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Concentration Effect Of Cadmium Chloride For Inducing Testicular Degeneration in male wistar rats

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Abstract

Infertility was the worldwide problem, in which reproductive vigor is deteriorating has an effect on the physical and social well and being of an infertile double couple The male considers 50% of causes infertility defects in world Testicular degeneration considered most of the problems of infertility and sperm production overall sperm are damage and defect in its function such as general and progresses motility, viability and concentration of the sperm in the eventual loss of capitation on fertility. Present studies amid for determine that cadmium chloride can Inducing testicular degeneration in male wistar rats.

Concluded: -

Present study concludes I.P injection of CdCl2 in concentration 1,2,3 mg/kg B.W once/week for two-week interval show graded decrease in fertility of rats as well as 1-mg doses of CdCl2 showed causes reversable testicular degeneration in the male rats

Introduction: -

Cadmium (Cd) is a heavy metal toxic in both environmental and vocational concerns, has found its relevance in several industrial processes such as in electroplating and manufacturing of paint pigments, batteries, plastic, and fertilizers. Cadmium it is induced decline in reproductive function by causes testicular toxicity, and degeneration (Imafidon *et al.,* 2016). Cadmium chloride ($CdCl_2$) causes loss of cell-polarity and mis-orientation in sperm development and maturation indeed damage in all diffracts stage of spermatogenesis stage by causes change in

structure of cells and cell death in both bird and mammal's (Marettová *et al.*, 2015). Scientists observed that exposure to $CdCl_2$ decreased the body weight as well as the absolute and relative weights of the testis, epididymis, liver and kidney (Nna., 2017). As well as, scientists has been reported the exposure to the $CdCl_2$ induced insufficiency in fertility of both male (humans and rodents) (Benoff *et al.*, 2000), which it has been reported that as low as 1-2 mg $CdCl_2$ for every 1 kg body weight, induced damage in testicular (germ cell and spermatids) without every change in pathological in other organs (Prozialeck *et al.*, 2006).

It has been reported the uses of $CdCl_2$ in high level such as 5mg for each 1 kg body weight lead to reduction and necrosis of seminiferous tubular lumen therefore causes sloughing and detachment of spermatogenic cells ,which spermatogenic cells support and nurture by Sertoli cells present in the seminiferous tubular lumen, therefore spermatogenic cell cannot survive longer without supporting and thus undergo apoptosis (Bekheet., 2010). Researcher believed that cadmium toxicity has two effects are; pre–testicular, testicular and post–testicular effects. The pre–testicular effects contain inhibit of the HPG axis which leads to decrease in levels of follicle stimulating hormone FSH)in blood (Mayorga *et al.*, 2000). Testicular and post–testicular effect is cadmium toxicity are reported in; daily sperm production, defect in sperm viability, sperm morphology, sperm motility decreased in marker steroidogenic enzymes and antioxidant enzymes, and changes in the histological testis (Jahan *et al.*, 2014). Cadmium chloride induced its effect by reactive oxygen species (ROS) stress which, acquired of apoptotic tolerance renders cells damaged to proliferate with ingrained of DNA oxidative lesions, potentially leading to tumorogenesis then oxidative stress by increase of lipid peroxidation or by changing in intracellular glutathione levels. Also it is affects the ubiquitin Adenosine triphosphate depend the proteolytic pathway. (Patra *et al.*, 2011.) Decreased in Leydig cell viability are Another mechanism of cadmium toxicity as mediated are possible resulted (Marettová *et al.*, 2015).

Material and mothodes :-

Experiment animals

A total of fifty of adult male rats were use in this study . Their body weight ranged between 250-350 grams were housed in clean cages were kept in the animal house at the faculty of veterinary medicine /university of Kufa. Animals had ad-libitum to fed and water during the

experiments day and the animals were maintain at about two weak for adaptation before starting the experiment.

Experiment design: -

Thirty fifty adult males' rates divided into four equal group. Three groups treated with $CdCl_2$ in a concentration (1, 2 and 3) mg/kg body weight intraperitoneal injection intraperitoneally, one /week for two weeks the studies parameters were measured percentage. After 14 days of the experimental days five animals from each group will be scarified and testis will be taken for measuring the fertility parameters via measured the following parameters:

1. General motility%
2. Individual (progressive) motility %
3. Viability %
4. Acrosomal damage %
5. Sperm Concentration (SPM/No.)
6. Testis will be taken for histopathology examination

Studies parameters: -

1. Prepares parameters: -

Immediately bilateral the testis and epididymis of scarified animals were taken at different intervals along the experiments from anaesthetized animals using I.M. injection of Ketamine 90 mg/kg B.W and Xylazine 40 mg /kg B.W. for studies parameters done at day 14,21,42 of the experimental day.

2. Epididymal spermatozoa

The left tail epididymis was rinsed and incubated in 2 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).

3. Sperms motility %

To evaluate the sperm general and progressive motilities%, 10µl of the semen suspension was placed on a dry and warm slide and examined at 400× magnifications using a Computer Assisted Sperm Analysis (CASA; Genex laboratories; Florida, USA).

4. Sperms concentration (SPM/Ml)

Ten µl of semen suspension was added to 9990 µl of counting solution, so the dilution factor was 1:1000. The counting solution contains (normal saline 95%, formaldehyde 4%, eosin stain 1%; (Smith and Mayer., 1955). The sperm concentration was determined using a Neubauer hemocytometer as previously mentioned by (Yokoi *et al*., 2003).

5. Sperms viability%

Values of sperm viability and normal sperm morphology were examined with eosin-nigrosin(EN) dye (Felipe-Pérez *et al.,* 2008) and Murcia-Robayo *et al.*, (2018). In short, a 10 µL drop of raw semen was added to 30 µL of EN, and stir for 10 second, then after, the mixture was smeared on a dry warmed slide and left to arid on slides warmer at temperature 45°C (Baiee *et al.,* 2018).

Then, the slides were read under microscope at ×40 magnifications; either 200 spermatozoa or five microscopic fields were calculated. Furthermore, the pink stained spermatozoa were considered dead while unstained spermatozoa were being alive.

6. Sperms Acrosomal integrity %

Acrosome integrity% will be investigated using semen smear stained with (EN) stain and evaluated under a 1000× of phase contrast microscope at enlargement oil immersion (Kaka *et al.,* 2015). A total of two hundred sperm will be tested for either detached or intact acrosome.

7. 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 5 micrometers 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.

8 Statistical analysis

Statistical analysis of the experimental results was conducted according to Graphpad prism 8. were T-test and (one-way ANOVA) was used to assess the significance of differences between groups and within times. The data were expressed as mean ± standard errors (SE) and (P value<0.05) was considered statistically significant LSD was carried out to test the significant level among means of treatment (Prism., 2019)

The Results :-

The data obtained from study (Table -1) demonstrated that I.P injection of $CdCl_2$ in concentration 1,2,3 mg/kg B.W once/week for two-week interval show graded decrease in sperm general of progress motility, live viability and concentration % and increased in dead viability. Also the figure (1) in A and B of histopathological study of testis shows a accumulations of homogenous, eosinophilic material in the ductuli efferentes of seminiferous tubules in rats since injection with 1mg $CdCl_2$ which induction testicular degeneration, while in figure (2) in A and B showed damage in the testicular septa and clear sloughing of germinal epithelium and leydig cell as well as blood vessels congestion in rats which are injection with 2 and 3 mg/kg of $CdCl_2$ in one /week for two weeks compared with control rets injection with normal saline as showed in figure (3). Additionally, for microscopic evaluation in figure (4) which shown the compared between the effected of $CdCl_2$ in three doses with the control.

Table 1 sperm parameters of wister rats

	Control	1 mg	2 mg	3 mg

General Motility	77.5 ± 2.5	40 ± 5	20 ± 5	18 ± 2
Progress Motility	72.5 ± 2.5	35 ± 5	15 ± 5	12 ± 2
Live viability	70 ± 5	34.5 ± 2.5	31.5 ± 1.5	32.5 ± 1.5
Dead Viability	30 ± 5	65.5 ± 2.5	68.5 ± 1.5	67.5 ± 1.5
Acrosome integrity	0.25 ± 0.25	1 ± 0.25	2.25 ± 0.25	1.5 ± 0.5
Concentration	9.25 ± 0.25	6.25 ± 0.25	3.5 ± 0.5	3.5 ± 0.25

Values are expressed as the means and error bars represent standard error (SE)
injection of $CdCl_2$ 1mg/kg , 2mg/kg and 3mg/kg B.W. I.P. once /week for two weeks

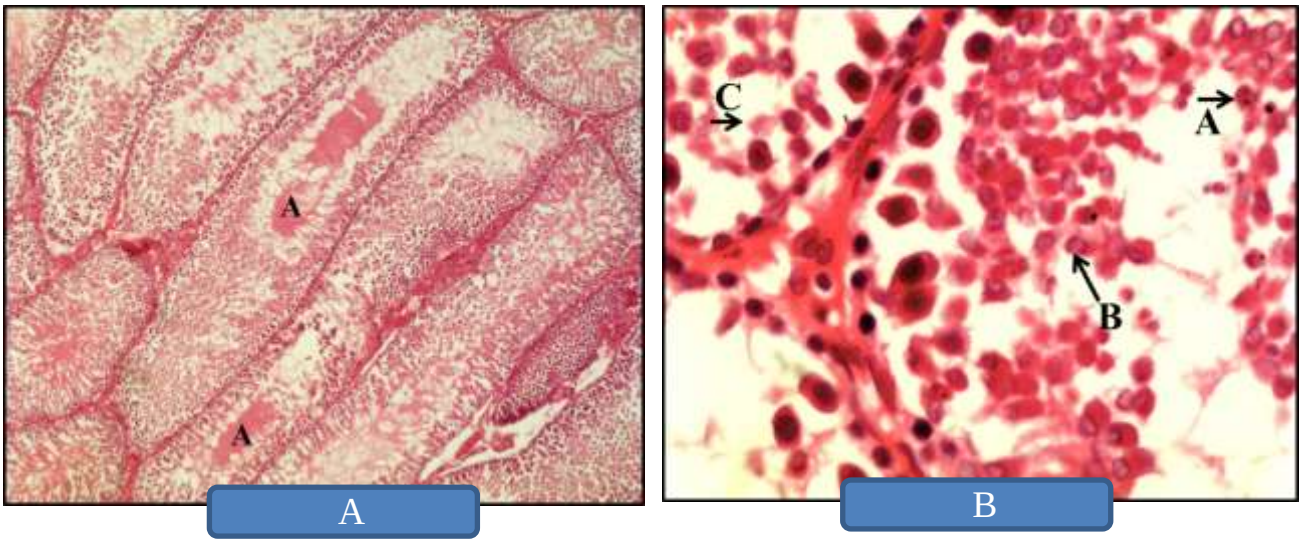
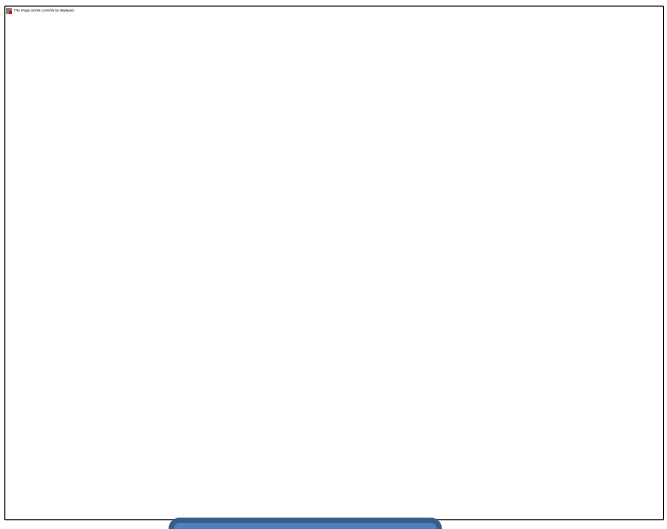


Fig. 1 :.

Induction 1 mg of $CdCl_2$. A. The histopathological section of testis shows a perivascular accumulation of homogenous, eosinophilic material in the ductuli efferentes of seminiferous tubules (testicular amyloidosis). The section is stained with H&E stain.The section is cuptured with 20x magnifier scale.

The histopathological section of testicular tissue shows the necrotic cells with pyknotic nucleus as a black, decreased in size and rounded. (B). Many tiny fragments in nucleus of dead cell can be seen in this section. (C). Karyolysis of the nuclear material appear as



A B

Fig. 2 :

. Induction 2 mg of $CdCl_2$. A. The histopathological section of testis shows cleat testicular septa damage with loosing of germinal epithelia (spermatogenic and Sertoli cells). The section is stained with H&E stain. The section is cuptured with 20x magnifier scale.

. Induction 3 mg of $CdCl_2$. A. The histopathological section of testis shows damage in the testicular septa and clear sloughing of germinal epithelium and interstitial cells of Leydig

Fig. 3 : The histological section of testis in control group shows no significant occupied lesion (SOL). The rats in this group injected (normal salain). The section is stained with H&E stain. The section is cuptured with 20x magnifier scale.

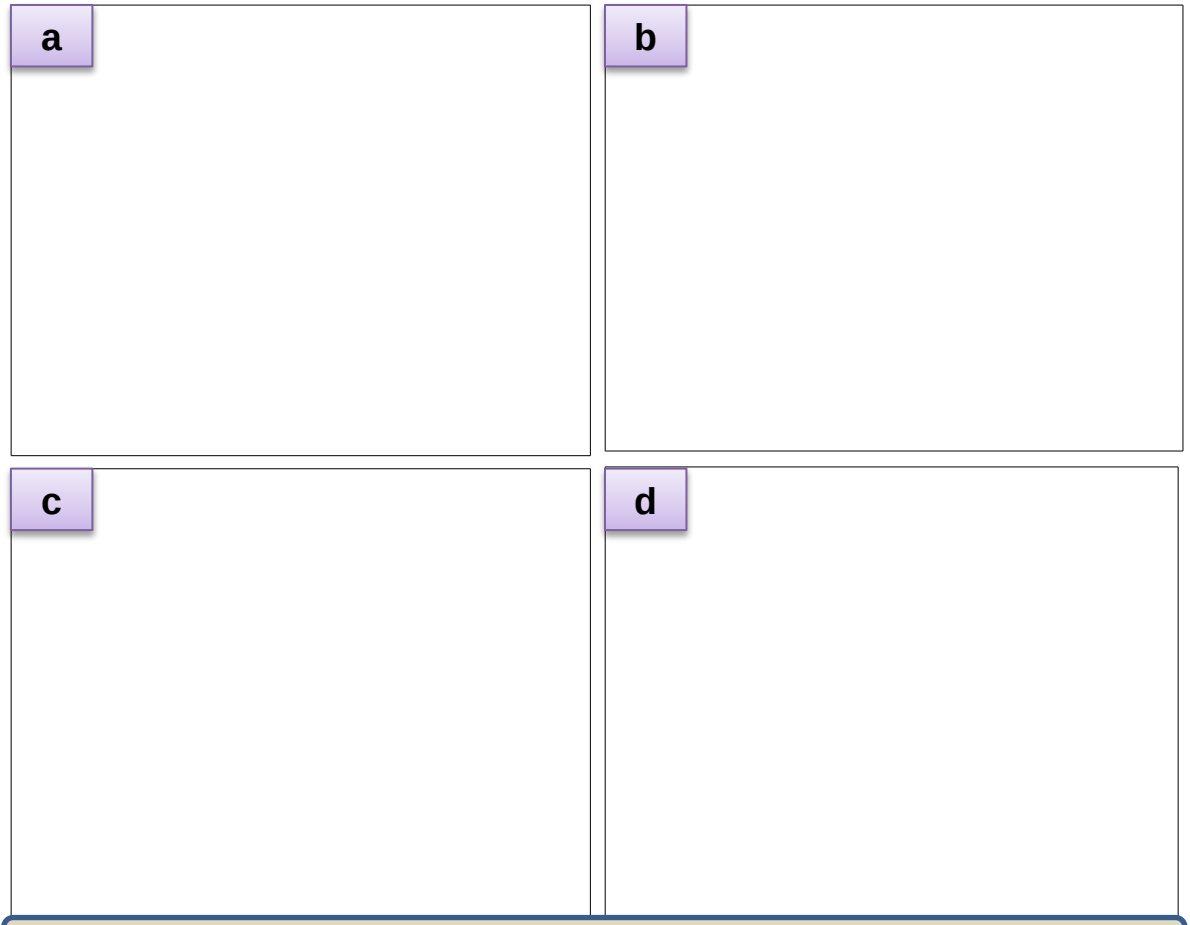


Fig 4: Microscopic evaluation of pilots' studies
1. Negative Control (injection normal slain)
2. injection of CdCl2 1mg/kg B.W once /week for two weeks
3. injection of CdCl2 2mg/kg B.W once /week for two weeks
4. injection of CdCl2 3mg/kg B.W once /week for two weeks

Desiccation :-

induced testicular degeneration in male rats by using cadmium chloride ($CdCl_2$) as mentioned in the literature review and prior studies (Medina *et al.*, 2017; Gondwe *et al.*, 2019 and Khafaga *et al.*, 2019). Because of variation and present a wide range of concentration and experimental days in different studies of cadmium chloride induced reversible testicular degeneration (De Souza *et al.*, 2010; Jahan *et al.*, 2010; Zaid et al., 2018 and Nna *et al.*, 2017), a pilot study was applied for determining the adverse dose induced reversible testicular

degeneration , According to pilot test was found to be equal to 1mg/kg B.W. I.p. one /week for two weeks induced testicular degeneration in male rats.

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 (Amara *et al.,* 2008; Mohammed et al., 2018 and Jahan *et al.,* 2014) through induced its effect by reactive oxygen species (ROS) stress which, acquired of apoptotic tolerance renders cells damaged to proliferate with ingrained of DNA oxidative lesions potentially leading to degeneration in the end oxidative stress by the increase of lipid peroxidation or by changing in intracellular glutathione levels. membrane integrity decreased, mostly in the part of flagellum mitochondrial is supported by researchers of deformation of spermatozoa mitochondrial after cadmium exposure to sperm (Akunna *et al.,* 2017). Also, it affects the ubiquitin adenosine triphosphate (ATP) depend on the proteolytic pathway, which ATP the most important energy source for sperm mortality and viability (Patra *et al.,* 2011). seminal fluid is contain high concentration of zinc, which it has important role in faunctional properties in sperm since scientists suggested that zinc play multifaceted and important role as anti-inflammatory factor and involved oxidative metabolism in sperm (Fallah *et al*., 2018). Among the mechanisms of toxicity of cadmium chloride on testes is; a failure in blood circulatory because of vascular damage and drop in zinc utilization by spermatogenic cells in the spermatogenesis process due to action of cadmium which is competitive to Zn (Amara *et al.,* 2008), which Zn effects on lipid flexibility and sperm membrane stabilization (Ali *et al.,* 2007). It also has a regulated role in capacitation and the acrosome reaction of sperm and is essential for conception and embryonic implantation (Cutini *et al*., 2020). Overall, in the present study the results figures (4-2), (4-3), (4-4), and (4-5) indicate that cadmium chloride causes reversible testicular degeneration after 14 days which show decreased in motility, viability and concentration of sperm, all these results accordance prior studies (Nna *et al*., 2017) which found that cadmium cause significantly decreased in all motility, sperm count, viability and after 15 to 30 days of exposure to $CdCl_2$ which cause destroyed the sperms, Leydig and Sertoli cells, also caused increasing the death of cells and reduction in the germinal layer which decrease the thickness in the seminiferous tubules (Mahmoudi *et al.,* 2018).

Cadmium chloride induced oxidative damage reflected on morphology of sperm and various structural changes (Ma *et al.,* 2013) by change the gross antioxidant capability which that leaded to the oxidative-damage therefore causes damage in DNA and protein of spermatozoa and decrease the concentration sperm, all of that by increase in lipid peroxidation. at lower cadmium chloride doses (dose-dependent) the effects of oxidative damage effect on germ cell, which destroyed the spermatogonia and lead to decrease acrosomal integrity and sperm concentration (Mahmoudi *et al.,* 2018 and Marchiani *et al.,* 2019) However, the toxic effect of $CdCl_2$ became evident considerable reduction of these parameters in all effective concentrations which increase in the lipid peroxidation indicated the harmful effect of cadmium on the spermatozoa membrane integrity as showed in rabbit (Roychoudhury *et al.,* 2010) and in bull (Arabi *et al.,* 2007 and Fitriawan,. 2017) and in human sperm (Roblero *et al.,* 1996) when used the cadmium chloride in different effected dose. In our result the figure (4-4) and (4-5) show significant increased acrosomal damage and decreased in sperm concentration due to effect of cadmium chloride after 14 days of injection intraperitoneally according to our pilot study in dose 1 mg/kg body weight and these findings are similar to those reported from animal studies by uses cadmium chloride orally given $CdCl_2$ at 5 mg/kg bw for 30 days in rats (Adamkovicova *et al .,* 2016), by effective injection cadmium chloride in mouse (Oliveira *et al.,* 2009), in goat sperm (Mao *et al.,* 2018) and research involving humans (Wang *et al.,* 2016 and Marchiani *et al.,* 2019)

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