EFFECTS OF NIFEDIPINE ON THE TESTES OF MALE WISTAR RATS (*Rattus Novergicus*)

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ABSTRACT

Calcium ions are of great fundamental importance in several physiological processes in all animals, therefore a lack or absent of it leads to series of critical pathologies, including infertility, cardiac hypertrophy, arrhythmia, and post-ischemic brain damage. It's known that certain categories of drugs called calcium channel blockers (CCBs) or calcium ion antagonists and only induce their primary function by obstructing the free flow of Ca^{+}. Some of these drugs include Nifedipine, amlodipine, felodipine, Diltiazem and others.Concurrently, Nifedipine is one of the most widely used CCB that belong to a class of antihypertensives used in the treatment of hypertension. The aim of this study was to investigate and elucidate the effects of different doses of Nifedipine on the male reproductive parameters such as Morphological changes of the testis (histological changes), Sperm count, Sperm motility, and Endocrine function of the testis (serum testosterone).Seventeen adult male wistars were randomly divided into 3 groups, the control group (n=5) were treated with 2ml of distilled water, the 2nd group (n=6) were treated orally with 0.3mg/kg body weight of Nifedipine while the 3rd group (n=6) were treated orally with 0.6mg/kg body weight of Nifedipine. The epididymal sperm count, motility, morphology and progressivity of the spermatozoa were assessed and the serum levels of testosterone, oestradiol, progesterone, and the pituitary gonadotropins were estimated. More so, the testes were also fixed and processed for histo-architecture analysis, haematological parameters were also assessed. We therefore conclude that Nifedipine did not have advert effects on the male reproductive health of treated rats, as it increased the sperm counts, stimulate spermatogenesis and testosterone production and no remarkable change in the hist-architecture.

Keywords: Nifedipine, sperm count, testosterone, pituitary gonadal-axix hormones, fertility

No: of Tables: 4 **No: of Figures :14** **No: of References: 15**

INTRODUCTION

The major function and extensive usage of calcium channel blockers (CCBs) is to manage or treat hypertension, which is the constant increase in the arterial pressure beyond a specific level (i.e. blood pressure above 115/75 mmHg). However they could also be used for the management of migraine headache, angina and arrhythmias (Morakinyo AO et al., 2011). Because hypertension is classified as a worldwide epidemic disease, it's therefore requiring potent medication suchas NIfedipine. It wasalso, estimated that 20% of adult males,approximately 1 billion suffer from hypertension, which also contribute to about 7.1 million death per year (Rollet al., 2013).During the cause of it usage, it was however claimed, that it induces infertility, which is the inability to obtain a pregnancy after 12 months of unprotected sexual intercourse(Olayemi, 2010).Although, infertility may affects both males and females. However, malefactor infertility (MFI) has shown more incidence rates and contributing about 50% of major infertilities. Although MFI parameters could be measured in several ways, however, the major ones includes, sperm count, sperm motility and sperm morphology (Zhao and Brezina, 2011). Consequently, a myriad of literature have proved, several factors with its detrimental effects on male fertility. However, this article has critically observed series of studies and a little is known about calcium blockers' effects on male fertility.

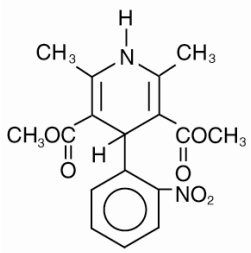

Figure 1: Structural representation of Nifedipine

Nifedipine is a calcium channel blocker (CCB), predominantly used as a vasodilator and has its chemical formula as dimethyl 2, 6-dimethyl-4-(2-nitrophenyl)-1, 4-dihydropyridine-3, 5-dicarboxylate with a molecular formula $C_{17}H_{18}N_2O_6$ and molecular weight of 346.3. Nifedipine belong to a group called the dihydropyridine which consist of (nifedipine, amlodipine, felodipine, isradipine, nicardipine, and nisoldipine).

Nifedipine and its infertility effects have been argued in several literatures. In a review by Brezina PR, et al. 2012, after certain examinations with respect to sperm motility, sperm morphology and phospholipids it was claimed that Nifedipine significantly prevent the uptake of Ca^+ , prevent the sperms from binding to the egg by altering the lipid bilayer system of the sperm plasma membrane. Interestingly, this also corroborate Blomberg Jensen 2014,who claims that having a high amount of intracellular Ca+ initiate sperm capacitation, motility, improve acrosome reaction and enhance easy binding to the egg, however using Nifedipine cause a reduction in the intracellular Ca+. Concurrently, a study however, refutes that Ca+ antagonists cause a reduction in sperm motility but rather increases semen motility (Brezina PR, et al. 2012).

For fertilization to occur acrosome reaction needs to take place, however, (Olayemi 2010) claimed that Nifedipine hinder the occurrence of this process thus, leading to infertility.

Its' interesting that several studies conducted with regards to (CCBs) including Nifedipine and its members of the dihydropyridine group have proven to have a deleterious effects on the MFI.

A study conducted bylranloye BO, et al., 2009, with Nifedipine at different doses (i.e. 0.57 mg/kg and 0.57 mg/kg) shows detrimental effects on the sperm functions and testosterone secretions.Consequently another study corroborating this, however, on Amlodipine at different doses (i.e. 0.056mg/kg and 0.114mg/kg) this gave a change in the structure of the testis and alteration in the histological architecture of the testis (Adefule A et al., 2012).

It was however, reported that CCBs could induces their effects by not actually affecting the testicular hormones (gonadal axis hormones remain unchanged), but rather instigate a reduction in epididymal sperm count and motility (Morakinyo AO, et al., 2009). Therefore hormonal analysis might fail to give substantial evidence on the MFI.

Since, Ca+ plays a crucial role in fertilization and also engaged in the main functions of spermatozoa such as maturation, motility, and the acrosome reaction (AR), an exocytotic process essential for fertilization in many species (Darszon et al., 2011). However using Ca+ antagonists substances require immediate attention and therefore aimed to look into these issues as clearly mentioned above in our aims.

MATERIALS AND METHODS

All experiments were carried out with sexually mature albino rats of Rattus Novergicus strain weighing approximately 140g-160g, obtained from the Central Animal House of the College of Medicine of the University of Ilorin. The rats were housed in wired net cages and are well ventilated with photoperiod controlled (12 hour light: 12 hour dark) animal house for at least two weeks prior to use in experimental protocols. The rats were fed on commercial standard pellet diet (Livestock Feeds, Lagos, Nigeria) and water *ad libitum*. Generally, the study was conducted in accordance with the internationally accepted guidelines for laboratory animal use and care. The experiments reported here were approved by our institutional ethics committee.

Drugs, Doses and Design

We utilised5 Tablets of Nifedipine each weighing 60mg(Bayer Schering Pharma) in this study. The drug was slightly crushed and dissolved in distilled water for about 4 days to achieve total dissolution. And dosing formulations were preparedsince the therapeutic use of Nifedipine is between 20mg to 90mg and since normal physiological man weighs 70kg, therefore: $\frac{20mg}{70kg} = 0.3mgkg^{-1}$ therefore the minimal dose given was 0.3mg/kg while the maximal dose group was multiply by 2 therefore 2*0.3mg/kg $= 0.6mgkg^{-1}$

Seventeen male rats were divided into three (3) low dose groupsconsist of 6 rats (0.3mg/kg body weight. The high dose group consist of 6 rats (0.6mg/kg body

weight) and the control group consist of 5 rats, received2mls of distilled water. Treatment was done intra-gastrically daily via oral cannula for 25 days. At the end of drug-treatment period, the animals were anesthetized using intraperitoneal ketamine hydrocloride injection 20mg. then thoracolaparactomy was performed to open the thorax and the abdomen All surgeries were performed under sterile conditions, the epididymis was collected, blood was taken from the heart through the right ventricle, total body perfusion was done by using normal saline firstly to remove the blood from vascular space followed by the introduction of 4% paraformaldehyde for general body fixation which is then followed by the removal of necessary organs.

The epididymis, testes, seminal vesicles and prostate were obtained from each animal for semen analysis, biochemical and morphological analyses.The collected blood was centrifuged at 2000 rpm for 20 min. The supernatant was subsequently collected and analysed for hormonal bioassay (testosterone, LH, FSH).

Sperm Analysis

Sperm analysis was done on sample derived from the cauda epididymis by conventional methods. Succinctly, sperm motility was assessed by placing 10ul of sperm suspension on slide for microscopic evaluation at a magnification of 40X.Epididymal sperm were obtained by mincing the epididymis with anatomical scissors in 5 ml of physiological saline. Sperm counts were carried out using the Neubeurs Haemocytometer counting chamber and each chamber of the Neubauer haemocytometer was loaded with 10ul of diluted sperm and allowed to stand or settle for 5 minutes. Counting was done under a light microscope at 40X magnification and expressed as million/ml of suspension.

Progressivity was graded as follows:**A** – Excellent forward directional movement (EFDM),**B** – Good forward directional movement (GFDM),**C** – Fair forward directional movement (FFDM),**D** – Poor forward directional movement (PFDM)**(W.H.O,** 1976**).**

Tissue Processing For Microscopy

The testis was harvested and fixed in both 4% paraformaldehyde and formosaline solution for histopathological studies. The summary of the steps used in fixation are: fixation, dehydration, and clearing. Infiltration/Impregnation, Embedding, sectioning, staining and mounting. Histological processing was done as essentially as described by Akpantah et al., 2003.

Hormonal Assay Procedures

Similar procedure is required for all the hormones tested for, which is analysed usingImmunoenzymometric assay following the manufacturer's instruction.

Statistical Analysis

Data were analysed by analysis of variance (ANOVA) and were presented as Mean ± SEM. Using SPSS.

RESULTS

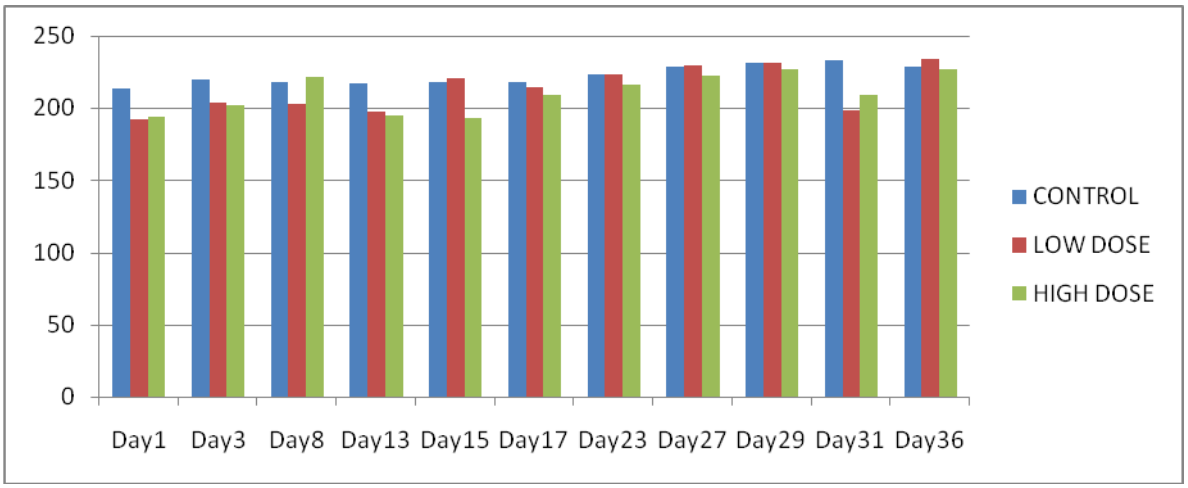

Figure 2: Bar chart showing mean body weight of each group before, during and prior to administration.

SEMEN ANALYSIS

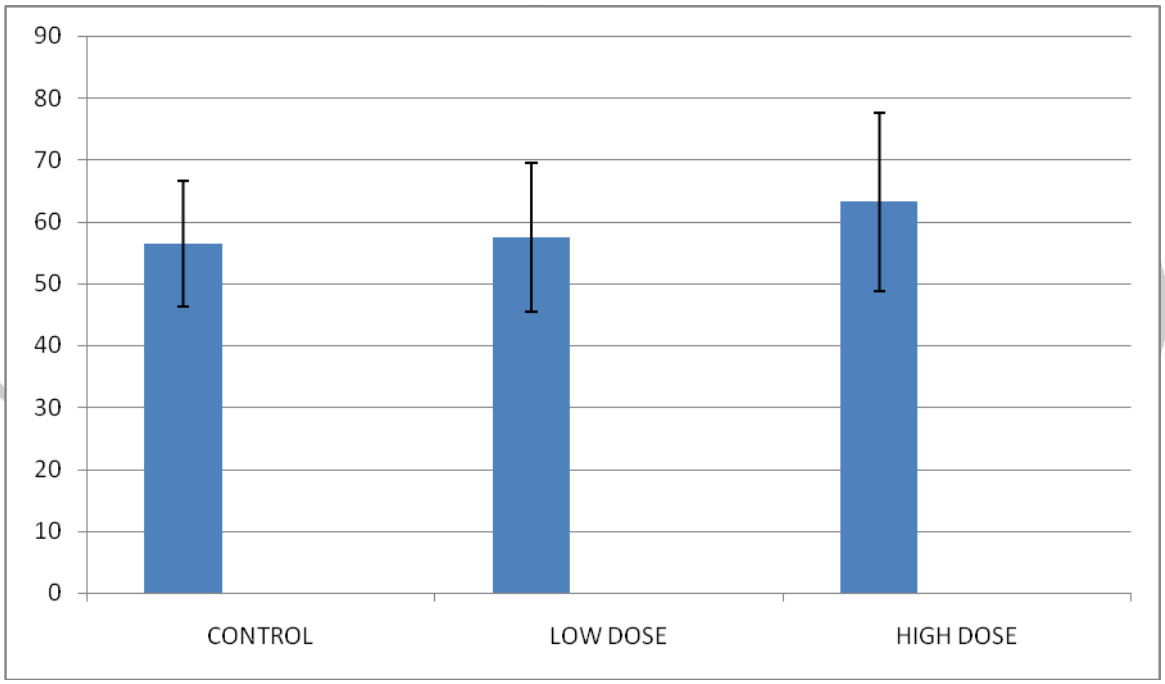

Figure 3: showing the sperm count of the animals after sacrifice; Sperm count (x10^6/ml):

Table1: showing the sperm count of the animals after sacrifice; Sperm count (x10^6/ml):

GROUP	MEAN±SEM
CONTROL	56.4000±10.19902
LOW DOSE	57.5333±11.99841
HIGH DOSE	63.1667±14.46554

WEIGHTS OF THE TESTES AFTER SACRIFICE

Table 2: showing the Mean Value of testicular weight Value expressed as mean ± SEM; p<0.05.

Groups	RIGHT (g)	LEFT
CONTROL	1.1200±0.08602	0.8200±0.22226
LOW DOSE	1.0000±0.14376	0.8500±0.13601
HIGH DOSE	1.1500±0.18753	1.0500±0.16073

PROGRESSIVITY

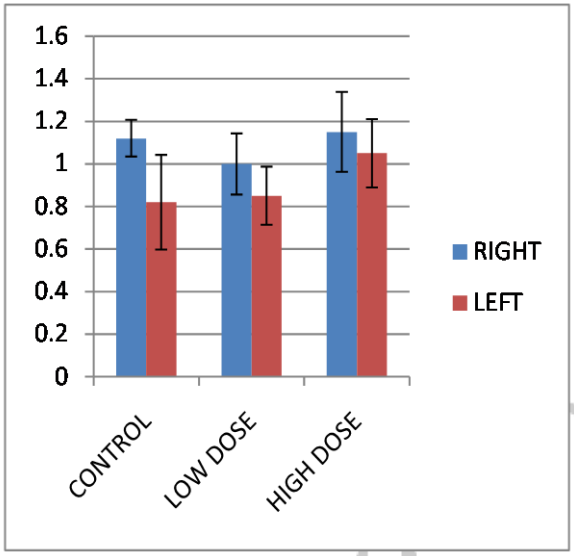

Figure 4:: showing the testicular weight of theanimals after sacrifice

Table 3: showing the progressivity of each animal in the three groups (*): pathological (diseased)

CONTROL DOSE GP	M3	M6	M9	M11	M17	
	BAD*	A	B	A	B	
MINIMAL DOSE GP	M1	M2	M4	M8	M10	M15
	D	B	A	D	A	B
MAXIMAL DOSE GP	M5	M7	M12	M13	M14	M16
	B	D	B	A	A	A

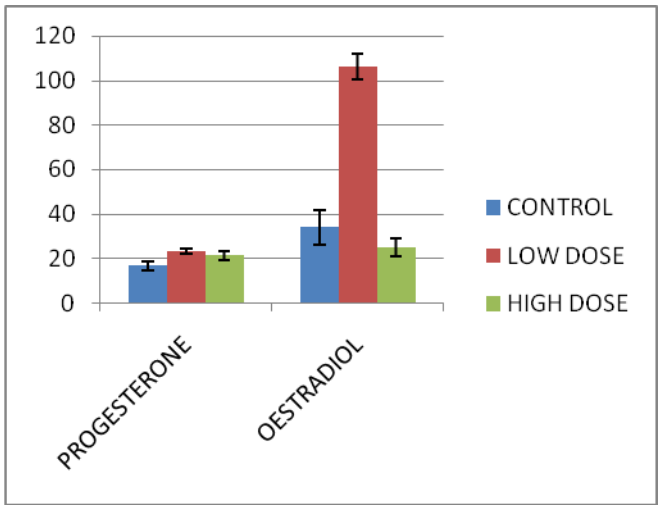

Figure 5:: showing the hormonal analysis of the animal in the three groups

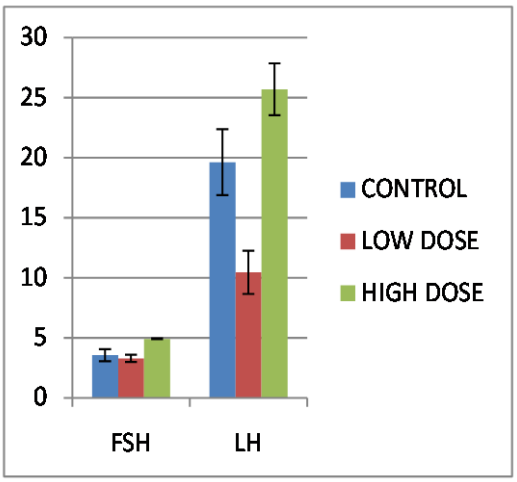

Figure 6:: showing the hormonal analysis of the animal in the three groups

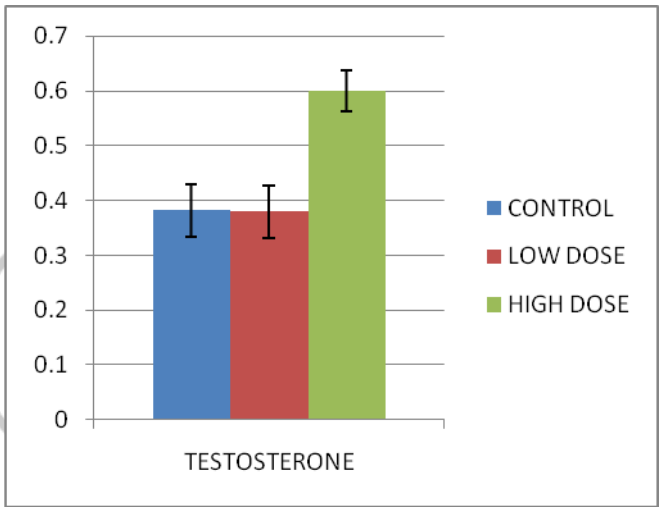

Figure 7: showing the hormonal analysis of the animal in the three groups

Table 4: showing the hormonal analysis of the animal in the three groups.

1. HORMONES	CONTROL	LOW DOSE	HIGH DOSE
PROGESTERONE	16.68±1.97646	23.1500±1.12153	21.2933±1.82049
OESTRADIOL	33.7600±7.69919	1.0625E2±5.68258	25.0000±3.87298
FSH	3.5600±0.50359	3.3000±0.29439	4.9200±0.05060
LH	19.6250±2.75397	10.4500±1.80555	25.7000±2.15870
TESTOSTERONE	0.3825±0.04779	0.3800±0.04761	0.6000±0.03651

HISTOLOGICAL OBSERVATION

Two different magnifications where used for each group, so as to obtain a detailed histological sequence of the seminiferous tubules.

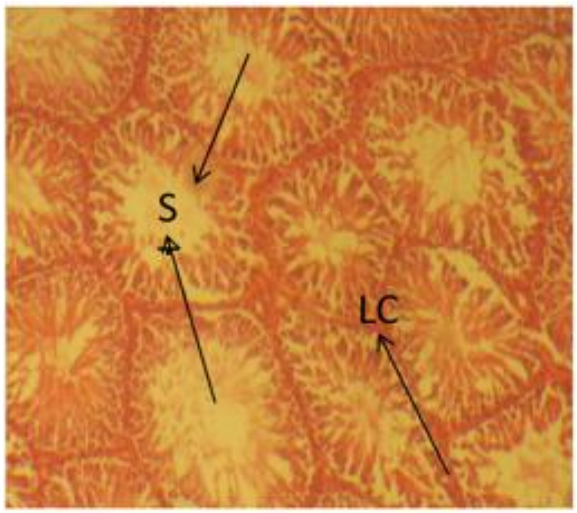

Figure 8: Photomicrograph of the transverse section of the testis of a rat in the control group (control grp, H&E x256) ST: Seminiferous Tubule, LC: Leydig cell

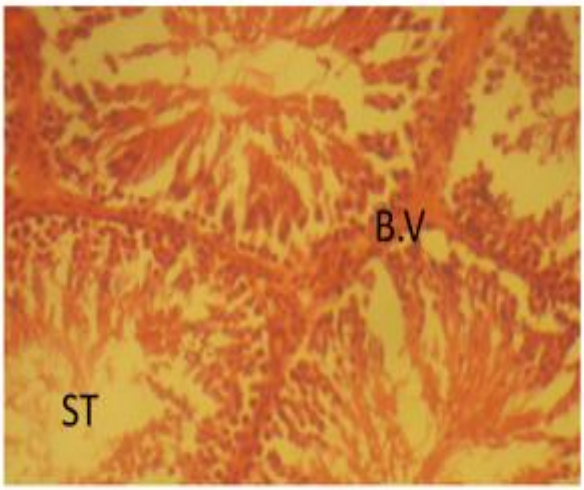

Figure 9: Photomicrograph of the transverse section of the testis of a rat in the control group (control grp, H&E x640) ST: Seminiferous Tubule, B.V: Blood vessel

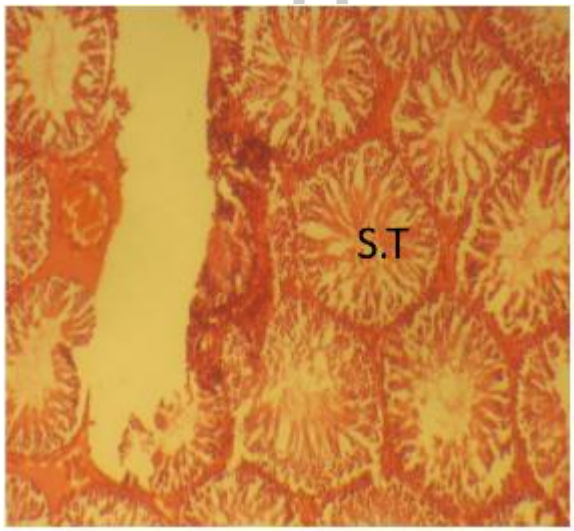

Figure 10: Photomicrograph of the transverse section of the testis of a rat in the low dose group (H&E x256)ST:

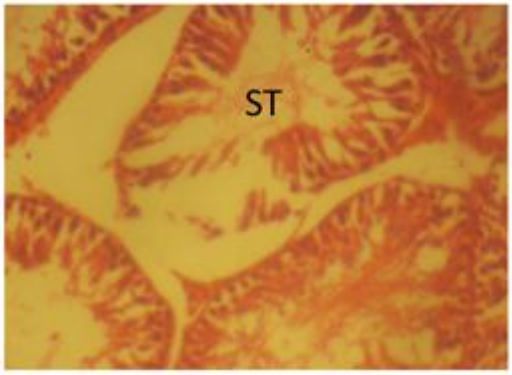

Figure 11: Photomicrograph of the transverse section of the testis of a rat in the low dose group (H&E x640)

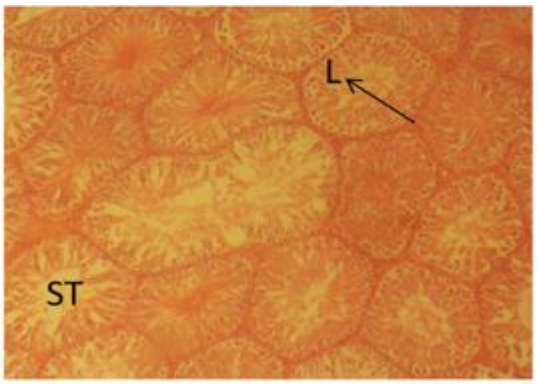

Figure 12: Photomicrograph of the transverse section of the testis of a Wistar rat in the high dose group (H&E x256)SP: Seminiferous tubule, L: Leydig cell

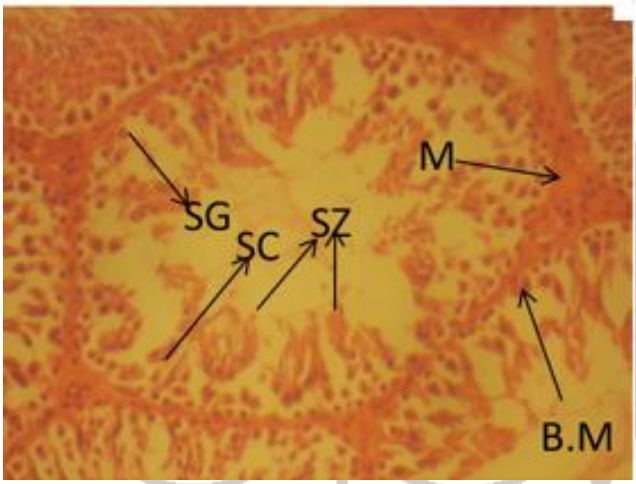

Figure 13: Photomicrograph of the transverse section of the testis of a rat in the high dose group (H&E x640) ST: Spermatids, SC: Spermatocytes,

SG: Spermatogonium, M: Myoid cells

B.M: Basement Membrane

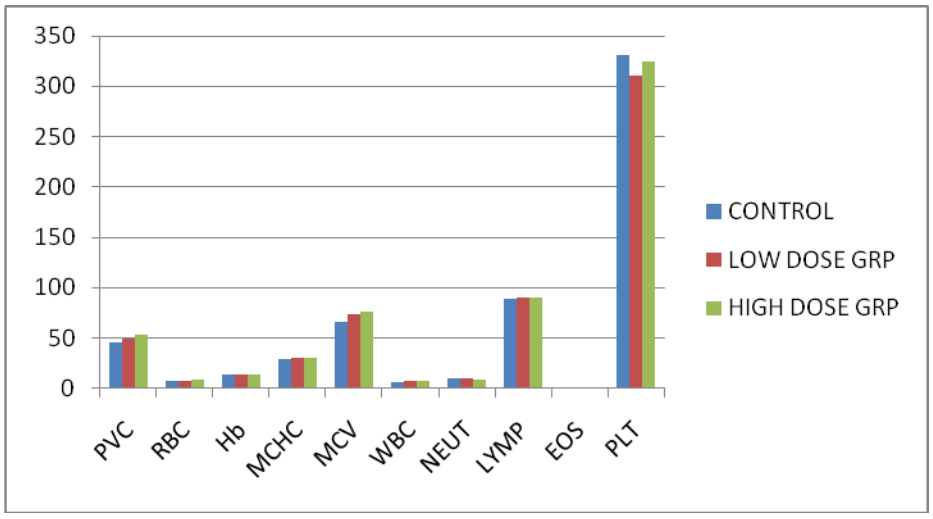

Figure 14: Chart showing the haematological analysis

DISCUSSION

After random selection of 17 male wistar rats into three different groups: control group given 2ml of distilled water, low dose group given 0.3mg/kg and high dose group given 0.3mg/kg. There was no significant difference in the body weight between the control and the treated groups (p<0.05). This was however, in contrary with the report of Olivari et al, which reported that the weight was significantly less after Nifedipine therapy.Furthermore, testicular weight was measured among the groups and compared, it was observed that there was no significant difference in testicular weight (p=0.528 and 0.375).

Weight changes may indicate physiological abnormalities in the function of certain organs or systems, which could further develop into serious health complications. Decrease in testicular mass is an index of reproductive toxicity and could indicate atrophic and degeneration of tissue (Metwally et al., 2011). It was thus, important that proper weight monitoring was done as one of the steps to ensure that the rats were in good health. This served to show also that patient undergoing Nifedipine therapy will very unlikely experience drastic and unhealthy weight changes.Sperm count was important to analyze the toxicity of the drug in study on the spermatozoa production in the gonads of the rats. Damage to cells involved in spermatogenesis or lowered sex hormones could be reasons behind changes in sperm production(Srinivasa & AnnGie 2013).Rats in the control and treated groups (low dose and high dose) had a mean sperm count of 56.4000±10.19902x10^6/ml, 57.5333±11.99841 x10^6/ml and 63.1667±14.46554 x10^6/ml. respectively, Despite a higher sperm count in the treated groups, the difference between the three groups was insignificant (p=0.726) to draw a conclusion that the drug affects sperm numbers, by increasing the quantity of sperms based on the dose given, thus, low dose increase slightly and at a higher dose, increases highly.Motility between rats of control and treated groups were similar to each group. Clearly, sperm motility was not affected by the treatment of Nifedipine. Furthermore, sperm quality depends on certain variables such as: the count, motility, progressivity and morphology. While the increase in sperm numbers will essentially increase the chances of thesperm fertilizing the oocyte, this relation will not be of importance if sperm motility was low. It is important that the sperm is motile in a progressive manner to be able to move up the cervix and along the fallopian tube to eventually encounter and fuse with an oocyte. Low sperm motility is often attributed to chemical-induced testicular toxicity.(Metwally et al., 2011).The three groups showed the same order in the number of abnormal sperm according to types of abnormality – sperm with banana-like form, folded on itself, without a hook, amorphous and lastly double-tailed. No sperm of the last category was observed.

However, sperm morphology influences the degree of its motility. The energy required for the sperm to be motile is

generated in the axoneme, whereas the tail is required for the progressive movement of the sperm. Hence, abnormalities in the anatomy of the sperm will largely impair its movements. An individual's sperm morphology may also reveal the overall health of the testes since its production is within the testis(Carreira et al., 2012)

The close-level of normality in the treated and control groups showed that Nifedipine does not affect the morphology of sperm, rather it slightly increases the quantity of sperm, the overall sperm analysis showed that Nifedipine did not adversely affect, if at all, the quality of sperm.

The testosterone level was significantly increased (p=0.012) in the high dose group when compare with the control. As testosterone is required in the later stages of spermatogenesis, this could possibly be one of the factors behind a slightly high mean sperm count in the treated group. The comparative mean concentration of testosterone between the control and treated (higher dose group) also indicates that Leydig cells were not damaged or adversely affected by Nifedipine.

The Follicle Stimulating Hormone level (FSH) was statistically significantly (p=0.028) increased in the higher dose group when compared with the control group, and would require a larger sample size to test its accuracy. It has been proposed that FSH levels increase as a result of seminiferous tissue damage due to higher inhibin secretion (Martin-du Pan R 2011) FSH is inversely proportional to spermatogonia population but the concentration of FSH is higher in the treated group, which also had a higher sperm count than the control group. Which value was statistically significant, this could be due to a higher incidence of spermatogonia which do not mature to become spermatozoa in the treated group. Elevated FSH concentration could also point to germinal epithelial damage and can be linked severe oligospermia or azoospermia of bad prognosis(Martin-du Pan R 2011).

The Luteinizing Hormone (LH) level was significantly increased (p=0.037) in the higher treated dose group compare with the control group LH is an important hormone as it stimulates Leydig cells to produce testosterone. The higher testosterone level in the treated group (higher dose) might have indicated that LH levels in the treated group were higher than the control too. This is, however, not the case. First of all, LH concentration of an individual cannot be determined with just a single test. Instead, it has to be done several times over a period of time as LH is secreted in bursts which vary from 30 minutes to 480 minutes. Secondly, it has a short half-life. Therefore, a single determination of LH can only be 50% accurate (Martin-du Pan R 2011).

High levels of LH may indicate hyperthyroidism or androgen resistance syndrome (Martin-du Pan R 2011) Despite some fluctuations with the treated groups in sperm count, testis weight and hormone levels, the difference could be used to draw a conclusion on such effects as they were statistically significant. And above hormone results

were in contrary with the work of(Almaida et al., 2000),who claimed,that calcium channel blockers had effect on the testicular weight, in that calcium channel blockers suppress spermatogenesis(Almaida et al., 2000),Also discovered was that calcium antagonist (amlodipine) used in the treatment of hypertension decreased the plasma follicle-stimulating hormone (FSH) and testosterone but not luteinizing hormone (LH) (Rabia et al., 2008)
Progesterone level is significantly increased (p=0.049) in the treated group (higher dose) compared with the control group.

The oestradiol level was highly significant (p=0.049) in the treated group (low dose) compared with the control.

Overall, it's surprising and interesting to realised that the findings of this research was in contrary with that of Iranloye et al, that claimed, that Nifedipine appears to have a deleterious effect on sperm functions in male rats which was not facilitated by a change in testosterone secretion. (Iranloye et al., 2009).

Histologically, using a light microscope at different magnifications it was observed that Nifedipine had a progressive destructive effect on the seminiferous tubule, however, the leydig cells which seem to be the testosterone producing site was unaltered. Thus the steroidogenesis process was left undisrupted, since there no disorganization, degeneration or destruction of the testicular histo-architecture. This findingstherefore corroborated (Morakinyo et al., 2011). However, perhaps if a more powerful microscope was used better and meaningful conclusion could be arrived at, histologically.

Haematologicallythe PCV was significantly increased (p=0.049) in the treated group (higher dose) while the level all other haematological parameters were statistically insignificant.

CONCLUSION

Many literatures had reported that antihypertensives especially calcium blockers have deleterious effects on the male reproductive functions. On the contrary, the results of our findings have proven otherwise. Nonetheless, findings appeared to be limited histologically. Nifedipine appeared to have shown damage on the testicular architecture.

In any case, the present study has shown with substantial evidence despite the limited microstructural analysis that CCB does not induce antifertility effects in the males.

RECOMENDATION

It is recommended that further work should be done to elucidate the toxicity effects of Nifedipine since; fertility seems to be a very critical issue in many homes. More so, more attention should be pay to testicular morphology of the testis in male adult wistar rats since disruption in it structure leads to unwanted effects..

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