ISSN: 2536-7153 SJMLS

Sokoto Journal of Medical Laboratory Science 2022; 7(3): 114 - 122

SJMLS - 7(3) - 010

Sperm Parameters and Histological Changes in Testes of Cadmium-exposed Rats Treated with *Hibiscus Sabdarrifa L.* Anthocyanins

*Osuvwe C. Orororo [1], Samuel O. Asagba [2], Egoamaka O. Egbune [2], Ofejiro I. Efejene [3]
Department of Chemical Sciences, Edwin Clark University, Kiagbodo, Delta State, Nigeria [1], Department of Biochemistry, Delta State University, Abraka, Nigeria [2], Department of Pharmacology, College of Medical and Health Sciences, Novena University, Ogume, Delta State, Nigeria [3].
Author for Correspondence *. osuvwec@yahoo.com/+234-806-230-6783/ORCID No: 0000-0002-9217-1530. https://dx.doi.org/10.4314/sokjmls.v7i3.11

Abstract

Given increase in infertility due to environmental pollutants, this study examined changes in sperm parameters and testicular histology of cadmium-exposed rats treated with *Hibiscus sabdarrifa* L. anthocyanins (HSA). Adult male Wistar rats were divided into 9 groups: A (Control), B (Cd), C (HS Aq Extract), D (Low Dose HSA), E (High Dose HSA), F (Low Dose (HSA Pre Cd), G (High Dose HSA Pre Cd), H (Low Dose HSA Post Cd), I (High Dose HSA Post Cd) and treated in two experimental periods (5 days acute toxicity study and 15 days chronic toxicity study). Exposure to Cd alone significantly (p<0.05) reduced sperm volume, motility, morphology and count for both treatment periods compared to the control, and rats administered high and low doses of HSA alone. However, the administration of high and low doses of HSA to Cd-exposed rats significantly altered (p<0.05) these parameters compared to rats exposed to Cd alone. Sections of the testes from control showed several seminiferous tubules disposed within an interstitium composed of interstitial cells of Leydig indicating normalcy. Conversely, section of Rats testes from rats exposed to Cd showed several small sized seminiferous tubules with predominantly roundish architecture indicating great congestion of interstitial blood vessels. The study showed that *exposure to Cd induced alterations in sperm parameters and also caused histological changes in the testes of rats. However, the* administration of high and low doses of HSA to Cd-exposed rats before and after Cd exposure significantly altered these parameters compared to rats exposed to Cd alone.

Keywords: *Sperm, Testis, Anthocyanins, Cadmium*

Introduction

It is an established scientific fact, and a serious global concern, that heavy metal contamination of the environment is a threat to human, animal and plant survival due to the ability of these metals to bio-accumulate and cause toxic effects in tissues (Samatha *et al*., 2020). One heavy metal that has gained global attention in recent years is cadmium (Cd), which is prevalent in the living environment due to its various industrial applications (*Das and Al-Naemi, 2019)*. Cd has been reported to affect several organs including the liver, kidneys, brain and testes, with the testes being extremely sensitive to Cd toxicity (*Genchi et al*., 2020). Cd has been linked to infertility as it can modify sperm progress motility, viability and other post-testicular events and has been implicated in the declining rate of fertility in developing countries (*Zhao et al., 2017)*. This metal can decrease testosterone levels in rats (*Monsef et al*., 2010; Orororo *et al*., 2018)**.** Akunna *et al*. (2017) also reported reduction in count, motility and normal spermatozoans due to Cd exposure. It has been reported that about 15% of couples suffer from infertility with the males accounting for approximately half of the cases (Latif *et al*., 2017). Apart from child bearing, infertility in males has other implications on the general population as it has been linked with greater occurrence of death (*Eisenberg et al*., 2016). Finding antidotes to Cd-induced toxicity including infertility is therefore a worthwhile endeavour.

The use of plants and their products in the treatment of ailments, though as old as man, has continued to receive scientific attention as many plants are been screened for their antioxidant

potentials (Onoja *et al*., 2021). Secondary plant metabolites such as flavonoids, saponins, alkaloids, terpenes, anthocyanins and vitamins are powerful antioxidants that help in eliminating free radicals and protect cells from the harmful effects of toxins (*Al-snafi, 2018; Chaves et al*., 2020).

*Hibiscus sabdariffa L. (*roselle) is rich in anthocyanins and has long been used in folk medicine in the treatment of various ailments including infertility (*Al-snafi, 2018*). *Hibiscus sabdariffa L. anthocyanins (HSA)* have been shown to ameliorate Cd-induced oxidative stress in the testes and changes in male reproductive hormones (Orororo *et al., 2018*a; *Orororo et al., 2018b)*, but studies on its influence on sperm parameters and histology of the testes are rare. Thus, this study was designed to examine sperm parameters and histological changes in testes of cadmium-exposed rats administered HSA.

Materials and Methods

Preparation of H. sabdariffa anthocyanins

Iyare and Adegoke (2008) documented the preparation of an aqueous extract of *H. sabdariffa* calyces. Anthocyanins from *H. sabdariffa* L. calyces were isolated using the technique of Hong and Wrolstad (*1990)*. Drust and Wrolstad's (*2001)* technique for HPLC analysis and identification of isolated anthocyanins was used. Details can be found in our earlier report (*Orororo et al., 2018)*.

Ethics approval

Ethical approval for this research was obtained from the ethical committee of the Delta State University, Abraka, Nigeria (DEL/FOS/ 2016/06/07) and laboratory animals were handled in accordance with the laboratory principles of handling and care of experimental animals.

Experimental animals

One hundred and twenty-six (126) adult male Wistar rats with average weight of 185g were used for the study. The rats were handled in accordance with international protocols for the handling of laboratory animals.

Experimental Design and Treatment of animals

Experimental rats were divided into 18 groups with seven (7) rats in each group. Nine groups were used for 5 days acute toxicity study while the other nine groups were used for the 15 days chronic toxicity study. Details are shown in Tables 1 and 2.

Table 1: Five (5) Days Acute Toxicity Study

GROUPS	DESCRIPTION
A	Control
B	Cd (5mg/kg b w)
C	Aq Extract (1g/kg b w)
D	Low Dose Anthocyanins (1g/kg b w)
E	High Dose Anthocyanins (3g/kg b w)
F	Low Dose HAS (1g/kg b w) for five consecutive days before a single dose of Cd (5mg/kg b w)
G	High Dose HSA (3g/kg bw) for 5 consecutive days before a single dose of Cd (5mg/kg bw)
H	Low Dose Cd (A single dose of Cd, 5mg/kg bw) on the first day then HSA (1g/kg bw) for 5 consecutive days
I	High Dos e Cd (A single dose of Cd. 5mg/kg bw) on the first day then HSA (3g/kg bw) for 5 consecutive days

Table 2: Fifteen (15) Days Chronic Toxicity Study

GROUPS	DESCRIPTION
A	Control
B	Cd (at a dose of three mg per Kg body weight) for 10 days
C	Aq Extract (1g/kg b w) for 10 days
D	Low Dose Anthocyanins (1g/kg b w)
E	High Dose Anthocyanins (3g/kg b w)
F	Low Dose HSA (1g/kg bw) for straight ten days then Cd (dosage as in group B) for the next five days
G	High Dose HSA (3g/kg bw) for 10 successive days then Cd (dosage as in group B) for the remaining five days
H	Low Dose Cd (3mg/kg bw) for the first five consecutive days then HSA (1g/kg bw) for the remaining ten days
I	High Dose Cd (3mg/kg bw) for the first five consecutive days then HSA (3g/kg body weight) for the remaining ten days

The rats were sacrificed when the experimental periods elapsed and samples (blood and testes) were obtained and processed for laboratory investigations.

Determination of Sperm Characteristics

Ekhoye *et al*. (2013) reported that sperm characteristics were assessed using the approach of Bearden *et al*. (1997). Spermatozoas were retrieved by cutting the cauda epididymis into tiny pieces using scissors. They were homogenized in a preheated Petri plate with 5 mL physiological saline and incubated at 370C for 2 minutes. After stirring the solution, one drop was put on a warmed microscope slide. A 22 × 22 mm cover slip was put over the droplet, and the proportion of motile sperm was measured using a light microscope at 400 x magnification. Other slides were produced and stained with 1% eosin B and 5% nigrosine in 3% sodium citrate dehydrate solution before being viewed at 400x magnification. The same solution was used for sperm counting with a Neubauer haemocytometer, as Strader *et al*. (1998) reported.

Histopathological Examination

Parts of the testes were removed and treated in a 10% formalin solution. Following that, the fixed tissues were treated, paraffin embedded, and sectioned (5-6). A light microscope was used to examine the sections, which were stained with haematoxylin and eosin.

Data Analysis

Data obtained for biochemical assay were subjected to differential statistics and the one-way analysis of variance (ANOVA) with the aid of the Statistical Package for Social Sciences (SPSS) software and are presented as Mean ± SD with difference between means considered significant at $p < 0.05$.

Results

Influence of H. sabdariffa anthocyanin on reproductive parameters/indices of Cd-exposure rats

Table 3 depicts the influence of anthocyanins from *H. sabdariffa* on sperm parameters of rats exposed to cadmium. Exposure to Cd alone (Group C) significantly ($p<0.05$) reduced sperm volume, motility, morphology and count for both treatments compared to the control (Group A), rats maintained on HS aqueous extract (Group C) and rats administered high and low doses of HSA alone (Groups D and E). In addition, administration of high and low doses of HSA to Cd-exposed rats before and after Cd exposure, significantly altered ($p<0.05$) these parameters compared with rats administered only Cd (Group B). In this regard, it was observed that high dose of the HSA produced better results compared to the low dose. This can be seen in the values obtained for sperm volume and sperm count in rats intoxicated with Cd and treated with high dose HSA (Group I) for both acute and sub-chronic treatments.

Table 3: Influence of anthocyanin from *H. sabdariffa* on sperm parameters of rats exposed to Cd

Groups	Sperm Parameters/ Characteristics			
	Volume (ml)	Motility (%)	Morphology (%)	Count (x1006cells/ml)
Acute Exposure				
A	1.10 ± 0.08a	79.34 ± 3.24a	50.02 ± 2.15a	54.40 ± 4.10a
B	0.68 ± 0.09b	45.88 ± 2.50b	25.04 ± 2.60b	34.56 ± 3.97b
C	1.09 ± 0.10a	75.28 ± 3.10a	48.01 ± 2.50a	55.00 ± 4.26a
D	1.00 ± 0.11a	76.34 ± 2.67a	46.03 ± 2.42a	52.34 ± 3.58a
E	1.02 ± 0.19a	74.33 ± 3.00a	47.05 ± 2.54a	50.03 ± 3.48a
F	0.82 ± 0.09c	55.56 ± 2.50c	35.12 ± 2.08c	43.78 ± 2.59c
G	0.84 ± 0.09c	64.00 ± 3.20d	34.23 ± 2.14c	46.88 ± 2.75c
H	0.80 ± 0.06c	60.90 ± 3.04d	36.43 ± 2.32c	49.82 ± 2.82c
I	0.98 ± 0.05a	65.08 ± 3.20d	38.56 ± 2.33c	50.00 ± 2.64a
Sub-chronic Exposure				
A	1.22 ± 0.08a	81.45 ± 1.20a	55.55 ± 1.89a	58.00 ± 4.10a
B	0.80 ± 0.09b	40.56 ± 2.00b	22.04 ± 2.10b	30.50 ± 3.97b
C	1.10 ± 0.10a	80.30 ± 2.45a	50.07 ± 2.30a	56.09 ± 4.26a
D	1.40 ± 0.11a	79.06 ± 2.55a	48.22 ± 1.99a	54.64 ± 3.58a
E	1.20 ± 0.19a	77.55 ± 1.60a	49.06 ± 1.86a	53.30 ± 3.48a
F	1.20 ± 0.03a	58.60 ± 1.80c	42.05 ± 2.04c	53.80 ± 2.59a
G	1.04 ± 0.09a	60.66 ± 3.00c	47.44 ± 2.11a	50.70 ± 2.75a
H	1.06 ± 0.06a	61.08 ± 3.00c	40.23 ± 0.98c	50.88 ± 2.82a
I	1.18 ± 0.05a	63.56 ± 2.50c	48.56 ± 1.00a	50.92 ± 2.64a

Values are presented as mean ± standard deviation (SD). n=3. Significant differences exist between values within the same column, but with unlike superscripts. ($P<0.05$) within the same treatment/exposure time. **Groups: A** (Control), **B** (Cd), **C** (Aqueous Extract), **D** (Low dose Anthocyanin), **E** (High Dose Anthocyanin), **F** (Low dose Anthocyanin Pre-Cd), **G** (High Dose Anthocyanin Pre-Cd), **H** (Low dose Anthocyanin Post- Cd), **I** (High dose Anthocyanin Post-Cd).

Influence of anthocyanins from H. sabdariffa the Histology of the Testes of Rats exposed to Cd

The influence of *H. sabdariffa* anthocyanin on the histology of the testes of Cd-exposed rats is shown in Figures 1 (acute toxicity) and 2 (chronic toxicity).

Section of the rat testes from Groups A (control); C (aqueous extract), D (Low dose Anthocyanin), E (High Dose Anthocyanin) and G (High Dose Anthocyanin Pre-Cd) showed several seminiferous tubules (ST) disposed within an interstitium composed of interstitial cells of Leydig (L). The tubules were lined by a germinal layer of cells ranging from spermatogonia (A) to spermatocyte (B and C) and spermatids (D) inter-mixed with Sertoli cells (S) indicating normalcy. This was observed for both the acute and sub-chronic treatments.

Conversely, section of rats testes from Groups B (Cd alone) and H (Low dose Anthocyanin Post-Cd) showed several small sized seminiferous tubules (ST) with predominantly roundish architecture indicating great congestion of interstitial blood vessels (BV). Again, this was observed for the two treatments.

Also, sections of the rats testes from Groups F (High Dose Anthocyanin Pre-Cd) and I (High dose Anthocyanin Post-Cd) for the sub-chronic treatment showed variably sized seminiferous tubules within a loose connective tissue. The germinal (G) layer however was intact indicating moderate degeneration of seminiferous tubules and loss of interstitial cells of Leydig.

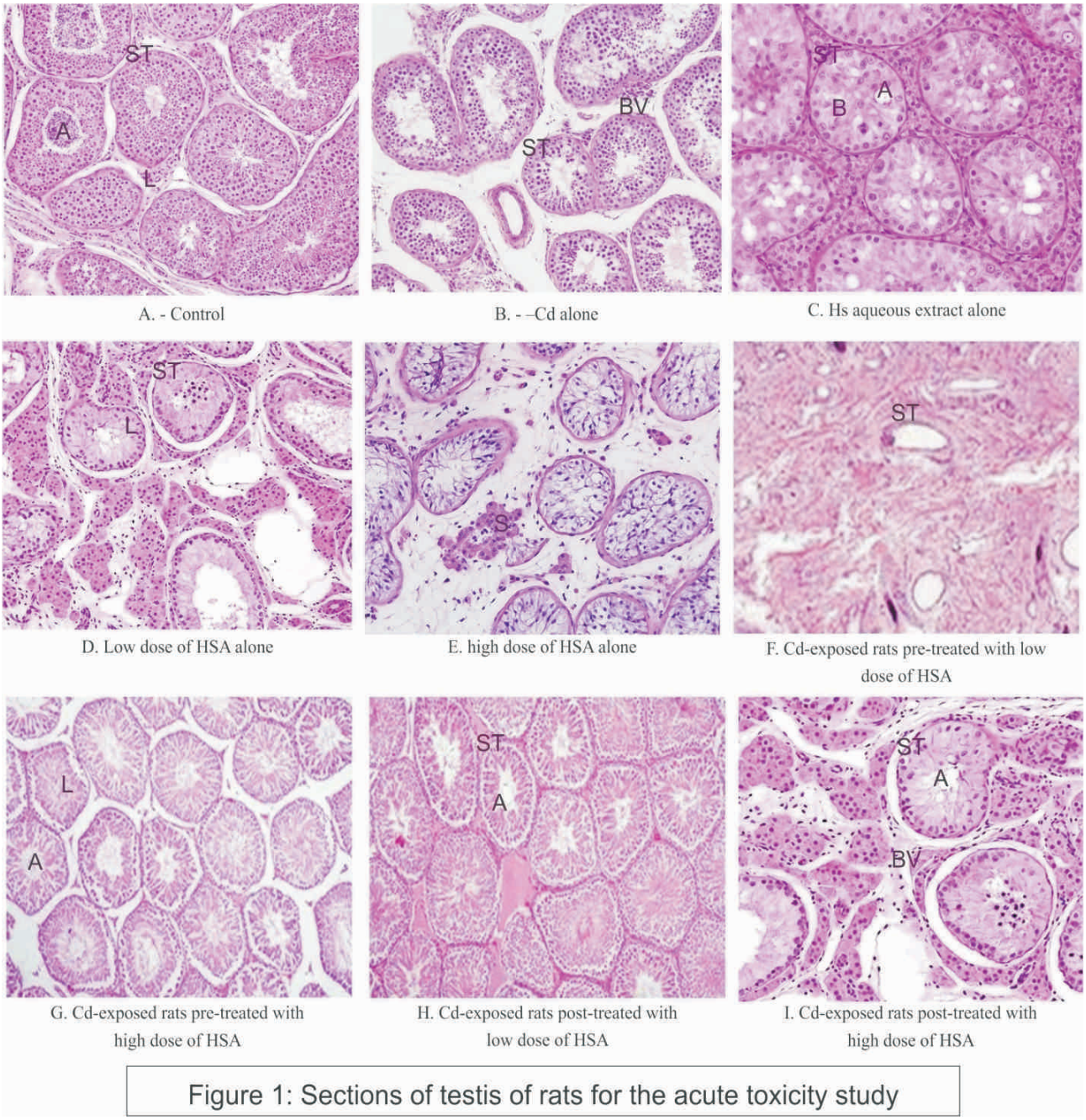

Figure 1: Sections of testis of rats for the acute toxicity study

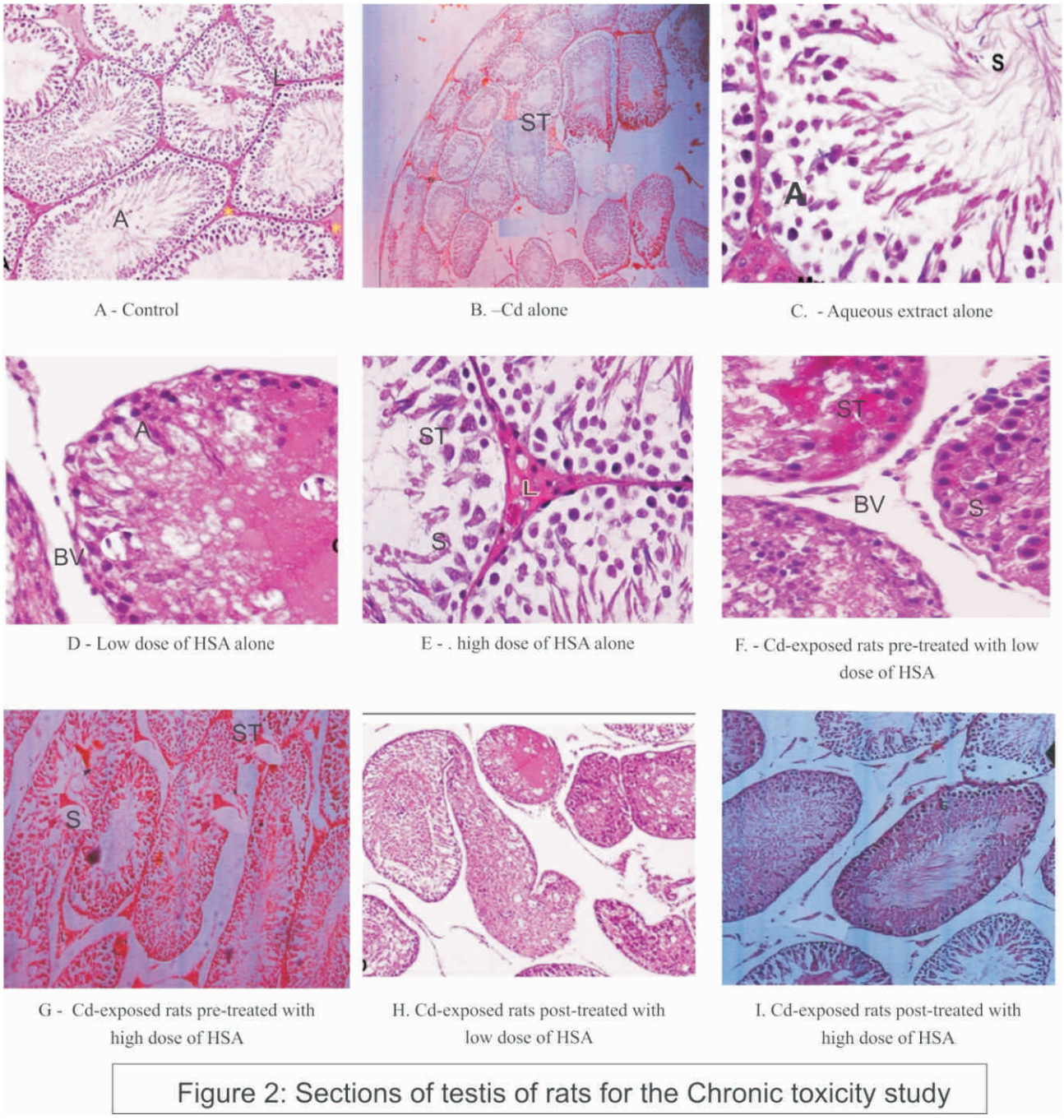

Figure 2: Sections of testis of rats for the Chronic toxicity study

Discussion

Influence of H. sabdariffa anthocyanin on Cd-induced Changes in Semen Parameters

Heavy metals generally have been observed to cause damages to reproductive organs. For this reason, the reproductive toxicity potentials of chemical pollutants have gained more attention recently as endocrine disruptive chemicals are increasingly accumulating in the environment (Kumar *et al*., 2016). As noted by Cheng *et al*. (2012), Cd disrupts endocrine system and is toxic to the reproductive system. It affects male fertility in many ways such as alteration of hypothalamic-pituitary-testicular signal relationship (Lafuente, 2013), direct spermiotoxic effects, interruption of blood-testis functional structures and serving as barrier to blood-epididymis relationship (Kumal *et al*., 2016; *Dub'e et al., 2018)*. All these lead to impairment of spermatogenesis and infertility.

Thus, Cd-induced changes in sperm parameters observed in this study (Table 3) are not surprising. Exposure to Cd alone significantly

(p<0.05) reduced sperm volume, motility, morphology and count for both acute and sub-chronic exposure compared to the control, HS aqueous extract group and rats given high and low doses of HSA alone. This agrees with the finding of Ekhoye *et al*. (2013) who reported that administration of Cadmium Chloride badly affected semen quality parameters. Mohamed *et al*. (2014) who studied the ameliorating effect of zinc and vitamin E on Cd-induced toxicity in the testes of adult albino rats also observed Cd-induced reduction in sperm quality, morphology and motility. In a similar study conducted by Qadori *et al*. (2012), Cd toxicity lowered sperm count and elevated the incidence of abnormal sperms and testicular lipid peroxidation.

Reduction in count, motility and normal cells due to Cd exposure has also been reported by Akunna *et al*. (2010) and Adamkovicova *et al*. (2016). It has been revealed that Cd-induced reactive oxygen species also disrupt Leydig cell mitochondria which leads to decline in testosterone synthesis and hence spermatogenesis (*Huang and Liu, 2004)*. The considerable decrease in sperm count, motility, and morphology seen in this research after Cd injection might be attributed to spermatogenesis impairment caused by decreased testosterone release.

In this study also, administration of high and low doses of HSA to Cd-exposed rats before and after Cd exposure, significantly ameliorated (p<0.05) Cd-reduced sperm parameters compared to rats intoxicated with Cd alone in a dose dependent manner. This can be credited to the antioxidant capability of the extract which countered Cd-induced damages to sperm.

Effect of anthocyanin from H. Sabdariffa on Cd-induced Changes Histology of Tissues

Histopathology is seen as a very reliable measure for the assessment of toxic effects of chemicals on cells and tissues (*Creasy et al., 2001; Lanning et al., 2002)*. In this regard, Awobajo *et al*. (2010) also noted that the use of histopathological assessments is prominent in diagnosing male reproductive risk and listed the organs that are often evaluated to include the testes, epididymis and prostate. Therefore, histological examination was carried out to determine the degree of tissue damage produced by Cd toxicity and the possible preventive and curative effects of pre-treatment and post-treatment with high and low doses of HSA respectively.

Sections of the testes from control rats, those administered Hs aqueous extract and high and low doses of HSA, showed the normal structure of the testis. Several seminiferous tubules (ST) disposed within an interstitium composed of interstitial cells of Leydig (L) were observed and the tubules were lined by a germinal layer of cells ranging from spermatogonia (A) to spermatocyte (B and C) and spermatids (D) inter-mixed with Sertoli cells (S) indicating normalcy.

Sections taken from the testes of cadmium chloride-treated rats, on the other hand, revealed several small sized seminiferous tubules (ST) with predominantly roundish architecture, indicating significant congestion of interstitial blood vessels (BV), degeneration of seminiferous tubules, and loss of interstitial cells of Leydig. This congestion may explain the decrease in testes/body weight ratio recorded for this group and is not surprising given the fact that the testes has been shown to be very sensitive to even low doses of Cd. Similar findings were reported in previous studies (*El-Shahat et al., 2009; Kamel et al., 2011Onoja et al., 2021)*.

The nuclei of Leydig cells in rats exposed to Cd looked smaller and unevenly delineated in the current investigation. These findings are consistent with the nuclear shrinkage of Leydig cells described by Blanco et al. (2007) and De Souza-Predes *et al*. (2010), who also discovered a decreased quantity of endoplasmic reticulum in Leydig cells. All of these explains the reduction in sperm production and sperm qualities seen in Cd-treated rats compared to controls.

Conclusion

Exposure to Cd caused alterations in sperm parameters and also caused histological changes in the testes of rats. However, the administrations of high and low doses of HSA to Cd-exposed rats before and after Cd exposure significantly altered these parameters compared to rats exposed to Cd alone. The ameliorative effects of HSA were dose dependent as the high dose of HSA produced better results compared to the low dose.

Conflict of Interest:
The authors declare that they have no conflict of interest.

Authors' contributions
This research was initiated, projected and developed by OCO and SOA. OCO and OIE wrote and edited the manuscript for publication.

References

Adamkovicova, M., Toman, R., Martiniakova, M., Omelk, A.R., Babosova, R., Krajcovicova, V., Grosskopf, B., Massanyi, P. (2016). Sperm motility and morphology changes in rats exposed to cadmium and diazinon. Reproductive Biology and Endocrinology; 2016: 14-42.

Akunna, G.G., Obikili, E.N., Anyawu, G.E., Esom, E.A. (2017). Evidences for spermatozoa toxicity and oxidative damage of cadmium exposure in rats. Journal of Pharmacology and Toxicology; 12:50-56.

Al-snafi, A.E. (2018). Pharmacological and therapeutic importance of Hibiscus sabdariffa- A review. International Journal of Pharmacy Research; 10 (3): 451-475.

Awobajo, F.O., Raji, Y., Akinloye, A.K. (2010). Histomorphometric Changes in the Testes and Epididymis of Wistar Strain Albino Rats Following Fourteen Days Oral Administration of Therapeutic Doses of Some Antibiotics. International Journal of Morphology; 28(4):1281-1287.

Bearden, H.J., Fuquay, J.W. (1997). Semen evaluation. Applied animal reproduction. New Jersey 7 Prentice Hall: 168-182.

Blanco, A., Moyano, R., Vivo, J., Flores-Acuna, R., Molina, A., Blanco, C., Aguera, E., Monterde, J.G. (2007). Quantitative changes in the testicular structure in mice exposed to low doses of cadmium. Environmental Toxicology and Pharmacology; 23:96–101.

Chaves, N., Santiago, A., Alias, J.C. (2020). Quantification of the antioxidant activity of plant extracts, analysis of sensitivity and hierarchization based on the method used. Antioxidants (Basel); 9(1):76.

Cheng, Y.T., Wu, C.H., Ho, C.Y. (2012). Catechin protects against ketoprofen-induced oxidative damage of the gastric mucosa by up-regulating Nrf2 in vitro and in vivo. Journal of Nutrition and Biochemistry; 24:475–483.

Creasy, D.M. (2001). Pathogenesis of male reproductive toxicity. Toxicology and Pathology: 29(1): 64–76.

Das, S.C., Al-Naemi, H.A. (2019). Cadmium Toxicity: Oxidative Stress, Inflammation and Tissue Injury. Occupational Disease and Environmental Medicine; 7:144-163.

De Souza-Predes, F., Diamante, M.A., Dolder, H. (2010). Testis response to low doses of cadmium in Wistar rats. International Journal of Experimental Pathology; 91:125-131.

Drust, R.W., Wrolstad, R.E. (2001). Separation and characterization of Anthocyanins by HPLC. In current Protocols in Foods Analytical Chemistry, Wrolstad, R. E., Eds., John Wiley and Sons: New York, 2001: 1-13.

Dub'e, E., Hermo, L., Chan, P.T., Cyr, D.G. (2013). Alterations in the human blood-epididymis barrier in obstructive azoospermia and the development of novel epididymal cell lines from infertile men. Biology and Reproduction; 83:584-596.

Eisenberg, M.L., Li, S., Cullen, M.R., Baker, L.C. (2016). Increased risk of incident chronic medical conditions in infertile men: analysis of United States claims data. Fertility and Sterility; 105: 629–636.

Ekhoye, E.I., Nwangwa, E.K., Aloamaka, C.P. (2013). Changes in some testicular biometric parameters and testicular function in cadmium chloride administered Wistar rats. British Journal of Medicine and Medical Research; 3:2031-2041.

El-Shahat AER, Gabr A, Meki AR, Mehana ES. Altered testicular morphology and oxidative stress induced by cadmium in experimental rats and protective effect of simultaneous green tea extract. Int J Morpho; 2009; 27:757–764.

Genchi, G., Sinicropi, M.S., Lauria, G., Carocci, A., Catalano, A. (2020). The Effects of Cadmium Toxicity. International Journal of Environmental Research and Public Health; 17:3782-3790.

Hong, V., Wrolstad, R.E. (1990). Use of HPLC separation/photodiode array detection for characterization of anthocyanins. Journal of Agricultural Food Chemistry;38: 708-715.

Huang, B.M., Liu, M.Y. (2004). Inhibitory actions of lead on steroidogenesis in MA-10 mouse leydig tumor cells. Archives of Andrology; 50(1): 5-9.

Iyare, E.E., Adegoke, O.A. (2008). Maternal consumption of an aqueous extract of Hibiscus sabdariffa during lactation accelerates postnatal weight and delays onset of puberty in female

offspring. Nigerian Journal of Physiological Science; 23(1-2):89–94.

Kamel, M.L., Abeer, H., El-Razek, A., Kawkab, A., Ahme, F., Gehan, M.K. (2011). Exposure of Adult Male Rats to Cadmium: Assessment of Sexual Behaviour, Fertility, Aggression as well as Anxiety like Behaviour with Special Reference to Biochemical and Pathological Alterations. Life Science Journal; 8(2): 106-119.

Kumar, B.A., Reddy, A.G., Kumar, P.R., Reddy, Y.R., Rao, T.M., Haritha, C. Protective role of N-Acetyl L-Cysteine against reproductive toxicity due to interaction of lead and cadmium in male Wistar rats. Journal of Natural Science and Biological Medicine; 4 (2):414-419.

Lafuente, A. (2013). The hypothalamic-pituitary-gonadal axis is target of cadmium toxicity. An update of recent studies and potential therapeutic approaches. Food Chemical Toxicology; 59:395-404.

Lanning, L.L., Creasy, D.M., Chapin, R.E. (2002). Recommended approaches for the evaluation of testicular and epididymal toxicity. Toxicology and Pathology; 30(4):507–520.

Latif, T., Kold Jensen, T., Mehlsen, J., Holmboe, S.A., Brinth, L., Pors, K., Skouby, S.O., Jørgensen, N., Lindahl-Jacobsen, R. (2017). Semen quality as a predictor of subsequent morbidity: A Danish cohort study of 4,712 men with long-term follow-up. American Journal of Epidemiology; 186: 910–917.

Mohamed, S., Othman, A.N., Zaki, H.S., Abdel, A.E. (2014). Moneim Effect of Physalis peruviana L. on Cadmium-Induced Testicular Toxicity in Rats. Biological Trace Element Research; 159:278–287.

Monsefi, M., Alaee, S., Moradshahi, A. (2010). Cadmium-induced infertility in male mice. Environmental Toxicology; 25:94–102.

Onoja, R.I., Chinwe, U.C., Ugwueze, E.U., Anyogu, D.C., Obidah, W., Emesiani, B.I. (2021). Effect of Thymus vulgaris leaf extract on cadmium-induced testicular toxicity in rats. Bulletin of National Research Centre; 45:125.

Orororo, O.C., Asagba, S.O., Tonukari, N.J., Okandeji, O.J., Mbanugo, J.J. (2018). Cadmium-Induced Testicular Damage in Wistar Rats: Protective Effects of Hibiscus sabdariffa L. Anthocyanins. International Journal of Biochemistry Research and Review; 21(4): 1-8.

Orororo, O.C., Asagba, S.O., Tonukari, N.J., Okandeji, O.J., Mbanugo, J.J. (2018). Effects of Hibiscus Sabdarrifa L. Anthocyanins on Cadmium-Induced Oxidative Stress in Wistar Rats. Journal of Applied Science and Environmental Management; 22 (4): 465 – 470.

Orororo, O.C., Asagba, S.O., Tonukari, N.J., Okandeji, O.J., Mbanugo, J.J. (2018). Hibiscus sabdarrifa L. Anthocyanins-Induced Changes in Reproductive Hormones of Cadmium-Exposed Rats. International Journal of Science Research Publication; 12(4):308-311.

Qadori, Y.T., Mahera, N., Al-shaikh, D. (2012). Effects of high and low dose of cadmium chloride on male Reproductive system in mice. Journal of Faculty of Medicine Baghdad; 53(1):1-5.

Samatha, K., Girish, B.P., Sreenivasula, R.P. (2020). Embryonic cadmium exposure of male rats alters reproductive functions at adulthood, but without overt alterations in developmental and behavioral outcomes and metabolism. Toxicology Research Application; 4:1–11.

Strader, C.D., Hwa, J.J., Van, M.H., Parker, E.M. (1998). Novel molecular targets for the treatment of obesity. Drug Discovery; 3: 250–256.

Zhao, L.L., Ru, Y.F., Liu, M., Tang, J.N., Zheng, J.F., Wu, B., Gu, Y.H., Shi, H.J. (2017). Reproductive effects of cadmium on sperm function and early embryonic development in vitro. PLoS ONE;12: e0186727.

Citation: Osuvwe C. Orororo, Samuel O. Asagba, Egoamaka O. Egbune, Ofejiro I. Efejene. Sperm Parameters and Histological Changes in Testes of Cadmium-exposed Rats Treated with *Hibiscus Sabdarrifa L.* Anthocyanins. ***Sokoto Journal of Medical Laboratory Science*; 7(3): 114 - 122.**
https://dx.doi.org/10.4314/sokjmls.v7i3.11