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PROTECTIVE EFFECT OF α-TOCOPHERAL ON BIOCHEMICAL AND HISTOLOGICAL ALTERATIONS INDUCED BY CADMIUM IN RAT TESTES

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Abstract : Cadmium (Cd) is a potential environmental pollutant and causes severe damage to reproductive organs in adults including ovary and testes. Of all antioxidants α-tocopheral is considered to be most potent chain breaking antioxidant. Our aim was to study the effect of α-tocopheral on biochemical and histological alterations induced by Cd in testes of rats. Group 1 served as control, while groups 2 and 3 received subcutaneous injections of $CdCl_2$ (3 mg/kg b.wt) once a week for four weeks. Group 3 in addition received α-tocopheral (75 mg/kg b.wt.) orally, daily for six weeks. Cadmium caused testicular tissue biochemical alterations such as significant increase in MDA, a peroxidation marker, decrease in antioxidant markers viz SOD, CAT and GSH and functional markers viz ALP and LDH. Histological alteration induced by Cd consisted of desquamation of basal lamina, shrunken tubules, generalized germ cell depletion with multinucleated gaint cells, degenerating Leydig cells, vascular congestion, interstitial edema and significant reduction in spermatodynamic count. α-tocopheral significantly reversed all the Cd induced alterations. These results indicate that α-tocopheral has a protective effect against Cd indced biochemical and histological alterations in rat testes.

Key words : cadmium, α-tocopheral, testes, histological markers, biochemical markers

INTRODUCTION

Cadmium (Cd) is a noxious environmental contaminant of great concern (1). Cadmium causes severe damage to embryos and the reproductive organs including the ovary and testis, which are highly sensitive to Cd toxicity (2).

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The metal is currently believed to cause most of its toxic effects by mechanism(s) related to its ability to generate free radicals at a rate high enough to overwhelm the natural antioxidant defense systems of the body (3). Cadmium increases the production of free radicals and causes peroxidation of lipids, proteins and nucleic acids (4) by reducing the activity of Cu-Zn superoxide dismutase (Cu-Zn SOD) (5), reduced glutathione (GSH) (6) and catalase activity (7).

Cadmium exposure to adult male rats decreases body weight, paired testicular weight, relative testicular weight, testicular total antioxidant capacity and protein levels along with significant decrease in activities of testicular tissue enzymes such as LDH and ALP (8). Ola-mudathir et al (9) observed that Cd administration caused a marked reduction in testicular tissue functional marker alkaline phosphatase (ALP) along with testicular tissue antioxidants.

Of all antioxidant defenses found in the fat soluble cellular membrane, α-tocopheral is considered to be most potent chain breaking antioxidant (10). Administration of á-tocopheral at the dose of 75 mg/kg body weight orally for 4 weeks effectively protected rat testes and prostrate by reversing the Cd induced alterations in lipid patterns (11).

The detailed information on effect of acute toxic dose of Cd on the biochemical and histological alterations of the testes of rats and the ameliorating effect of α-tocopheral has to be well established. Hence, the present study was aimed to investigate the acute effect of Cd induced testicular toxicity and the protective effect of α-tocopheral in rats.

MATERIAL AND METHODS

Experimental design

Thirty adult Wister strain albino male rats of 60 days age, with average body weight of 150 g were obtained from M/s Mahaveer Enterprises, Hyderabad (Regn. No. 146/1999/CPCSEA). The animals were weighed and maintained in the lab animal house as per the guidelines of CPCSEA. Animals were divided into 3 groups (n=10) and various treatments were given for 6 weeks as follows.

Group 1: Control-1.0 ml of distilled water subcutaneously once a week and 1.0 ml of distilled water daily through oral route.

Group 2: Cd toxic-$CdCl_2$ dissolved in distilled water and administered at the dose of 3 mg/kg b.wt. SC once a week, starting from day one, once a week for four weeks.

Group 3: Cd toxic treated with α-tocopheral-$CdCl_2$ at the rate of 3 mg/kg b.wt. SC once a week for four weeks and Vitamin E at the rate of 75 mg/kg b.wt, daily, orally for six weeks.

The dose of Cd to induce oxidative stress in this experiment was selected as per Adaikpoh et al (12) and α-tocopheral as per Adaikpoh and Obi (11).

Body weight gain and organ weight

Body weight was measured before and after the experimental period. At the end of

the experiment, the animals were sacrificed and testes were dissected out and weighed individually.

Biochemical estimation

The right testes were homogenized individually to make 10% homogenate to study various biochemical parameters. Antioxidant markers viz SOD was estimated by the method of Madesh and Balasubramanian (13); catalase activity was determined by the method of Calliborne (14) and GSH was estimated Moran et al (15) method. Malondialdehye, a peroxidation marker was estimated by the method of Subramanian et al (16). Testicular functional marker enzymes viz LDH and ALP were estimated by using the standard kits from Diagnosticum Zrt, Hungary and Transasia Bio-medicals Ltd, respectively. Total protein in testicular tissue homogenate, was quantified as per Lowry et al (17).

Histological study

The testes were fixed in Bouin's fixative, embedded in paraffin and 5 µm thick sections were stained with routine Hematoxtylin-Eosin. Histological changes were examined with optical microscope.

Morphometry and spermatodynamic count

Quantitative analysis of spermatogenesis was carried out from 5 perfect transversely cut tubules from each testis of respective groups. The relative number of spermatogonia, resting spermatocytes, pachytene spermatocytes, and spermatids were counted at 400x magnification (18). Seminiferous tubular diameter was determined at 400x magnification by ocular micrometer. Leydig cell population was counted per field from the sagital plan area of the section at 400x magnification.

Statistical analysis

The data were subjected to statistical analysis by applying one way ANOVA using statistical package for social sciences (SPSS) 12th version. Differences between means were tested using Duncan's multiple comparison test and significance was set at $P<0.05$.

RESULTS

Body weight gain

The average body weight gain at the end of the experiment showed significant reduction in group 2 compared to control. However, the body weight gain of group 3 was increased significantly compared to group 2 (Table I).

Testes weight

A significant reduction in absolute and

TABLE I: Body weight gain and absolute and relative testes weights of different groups of rats.

Groups	*Body weight gain (g)*	*Testes weight*	
		Absolute (g)	*Relative (g)*
1 Control	112.30±3.52	2.47±0.06	9.98±0.31
2 Cd toxic	71.70±3.30*	1.09±0.02*	5.27±0.22*
3 Cd toxic treated with α-tocopheral	99.1±5.84#	2.16±0.041#	8.50±0.79#

Values are mean±SEM (n=10) One way ANOVA (SPSS 12.0)
*Significantly differ from control ($P<0.05$);
#Significantly differ from Cd toxic group ($P<0.05$).

relative testes weight in group 2 compared to group 1 was observed, while a significant increase in testes weight was observed in group 3 compared to group 2 (Table I).

Antioxidant markers of testicular tissue

The activities of SOD (units/mg protein) and catalase (mM H_2O_2 utilized/min/mg of protein) and concentration of reduced glutathione (μM/mg protein) in testicular tissue of cadmium treated group 2 rats were significantly (P<0.05) lesser than control group. However, upon treatment with α-tocoferal in group 3, the antioxidant markers were significantly (P<0.05) reversed towards normal level compared to group 2 (Table II).

Testicular tissue peroxidation markers

The concentration of MDA (nM/g protein) in testicular tissue of group 2 was significantly (P<0.05) increased as compared to control, whereas in group 3 it showed a significant (P<0.05) decrease as compared to group 2 (Table II).

Functional markers of testicular tissue

The concentrations of LDH (IU/mg protein) and ALP (U/mg protein) were significantly decreased (P<0.05) in group 2 as compared to group 1 whereas a significant increase was observed in group 3 compared to group 2 (Table II).

Histological findings

In the normal control, histoarchitecture of the testes showed an organized distribution of the different types of cells in the seminiferous epithelium of all the tubules. Sertoli cells and spermatogonia were observed in the basal compartment, whereas the spermatocytes and the spermatids were observed in the adluminal compartment. The peritubular basement membrane was intact. The interstitial tissue showed normal Leydig cells and intact blood vessels (Fig. 1).

Testes of Cd treated group (2) showed an alteration in the histoarchitecture and the histological injury was characterized by necrotic changes in both the seminiferous tubules and the interstitial tissue. Peritubular basement membrane was desquamated and basal lamina was not seen in some tubules. Destruction of the germ cells in the seminiferous epithelial layer,

TABLE II: Effect of α-tocopheral on Cadmium treated rat's testicular tissue peroxidation markers, antioxidant markers and functional markers.

		Peroxidation marker	*Antioxidant markers*			*Functional markers*	
S. No.	*Groups*	*MDA (nM /g protein)*	*SOD (U/mg protein)*	*CAT (mM H_2O_2 utilized/ min/mg protein)*	*GSH (μM of GSH/ mg protein)*	*LDH (IU/mg protein)*	*ALP (U/mg protein)*
1.	Control	81.25±4.15	13.42±0.8	2.37±0.10	57.75±2.21	25.11±0.98	53.30±1.82
2.	Cd toxic	138.78±2.79*	4.73±0.56*	1.04±0.06*	31.47±1.60*	6.19±0.27*	22.64±0.87*
3.	Cd toxic treated with α-tocopheral	89.72±2.68#	9.49±0.57#	1.92±0.05#	51.89±2.96#	15.85±0.53#	42.13±1.87#

Values are mean±SEM (n=10) One way ANOVA (SPSS 12.0).
*Significantly differ from control (P<0.05); #significantly differ from Cd toxic group (P<0.05).

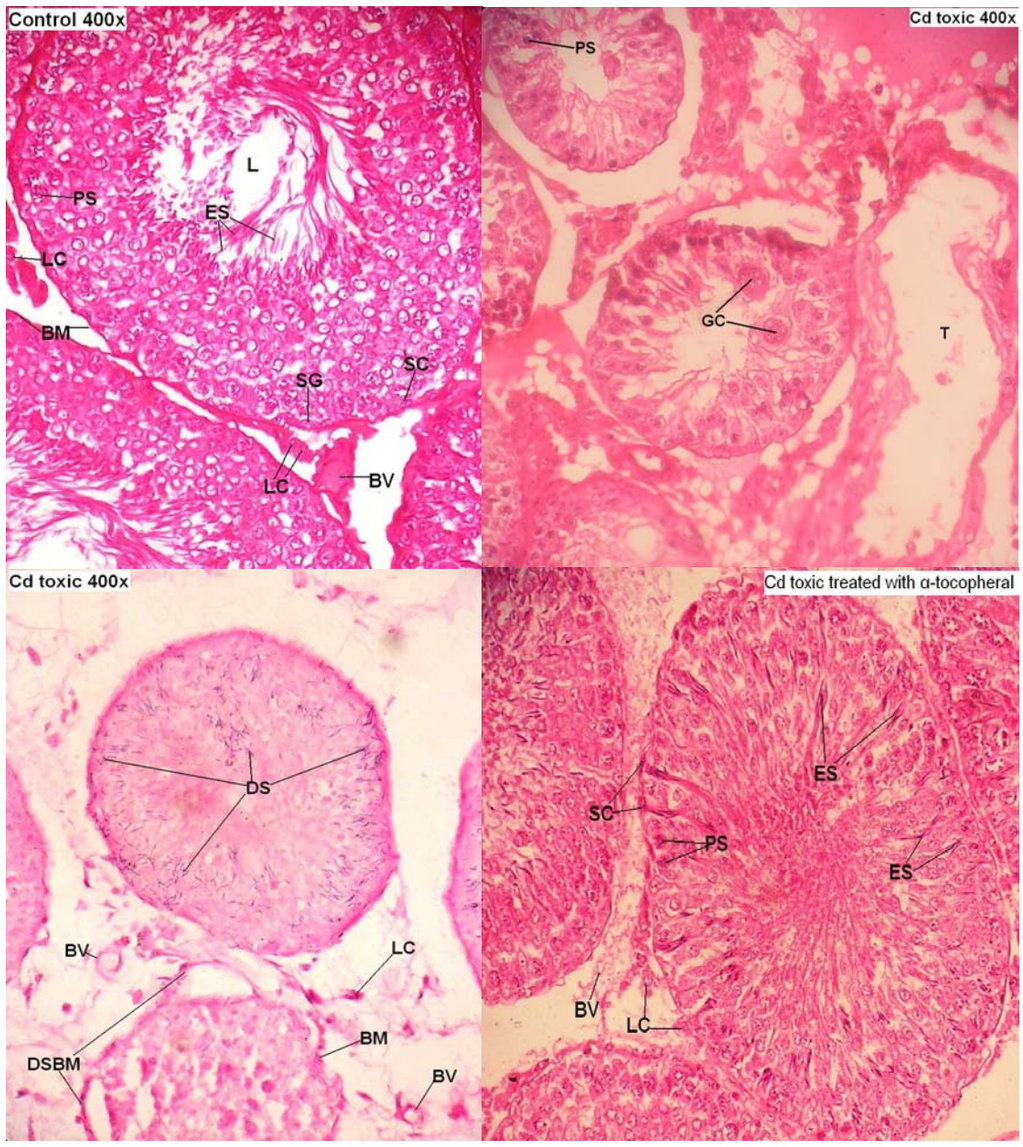

Fig. 1: Photomicrograph of the testes of Control: showing an organized distribution of various types of cells in the seminiferous epithelium of the tubule and the Leydig cells and intact blood vessels in the interstitial tissue. H&E 400x. Cadmium Toxic: showing alterations in the normal histoarchitecture, desquamated basement membrane, ruptured blood vessel, degenerating Leydig cell and tubules with dead spermatozoa dispersed in all directions. Cadmium Toxic treated with α-tocopheral: Showing almost normal architecture.

ST-Seminiferous Tubule; SC-Sertoli Cell; SG-Spermatogonium; LC- Leydig Cell; L-Lumen; PS-Pachytene Spermatocyte; ES-Elongated Spermatids; BV-Intact Blood Vessel; BM-Basement Membrane; DS-Dead Spermatids; DSBM-Desquamated Basement Membrane; GC-Gaint Cell; T-Tubule with depleted germ cell.

TABLE III : Effect of α-tocopheral on Cadmium treated rat's testicular tissue histomorphometry.

S. No.	Groups	Seminiferous Tubular diameter (μm)	Leydig cell count/ field	Spermatodynamic count per tubular cross section in each slide ten nearly round seminiferous tubule was counted at 400x			
				Spg	R-Rpcyt	Pchyt-Spcyt	Spermatid
1.	Control	346.13±14.60	10.50±0.84	38.00±0.93	71.33±1.62	86.83±2.31	127.50±2.5
2.	Cd toxic	192.58±9.53*	5.16±0.70*	23.50±0.88*	55.33±1.54*	66.66±2.10*	103.83±1.97*
3.	Cd toxic treated with α-tocopheral	258.42±5.02#	7.50±0.6#	34.16±1.60#	67.00±1.39#	80.50±1.40#	114.16±1.95#

Values are mean±SEM (n=10) One way ANOVA (SPSS 12.0)
*Significantly differ from control (P<0.05); #significantly differ from Cd toxic group (P<0.05)
Spg: Spermatogonia, R-Spcyt: Resting Spermatocyte, Pchyt-Spcyt: Pachytene Spermatocytes.

pyknosis and destruction of nuclei were observed, resulting in germ cell depletion. The sections showed some shrunken tubules with multinucleated giant cells. Cadmium also destroyed the supporting Sertoli cells and some of them showed cytoplasmic vacuolization. Some tubules appeared with various stages of scattered spermatogonic cells, especially the spermatids losing their characteristic adluminal location and being oriented in different directions between the spermatogenic cells. In the interstitial tissue, degenerating Leydig cells, vascular congestion and interstitial edema were observed (Fig. 1).

The histoarchitecture of group 3 testes appeared near normal. The tubules and the interstitial tissue showed no signs of histological injury. The basement membrane around the tubules was intact. The Sertoli cells and spermatogenic cells were observed in an organized way. The interstitial tissue showed the normal Leydig cells and intact blood vessels (Fig. 1).

Morphometric study and spermatodynamic count

Cadmium administration significantly reduced the seminiferous tubular diameter compared to control, whereas simultaneous administration of α-tocopheral along with Cd significantly lessened the reduction of tubular diameter compared to group 2. Counting of various cell types of spermatogenesis such as spermatogonia, resting spermatocytes, pachytene spermatocytes and spermatids revealed their significant decrease in Cd group compared to control. However, group 3 showed a significant increase in their number compared to group 2 (Table III). The number of Leydig cells was also significantly decreased in group 2 compared to group 1, while in group 3 the count was significantly increased.

DISCUSSION

Cadmium is a potential environmental pollutant (1) and testes are highly susceptible to the toxicity compared to other vital organs of the body (19). In the present study, Cd administration significantly reduced the body wt gain compared to control group which may be due to oxidative stress induced by Cd on all vital organs (20).

In our study, Cd administration induced

tissue peroxidation that was indicated by significant increase in peroxidation marker (MDA), and significant decrease in antioxidant markers i.e SOD, CAT & GSH of testicular tissue. The tissue peroxidation there by disrupted the Sertoli cell functions such as structural support, nutrient supply regulation of paracrine factors & blood testes barrier (21). Consequently, Sertoli cells were vacuolated and the spermatogonia were separated from Sertoli cells and the basement membrane.

Spermatodynamic count indicated significant reduction in number of spermatogonia, resting spermatocytes, pachytene spermatocytes and spermatids. Cd induced the vascular congestion and there by produced interstitial edema. Cadmium also induced the necrosis of leydig cells and reduction of their number, resulting in reduction of testosterone production and there by protective effect on Sertoli cells. In the present study overall Cd induced adverse effects observed was peroxidative damage and reduction in absolute and relative testicular weight which was supported by observation of Sadik (8).

Administration of α-tocopherol to Cd intoxicated rats, exhibited a significant protective effect that was revealed by significant reduction in peroxidation marker (MDA), and significant increase in antioxidant markers (SOD, CAT and GSH). The protective effect was further evidenced by restoration of testicular tissue function to nearly normal as indicated by significant increase in testicular tissue function markers i.e LDH & ALP along with no signs of histological injury to tubules and interstitial tissue and restoration of various spermatodynamic counts compared with Cd intoxicated group.

In conclusion the results of present study enunciated that Cd induced toxicity in testicular tissue can be attributed to the excessive generation of free radicals and impairment of antioxidant defenses. Impaired testicular tissue function was revealed by significant alteration in testicular functional markers, peroxidation markers, spermatodynamic count and histomorphometry. Administration of α-tocopherol countered the Cd induced testicular toxicity.

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