Protective effect of Selenium on the testes and spermatogenesis in acute and long–term cadmium treated male Swiss mice

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الملخص

التأثير الوقائي لكلوريد السيليلينيوم ضد التأثير السمي لكلوريد الكادميوم على عوامل النطف وتركيب الخصية والأنزيمات المضادة للأكسدة في ذكور الفئران السويسرية تم بحثه في هذه الدراسة . إذ حقن بجرعة تعادل ٠.١٥ ملغم من وزن الجسم من كلوريد الكادميوم تحت المسراق أدى خفض في عدد النطف وارتبط هذا التأثير معنويا بزيادة نسبة النطف المشوهة والميتة واظهر. الكادميوم انخفاضاً معنوياً في أوزان الخصي والبربخ بالمقارنة بحيوانات السيطرة . بينما كان هناك نقصان في أوزان الأعضاء أعلاه اقل حدة في المعاملة طويلة الأمد . أظهرت فترة النقاهة تأثير استرجاع ي في كلا فترتي التعريض . التأثير النسجي الامراضي في الخصية مؤشر من خلال اختزال قطر الأنابيب المنوية والمظهر غير الطبيعي لشكل الخصي نتيجة انسلاخ الخلايا الجرثومية والتكاثرية وكانت أكثر وضوحا بعد مرور ٧٢ سعة من المعاملة بالكادميوم.

كان تحفيز لعملية أكسدة الدهون في نسيج الخصية من خلال الانخفاض المعنوي لفعالية الأنزيمات المضادة للأكسدة مثل البيروكسيديز والكتاليز في حيوانات التجربة.

إن إعطاء كلوريد السيليلينيو م كمزيج مع حجم مكافئ لكلوريد الصوديوم بالجرعات المذكورة أعلاه أدى إلى استعادة جزئية لوزن الأعضاء المذكرة أعلاه (الخصي والبربخ) مع زيادة عدد النطف الكلي وانخفاض في نسب النطف المشوهة والميتة مع زيادة ملحوظة لفعالية الأنزيمات المضادة للأكسدة.

Abstract

The protective effect of selenium chloride ($SeCl_2$) against cadmium chloride ($CdCl_2$), induced toxicity on sperms parameters, testes

architecture and antioxidant enzymes in male Swiss mice was investigated. Intraperitoneal injection of $CdCl_2$ (0.15 mg) dose in mice reduced total sperm count and significantly associated with increased sperm abnormality and death sperm percentage when compared with controls. The effect of Cd-treated mice showed a significant decrease in weight of testes and epididymides as compared with control groups. The degree of organ weight loss (testes and epididymides) were less severe in long-term treatment. Long-term exposure of both cadmium-injected mice had similar effect as acute exposure but with lower efficacy. Recovery period showed restoring effects in both exposure periods. The histopathological testes changes indicated by the reduction of seminiferous tubule diameter and testes architecture appearance due to the epithelial sloughing and elimination of germ cells were more pronounced at 72h/ Cd treatment. Substantially proves the ongoing damage effect of cadmium on developing germ cells. Long-term exposure of a batch of cadmium –injected mice had a similar effect as acute exposure but with lower efficacy. In addition, Intraperitoneal injection of $CdCl_2$ (0.15 mg) also stimulate lipid peroxidation in testicular tissues, indicated by significant decrease in antioxidant enzymatic activity such as peroxidase and catalase in the experimental mice group.

Administration of $SeCl_2$ in combination with equal volume $CdCl_2$ at the above mentioned doses led to partially organs weight resorted, elevated sperm count and reduction in the percentage of abnormal and dead sperm population along with significant increase in antioxidant enzymatic activities. The protective action and the antagonistic effect of Se at different periods against cadmium –induce toxicity are discussed.
Keywords: Sperm, cadmium, selenium, antioxidant enzymes.

Introduction

Environmental pollution may elicit both adaptive and adverse responses in animal at different structural level (tissue and organs). The reaction depend on a variety of factors, such as contaminant concentration and rate of exposure, the susceptibility of organisms (1).Cadmium is extremely hazardous to life and has been involved in historic poising episodes of human and animal population and it is a serious lethal occupational and environmental toxic, known for its high toxicity,which may affect living systems in various ways (2). Acute and chronic exposure to cadmium by different route may lead to many organs disorders, especially male testes and accessory glands (3). Some studies in rodents showed that contaminant induced a decrease in the testis weight associated with morphological changes, and sperm count and motility (4,5) Germ cell apoptosis occurs normally to remove abnormal spermatogenic cells and to maintain normal quantity and quality of sperm afterwards (6,7). Germ cell apoptosis is also induced by many factors including hormone deprivation, heat, radiation and toxicants (8,9).

Lipid peroxidation has been known as a marker of free radical damage in lipid molecules. The higher membrane lipid content of testes is presumed to make them more vulnerable to oxidative stress (10). At cellular level cadmium exposure leads to protein denaturation and lipid peroxidation (11). One mechanism by which metal ions produce injury is assumed to be through generation of free radical and lipid peroxidation, because co-treatment with antioxidant greatly reduce the Cd–induced toxicity (12). ROS generated in tissues are effectively scavenged by the antioxidant defence system, which constitute antioxidant enzymes such as peroxidase and catalase (13).

It has been reported that antioxidant enzymes evoke varied response that will depend on Cd frequency of exposure–dependent (14). The synergistic or antagonistic interaction between metal pollutants also revealed to find their toxic effect (1). Both the antioxidant and toxic roles of selenium at low and high concentration respectively have been extensively studied in mammals (15,16), in particular its protective and beneficial effects at low concentration in terms of tissues damage reducing capacity is well known in large number of mammalian studies(17).

This study was carried out to demonstrate the adverse effect of Cd on testicular gametogensis activities and its protection by selenium co-administration. Moreover, attempts have been made to study the Cd-induced testicular oxidative stress on the antioxidant enzymatic activities and its correction by Selenium co-administration.

Materials and Methods

The Intraperitoneal Cd-injection dose of 0.15 mg in this study was derived from the LD_{50} calculated for a single Intraperitoneal dose of cadmium in the form of $CdCl_2$ in mice was 6.75 mg/ body weight (18). Forty Swiss mice males (aged at least 2months) with average initial body weight of about 25g were used in this study. the animals were housed at laboratory conditions (22±1C^{o}) in plastic cages(one per cage)and allowed free access to drinking water and commercial rodent chow. The mice were randomly divided into the following experimental groups (5 animals each):

a) control group receiving only distilled water. b) second group 0.15mg cadmium chloride $CdCl_2$. c) third group Equal mixed volume of $CdCl_2$.and $SeCl_2$(net dose for both was 0.15mg). d) group fore recovery group (for each treatment mice left after initial injection to the end of experiment. In three of the following groups(b, c and d), each mouse was dosed via Intraperitoneal injection once per treatment period. For the recovery groups, each were left to the end of each experiment after initial dosing. Mice injected once per treatment.

Mice before sacrificed kept at the following designed experimental periods:

1- acute treatments, 24,48 and 72 hours and 28 days recovery period (received no treatment after later treatment)
2- Long –tem treatment, 4, 6, 8 weeks and 8 weeks recovery period (received no treatment after initial treatment)

Before each experimental period, mice were weighed and at the end of each experiment, the animals weighed and then sacrificed by ether over dose. The testes and epididymides were dissected out rinsed in ice cold saline, weighed and plotted dry.

For sperm count Skamoto and Hashimoto[19], Method was used.Briefly, sperm were isolated from the epididymis by teasing a part each epididymis. 0.1ml of semen fluid was diluted by1ml formal bicarbonate (5%), mixed gently, then applied to haemocytometer and left for 5 min. before examination. For alive, abnormal and dead sperm count from one droop of the semen sample a film slide was prepared, then stained by Eosin-Nigrosine stain. Sorensen [20] equation was used as follow to determine each number:

$$\text{Alive sperm\%} = \frac{\text{Number of stained sperms}}{\text{Total sperm (stained and not stained)}} \text{ X } 100$$

Histological examination

One testes from each mouse were fixed in buffered neutral formalin according to method used by Luna[21], dehydrated in ethanol series and xylene and stained with hematoxylin and eosin (H&E) stain.

Preparation of tissue extracts for enzymes assay

Tissue extracts were prepared according to Gerardo *et al* [22]., method. Briefly At the end of the experiment, all animals were killed and testes tissues was placed into containers and stored at -80◦C for antioxidant enzymes assay. Approximately (100 mg tissue was sliced into pieces and tissue homogenized in ice-cold phosphate buffer (50 mmol/l, pH.7.0, containing 0.1 mmol /l EDTA to give a 5 % (w/v) homogenate, and centrifuged at 3000 g for 10 mints at 0 $^{\circ}$C. The supernatants were then separated and used for enzyme assay.

Biochemical assay

The lipid peroxidation assay in the form of peroxidase activity was performed according to Chance *et al* [23.], method, Briefly, the reaction mixture contained 5 mL of 50 mM sodium phosphate buffer, PH(7.0), 0.1 mL of 20 mM guaiacol and 0.02 ml of 40 mM hydrogen peroxide (H_2O_2).

The reaction started by adding 0.2 ml 0f enzyme extract (prepared as described above). Enzyme activity determination was performed at 20°C by measuring the rate of color development at 470 nm using Shiamdzu-SP1650-double beam spectrophotometer. The coefficient (Σ_{470}) of tetraguaicol is 26.6 Cm^{-1} mM^{-1} (15).

Catalase (CAT) activity of tissues is determined according to Aebi [24.,] method. The decomposition of H_2O_2 is followed directly by the decrease in absorbance at 240nm. The differences in absorbance per unit time were used as measure of CAT activity. The enzyme activity is given in U mg-1 protein.

Statistical analysis: Data are expressed as means ± standard deviation (SD), the values analyzed by one-analysis of variance (ANOVA) followed by the Duncan's test (using SPSS for Windows). $p < 0.05$ were considered statistically significant.

Results

The weights of testes and epididymis in the acute 0.15 mg/Cd treated mice showed a significant reduction ($p<0.05$) by comparison with the corresponding control groups. Testes and epididymides weight exposed to Cd/Se equal volume combination gradually increased, in which weight were still significantly lower

Table (1). Effects of acute treatment on Testes and epididymis weight of mice exposed to 0.15mg/Cd and 0.15mg/Cd-Se combination.

Organs	Control	Exposure Duration						
		24 h		48 h		72 h		Recovery
		Cd	Cd+Se	Cd	Cd+Se	Cd	Cd+Se	
Testes Wt/mg	223± 2.5	165*± 3	219**± 2.1	94*± 1.3	198**± 2.1	173*± 2.15	182**± 6	206***± 6.4
Epididymis Wt/mg	166± 1.5	138**± 1.1	143± 4	137± 1	148± 1.7	120*± 1	138**± 1.2	144***± 2.2

The data represent mean ± SD. Asterisks (*P < 0.05) indicate significant difference from control. Asterisks (**P<0.05) indicate significant difference from /Cd. Asterisks (***P<0.05) indicate significant difference from later Cd treatment.

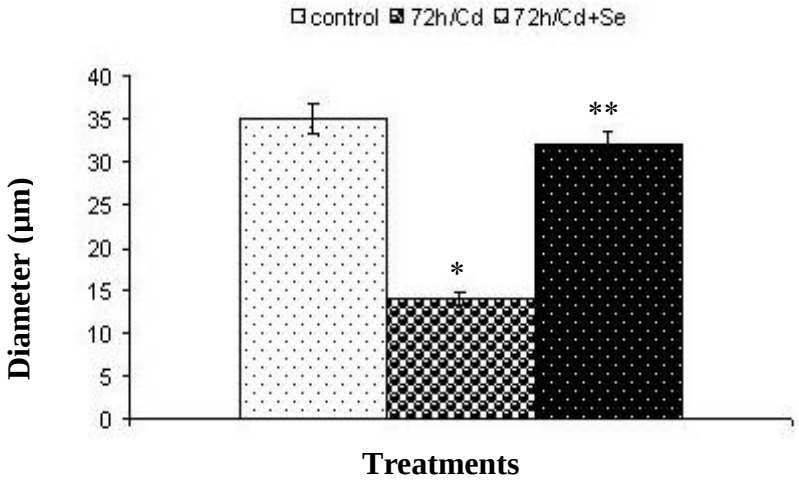
The degree of both organ weight loss were lees severe in long-term treatment, Recovery period showed restoring effects in both exposure periods. (Table2).

Table (2). Effects of acute treatment on Testes and epididymis weight of mice exposed to 0.15mg/Cd and 0.15mg/Cd-Se combination.

Organs	Control	Exposure Duration								
		4 Weeks			6 weeks			8weeks		
		Cd	Cd+Se	Rec.	Cd	Cd+Se	Rec.	Cd	Cd+Se	Rec.
Testes wt/mg	223 ±2.5	104* ±0.021	112** ±1.7	101*** ±1	131* ±4.9	132** ±4	122*** ±3	133* ±1.52	146** ±1.4	152*** ±7.2
Epididymis wt/mg	166 ±1.5	61 *±1	74** ±1.6	77** ±1	67* ±1.8	73*** ±1.5	78*** ±1	77* ±1	96** ±1	102*** ±2

The data represent mean ± SD. Asterisks (*P < 0.05) indicate significant difference from control. Asterisks (**P<0.05) indicate significant difference from /Cd. Asterisks (***P<0.05) indicate significant difference from initial Cd treatment.

In acute treatment mice were injected intraperitoneally with 0.15mg $CdCl_2$, the diameter of seminiferous tubules was significantly reduced ($p<0.05$) with a narrow or no lumen compared with controls. The seminiferous tubules diameter of mice exposed Cd/Se combination was significantly increased ($p<0.05$) and was almost recovered to normal value as compared with those of Cd exposed. (Fig. 1).

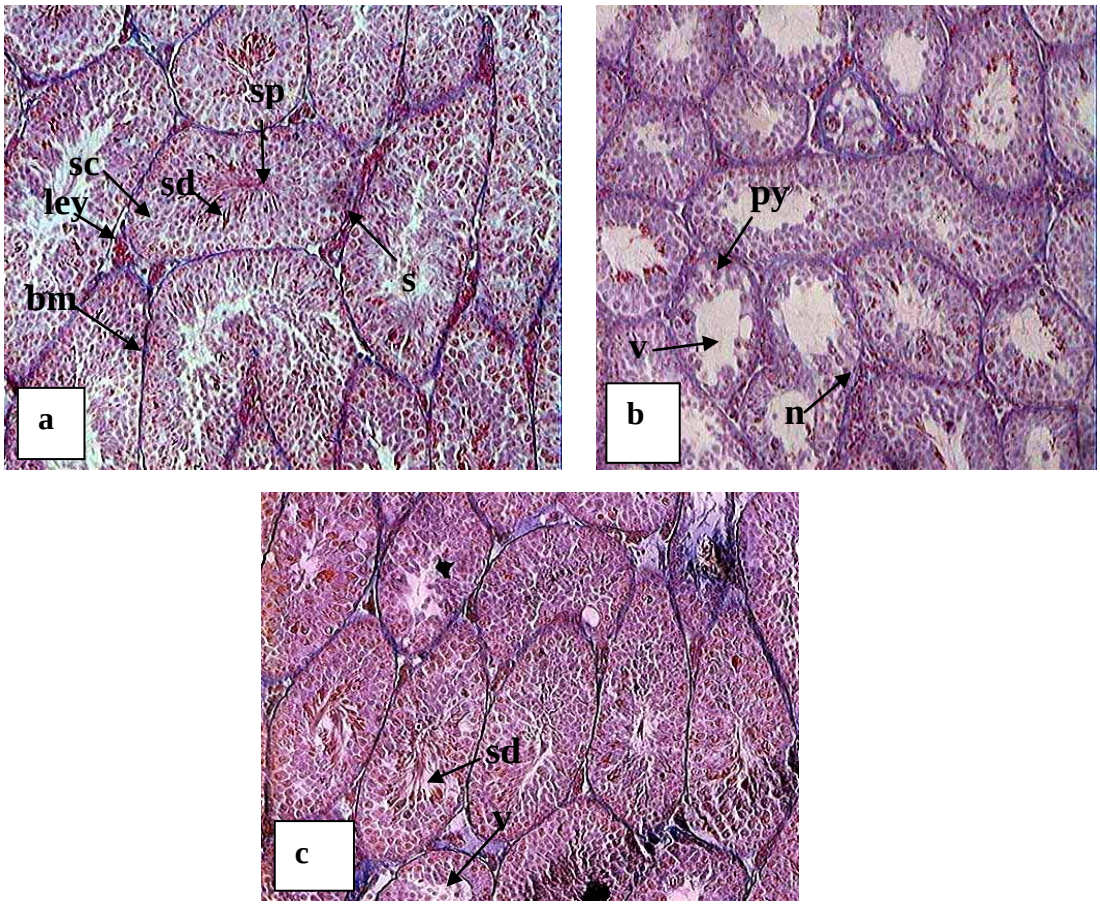


Fig(1). Seminiferous tubule diameter of mice testes exposedto72hCdandCd/Se combination.

(*P < 0.05) indicate significant difference from control. Asterisks (**P<0.05) indicate significant difference from /Cd.

Histopathological examination showed collapsing of seminiferous tubules, which causing an increase in intertubular space. On the other hand connective tissue necrosis had occurred as well as detachment of basement membranes (fig.2b), as compared with control as in Fig(2a).

Other changes including the disappearance of germ cells (leydig) in the interstitial tissue. Spermatogenic cells showed pyknosis, degeneration and partial necrosis. Results from this study revealed disarrangement and vacuolization of spermatogenic.. The changes were more pronounced in the 72h Cd-exposed mice group.



Fig(2). Section in mice testes (a- untreated control, b- Cd treated, c- Cd/Se combination treated).

(BM: Basement membrane; SG: spermtogonia; SC: sepermatocyte;SD: spermatid; SP: sperm; Ley: leydig cells; Pyknosis; V: vacuolization, N: necrosis)

Histopathological changes were partly recovered on Cd / Se combination exposure, the recovery represented by normal growth and renewal of spermatogenesis in different developmental stages.There were a few elongated spermatids and occasionally round spermatids clearly showed as in Fig.(2c).

Results demonstrated the reduction in sperm count, due to Cd - treatments. It revealed a significantly reduced sperm count in Cd –treated mice in all samples taken at different time after the treatment, acute in Fig (3) and long-term in Fig 4), in comparison to Cd- treated groups.

Administration of Cd decreased the epididymal sperm count in period-dependent manner. The degree of reduction was less sever in both Cd / Se combination and recovery treatments.

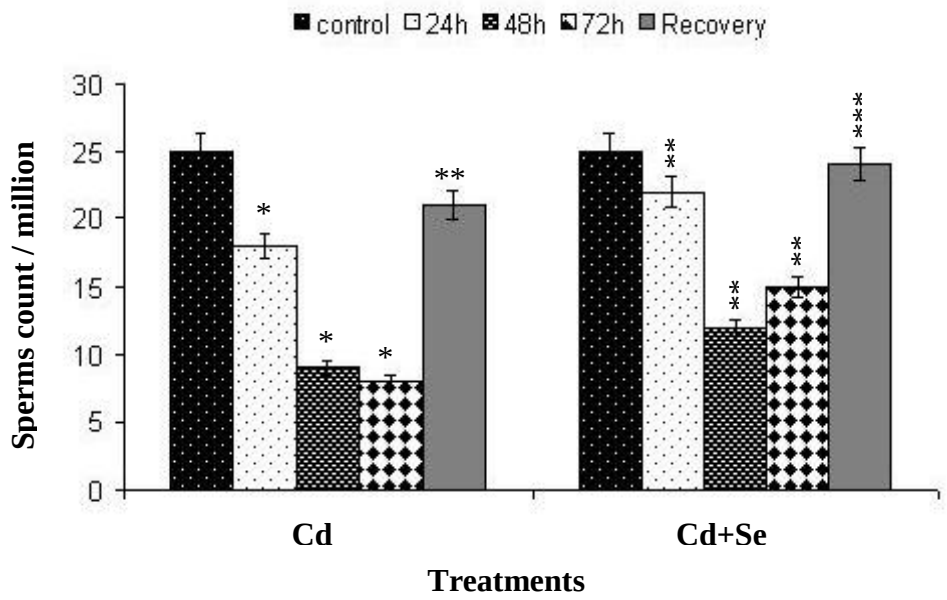


Fig (3). Sperms count in acute Cd and Cd/Se combination treated mice.

The data represent mean ± SD. Asterisks (*P < 0.05) indicate significant difference from control. Asterisks (**P<0.05) indicate significant difference from /Cd. Asterisks (***P<0.05) indicate significant difference from later Cd/Se treatment.

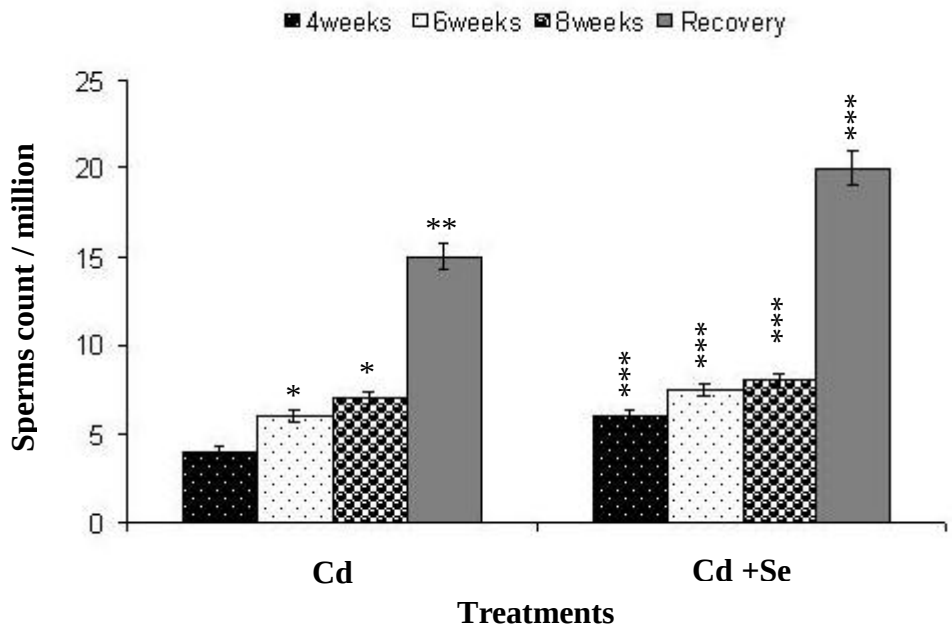


Fig (4). Sperms count in long-term Cd and Cd/Se combination treated mice.

The data represent mean ± SD. Asterisks (*P < 0.05) indicate significant difference from control. Asterisks (**P<0.05) indicate significant difference from /Cd. Asterisks (***P<0.05) indicate significant difference from initial Cd/Se treatment.

The abnormal and dead sperm percentage increased significantly ($p< 0.05$) in both acute (fig.5) and long-term (fig.6) in Cd exposure.

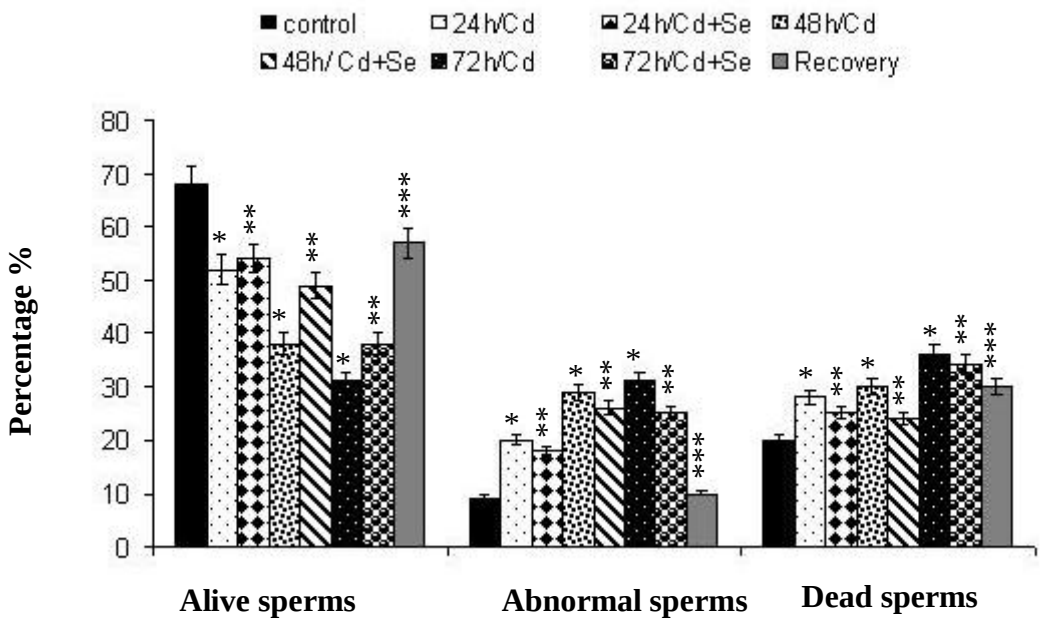


Fig (5). Sperm alive, sperm abnormal and sperm death percentage in acute Cd and Cd/Se combination treated mice.

The data represent mean ± SD. Asterisks (*P < 0.05) indicate significant difference from control. Asterisks (**P<0.05) indicate significant difference from /Cd. Asterisks (***P<0.05) indicate significant difference from later Cd treatment.

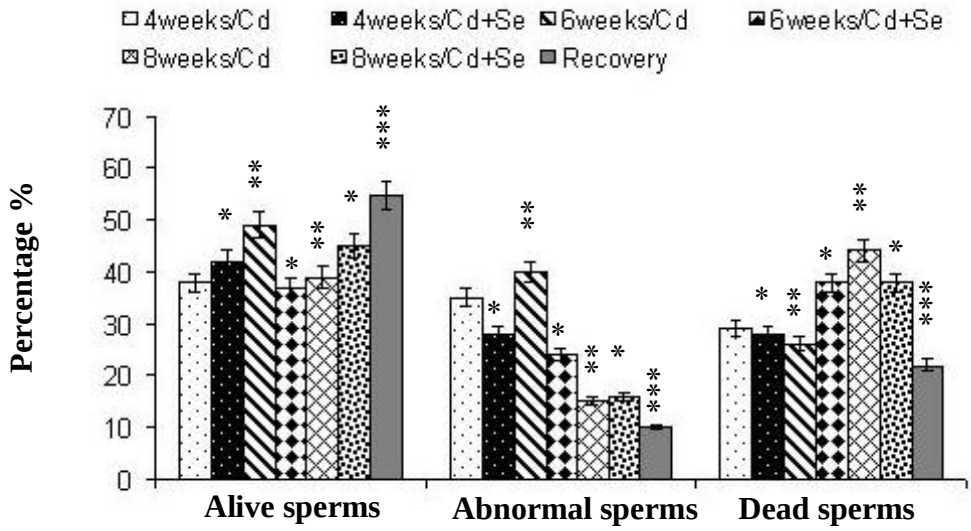


Fig (6). Sperm alive, sperm abnormal and sperm death percentage in long-term Cd and Cd/Se combination treated mice.

The data represent mean ± SD. Asterisks (*P < 0.05) indicate significant difference from control. Asterisks (**P<0.05) indicate significant difference from initial Cd. Asterisks (***P<0.05) indicate significant difference from initial Cd treatment.

The simultaneous co-se supplementation of a group of cadmium chloride-treated mice, is associated with a significantly higher sperm count and a lower percentage of abnormal sperm population along with a concomitant lower testicular injury, compared to the Cd-treated rat group.

The sperms morphological changes mainly appeared in head and tail deformation (fig 7a-AB). It was more pronounced in 72h/Cd exposure and distinguishable from normal appearance by flatten hooked or balloon head shaped and curly tail. (fig 7b – H and B).

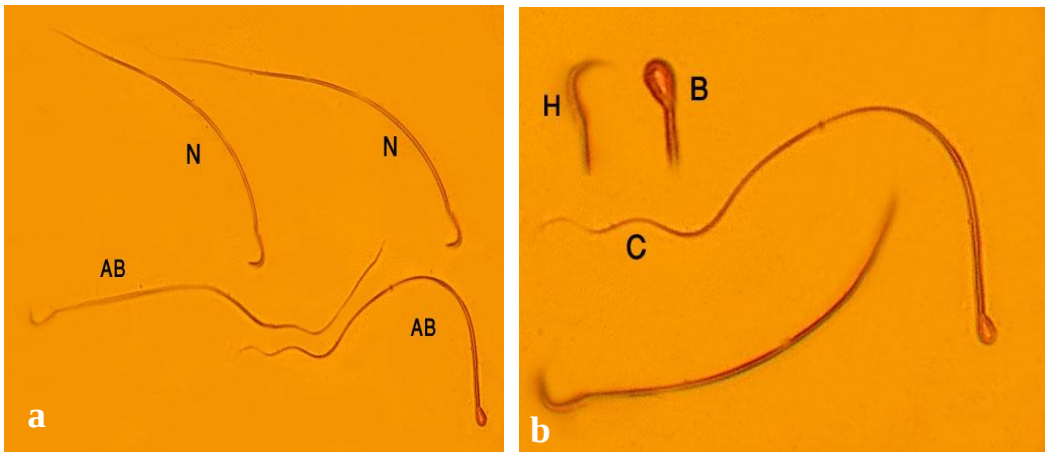


Fig (7): Sperms morphology of mice exposed to 72h Cd.

In the present study,cadmium-treated mice showed decrease in the level of both antioxidant enzymatic activities, catalase and peroxidase (Tables 3). Mice receiving only cadmium chloride had significant decrease in both testes peroxidase and catalase activities, due to acute Cd exposure.

Se co-administration with Cd to mice resulted in a significant elevation in the activities of testicular enzymatic activity with same normalizing trend, but it was less pronounced than that of control.Mice group left with no further treatment after the initial injection exhibit significant elevation in the activities of testicular antioxidant enzymes as compared to Cd treated group.

Table (3): Effect of Cadmium and cadmium–selenium combination) on catalase activity(U/mg/protein) in male mice

Treatments	Enzyme activity (U/mg/protein)	
	Peroxidase	Catalase
Cont	$2.8 \times 10^{-3} \pm 0.214 \times 10^{-4}$	274.09 ± 21.6
72 Cd	$2.18^{*} \times 10^{-3} \pm 0.752 \times 10^{-4}$	103.88* ± 2.5
72 Cd + Se	$2.428^{**} \times 10^{-3} \pm 0.196 \times 10^{-4}$	152.25**± 2.6
8 weeks Cd	$1.516^{*} \times 10^{-3} \pm 0.57 \times 10^{-4}$	98.98* ± 2
8 weeks Cd + Se	$2.445^{***} \times 10^{-3} \pm 0.94 \times 10^{-4}$	99.2***** ± 2

The data represent mean ± SD. Asterisks (*P < 0.05) indicate significant difference from control. Asterisks (**P<0.05) indicate significant difference from /Cd. Asterisks (***P<0.05) indicate significant difference from initial Cd treatment.

Discussion

In the present study, the male mice were administrated Cd at the LD_{50} dosages in order to evaluate the Cd-induce oxidative stress on epididymal sperm of male mice is time dependent. Two month old mice were selected in order to evaluate this effect through a complete spermatogenic cycle, which take approximately 45 days (25).

Body weight of the Cd-treated animals did not show significant changes, indicating that the general metabolic condition of the animals was with the normal range. The significant testes and epididymis weight reduction (Table1) may be due to Cd specific toxicity effect on the target organ and not the result of its general toxicity. This is in constituent with other investigation results demonstrated that the treatment by different oxidant substances resulted in significant reduction in relative organs weight (testes, epididymis, prostate and seminal vesicle in which subsequently affect the sperm production and motility (26).

Also our preliminary study shows that the testes weight of mice exposed to Cd was reduced with corresponding histopathological changes, Epididymal toxicity that manifested as decrease in the diameter of seminiferous tubule (fig.1.), this may be due to nicrosis and sloughing of epithelium indicating retarded testes and impaired spermatogenesis. This result in agreement with similar investigation results on rats testes(13).

Environmental contaminants, has been shown to interfere with normal reproductive processes and causing testicular abnormalities such as, decreased sperm count and motility (27). The observed sperm count decrease in the Cd treated mice in the present study may be due to direct interaction of ROS with sperm cell membrane, as well it was consistent with results of previous similar studies speculation in that oxidative damage to polyunsaturated fatty acids of cell membranes result in impairment of membrane fluidity and permeability leading to efficient damage of germ cells, spermatozoa and mature sperm (28-30). It is likely that epididymal sperms are a target of metals -induced toxicity. Mice sperms abnormalities may be due to chemical mutagens including cadmium (31,32). In the light of this, ($CdCl_2$), may considered as potent mutagen in mice causing formation abnormalities in both sperm head and tail Fig(7)

In the present study, cadmium-treated mice showed a significant increase in the level of lipid peroxidation in testes of Cd-treated mice and it was indicated by the reduction of peroxidase and Catalase activities(Table3). It has been reported that antioxidant enzymes evoke varied response that will depend on Cd frequency of exposure–dependent (14).Our finding in the present study very much in agreement with this concert. One mechanism by which metal ions produce injury is assumed

to be through generation of free radicals and lipid peroxidation (33. Free radicals or ROS generated in tissues in response to Cd toxicities, are effectively scavenged by epididymis antioxidant defense system which constitute antioxidant enzymes such as catalase and peroxidase, to protect spermatozoa from oxidative injury(34). The present study is in comply with previous studies speculation in that environmental contaminants induces excessive ROS generation which in turn may play important role in the defense mechanisms(35). The reduction and inhibition in Peroxidase and CAT activities parallel to metal exposure, which suggested that the production and accumulation of H_2O_2 is enough to cause Peroxidase and CAT to be poisoned, or possible mechanisms may include direct metal-mediated structural alteration of the enzymes and the depression of CAT synthesis(36). Catalase is responsible for the reduction of H_2O_2 by converting hydrogen peroxidase to water and molecular oxygen(37). This in turn resulted in the elimination of ROS generated due to intracellular Cd- toxification and considered an efficient protective enzyme against lipid peroxidation (38).

Results of the present study showed that the simultaneous Se/Cd administration to mice resulted in a significant elevation in the activities of testicular enzymatic activity. Selenium is one of several anti oxidative substance, which have been postulated to minimize testicular cytotoxic effects in animals treated with xenobiotic (39,40).

The protection in gametogensis activity after se co-administration in Cd-treated mice may be the result of restoration of testicular gametogensis (41), which may be due to the direct stimulatory effect of selenium nutrient on the enzymes (42,43). It may also be due to the antioxidant effect of selenium against oxidative stress induced by metal (44). The latter possibility is likely supported by results of other studies in restoring the facts that restored the testicular peroxidase and catalase activities(26)

The results of the present study revealed, that the long-term treatments as well the mice left to recover normally with out further treatment after each initial Cd–injection in the corresponding experimental periods, could outline useful remarks in that there were partial recovery of different parameters values in this study (sperm count,sperm abnormality and testes architecture, in addition to the antioxidant enzymatic activities). This may be due to the ability of the animals to adjust certain physiological process to compensate the toxic effect of metal with prolonged periods of exposure.

From results of the current study one may draw a conclusion that although the acute Cd exposure has deleterious effects on testes architecture and function, the prolonged period of exposure allow the animal adjust organ function to compensated the toxicity.

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