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Biotin Supplementation Improved Reproductive Parameters Following Lead Induced Testicular Toxicity in Male Wistar Rats

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ABSTRACT

Lead acetate, a prominent heavy metal, is released into the environment by various industries like paint, ceramics, lead-containing pipes, and plastics. Exposure to lead acetate has adverse effects on numerous organs in the body, with a particular impact on the testes due to its distinctive vascular system. The present study investigated the potential protective effects of biotin on lead acetate-induced testicular damage in Wistar rats. Twenty (20) male Wistar rats were divided into 4 groups: control (animal feed daily with water); negative control (lead acetate 60 mg/kg); Positive control group(high-dose Biotin (80mg/kg) and the treatment group Lead acetate (60mg/kg)/high-dose Biotin (80mg/kg). After 28 days of administration, blood samples were collected for hormonal assay, and semen from the epididymis for semen profile. Testicular samples were also collected for histopathological studies. Results showed that lead acetate administration significantly decreased the sperm count, motility, viability, and altered histology of the testis (testicular damage, necrosis of seminiferous tubules, and loss of spermatid) compared to the negative control. However, the treatment group showed significantly improved histology of the testis, and increased sperm count, motility, and viability. From the results of this study, it could be concluded that biotin supplementation could provide a promising ameliorative effect against lead acetate-induced testicular toxicity.

KEYWORDS: Lead Acetate, Testicular damage, Biotin, Sperm parameters

ARTICLE DETAILS

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INTRODUCTION

Environmental pollution has continued to impose several health challenges globally thereby increasing economic burden on countries around the world (Tong et al 2000; Landrigan and Fuller, 2014; Landrigan et al. 2018). Lead is a widespread environmental contaminant, which play an etiological role in many human pathological conditions via the depletion of antioxidant defense mechanisms and genotoxic effects (Flora et al., 2012). The toxicity of lead has been reported decades before now (Needleman, 1988). Detection of environmental contaminants in human tissues, together with reports of a global decline in semen quality, further fueled speculation that human infertility rates are increasing and environmental toxicants are potentially important causal agents associated with this change (Foster, 2003; Krzastek et al., 2020).

Lead occurs naturally in the environment. However, most of the high levels found throughout the environment come from human activities. Environmental levels of lead have increased more than 1000-fold over the past three centuries as a result of human activity. The greatest increase occurred between the years 1950 and 2000, and reflected increasing worldwide use of leaded gasoline (Agency for Toxic Substances and Disease Registry [ATSDR], 2007). Lead does not have any detectable beneficial biological role, however on the contrary its detrimental effect on physiological, biochemical and behavioral dysfunctions have been documented in animals and humans by several investigators. Heavy metals poisoning is a major public health problem in the world. Lead is one of the metals that cause death and diseases especially in developing countries (Needleman, 2004). Its toxicity can originate from

contaminated air, water, food and dust. It is most common in children (Dapul & Laraque 2014).

Lead can cause disease in different organs in the body heavy metals may compromise male reproduction, as demonstrated by epidemiological and animal studies (Raj & Das 2023). According to a medical humanitarian organization, a toxic concentration of lead was associated with illegal mining of gold ore in Northern part of Nigeria (Zamfara State) which led to contamination of soil and household dust. This caused an estimated mortality rate as high as 40% among children and claimed over 400 lives (Tirima et al., 2016).

Searching for protective therapeutic agents against lead-induced reproductive toxicity has been a great interest in scientific research. Reproductive consequences of lead exposure are widespread (Patrick, 2006), affecting almost all aspects of reproduction (Kumar, 2018). Lead induces decreased sperm count, motility and increased morphological abnormalities in animals (Hsu et al., 1998; Vigeh et al., 2011; Akano et al. 2024).

Biotin is a water-soluble vitamin that is classified as a B-group vitamin. In mammals, biotin serves as an essential co-factor for four carboxylases involved in fatty acid synthesis, branched-chain amino acid metabolism, and gluconeogenesis (Gravel & Narang 2005; Solvik & Strand 2024). It is also crucial for maintaining reproductive functions and normal embryonic development (Dakshinamutri & Chauhan 1989; Sawamura et al., 2015). However, few studies have examined the effects of excessive amounts of biotin during growth periods in mammals.

Lead represents a significant ecological and public health concern due to its toxicity and its ability to accumulate in living organisms (Raj & Das 2023). Earlier studies have demonstrated that lead can pass through the blood testis barrier, accumulate in the testis and/or epididymis and seriously affect the spermatogonia, primary spermatocytes, spermatids or spermatozoa (germinal cells different levels of differentiation) (Hassan & Alam 2014). Several studies assessed the genotoxic effect of lead acetate (LA) by means of chromosomal aberrations and micronucleus test. Regarding the induction of chromosomal aberrations, LA induced significant increase of aberrant cells and numerical distortion in bone marrow cells of Wistar rats. Additionally, Abd El-Monem (2012) and El-Alfy et al., (2016) detected a significant increase of structural chromosomal aberrations in bone marrow cells and primary spermatocytes of albino mice bone marrow cells and primary spermatocytes of albino mice treated with LA. Further, LA proved to be a potent micronuclei inducer in vivo and in vitro test system (Onyeso et al., 2015).

There is little study investigating effects of biotin supplementation on induced lead testicular toxicity aimed to investigate the effects of biotin supplementation on testicular toxicity in males Wistar Rats. Thus, this study address the effects of using biotin supplement on the male productive organs associated with the current rate of alarming infertility in our environment when exposed to heavy metals like lead. Thus, this study will highlights the effects of treating testicular toxicity relative to the reproductive parameters in males using biotin supplement.

METHODOLOGY

Procurement of Animals

A total of twenty (20) adult male Wistar rats weighing 140-190g were obtained from Animal House of the University of Port Harcourt. The rats were kept in clean disinfected wooden cages with saw dust as beddings in the Animal House, with 12hours light/dark cycle and 50-60% humidity at a temperature of about 30^{o}C and were allowed to acclimatize to the new environment for one week, with free access to clean water and animal feed. The rats were weighed using an analytical weighing balance at commencement of the experiment.

Drugs and Reagents

Drug and reagents used for this study includes; Biotin and Lead. Biotin were purchased from Alpha Pharmacy and Stores, a registered pharmaceutical company in Port Harcourt, Rivers State. Leads were purchased from local chemical stores within Rivers State University.

Induction of Toxicity

Testicular Toxicity was induced in Male Wistar rats using lead acetate (PbCoo). The lead solution was prepared according to the method of (Onyeso et al., 2015) by diluting Lead acetate in water.

Experimental Design

On commencement of the experiment, the 20-adult male Wistar rats were randomly divided into six (4) groups of five (5) animals each. The protocol for administration of treatment to the experimental animals is as shown in the tabl

Table 3.1: Experimental Design

Group	No. of Rats	Administration/Treatment
Group 1 (Control)	5	Commercial Feed + Water *ad libitum*
Group 2 (Lead)	5	Commercial Feed + Water *ad libitum* + 60mg/kg of Lead for 4 weeks
Group 3 (Lead + Biotin)	5	Commercial Feed + Water *ad libitum* + 60mg/kg of Lead+ 80mg/kg of Biotin for 4 weeks
Group 4 (Biotin)	5	Commercial Feed + Water *ad libitum* + 80mg/kg of Biotin for 4 weeks

Sample Collection

After 4 weeks of treatment administration of Biotin and Lead, blood samples were collected from each group of the Wistar rats via the jugular vein into a plain sample bottle for hormonal assay and semen parameter profile.

Biochemical assay

The hormonal assay was done at the Department of Chemical Pathology, University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria. Using Microplate Reader Surgifield England(SM 300A) Machine.

Histological analysis

The testes of the adult male rats were harvested and fixed in 10% formalin for histological studies.

Ethical consideration

The study was carried out in accordance with the guidelines of the Organization for Economic Cooperation and Development (OED). The procedure and details of this study were approved by the Departmental Board of Human Physiology in the course of presentation of the proposal for this study.

Method of Data Analysis

Data obtained from the study were analyzed using the SPSS software version 25.0. One way analysis of variance (ANOVA) was used to analyze the means and significant differences. Comparisons between the groups were made using least significant difference (LSD) post Hoc tool. Differences at $P<0.05$ (95% confidence interval) were taken to be statistically significant.

RESULTS

Table 1: Effect of Biotin supplementation on semen parameters in lead-induced testicular toxicity in adult male Wistar rats

Parameters	Group 1 Control Feed and water	Group 2 Negative control Lead(60mg/kg)	Group 3 Lead(60mg/kg)/high-dose Biotin (80mg/kg)	Group 4 Biotin only
Appearance	Milky	Milky	Milky	Milky
Volume	0.27±0.03	0.15±0.29	0.27±0.03	0.20±0.00
PH	8.0	8.0	8.0	8.0
Viability	85±2.89	58.75±1.25	75±2.89	73.33±3.33
Viscosity	Normal	Normal	Normal	Normal
Normal Morph	85±2.89	58.75±1.25*	75±2.89	73.33±3.33*
Abnormal	15±2.89	41.25±1.25*	25±2.89*	26.67±3.33*
Actively Morph	80±2.89	53.75±1.25*	70±2.89	70±2.89
Sluggish	8.33±1.67	10±0.00	10±0.00	10±0.00
Dead	11.67±1.67	37.5±1.44*	20±2.89*	23.33±1.67*
Sperm count	450±28.87	117.5±11.82*	263.33±11.82*	283.33±44.09*

* Statistically significant at $p\leq0.05$ when compared to contro

Table 2: Effect of Biotin supplementation on reproductive hormones in lead-induced testicular toxicity in adult male Wistar rats

Group	FSH	LH	TET
Group 1	0.41±0.11	0.86±0.16	3.04±0.28
Group 2	0.16±0.15*	0.26±0.05*	0.71±0.06*
Group 3	0.18±0.04*	0.42±0.10*	2.95±0.48
Group 4	0.12±0.01*	0.24±0.03*	1.26±0.22*

* Statistically significant at p≤0.05 when compared to control

FSH = Follicle Stimulating Hormone

LH = Luteinizing Hormone

TET = Testosterone

Histology of the Testis

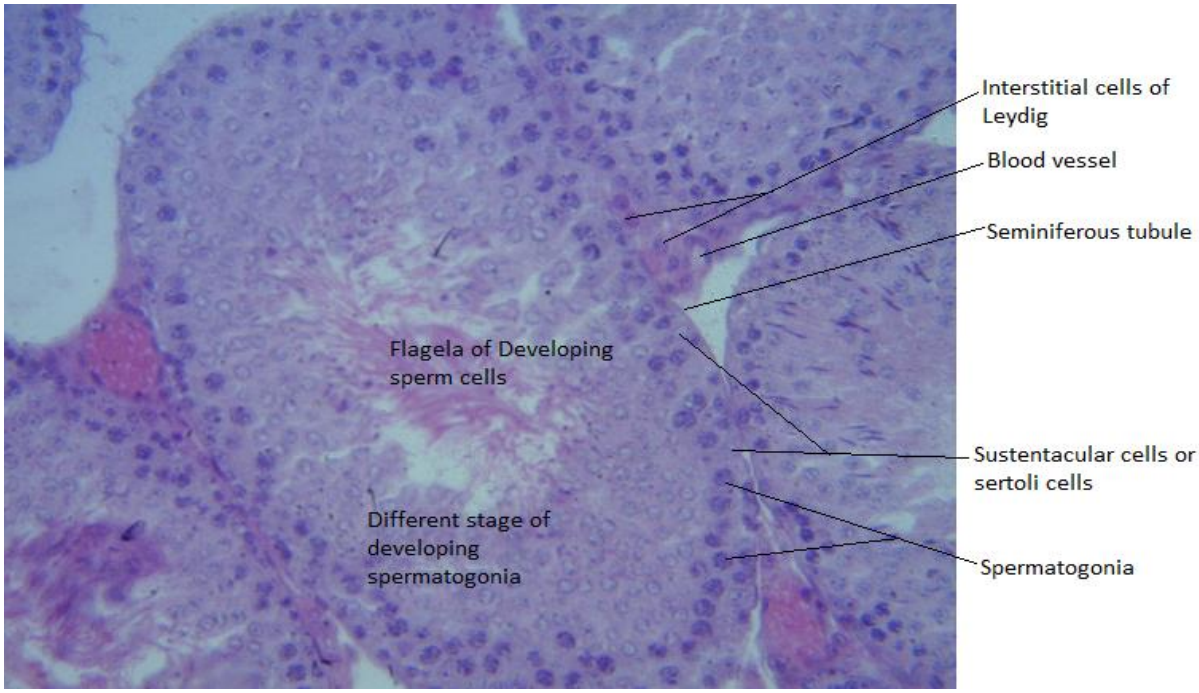
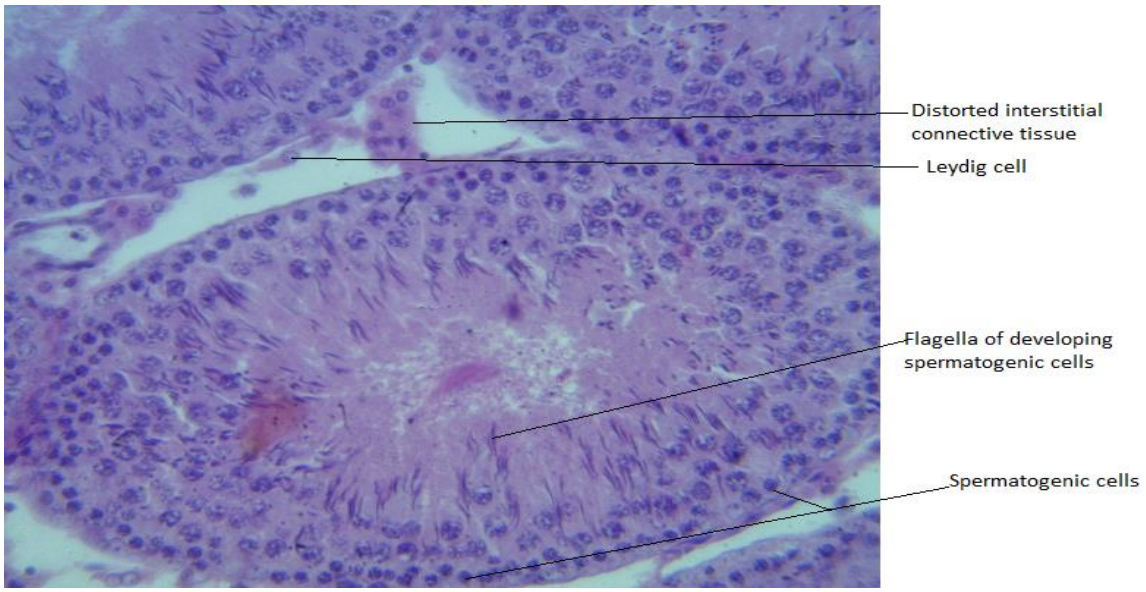
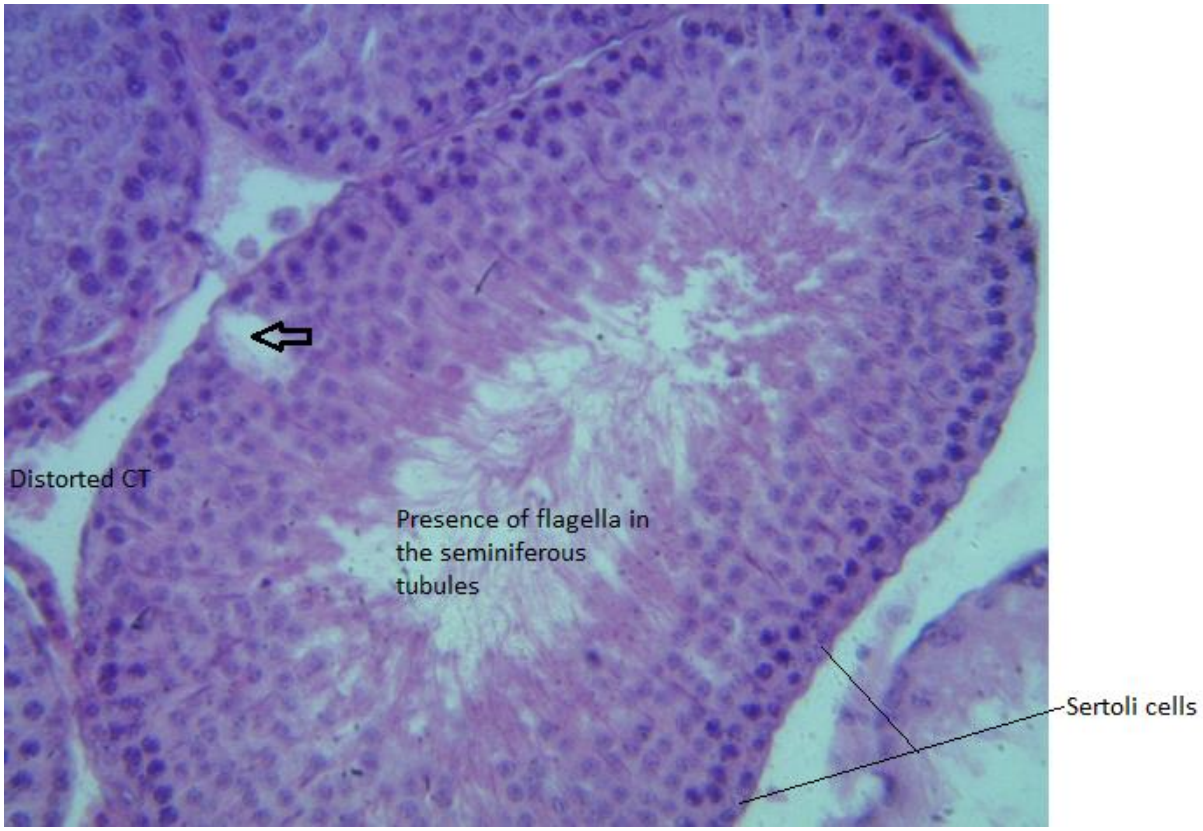


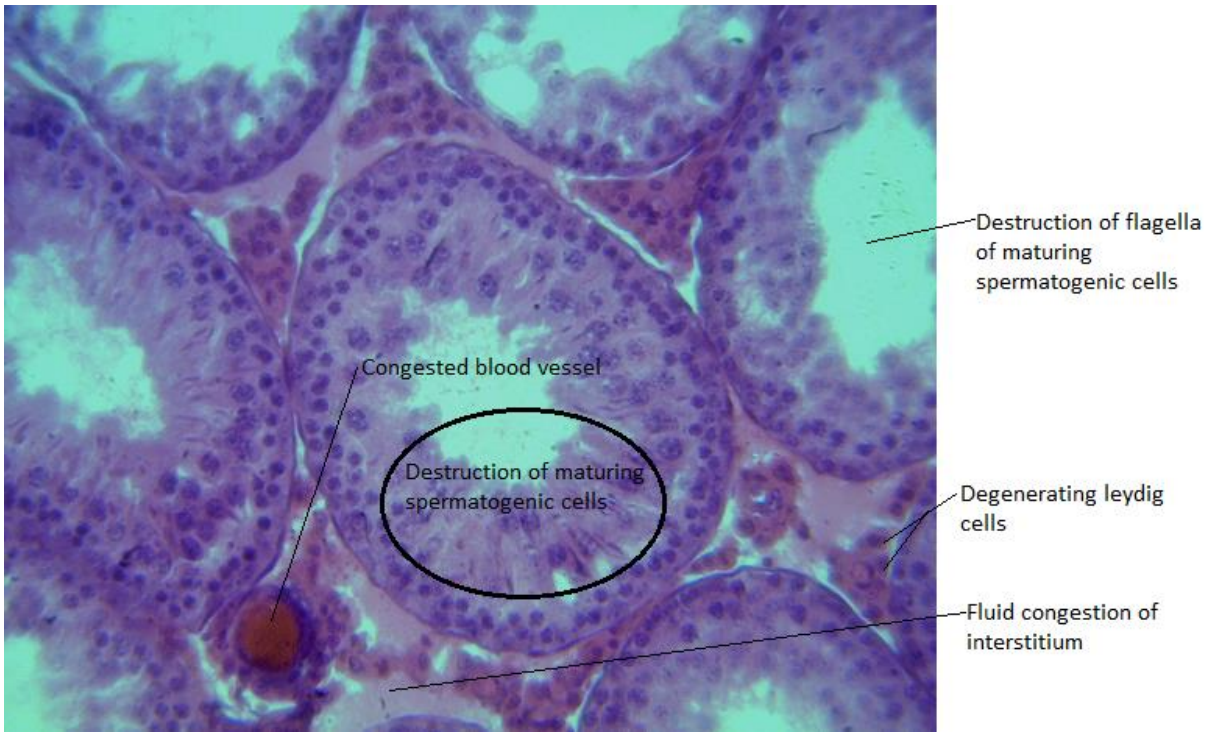
Figure 1: Photomicrograph of the testis showing well delineated cytoarchitecture features (Interstitial cells of Leydig, Blood vessels, sustentacular cells or Sertoli cells, developing spermatogenic cells). H & E, X400



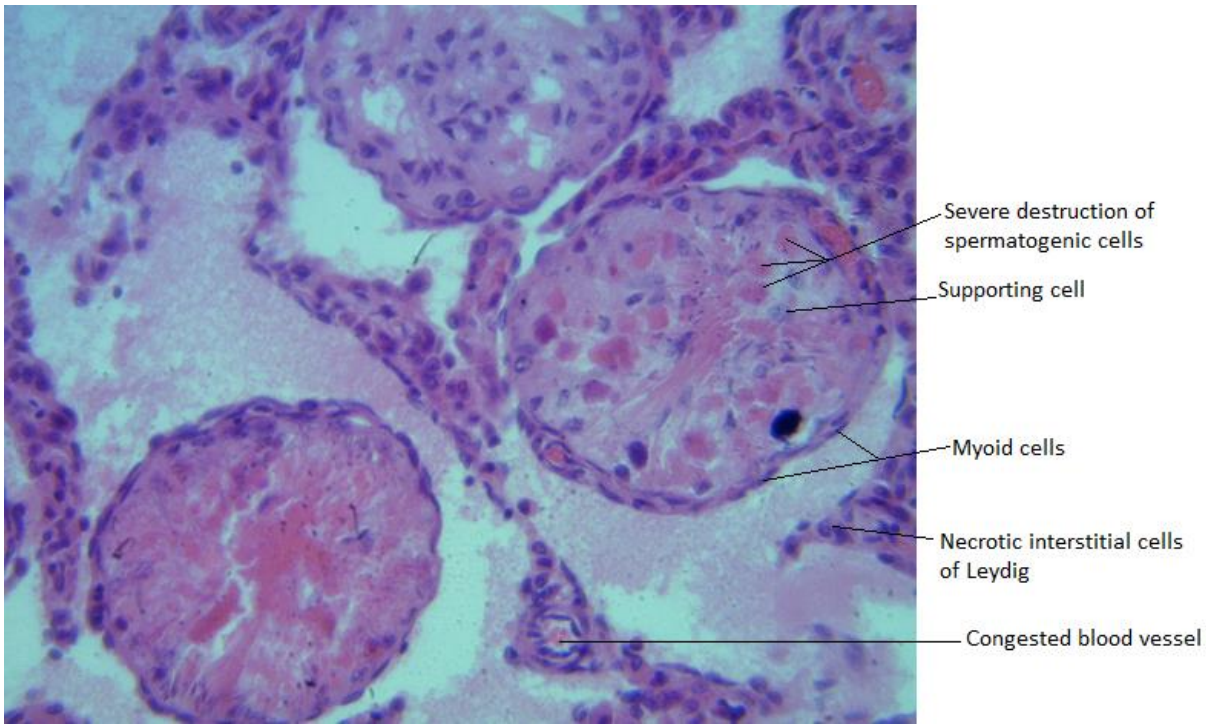
Photomicrograph of the testis showing distorted interstitial connective tissue with few observed Leydig cells. The cells in the seminiferous tubules do not show any observable histopathology. H & E, X400



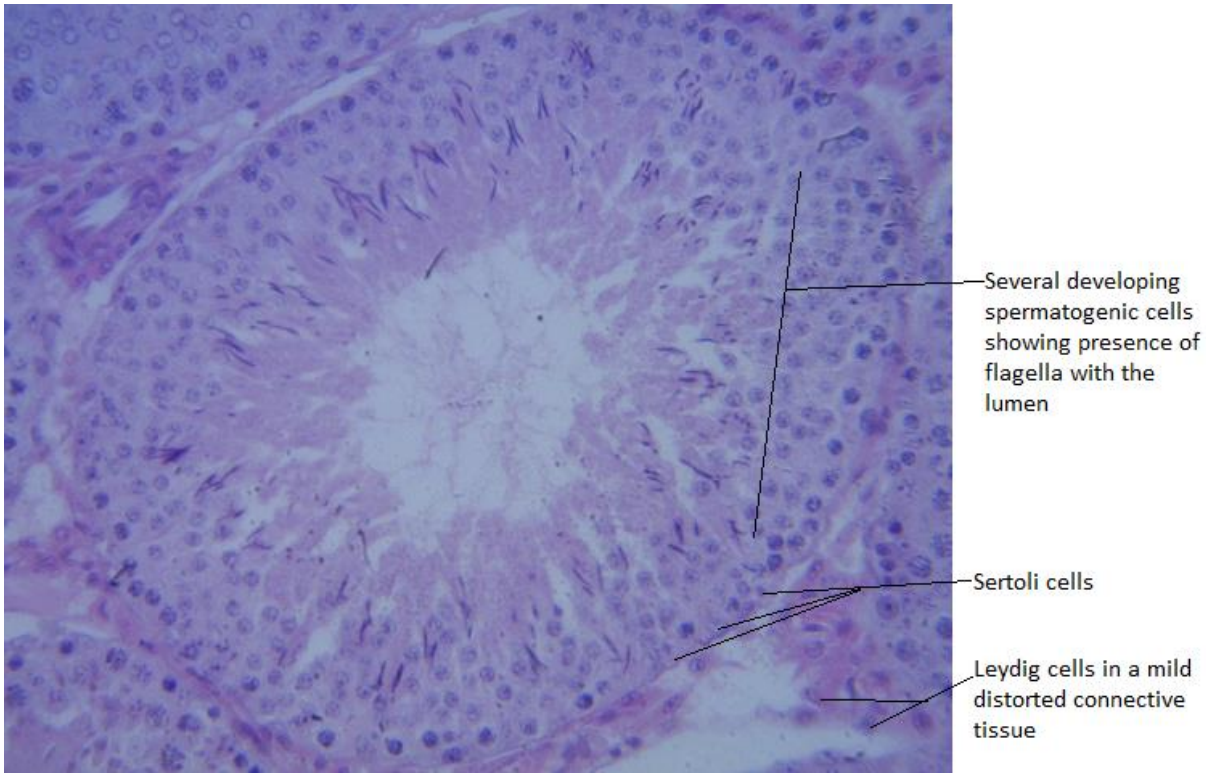
Photomicrograph of the testis showing distorted connective tissue (CT) within the interstitial space and vacuolation (black arrow) of the spermatogenic cell layer, H & E, X400



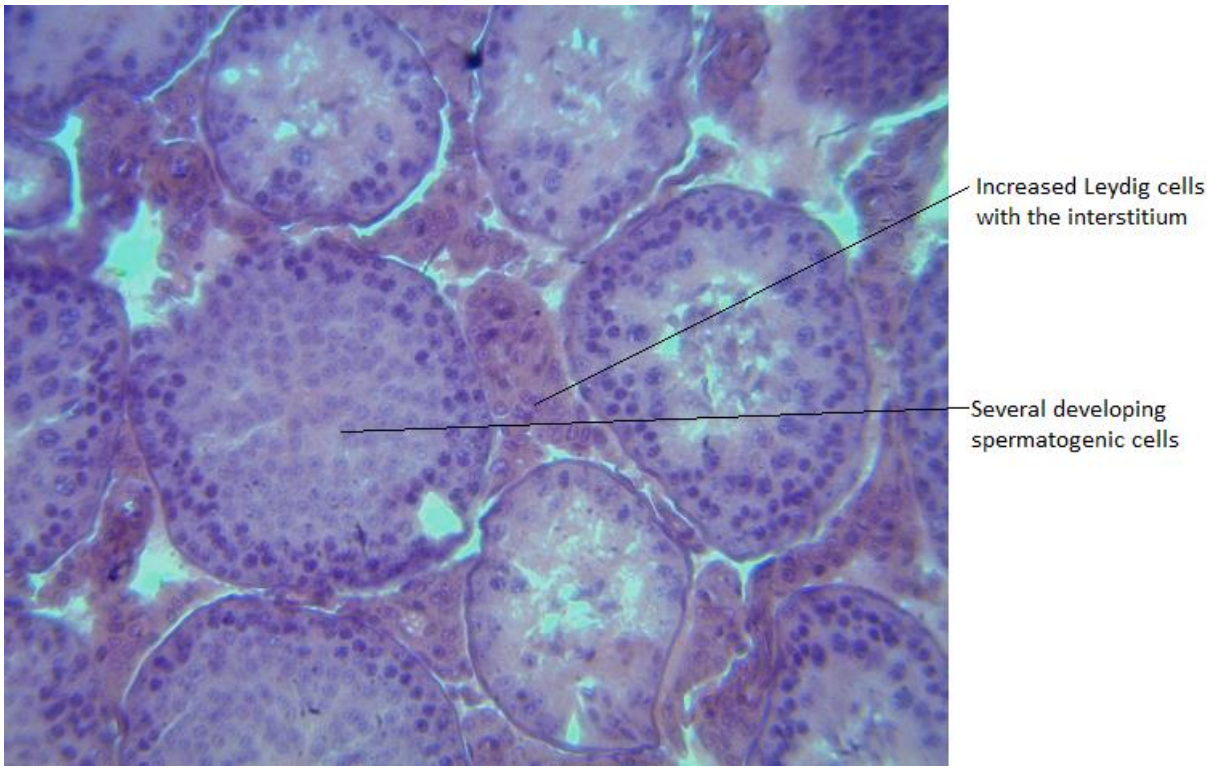
Photomicrograph of the testis showing destruction of flagella of maturing spermatogenic cells, degenerating Leydig cells and fluid congestion in the blood vessels. H & E, X400



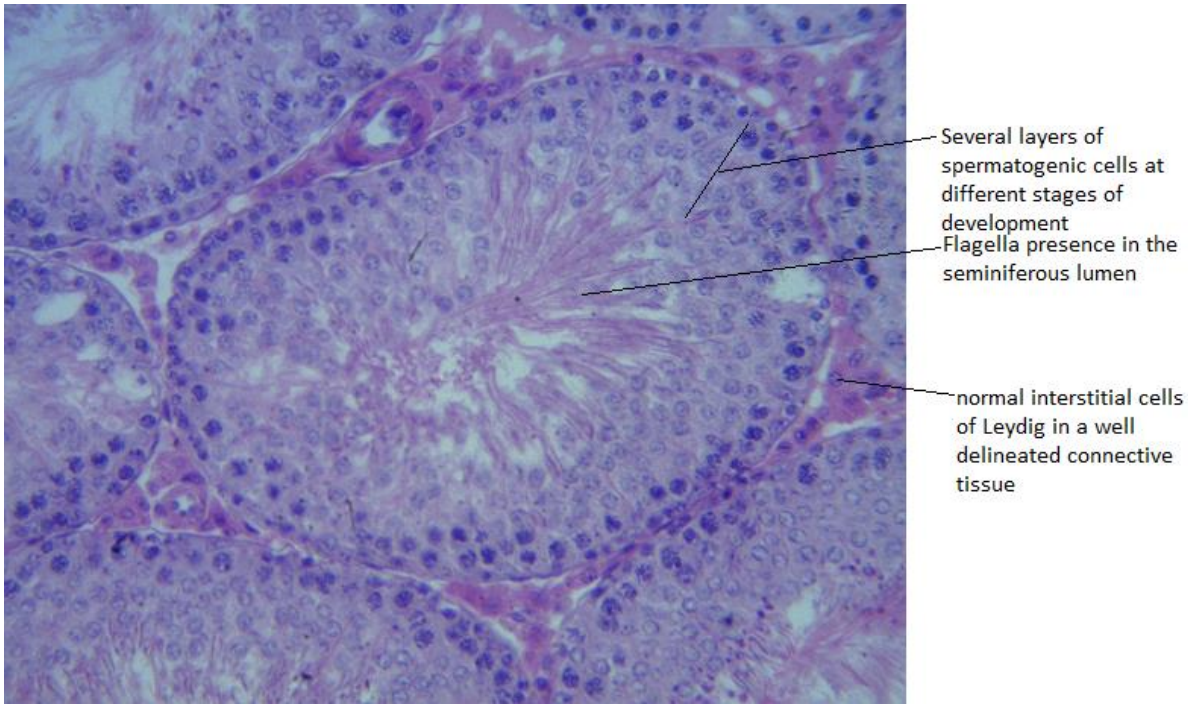
Photomicrograph of testis showing severe destruction of seminiferous tubule features. The spermatogenic cells are severely damaged with no trace of spermatogenic cells. Only pockets of supporting cells are visible. There is severe congestion of the blood vessels and connective tissue with appearance of Leydig cells necrosis. H & E, X400



Photomicrograph of the testis showing process of regeneration of destroyed spermatogenic cells, Interstitial cells of Leydig and connective tissue There are still pockets of interstitial tissue distortion . There is a process of recovery. H & E, X400



Photomicrograph of the testis showing process of regeneration of destroyed spermatogenic cells, Interstitial cells of Leydig and connective tissue. There is a process of recovery. H & E,400



Photomicrograph of the testis showing well structured cytoarchitecture and complete recovery of destroyed tissue. H & E, X400

DISCUSSION

This study was concerned with the evaluation of the effects of Biotin supplementation on reproductive parameters in Lead induced testicular toxicity in male Wistar rats. A number of studies reported that some factors, such as nutrients (Cheah & Yang 2011), stress (Zou et al., 2019; Li et al., 2020; Odetayo et al., 2024), and temperature (Hirano et al., 2022; Jorban et al., 2024), are involved in the regulation of spermatogenesis. Vitamins have an important role in the production of spermatozoa. For example, a deficiency in the water-soluble vitamin, vitamin B12, was shown to induce histological changes in the testes of rats, resulting in aplasia in sperm (Sawamura et al., 2015). A vitamin C deficiency also disturbs spermatogenesis; therefore, the dietary

prescription of vitamin C has been shown to protect germ cells from oxidative stress throughout spermatogenesis in humans (Angulo et al., 2011; Sawamura et al., 2015). Fat-soluble vitamins have also been associated with the endocrinological function and development of the testes. A previous study demonstrated that a vitamin D deficiency significantly reduced the mating ratio in rats, resulting in a low reproductive capacity (Nandi et al., 2016; Adamczewska et al., 2022). Vitamin A deficiency in rats has also been shown to lead to low serum testosterone levels and suppresses the ability of spermatogonia to differentiate (Hogarth et al., 2010; li et al., 2011). On the other hand, excess vitamin A levels in rats reduced the weight of the testes and enlarged the nuclei of spermatocytes (Sawamura et al., 2015). These Spermatogenesis is primarily dependent on testosterone, which has been linked to cell division and cell growth in spermatogenesis. Paulose et al. (1989) previously demonstrated that testicular and serum levels of testosterone were decreased in biotin-deficient rats. Thus, in addition to testosterone and follicle-stimulating hormones (FSH) for normal interactions in Leydig cells, Sertoli cells,and peritubular cells, biotin may be required in testicular development such as meiosis and sperm maturation (O'Shaughnessy et al., 2010). In the present study, it was observed that the testicular level of testosterone was not significantly decreased in the group 3 (e.i Lead+ biotin rats). Santillo et al.(2020) suggested that testosterone may not affect spermatogenesis directly. Song et al. (2011) demonstrated that the modification pattern of histone H3 was subjected to dynamic changes and was specific to a certain stage of germ cell differentiation during mouse spermatogenesis. Epigenetic effects on testicular function and spermatogenesis represents a new study field. Recently, a novel posttranslational modification has been identified: covalent binding of biotin to lysine residues in histones (Kothapalli et al.2005). Biotinylation of histones plays a role in cell proliferation, gene silencing and cellular response to DNA damage. Low levels of histone biotinylation have been linked to increased frequencyof retrotransposition events, suggesting a role for histone biotinylation in chromosomal stability (Chew et al. 2008).

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From the study it was observed that from table 4.2 on the effect of biotin on the reproductive hormones have shown that there were significant decrease in anterior pituitary hormones viz: (Follicle stimulating hormone FSH and Luteinizing hormone LH) when compared group C (i.e treated group of Lead+Biotin) to the control group A. From the analysis, it was observed that no significant difference in group C, which shown that Testosterone level was not affected in the present study. Thus, this is in agreement with the finding by (H. Sawamura et al., 2015) who reported long intake of high-dose Biotin inhibited spermatogenesisis in young rats with no significant difference in Testosterone level.

From the study, the results in table 4.1 on the effect of high-dose Biotin shows significant ($p<0.05$) decreased in semen parameters like sperm morphology, sperm count and increased in sperm death. This is in line with the finding of Pasten et al. (2019) who recently reported that the addition of pharmacological high dose biotin induced the increase of Spermatogonia layers and a loss of seminiferous tubules lumens in the testes.

CONCLUSION

It can be concluded from the current study that Biotin has ameliorative effects on the volume of sperms, PH of the sperms, viability of the sperm, viscosity of the sperm and Testosterone hormonal level associated with Lead induced testicular toxicity in adult male wistar rats.

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