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Hypothalamo-Pituitary-Gonadal Changes in Sleep Deprivation Induced with Kolaviron in Male Wistar Rats

O. O. Victor[1], M. O. Odigie[1*], A. A. Aigbiremolen[2], K. O. Gbagbeke[1], D. O. Ogbeide[1] and J. C. Igweh[1]

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State, Nigeria.
[2]Department of Pharmacology and Therapeutics, Faculty of Clinical Sciences, College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author OOV carried out the bench work, author MOO wrote and monitored the first draft of the manuscript, author AAA managed the literature searches, authors KOG and DOO managed the statistical analysis, author JCI designed and supervised the study.

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ABSTRACT

The Hypothalamo-Hypophyseal-Pituitary-Gonadal Axis (HPGA) is a neuroendocrine pathway that majors in influencing the biosynthesis and regulation of sexual hormones (Testosterone for males, Oestrogen and Progesterone for females). These hormones are Jermaine in the developments of primary and secondary sexual characteristics in humans. Several studies posit that sleep deprivation interferes with the HPGA to impede its roles in the regulation of these hormones. Though adequate sleep has been reported to maintain optimal functional levels in various systems, the alternative to adequate sleep has remained elusive. To this point, this study sought to investigate the effects of 'kolaviron extract' on the HPGA. Thirty (30) male Wistar rats were

**Corresponding author: E-mail: osgiedeprof@yahoo.com;*

randomly assigned into five (5) groups of six (6) rats each [A=Control, B=Sleep Deprived (SD), C=Kolaviron Extract (KE), D=KE+SD, and E=KE+SD]. While groups D and E respectively received 100 mg/kg and 200mg/kg of extract in a sleep-deprived state, group C was given 200 mg/kg of extract without sleep deprivation. Groups A and B were each administered 1ml of the vehicle (1% tween 80 solution) in normal-sleep and sleep-deprived states respectively. Following a two-week period of administration of test substance for 14 days, animals were euthanized, weights measured (weekly while administration lasted) and blood samples collected for immunoassay of hormones and semen analysis (from harvested testes). At $p < .05$, Analysis of variance test (ANOVA) found Kolaviron extract to have significantly normalized the changes observed in the hormone levels by sleep deprivation. It is recommended that further studies be made to check the possible impact(s) of *kolaviron* on other parts of the brain, with electron microscopy to evaluate any ultra-structural changes in the anterior pituitary and hypothalamus.

Keywords: Kolaviron; hypothalamus; pituitary gland; sleep.

1. INTRODUCTION

Sleep deprivation constitutes a biological stress implicated in many homeostatic alterations including weight loss, reduction in thymic weight, increased adrenal weight, elevated corticosterone and ACTH levels [1-3]. Several studies suggest that sleep deprivation interferes with the release of hormones via the hypothalamic-pituitary axis and the autonomous nervous system[2]. Sustained sleep deprivation leads to reduction in circulating anabolic hormones such as: growth hormone, prolactin, thyroid hormone, leptin and testosterone[2]. Although sleep deprivation is known to reduce serum testosterone, the exact mechanism is poorly understood.

The multiple effects of sleep deprivation and other medical challenges have made medical plants an alternative. Medical plants have ushered in some hope in health care delivery notwithstanding the advances in modern medicine [4-5]. Kolaviron, a major constituent of *Garcina kola,* is one of the numerous plant products and nutritional supplements with a wide range of medicinal value [6-8]. Its anti-oxidant action on lipoprotein has been reported [9]. Kolaviron may have an influence on male reproductive dysfunction as it is associated with oxidative stress damage [6]. This suggests that it could have effect on the hormones of the hypothalamic-pituitary-gonadal axis.

The acclaimed health effects of *G. kola* seed against liver and reproductive disorders in traditional medicine and its proven ability to suppress oxidative stress in different experimental models of organ toxicity increases curiosity into its effects in sleep deprived induced stress on the hypothalamic-pituitary-gonadal axis (HPGA). These affirm the fact that it could have an effect on the hormones of the HPGA, Considering its anti-oxidant capacity. Thus, it becomes necessary to determine its effects on the HPGA, particularly the pituitary and testicular organs.

2. AIM OF STUDY

This study aimed at investigating the effects of 'kolaviron extract' on the hypothalamo-pituitary-gonadal axis in sleep-deprived male Wistar rats. Specifically, study attempted to;

i. Determine the effects of Kolaviron on serum levels of Follicle Stimulating Hormones (FSH), Luteinizing Hormones (LH), and Testosterone in sleep-deprived wistar rat
ii. Examine the effects of Kolaviron on Semen Quality in sleep-deprived Wistar rat.

3. MATERIALS AND METHODS

3.1 Scope of Study

This study was limited to the effects of kolaviron on the hypothalamic-pituitary-gonadal axis in sleep-deprived male wister rats. Due to the invasive nature of the study, rat models (specifically wistar rats) were preferred. This was necessitated by the need to harvest an internal organ (testis), as well as proper standardization of experimental protocols.

3.2 Study Design

Thirty (30) male wistar rats (weighing between 190g – 240g) were obtained from the Central Animal House, College of Health Sciences, Delta State University. They were then housed in cages, provided with pelletized feed and water *ad libitum,* and acclimatized for two weeks before investigation. Animals were then randomly assigned into five (5) groups of six (6) rats each as follows;

Group A: Control group: Received 1ml of the vehicle (1% tween 80 solution)
Group B: Sleep-deprived (SD) group: Received 1ml of the vehicle (1% tween 80 solution)
Group C: Kolaviron group which received Kolaviron at 200mg/kg
Group D: Kolaviron and Sleep deprived (KV + SD) group which received Kolaviron at 100mg/kg
Group E: Kolaviron and Sleep deprived (KV + SD) group which received Kolaviron at 200mg/kg

The treatment materials were administered twice daily for a two-week period by oral gavage. The weights of animals were measured weekly while administration lasted for 14 days.

3.3 Resources and Sources

3.3.1 Plant materials

Seeds of *Garcinia kola* were purchased from the main market in Abraka, Ethiope East of Delta State, Nigeria. They were then authenticated in the Herbarium of the Department of Botany, Delta State University, Abraka campus. The seeds were peeled to remove the shell covering the pulp which was then chopped into small pieces and air-dried. Thereafter, the dried pulp was blended using a Marlex blender and the powdered samples stored in and placed at room temperature until it was used.

3.3.2 Ethical clearance

Ethical clearance was obtained from the Research and Ethics Committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State. All animals were treated in line with guidelines, stipulated by the National Institute for Health Guide on the Care and Use of Laboratory Animals (1985).

3.4 Procedure

3.4.1 Isolation of kolaviron

Extraction of kolaviron was achieved by the procedure previously described by Iwu (1985) and modified by Braide in 1990[10-12]. Also, 8.2kg of powdered samples (blended Garcinia kola) was weighed into a glass container and 5 litres of solvent (pure n-hexane) was added stirred at intervals of 2 hours and was left to stand for 72 hours. The process was repeated by adding another 2 litres of pure n-hexane to the plant shaft for another 72hours. This was done to properly remove the fat present in the *Garcinia kola*. The solvent (n-hexane) containing the crude fat was collected.

The solvent (n-hexane) containing the crude fat collected after 72 hours added together was concentrated using a rotary evaporator after being filtered, it was set at 40˚C and was further concentrated in a vacuum oven at a temperature of 40˚C and pressure of 600mm Hg. The *Garcinia kola* shaft (that is, defatted seeds) was spread and air-dried for 5 hours so as to remove the traces of n-hexane used. The defatted, dried marc was then repacked into a glass container and 5liters of solvent (methanol) was added stirred at intervals of 2 hours and was left to stand for 72 hours. The process was repeated by adding another 5 litres of pure methanol to the plant shaft for another 72hours. The solvent (pure methanol) containing the crude methanol extract was collected after 72 hours was concentrated using a rotary evaporator after being filtered it was set at 40˚c and was further concentrated in a vacuum oven at temperature of 40˚c and pressure of 600 mm Hg. The crude methanol extract was made into solution with methanol and equal volume of water was added. It was done in batches 200 ml of this mixture (methanol/water) was added 200 ml of chloroform and transferred into a separating funnel of 500ml and was carefully shaken and allowed to stay for 30 minutes for proper partitioning of the chloroform and mixture (methanol/water) layer. This process was repeated 4 times for proper extraction of kolaviron with the aid of chloroform. The chloroform fraction was collected and concentrated using a rotary evaporator it was set at 40˚c. The crude chloroform fraction was further concentrated in a vacuum oven at 40˚c in the pressure of 600mmHg as to properly remove any trace of solvent (chloroform). Percentage yield was calculated as follows

$$\% \text{ yield of kolaviron} = \frac{\text{weight of extract}}{\text{weight of plant sample used}} \times 100$$

3.4.2 Sleep deprivation induction

The Sleep deprivation chamber is a glass chamber (60cm x 60cm x 30cm) containing 16 multiple circulars galvanized iron platforms of about 0.6cm in diameter and 25cm in height, with water filled up to 1cm below the upper surface of the multiple circular form. The platforms are enclosed with wire mesh to enable the rat climb out of the water when it falls into it. The control chamber was designed in a similar manner but with a modified multiple galvanized iron platforms to prevent the animals from falling into water. Both the Control and sleep deprived rats were placed in the chamber to acclimate for about 4 hours (10.00 – 14.00h) of the last 3 days of acclimatization. At the onset of each paradoxical sleep episode, the sleep-deprived rats' fell into the water due to loss of muscle tonus and is thus awakened. All rats were placed back in their home cages for 4hours (sleep opportunity beginning at 10.00h). This particular time interval (10.00 – 14.00h) was chosen because paradoxical sleep is at its greatest episode here [13].The water in the glass chamber was changed daily and animals were allowed free access to feed and water throughout the 14days of paradoxical sleep deprivation period by placing pellets and water bottles on a grid located on top of the chamber.

3.4.3 Blood sample collection

At the end of the fourteenth (14) day, animals were euthanized by cervical decapitation with blood samples collected from the superior vena cava. The samples were centrifuged at 3000rpm for 15minutes with sera obtained and stored frozen. The animals were dissected with the testes removed, cleared of adherent tissues and weighed immediately using the electronic weighing balances. For each euthanized animal, testis was homogenized in 100mM Phosphate buffer (pH 7.4) and centrifuged at 3000rpm for 15minutes.

3.4.4 Immunoassay of hormones

Serum samples from experimental animals were assayed for serum concentration of testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH) corticosterone; and testicular interleukin 1beta level using Enzyme-Linked Immunosorbent Assay (ELISA). The hormonal assay was done at the Capitol Hill Hospital and Laboratory, Airport Road, Warri Delta State. Nigeria. ELISA kit was used for the immunoassay. Basically, the procedure for running the assays was the same.

3.4.5 Sperm analysis

The tests were carefully exposed and one was removed together with its epididymis. The caudal epididymis was dissected immediately. The epididymal fluid was collected from the caudal part and the sperm motility, morphology and sperm count was determined.

3.4.6 Percentage of sperm with progressive motility

The sperm motility evaluation was done immediately after epididymal fluid collection. It is expressed as the movement of spermatozoa which is a characteristic of good sperm. This method was determined in accordance with the conventional method of Zemjanis (1970). After, the epididymal fluid has been dropped on the slide, two drops of warm 2.9% sodium citrate were added to it. This was then covered with a coverslip and examined under the microscope using X40 objective lens with reduced light [14].

3.4.7 Percentage of sperm with normal morphology

This was carried out using Eosin/Nigrosin stain. The specimen used for the epididymal sperm motility was retrieved and the coverslip was quickly removed. To this, two drops of Eosin/Nigrosin stain were added and a smear was made and air dried. The observation was then made under the microscope using X40 objective lens.

3.4.8 Sperm counts

The caudal part of the epididymis removed was homogenized in 5ml normal saline or sodium citrate and the change in volume was measured. A further dilution of 1/200 was made and the sperm count was determined using the new improved Neubaur's counting chamber in the hemocytometer. The count was made in five different regions within the throma ruling. The sperms counted were expressed in million/ml suspension.

3.4.9 Acute toxicity test

Available acute toxicity studies suggest that Oral medium lethal dose (LD_{50}) of *Garcina Kola* extract is greater than 3,000 mg/kg body weight. More so, according to the American Society for Testing and Materials (1987), any chemical substance with LD_{50} estimate greater than 3,000-5,000 mg/kg (Oral route) could be considered of low toxicity and safe. Based on these, two concentrations (100 mg/kg and 200 mg/kg) of aqueous extract from *Garcina Kola* were used for the study.

3.5 Analytical Approach

Obtained data were expressed as mean ± Standard Error of Mean (SEM) and Statistical analysis was done using one-way analysis of variance (ANOVA), followed by least significant difference (LSD) test, Statistical software SPSS 20 was used to conduct the procedure. A p-level of less than 0.05 ($p \leq 0.05$) was considered as statistically significant

4. RESULTS

This study examined the changes in the hypothalamic-pituitary-gonadal axis in sleep-deprived male wistar rats, following administration of Kolaviron extract. To this point, results show changes in sperm count, percentage of sperm with progressive motility, percentage of sperm with normal morphology, testicular weight, testicular volume and serum testosterone levels.

It was observed from Fig. 1 that stress induced in rats via sleep deprivation decreased the body weight of the rats. This decrease in body weight was reversed in a dose-dependent manner following administration of graded dose of Kolaviron. These changes in body weight change were not statistically significant.

Fig. 2 shows that sleep-deprivation significantly ($p < 0.05$) decreased the sperm count. The sperm count was increased by the Kolaviron extract with no statistical significance. It was further observed that Kolaviron attenuated the sperm count level of the sleep-deprived rats in a dose-dependent manner, though significance ($p<0.05$) was recorded when compared to control and Kolaviron treated rats.

Fig. 3 shows changes in percentage of sperm with progressive motility of albino sleep deprived Wistar rats treated with Kolaviron. Sleep-deprivation caused a significant ($p<0.05$) decrease in percentage of sperm with progressive motility. This decrease was reversed following administration of Kolaviron in a dose-dependent manner. This reversed increase in percentage of sperm with progressive motility in sleep-deprived rats induced by Kolaviron was not significant when compared to control despite the increase.

Fig. 4 shows alteration in the percentage of sperm with normal morphology of Sleep deprived rats administered with Kolaviron extracts. It was observed that there was a significant ($p < 0.05$) decrease in percentage of sperm with normal morphology when compared to control and rats treated with Kolaviron. Administration of Kolaviron ameliorated the effect of sleep deprivation with dose-dependent rise in percentage of sperm with normal morphology as significance ($p<0.05$) was recorded in the sleep-deprived rats treated with 200 mg/Kg Kolaviron.

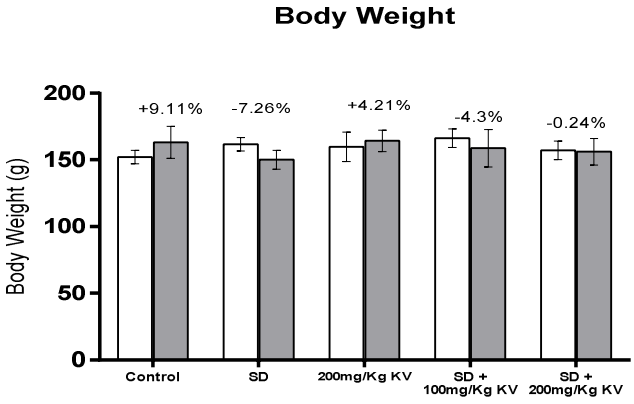

Fig. 1. Showing effect of kolaviron extract on body weight of sleep-deprived wistar rats (n=6)
**: p < .05 compared with control group;*

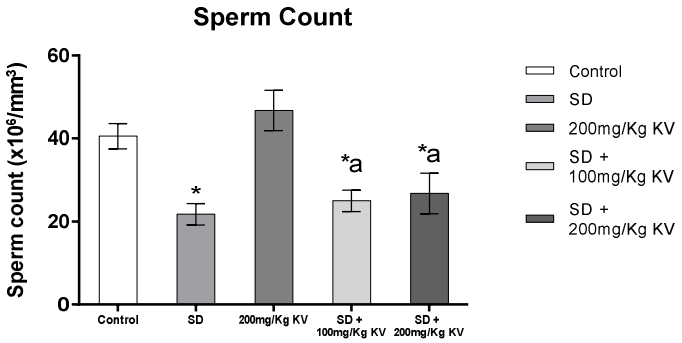

Fig. 2. Showing effect of kolaviron extract on sperm count of sleep-deprived wistar rats

**: p <0.05 compared with control rats;*
a: p < 0.05 compared with 200 mg/Kg Kolaviron treated rats;

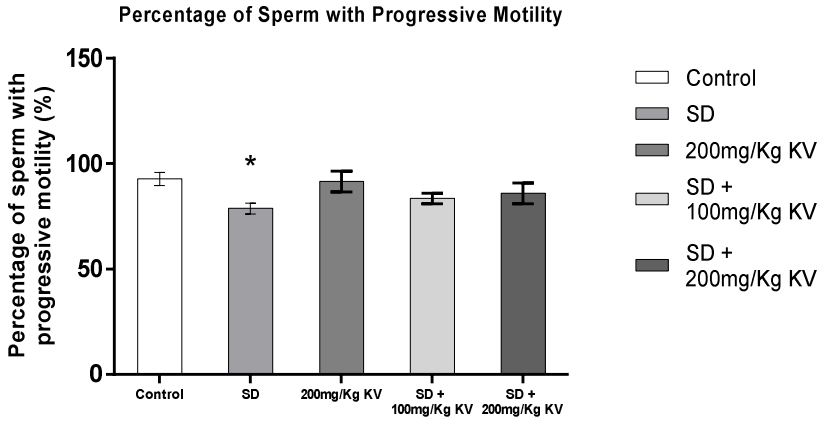

Fig. 3. Shows effect of kolaviron extract on percentage of sperm with progressive motility of sleep-deprived wistar rats

**: p < .05 compared with control group;*

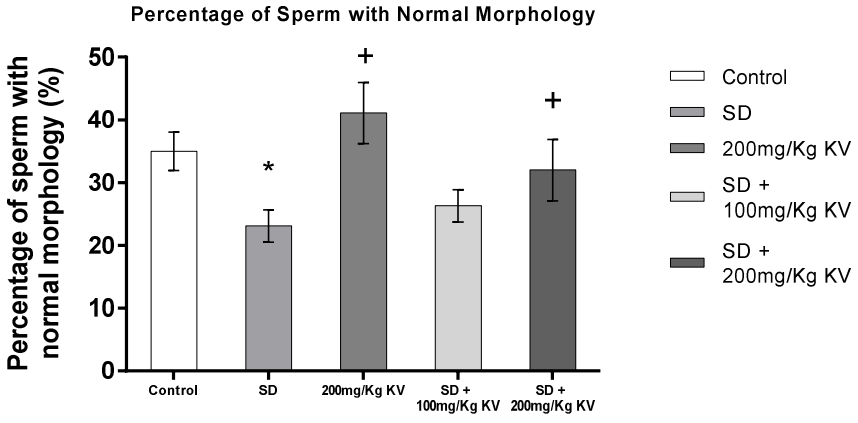

Fig. 4. Shows effect of kolaviron extract on percentage of sperm with normal morphology of sleep-deprived wistar rats (n=6)

**: p <0.05 compared with control rats;*
+: p < 0.05 compared with sleep-deprived rats;

Fig. 5 shows the changes in testicular weight following sleep deprivation in rats treated with Kolaviron extract. Testicular weight was significantly ($p<0.05$) reduced following sleep deprivation. This decrease in testicular weight was also significant ($p<0.05$) when compared to normal rats treated with 200 mg/Kg Kolaviron. Administration of Kolaviron had a minimal effect on reversing the detrimental changes of sleep deprivation as dose-dependent increase in sleep-deprived rats treated with Kolaviron was significant when compared to control and normal rats treated with 200 mg/Kg Kolaviron.

Fig. 6 shows that sleep deprivation had minimal effect on the testicular volume; hence there was no significance when compared to control. Administration of Kolaviron at different graded doses to the Sleep Deprived rats ensured the testicular volume of the rats remained within the control range.

Data from this Fig. 7 shows that stress induced by sleep deprivation caused a significant ($p < .05$) increase in serum cortisol level when compared to cortisol level in normal rats and rats treated with 200 mg/Kg Kolaviron. Further administration of graded doses of Kolaviron significantly ($p < .05$) decreased the cortisol level in a dose-dependent manner.

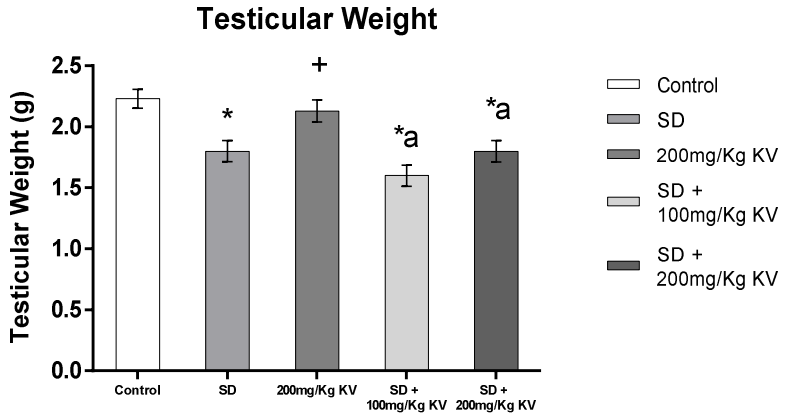

Fig. 5. Showing effect of kolaviron extract on testicular weight of sleep-deprived wistar rats (n=6)

**: p <0.05 compared with control rats;*
+: p < 0.05 compared with sleep-deprived rats;
a: p < 0.05 compared with 200mg/Kg Kolaviron treated rats;

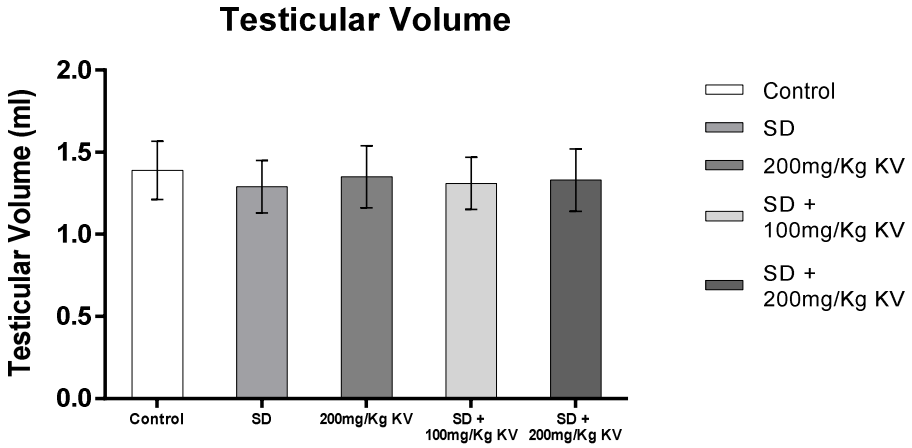

Fig. 6. Shows effect of kolaviron extract on testicular volume of sleep-deprived wistar rats (n=6)

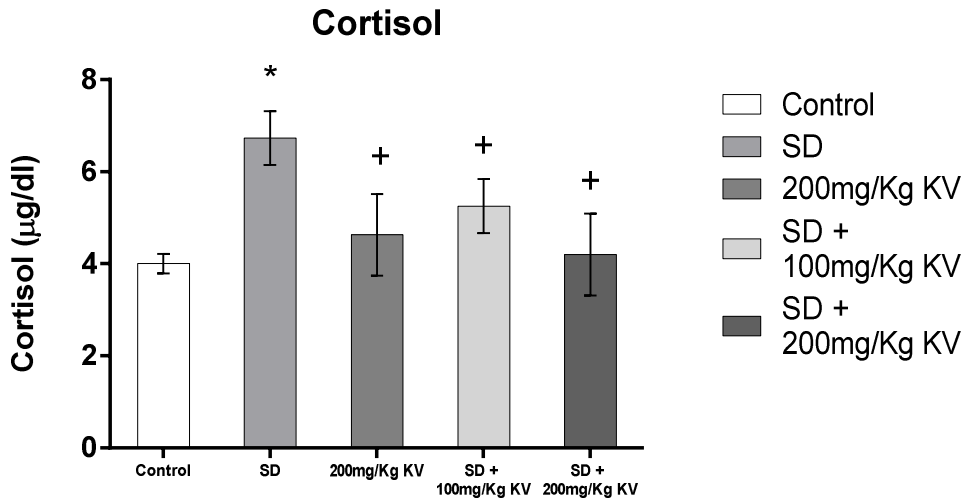

Fig. 7. Shows effect of kolaviron extract on serum cortisol level of sleep-deprived wistar rats (n=6)

**: p <0.05 compared with control rats;*
+: p < 0.05 compared with sleep-deprived rats;

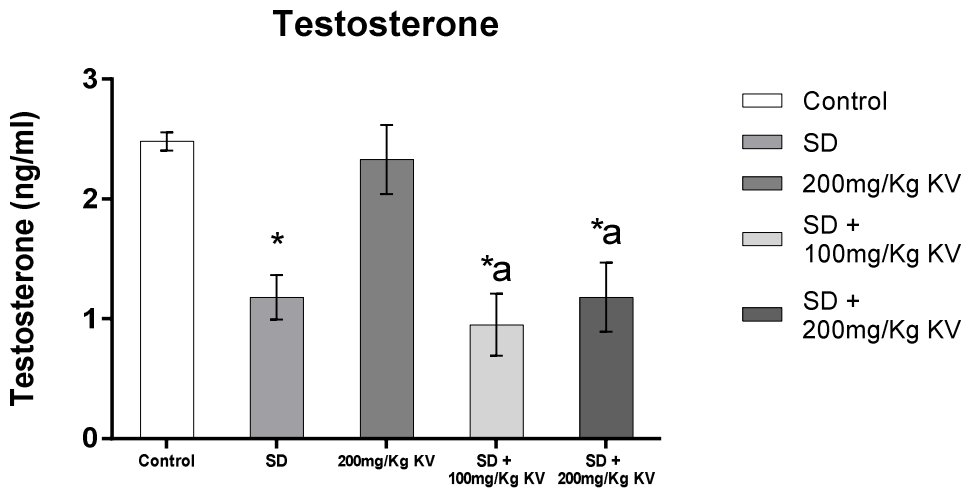

Fig. 8. Shows effect of kolaviron extract on testosterone level of sleep-deprived wistar rats (n=6)

**: p <0.05 compared with control rats;*
a: p < 0.05 compared with 200 mg/Kg Kolaviron treated rats;

From Fig. 8 above, Sleep deprivation significantly ($p<0.05$) decreased the testosterone serum concentration when compared to control and serum testosterone of normal rats treated with 200 mg/Kg. The insignificant effect was observed following administration of Kolaviron on the sleep-deprived rats as a dose-dependent increase in testosterone level in the rats with significance ($p < .05$) when compared to control and normal rats treated with 200 mg/Kg Kolaviron.

Fig. 9 shows that Sleep deprivation induced a decrease in serum FSH level when compared to control and normal rats treated 200 mg/Kg Kolaviron. Graded dose treatment of Kolaviron caused a dose-dependent ameliorating effect on FSH level of sleep-deprived rats. It was observed

that there was no significant change despite sleep deprivation and Kolaviron administration.

Fig. 9 shows that Sleep deprivation caused a decrease in serum LH concentration with significance ($p<0.05$) recorded in rats treated with 200 mg/Kg Kolaviron. Subsequent administration of different doses of Kolaviron had minimal changes on LH level of sleep-deprived rats as no significance was recorded with the LH level of rats deprived of sleep, though statistical significance ($p < .05$) was observed when compared to the LH level of control and rats treated with 200 mg/Kg.

5. DISCUSSION

Results from this study reveal a reduction in the brain weight of sleep-deprived rats when compared to control and kolaviron extract treated groups. The body weight of the two weeks (14 day) actively prolonged wakeful state of rat shows a relative body weight loss but not statistically significant. This Indicates that prolonged sleep deprivation mildly reduce and alter body weight. Similar effect was observed with organs as seen with the relative organ weight of the testis Control group.

A statistically significant reduction was found in sperm motility and count of the sleep deprivation group compared to those of the control group and the extract pre-treated groups. There were no significant decreases in sperm counts between the control and sleep deprivation groups. Also, sperm motility of subjects in the

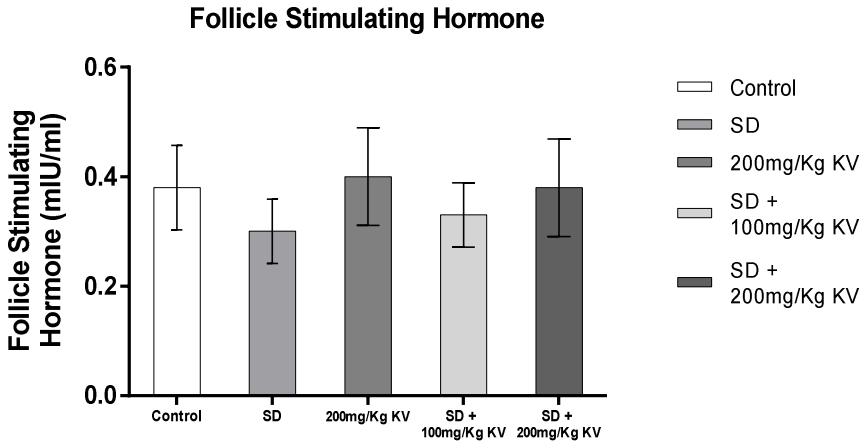

Fig. 9. Shows Effect of Kolaviron extract on serum Follicle Stimulating Hormone (FSH) level of Sleep-deprived Wistar rats

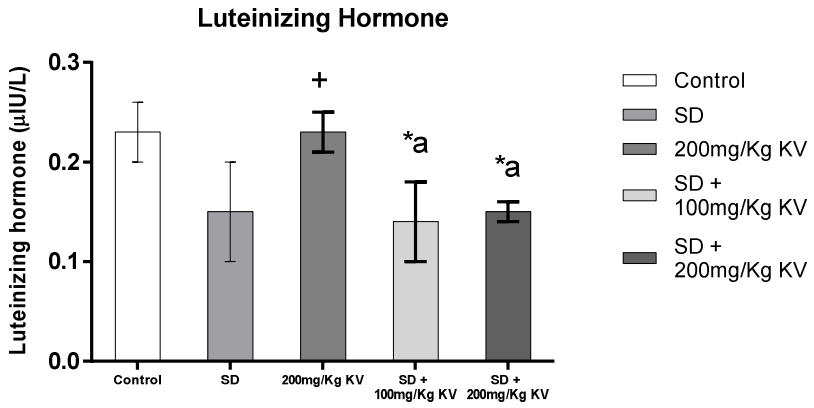

Fig. 10. Shows Effect of Kolaviron extract on serum Luteinizing Hormone (LH) level of Sleep-deprived Wistar rats (n=6)

sleep deprivation group decreased, while sperm motility of all subjects in the control and treated groups were within the normal range. The precise mechanism responsible for the differences in sperm motility between treated and sleep deprived groups is not known. However, sleep deprivation includes stress as an intrinsic part and induces many injurious health problems with endocrinologic, immunologic, and metabolic consequences.

Cortisol concentrations in the sleep deprivation groups were significantly increased, whereas testosterone concentrations in the sleep deprivation groups were significantly decreased compared with the control group. These results are in agreement with those of numerous studies that established changes in steroid hormone levels in sleep-deprived animals [15-16]. Furthermore, it has been shown that cortisol exhibits much higher glucocorticoid potency than corticosterone. However, whether cortisol is indeed present in laboratory rodents remains to be determined carefully in a special study using methods with greater specificity[17]. The increase in corticosteroid levels by stressful stimuli may inhibit the hypothalamus-pituitary-gonadal (HPG) axis leading to decreased testosterone secretion [17]. Additionally, declines in testosterone production may be associated with activation of the hypothalamus-pituitary-adrenal (HPA) axis inducing elevations in corticosteroid [18]. Consequently, it is presumed that during sleep deprivation, with some degree of stress, testosterone levels decrease via inhibition of the HPG axis caused by elevated corticosteroid. Cortisol levels increase via activation of the HPA axis. Under regulation by the hypothalamus, the pituitary gland produces pituitary gonadotropins such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH stimulates the Leydig cells, which have surface receptors for LH; the excited Leydig cells produce and secrete testosterone.

6. ADVANTAGE OF STUDY

Investigation of the role of kolaviron as an anti-fertility agent from this study will be useful in the holistic approach to the problem posed by sleep deprivation on human reproduction. This research work will be of great benefit as it will help to understand the possible mechanism of the effect of kolaviron and consumption of bitter kola. It is also expected to provide basic information for the treatment of infertility in Nigeria. Information from this study will be beneficial to health practitioners.

7. CONCLUSION

This study demonstrated that sleep deprivation negatively influences testosterone, luteinizing hormone and cortisol levels in serum and in rat testes. The administration of kolaviron extracts, therefore, poses as a good source of antioxidant activities to reduce the possible damage due to sleep deprivation. More so, Kolaviron showed the remarkable dose-dependent effect and caused significant beneficial effects at all levels, specifically at the hypothalamo-pituitary gonadal axis. It significantly improved reproductive functions in sleep deprivation.

8. RECOMMENDATIONS

Further works should be carried out with varied sample size and dosage. It is also recommended to expand the population to more than one species of animal. More so, effects of other constituents of *Garcinia kola* in sleep deprivation, effect of *kolaviron* on other parts of the brain and electron microscope evaluation of any ultra-structural changes in the anterior pituitary and hypothalamus should be further studied.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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