at different concentration ($p < 0.05$ is significant).

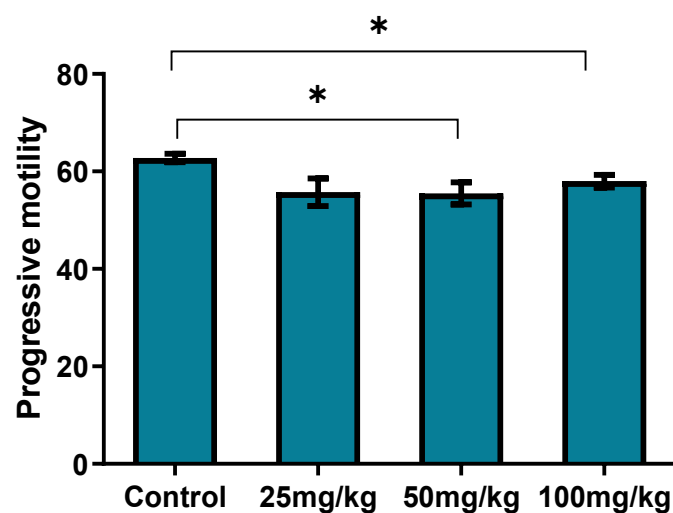


Figure 3. Sperm cell progressive motility of rats treated with *G. kola* extracts at different concentration ($p < 0.05$ indicates significant difference).

Collectively, these findings suggest that while moderate starvation may enhance sperm motility, administration of *Garcinia kola* ethanolic extract exerts a suppressive effect on sperm quality parameters in a dose-dependent manner.

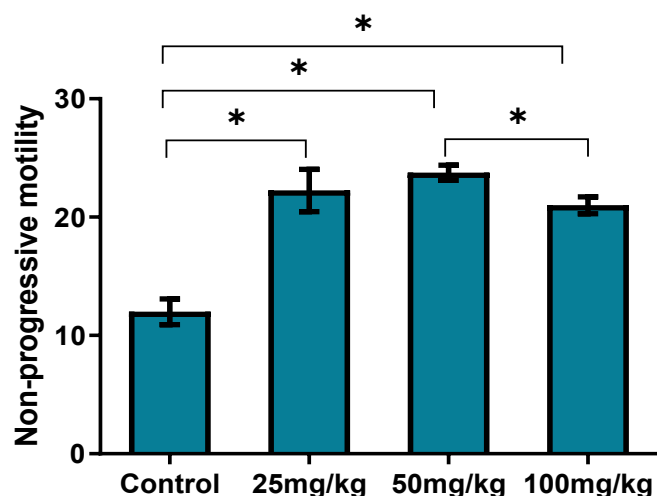


Figure 4. Non-progressive motility of Sperm cell group treated with *G. kola* extract at different concentration ($p < 0.05$ indicates significant difference).

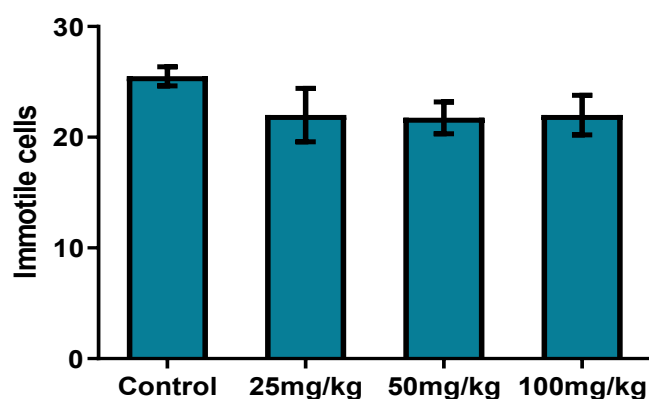


Figure 5. Immotile sperm cell of rats treated with *G. kola* ethanolic extract at different concentration (no significant different).

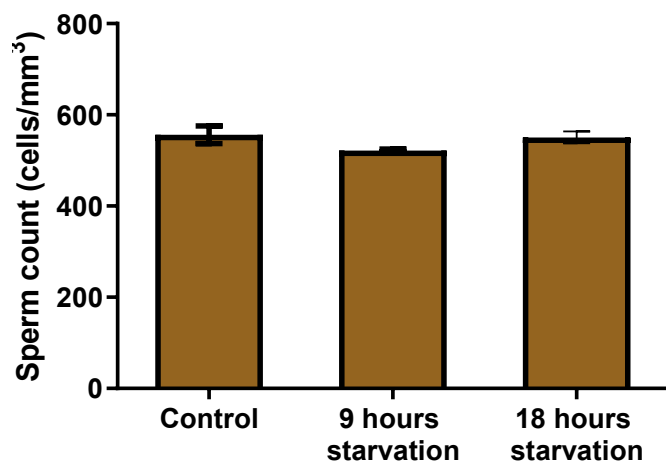


Figure 6. Effect of starvation hours on sperm count on normal diets (no significant different).

DISCUSSION

There was an absence of significant changes in both 9- and 18-hour fasting in sperm count and non-progressive motility of sperm among the group fed with a normal diet, which implies that there was no significant effect in both moderate and intense fasting on sperm count and non-progressive motility. A significant increase in the 18-hour fasting of progressive sperm motility in the group fed with a normal diet was observed. This could be attributed to improved spermatogenic activities in the testis and epididymis (Adienbo *et al.*, 2015). Hence, the significant decreases in both the 9- and 18-hour starvation of immotile sperm cells also portray the same improved spermatogenic activity.

Furthermore, there was a significant decrease observed in sperm count among the group treated with ethanolic extracts of *G. kola* at 25, 50 and 100 mg/kg compared with the Control. It could be due to a reduction in plasma testosterone levels associated with flavonoids and alkaloid extracts of *G. kola* (Christensen, 1975; Oyediji *et al.*, 2020). Also, the saponin components of the *G. kola* extract show the ability to permeate the blood-testis barrier, which could be a reason for the resultant changes in the seminiferous tubules microenvironment (Bloom and Fawcett, 1975; Baldessarini, 1980; Oyediji *et al.*, 2020). However, the significant decrease shown in the group treated at 100 mg/kg when compared with those treated at 50 mg/kg could be that beyond the 50 mg/kg of the treated extracts, a decline in non-progressive motility began to be observed (William, 2000; Bowman and Rand, 1985).

The absence of significant change observed for immotile sperm cells in groups treated with extracts of *G. kola* at different mg/kg when compared with the control gives the indication that no significant effect was displayed when the treated groups were compared to the control. This observation is congruent with Udoh and Patil (1992) study of anti-spermatogenic.

Conclusion

Prolonged administration of *G. kola* extract induces notable decreases in sperm profile and progressive motility, with converse increases in the non-progressive motility. On the other hand, significant increases were found in progressive motility but decreases in immotile sperm cells for prolonged starvation with normal diets. Males who venture into complicated fasting and are interested in giving birth should apply considerable caution since it may leave either positive or negative effects on the system, depending on its duration and severity.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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