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HISTOLOGICAL CHANGES IN RAT TESTICLES INDUCED BY DIFFERENT CONCENTRATION OF CADMIUM CHLORIDE

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ABSTRACT

Cadmium chloride considered the stimulates effect to production of reactive oxygen species and causes tissue damage in various tissue. Thus tis paper for investigation the effect of cadmium chloride and determine the effective tissue and reversible tissue on testicular tissue Fifty Wistar albino rats were used which they were divided into four groups control and three groups by injection intraperitoneally of different concentrations of cadmium chloride (0.5, 1, 2) mg/kg body weight one time in week for two weeks. The result showed significant different between groups according to histopathology and physiology parameters. In conclusion showed the 2 mg/kg body weight one time in week for two weeks causes irreversible toxicity of testis compared with other groups.

Keywords: pollutants; toxicity; physiology parameters

Introduction

Cadmium considered one of the most important causative factors that causes reproductive damages, since that cadmium chloride ($CdCl_2$) is environmental pollutant and source for environmental contamination, pesticides, fertilizers and industrial chemicals, which reason damages organs through different mechanism. Generally, $CdCl_2$ used in the industry of batteries, paints and plastics (Thompson *et al*., 2008; Niknafs *et al*., 2015).

Oxidative stress is the most common mechanism causes damage in the testicular tissue due to overproduction of reactive oxygen species and free radicals lead to a state of oxidative stress and resultant damages of lipids, protein, and DNA (Patra *et al*., 2011).Additionally, International Agency of Research on Cancer has stated cadmium have carcinogenic effects on most of organs such as liver and testis (Tomatis, 1976; Smith and Perfetti, 2020).

Reactive oxygen species (ROS) is unavoidable toxic byproducts of aerobic metabolism. As well as accumulation in ROS lead to Oxidative stress, which the over-accumulated ROS can react with different cellular components to cause oxidative cellular injury and cell death (Mittler, 2017). Furthermore, oxidative stress have been suggested as factors causing infertility in animal (Ommati *et al*., 2018), which sperm dysfunction is consequence to elevated testicular or seminal ROS as described by Sun *et al* (2018) and Yu *et al.* (2018). Additionally RSO lead to DNA damage, bio-membrane disruption and proteins denaturation of sperm.

Present studies showed that $CdCl_2$ cause cell death by creation necrosis in testicular tissue in mouse (Niknafs *et al*., 2015), dog (Kim *et al*., 2018) rats (Nna *et al*., 2017) and human (Skipper *et al*., 2018). The necrosis and atrophyin testicular tissue that induced by $CdCl_2$due to suppression of glutathione peroxidase activity (Gutyj *et al*., 2018). Additionally apoptosis in germ cells lead to decrease spermatogenesis, is altered by toxic materials. Factors such as viruses, anticancerdrugs, hormones, radiation and toxic materials, including cadmium, induce cell death in seminiferous tubules (Mahmoudi *et al*., 2018).

Thus, aim of this study was to investigate the effect of different dose of cadmium chloride on testicular in term of histological of testis and physiological parameters of sperm.

Material and methods

In this study 50 adult rats weighing (250-300 g) were obtained from (university of Bagdad-faculty of science) The animals were kept in animal houses for 1-weeks the rats were randomly divided into three groups and one group was considered as the control which injection of normal saline. Testicular toxicity was induced in rats of other three groups by injection intraperitoneally of different concentrations of cadmium chloride (0.5, 1, 2) mg/kg body weight one time in week for two weeks. After two weeks of the experimental days the animals of each group will be scarified and testis will be taken for histopathology examination and measuring the fertility parameters.

Sampling and histological test

Epididymal spermatozoa : The tail of epididymis was rinsed and incubated in two ml of normal saline at 37°C and cut into about 200 pieces using an anatomical micro-scissor to leak the spermatozoa from the epididymal tubules for further tests (Ngaha Njila *et al.,* 2019).

Sperms motility : To evaluate the motility percentage of sperm (general and progressive), placed 10µl of the semenon a dry and warm slide then covered by coverslip and was evaluated under 400× magnifications using a microscope (Genex laboratories; Florida, USA) (Ibănescu *et al*. 2016).

Sperm morphology : To evaluate morphology of rat's sperm about 20 µL of extender mixed with 20 µL eosin nigrosine stain on a warm slid and was smeared on slide and left to dry

at 45 °C. The stained slide was evaluated under a phase-contrast microscope at 400× magnification, and total of 200 sperm was calculated and evaluation normal and abnormal sperm

Histopathological study : Testis will be excised and opened longitudinally and preserved in 10% formalin solution till the preparation of histological sections. Several tissue sections were prepared according to (Lee and Luna., 1968). Carefully immediately the testis remove of tissue sample were be taken from organs and specimens are fixed by 10% formalin-buffered for forty-eight hours at room temperature. After procedures of fixation the tissues were graded dehydrated in alcohol concentration then clearing in two stages of xyline and implanted in liquid-paraffin for two hours at 56-degree temperature. The tissue was done at 5micrometers by microtome for sectioning. In the end, dewaxed and stained with Eosin and Harris Haematoxylin (H&E), and tissues section was studied using X4, X10, and X40 objective of light microscopy

Statistical analysis

The statistical analysis was performed using SPSS statistical software version 26 repeated measure two-way ANOVA test was conducted Tukey's multiple comparison test was applied to determine differences among the extenders to investigate the effects of different levels of omega-3on sperm quality during the study period. P-values < 0.05 were considered as statistically significant. Data are presented as mean ± standard error of the mean (SEM).

Results

Parameters

The table 1 illustrate the mean value of motility (General and progressive) and morphology percentage of sperm parameters after injection of different concentration of cadmium chloride after 14 day of injection.

Showed significant different between control and three group of concentration as well as showed that are significant different between high concentration of cadmium chloride 2 mg with 1 and 0.5 concentration of cadmium chloride on sperm motility as showed on figure 1.

Table 1 : Physiological parameters of sperm after two weeks

Groups	General motility%	Progressive motility%	Normal Sperm morphology%
Controls	81.5 ± 0.5 a	75 ± 2 a	94.5 ± 0.5 a
0.5	50 ± 1.5 b	45±1.2 b	60.5 ± 1.5 b
1	43.5 ± 1.5 b	37.5 ± 2 b	55.5 ± 0.5 b
2	28.5 ± 2 c	21 ± 1.5 c	20.5 ± 0.5 c

Histology

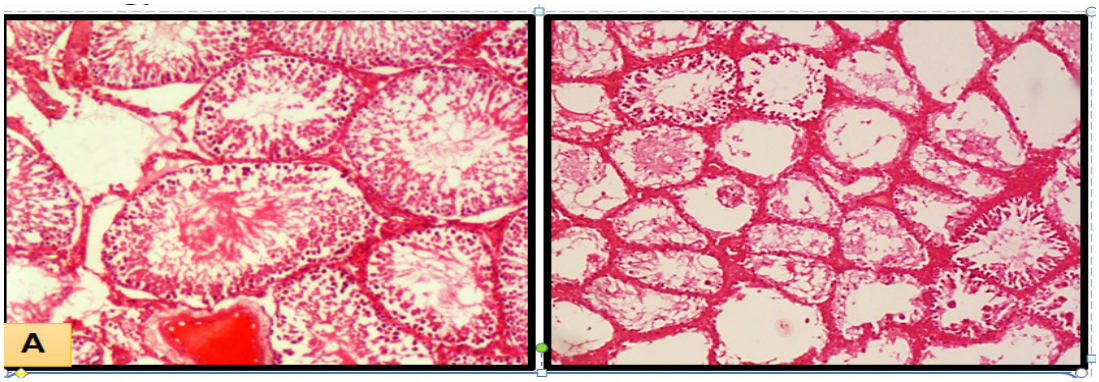

Fig. 2.A : injection with 0.5mg of cadmium chloride ; B. injection with 1mg of cadmium chloride: The histopathological section shows the damage (necrosis) in germinal epithelium layers cells (spermatogenic and Sertoli cells) of seminiferous tubules. Hemorrhage in the section of seminiferous tubules can be seen due to damage of testicular tissue and organized thrombus in the testicular blood vessels result in dilation of blood vessels and reduce the blood vessels wall thickness. The section is stained with H&E stain. The section is cuptured with 20x magnifier scale.

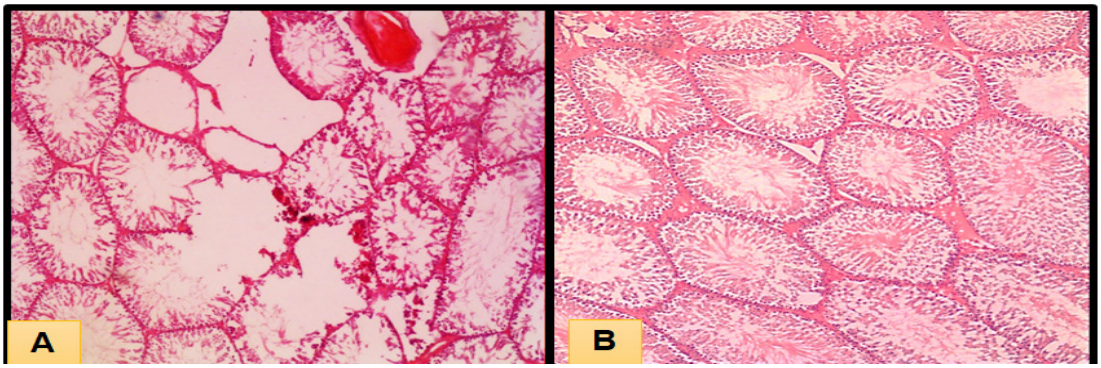

Fig. 3: A .The histopathological section of testis shows cleat testicular septa damage with loosing of germinal epithelia (spermatogenic and Sertoli cells); and B. The histological section of testis in control group

Discussion

An initial objective of this project was evaluated the effect of cadmium chloride on mammalian testis. Since our data suggest that administration of different concentration of cadmium chloride induced toxicity in testisticulat tissue and on some sperm parameters. The result found to be equal to 0.5, 1 and 2 mg/kg body weight intraperitonially injection in one /week for two weeks induced testiculartoxicity in different stage and forced as showed in past studies (Amara *et al.,* 2008 and Jahan *et al.,* 2010 and Nna *et al.,* 2017). Several studies have revealed that $CdCl_2$ causes delay daily sperm production (concentration of sperm), defect in sperm viability, sperm morphology, sperm motility and decreased in steroidogenic enzymes and antioxidant enzymes, and changes in the histological of testis (Jahan *et al.,* 2014; Gondwe *et al.,* 2019)

Reproduction is a network of sequential responsive actions of reaction processes between the gonads, pituitary gland and hypothalamus which regulates the mechanism of the gonadotropin release and in parallel with the production of gametes (Hanoune, 2008 and Herbison, 2005).

Subsequently, it regulates and plays a role in sperm parameters such as motility (López *et al.,* 2017). Therefore the hormone-based treatment for infertility work through the manipulation of hypothalamic-pituitary-gonadal (HPG) axis at level of the gonadotropin-releasing hormone (GnRH) or below (DCP *et al.,* 2015). Hormones are play a essential role in the directness and regulation of spermatogenesis, Gonadotropin-releasing hormone (GnRH) has a activitied effect on the anterior pituitary gland for secretion of LH snd FSH. Which FSH acts an important role in nutritive the sertoli cellsthus regulation the spermatogenesis. Furthermore, LH induced the leydig cells for secretion of testosterone hormone, several reports have shown that suggesting a ability to disruption of the HPT axis by toxicity of the $CdCl_2$ and which causes decreased Leydig cell count (Priya *et al.,* 2004; EL-Maraghy *et al.,* 2011 and Eleawa *et al.,* 2014). steroidogenic enzymes activities decreased as well as decreased in LH receptor expression due to decrease in Leydig cell count correspond to them and therefor destroyed in the spermatogenesis process and sperm parameters (Nna *et al.,* 2017 and Wu *et al.,* 2017). The evidence reviewed in this research seems to suggest a prominent role for cadmium chloride in testicular degeneration and sperm parameter in the first 14 days of the experiment.

The result of present study showed in pathological examination that $CdCl_2$ causes hemorrhage in the capillaries of testes, degeneration of the seminiferous tubules in the final causes testicular infarction and degeneration, as well as, the testis is extremely sensitive to $CdCl_2$ toxicity which researcher showed causes testicular edema, germ cell loss, hemorrhage, interstitial fibrosis and sterility in a few mammalian species (Hassanzadeh and Mortazavi, 2016 and Al-Okaily, 2017). histological examination of our pilot test the 0.5, 1, 2 mg/kg body weight intrapqeritonial injection of cadmium causes histological change in diffrent levels which showed degeneration in the interstitial cell, marked hypo spermatogenesis and decreased number of germ cell for induced testicular degeneration in 1mg/kg body weight this result agreed Prior studies (Medina *et al*., 2017; Saleh, 2019 and Gong *et al*., 2020) that have noted $CdCl_2$ causes testicular toxicity and degeneration in low does 1mg/kg B.W induced testicular degeneration in male rats.

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