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Histo-Architectural and Biochemical Changes in Kolaviron Induced Sleep Deprivation in Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author OOV carried out the bench work. Authors MOE and PEO managed the literature searches and performed the statistical analysis. Author MOO wrote and monitored the first draft of the manuscript. Author JCI managed and supervised the experimental protocol. All authors read and approved the final manuscript.

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ABSTRACT

Organisms survive by maintaining a dynamic equilibrium with their environment. Stress is a state of threat to this equilibrium, and adaptation to stress, or allostasis, confers a survival advantage. Sleep deprivation constitute a biological stress implicated in many homeostatic alterations including weight loss, reduction in thymic weight, increased adrenal weight, elevated corticosterone and ACTH levels. This study sought to investigate the effects of 'kolaviron extract' on the biochemistry and histo-architecture of selected visceral. Thirty (30) male Wistar rats were 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 200 mg/kg of extract in sleep deprived state, group C was given 200 mg/kg of extract without sleep deprivation. Groups A and B were each administered 1 ml of the vehicle (1% tween 80

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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 assay of biochemical stressors. Selected organs were also harvested for histological analysis. At $p < .05$, Analysis of variance test (ANOVA) returns that Kolaviron extract decreases oxidative stress resulting from sleep deprivation in brain (specifically the pituitary gland and hypothalamus) and testicular tissues. 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; stress; histo-architecture; 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 effects of sleep deprivation are cumulative; such that a mild reduction in sleep per night can over a period of time, result in significant functional deficits. Several studies have reported that sleep deprivation causes alteration in several aspects of physiological and behavioural function such as sexual behaviour, memory, attention, hormonal balance and immune system [3,4].

As a complex behavioural state occupying one–third of human life, sleep is often thought of as a passive condition, but it is in fact a highly active and dynamic process. Although the function of sleep has not fully been elucidated, it has been known for a number of years that sleep plays a restorative role in most organic functions. Studies have shown that sleep influences a vast array of behavioural and physiological functions, such as memory and cognitive ability, hormone secretion and glucose metabolism, and immune function. Though adequate sleep has been reported to maintain optimal functional levels in various systems, however, the numerous effects of sleep deprivation on both behavioural and physiological functions have been shown [5].

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 [6,7]. Kolaviron, a major constituent of *Garcina kola,* is one of the numerous plant products and nutritional supplements with a wide range of medicinal value [8-10]. Its anti-oxidant action on lipoprotein has been reported [11,12]. Kolaviron may have influence on male reproductive dysfunction as it is associated with oxidative stress damage [9]. 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 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.

1.1 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. Assess the effects of Kolaviron on Testicular oxidative stress marker in sleep deprived Wistar rat,
ii. Assess the effects of Kolaviron on histoarchitecture of the hypothalamus and testis in sleep deprived wistar rat.

2. MATERIALS AND METHODS

2.1 Scope of Study

This study was limited to the effect of kolaviron on the Histo-Architecture and Biochemical indicators of stress (Biomarkers of stress) in sleep-deprived male wistar rats. Due to the invasive nature of the study, rat models (specifically wistar rats) were preferred. This was necessitated by the need to harvest selected internal organs, as well as proper standardization of experimental protocols.

2.2 Study Design

Thirty (30) male Wistar rats (weighing between 190 g – 240 g) 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 1 ml of the vehicle (1% tween 80 solution)
Group B: Sleep-deprived (SD) group: Received 1 ml of the vehicle (1% tween 80 solution)
Group C: Kolaviron group which received Kolaviron at 200 mg/kg
Group D: Kolaviron and Sleep deprived (KV + SD) group which received Kolaviron at 100 mg/kg
Group E: Kolaviron and Sleep deprived (KV + SD) group which received Kolaviron at 200 mg/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.

2.3 Resources and Sources

2.3.1 Plant materials

Seeds of *Garcinia kola* were purchased from 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 to 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.

2.3.2 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.

2.4 Procedure

2.4.1 Isolation of kolaviron

Extraction of kolaviron was achieved by the procedure previously described by Iwu (1985) [13]. Also, 8.2 kg 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 defatting process was repeated by adding another 2 litres of pure n-hexane to the plant shaft for another 72 hours. 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 with 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 *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 5 liters 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 72 hours. 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 500 ml 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 600 mmHg as to properly remove any trace of solvent (chloroform).

Percentage yield was calculated as follows;

% yield of kolaviron =

$$\frac{\text{weight of extract}}{\text{weight of plant sample used}} \text{ X100}$$

2.4.2 Sleep deprivation induction

The Sleep deprivation chamber is a glass chamber (60 cm x 60 cm x 30 cm) containing 16 multiple circular galvanized iron platforms of about 0.6 cm in diameter and 25 cm in height, with water filled up to 1 cm 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 4 hours (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 14 days of paradoxical sleep deprivation period by placing pellets and water bottles on a grid located on top of the chamber.

2.4.3 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 3000 rpm for 15 minutes 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 100 mM Phosphate buffer (pH 7.4), and centrifuged at 3000 rpm for 15 minutes.

2.4.4 Histological analysis

One testis from each group was harvested immediately the animals were opened up. Testis was then fixed in Bouin's fluid before they were transferred into 10% formal saline to preserve its various constituents from any degeneration or analytic changes. The following processes were then carried out sequentially. The tissues were fixed in 10% formal saline for 48 hours, grossed, and cut into smaller pieces of 3 mm thick in pre-labelled tissue cassettes. They were then processed with Automatic tissue processor (LEICA TP1020) and passed through various reagents including alcohol (of various concentrations starting from 70%, 80%, 90%, 95%, and two 100% or absolute alcohol) for dehydration. While processing lasted for 12 hours, tissues were embedded in Paraffin wax by burying in a metal mold containing molten paraffin wax and allowed to cool and form tissue paraffin blocks, ready for microtome. The tissues were sectioned at 4microns using Rotary microtome (LEICA RT2115). Obtained sections were then floated in hot water bath and attached to pre-labelled slides. The sections were dried on hot plate and stained with Haematoxylin and Eosin for 20-40 minutes. While stained sections were washed in tap water for 1-5 minutes until sections turn blue ("bluing"), differentiate sections were soaked in 70% ethanol—containing 1% HCl for 5 seconds. This removed excess dye, allowing nuclear details to emerge.

2.5 Determination of Antioxidant Activities

2.5.1 Superoxide dismutase activity

According to Aline et al. (2013), this method was based on the ability of superoxide dismutase to inhibit the autoxidation of adrenaline (epinephrine) at pH 10.2 because; superoxide (O_2^-) radical generated by the xanthine oxidase reaction causes the oxidation of epinephrine to adrenochrome [14,15,16].

2.5.2 Reduced glutathione activity

According to Aline et al. (2013), this method was based on the development of a relatively stable

(yellow) colour when 5′, 5′–dithiobis-(2-nitrobenzoic acid) (DTNB) is added to sulfhydryl compounds. The chromophoric product resulting from the reaction of DTNB with reduced glutathione; 2 – nitro-5-thiobenzoic acid possessed a characteristic absorbance at 412nm and the amount of reduced glutathione in the sample is proportional to the absorbance at this wavelength.

2.5.3 Lipid peroxidase activity

The malondialdehyde (MDA) content of the lipid peroxidation was measured according to the method described by Aline et al. [12]. This assay principle is based on the fact that lipid peroxidation generates unstable lipid peroxides, which decompose to form a complex series of compounds including reactive carbonyl compounds. The polyunsaturated fatty acid peroxides produced generate malondialdehyde (MDA) upon decomposition. MDA forms a 1:2 adduct with thiobarbituric acid (TBA) that gives rise to a pink colour product when heated in acidic pH. This takes place at a maximum absorbance of 532 nm [17].

2.5.4 Acute toxicity test

Available acute toxicity studies suggest that Oral medium lethal dose (LD50) of *Garcina kola* extract is greater than 3,000 mg/kg body weight. More so, according to the American Society for Testing and Materials, any chemical substance with LD50 estimate greater than 3,000-5,000 mg/kg (Oral route) could be considered of low toxicity and safe [16]. Based on these, two concentrations (100 mg/kg and 200 mg/kg) of aqueous extract from *Garcina Kola* were used for the study.

2.6 Analytical Approach

Obtained data were expressed as mean ± Standard Error of Mean (SEM) with statistical analysis performed using one way analysis of variance (ANOVA), followed by least significant difference (LSD) test. A p-level less than 0.05 ($p < 0.05$) was considered as statistically significant.

3. RESULTS

Study examined the changes in the histo-morphology and biochemistry of sleep-deprived male wistar rats with Kolaviron extract administered as anti-sleep agent. To this point, results show changes in normal histo-architecture of testes and hypothalamus.

Chart 1 shows changes in testicular superoxide dismutase (SOD) of sleep deprived rats treated with graded doses of Kolaviron. Data show that sleep deprivation decreased the testicular SOD level with no statistical significance. This decrease was attenuated by graded doses of Kolaviron, with ameliorating effect proving insignificant as compared to rats that served as control.

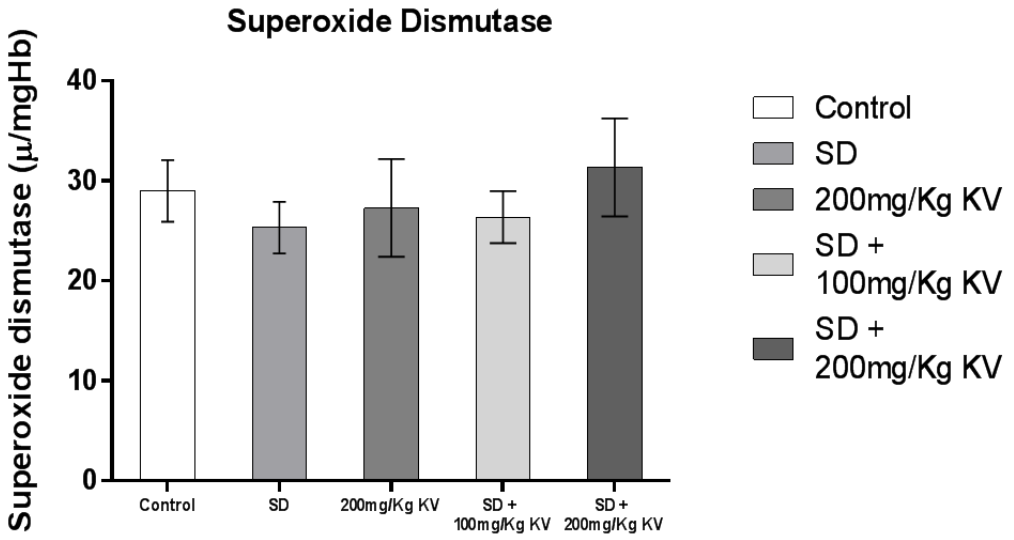


Chart 1. Showing effect of Kolaviron extract on testicular superoxide dismutase (SOD) level of sleep-deprived wistar rats (n=6)

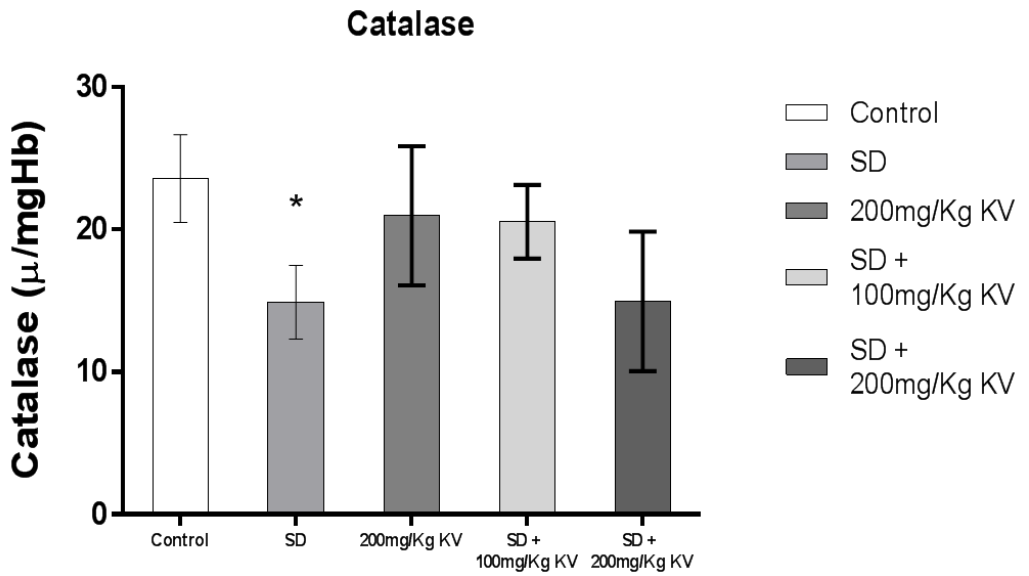


Chart 2. Showing effect of Kolaviron extract on testicular catalase (CAT) level of Sleep-deprived wistar rats (n=6)

**: p < 0.05 compared with control group*

Chart 2 shows changes in testicular catalase (CAT) level of sleep deprived rats treated with graded dose of Kolaviron. As seen, sleep deprivation decreased CAT activities of the testis, while subsequent administration of graded doses of Kolaviron reversed this effect which apparently was insignificant when compared to testicular CAT level of stressed rats.

From Chart 4, Sleep deprivation significantly ($p < 0.05$) decreased testosterone serum concentration when compared to control and serum testosterone of normal rats treated with 200mg/Kg. Insignificant effect was observed, following administration of Kolaviron on the sleep deprived rats as compared to control and normal rats treated with 200 mg/Kg Kolaviron.

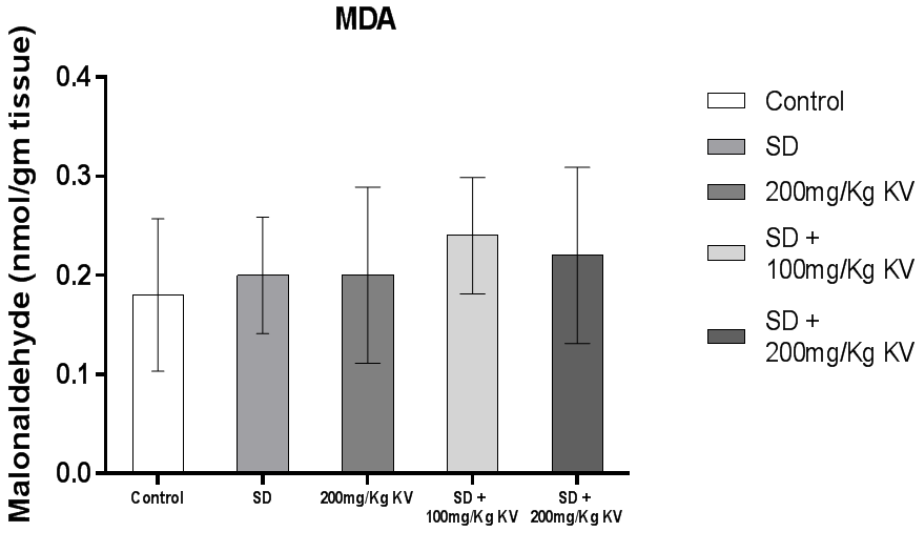
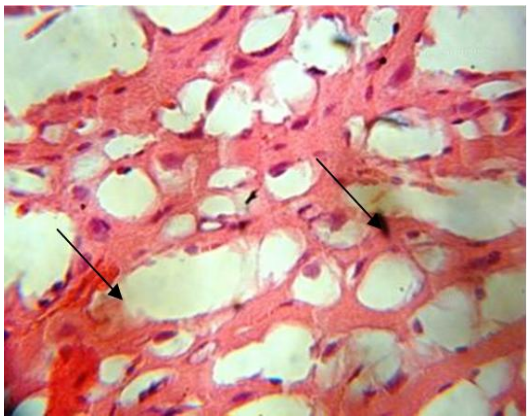
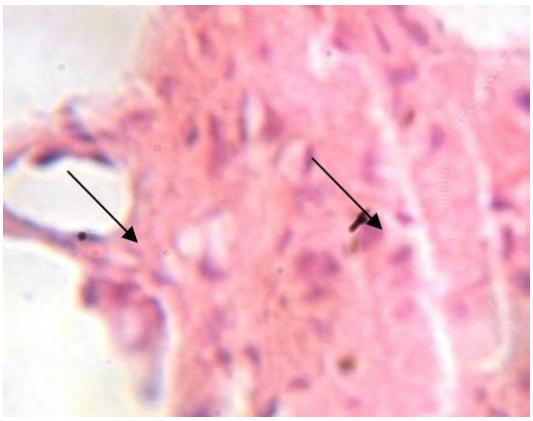


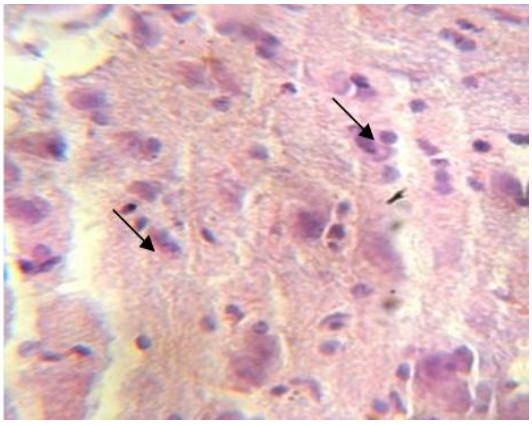
Chart 3. Showing effect of Kolaviron extract on testicular Malonaldehyde activities in sleep-deprived wistar rats (n=6)



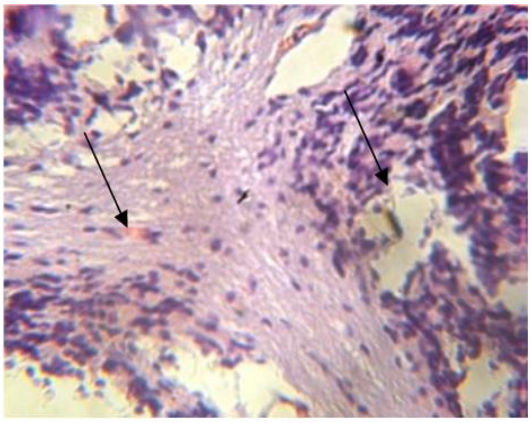
Group A. Hypothalamus reveals lesser vacuolization of dark cells and neurophil in hypothalamus in the granular layer and molecular layer. Fibrillary background. Haematoxylin and eosin. x 40 magnification



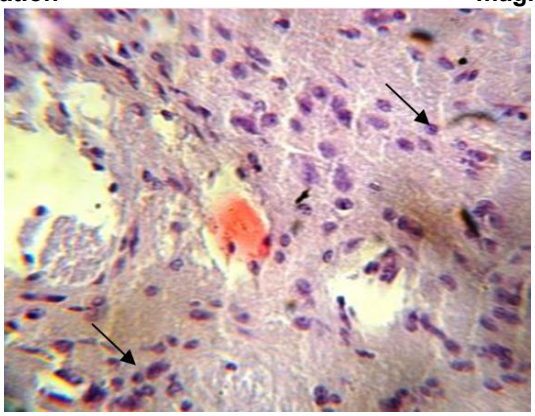
Group B. Hypothalamus reveals Vacuolization of dark cells and neuropil in hypothalamus of sleep deprived rat. Haematoxylin and eosin x 100 magnification



Group C. Reveals lesser vacuolization of dark cells and neuropil in hypothalamus in the granular and molecular layers. H and E x 100 magnification

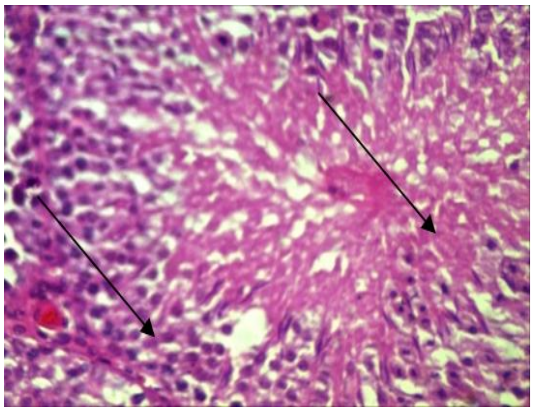


Group D. Reveals lesser vacuolization of dark cells and neuropil in hypothalamus in the granular and molecular layers. H and E. x 100 magnification

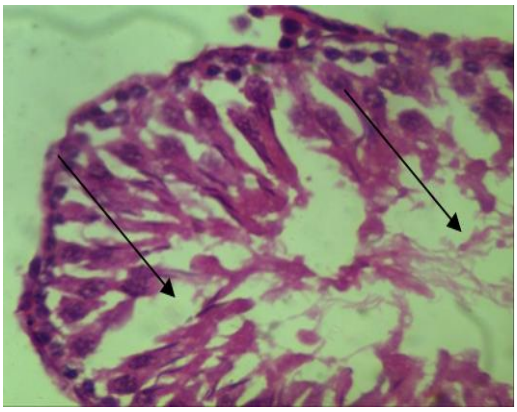


Group E. Reveals lesser vacuolization of dark cells and neuropil in hypothalamus in the granular and molecular layers

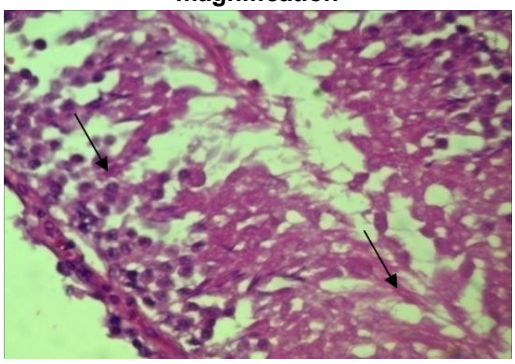
Fig. 1. Effect of Kolaviron on histology of hypothalamus of sleep deprived rats



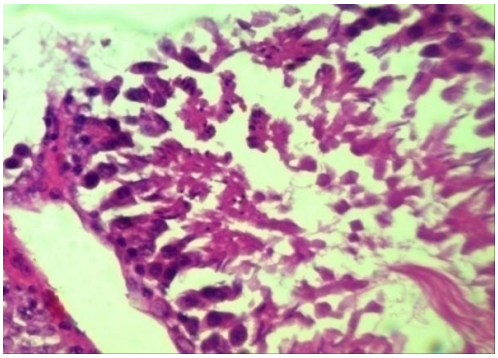
Group A. Shows seminiferous tubules lined by germ cells at different stages of spermatogenesis. Spermatozoa (arrow). The sinusoids are congested. H and E. x 400 magnification



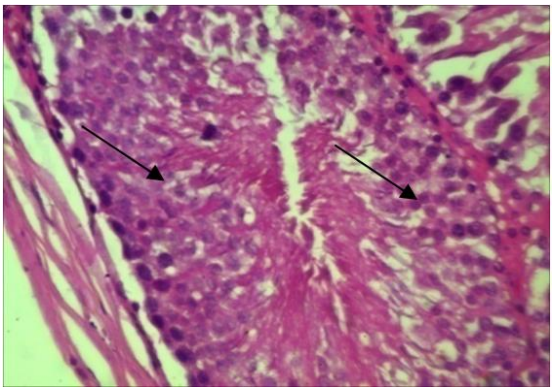
Group B. Shows semineferous tubules lined by germ cells at different stages of spermatogenesis. The sinusoids are congested. H and E. x 400 magnification



Group C. Shows seminiferous tubules lined by germ cells at different stages of spermatogenesis (Spermatozoa arrow). Sinusoids are congested with H and E. x 400 magnification



Group D. Shows semineferous tubules lined by germ cells at different stages of spermatogenesis (Spermatozoa arrow). The sinusoids are congested. H and E. x 400 magnification



Group E. Figure shows semineferous tubules lined by germ cells at different stages of spermatogenesis. Spermatozoa (arrow). The sinusoids are congested. Haematoxylin and eosin. x 400 magnification

Fig. 2. Effect of kolaviron extract on histology of testis of sleep deprived rats

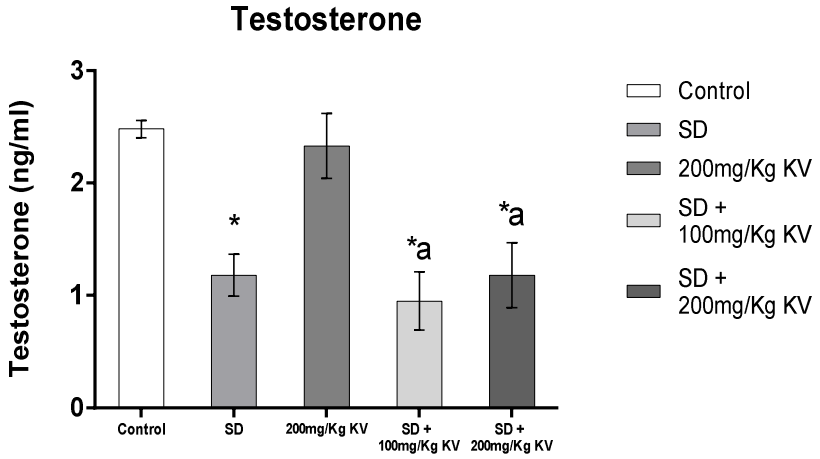


Chart 4. Shows Effect of Kolaviron extract on Testosterone level of Sleep-deprived Wistar rats (n=6)

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

4. DISCUSSION

Results from this study reveal a reduction in 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 rats show a relative body weight loss with no statistical significance when compared to initial and final body weights of control group (9.11%). Also, sleep deprived Group B rats had prolonged sleep deprivation that mildly reduced and altered body weights. Similar effect was observed with organs as seen in the relative organ weight of testis Control group. The Brain Control group, sleep Deprived group and Groups D and E respectively showed mild significant alterations ($p < 0.05$) upon comparisons.

Both acute and chronic sleep deprivation have been found to be intricately associated with changes in sleep patterns and cognitive deficits both in preclinical and in clinical studies [18,15,14,19,20]. In this study, sleep debt generated as a consequence of four (4) hours daily within the 14 days protocol significantly decreased Superoxide dismutase and calatase activities in the histology of the brain and testis as compared to sleep deprived control group. Subsequent treatment with the extract (100 mg/kg and 200 mg/kg) reversed the depleted reduced Superoxide dismutase and catalase activities. Malondialdehyde activity in brain and testis tissues increased in sleep deprived group when compared to control group. However, Treatment with the extract (100 mg/kg and 200 mg/kg) moderately decreased the malondialdehyde activity.

Superoxide dismutase, catalase and lipid peroxidation (malondialdehyde activity) can be used to understand the biochemical events of sleep deprivation in the studied animals. However little is known whether stress is an important consequence of sleep deprivation. In line with the acute sleep deprivation conditions, present study exposure to alternate days sleep deprivation (over a total period of 14 days) not only depicted increased lipid peroxidation and nitrite production, but also diminished glutathione reduction and antioxidant enzyme activities of superoxide dismutase as well as catalase in discrete encephalic areas of the hippocamapus as well and the cortex.

Detrimental effects of sleep deprivation on male reproductive function, including a decrease in sperm motility were further confirmed by abnormal testicular histopathological findings, as well as hormonal changes. There were no abnormal morphologies of seminiferous tubules in control group. On the other hand, spermatid retention in seminiferous tubules was observed in 20% of the sleep deprivation group, and in 50% of the sleep deprivation group respectively. In addition, atrophy of seminiferous tubules was found in 30% of the sleep deprivation group. These results indicate that prolonged excess

period of cortisol and suppression period of testosterone are related to the duration of sleep deprivation, leading to a more severe harmful effect on the histopathology of testis in male rats.

In our results, there were significant decreases in testosterone levels of sleep deprived group. These hormonal disruptions were the common cause of spermatoid retention and tubular atrophy which results in decreased fertility. Additionally, present study also depicts that sleep deprivation led to significant depletion in activities of mitochondrial respiratory enzyme complexes and cellular viabilities in both hippocampus and cortex which may manifest in situations of not only cellular energy deficits but also increased production of oxidative free radicals as well as activation of caspase dependent apoptotic responses.

It can be purported that vicious and complex mechanistic interplays between oxidative free radicals, mitochondrial respiratory enzyme complex insufficiencies, antioxidant activities, as well as activation of apoptotic responses may serve as cardinal interactive molecular cascades in sleep deprivation induced cognitive dysfunction. But still, confirmative molecular and sub cellular studies may be required to validate the above mentioned set of results. However, figures tried to elaborate the plausible molecular mechanistic interplays that may play cardinal role apart from other neuropathologies in sleep deprivation induced cognitive deficits.

Additionally, it was also suggested that oxidative stress, depleted enzyme complex activities/cellular survival, increased activity may cause neuromorphological and testicular alterations in specific regions which may manifest alterations in cognitive physiologies, thereby serving as cardinal etiologies in sleep deprivation induced cognitive dysfunction.

4.1 Advantage of Study

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

5. CONCLUSION

This study demonstrated that sleep deprivation influences oxidative stress markers. The administration of kolaviron extracts may therefore pose as a good source of antioxidant activity that reduces the possible damage due to sleep deprivation. More so, Kolaviron showed remarkable dose dependent effect and caused significant histo-morphological changes at all levels, specifically in the hypothalamus and testes. It also significantly improved metabolic parameters, whilst improving reproductive functions in states of sleep deprivation.

6. RECOMMENDATIONS

Further works should be carried out with varied sample size and dosage. It is also recommended that expansion be made on population. More so, on the effects of other constituents of *Garcinia kola* on sleep deprivation, effect of *kolaviron* on other parts of the brain, with electron microscope evaluation of any ultra-structural changes in the anterior pituitary and hypothalamus should be further. Further studies will also be required to assess whether sleep deprivation has effect(s) on mating, gestation and implantation, and whether sleep deprivation, or insufficient sleep, may influence male reproductive system, sperm and testis, in humans.

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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