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Preventive Role of Vitamin E and Vitamin C In Combination on Cadmium Induced Oxidative Stress on Rat Testis.

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ABSTRACT

Cadmium (Cd) is an industrial pollutant that affects the male reproductive system. The purpose of present study was to investigate the protective role of vitamin E & vitamin C on cadmium induced testicular damage. Rats were divided into four groups with 8 rats in each .The Gr. I rats were administered with the single dose of normal saline intraperitoneally. Group II received Vitamin E & Vitamin C orally for 30days.Group III received a single dose of 1mg/kg bw cadmium chloride and Group IV vitamin E and vitamin C for 30 days prior to cadmium administration. After the desired protocol, rats were sacrificed & both the testes were removed for biochemical & histopathological evaluation .In the present study, the level of lipid peroxidation (LPO) was significantly high & GSH& SOD were low in cadmium treated rats compared to normal control. Pre-treatment with vitamin E & C showed a protective effect by decreasing LPO & increasing GSH& SOD level. The morphological changes like atrophy of tubules, edema &decreased spermatogenesis in the testis of rats exposed to cadmium chloride. But, antioxidant showed the normal architecture of the testis .Thus, present study showed that pre-treatment with vitamin E & C combination was beneficial in protecting the testis from cadmium induced toxicity

Keywords: Cadmium, oxidative stress, antioxidants, lipid peroxide, glutathione

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INTRODUCTION

Cadmium (Cd) is an toxic pollutant that affects the male reproductive system [1]. There are very few studies showing the impact of environmental pollutants on human health in general or on human reproductive function in particular [2]. Cadmium is one of the most toxic heavy metals known to pollute environment[3]. Being widely used in industry, it affects human health through occupational and environmental exposure [4]. Cadmium is known to affect various organs like kidney, liver, bone and testis in human beings and experimental animals [5]. Testes are exquisitely sensitive to cadmium toxicity [6]. Exposure to cadmium metal is known to induce the formation of reactive oxygen species (ROS) like superoxide radical, hydroxyl ion and hydrogen peroxide From the literature it is evident that use of antioxidant in preventing cadmium chloride induced testicular damage is conflicting. The purpose of present study was to investigate the protective role of combined therapy of vitamin E & vitamin C on cadmium induced testicular damage.

METHODS AND MATERIAL

The present study was conducted following approval from Institutional Bioethical Committee and strict internationally accepted guidelines, for the usage of animals in experimental study were followed .Rats were segregated into 4 different groups(n=8).Number of rats in each group is 8. The normal control group (Gr. I) rats were administered with the single dose of normal saline intraperitoneally. Group II received Vitamin E (100mg/kg bw) & Vitamin C(30mg/kg bw) orally for 30days.Group III received a single dose of 1mg/kg bw cadmium chloride and Group IV received vitamin E and vitamin C for 30 days orally prior to 1mg/kg bw cadmium administration. After the desired protocol, rats were sacrificed & both the testes were removed for biochemical & histopathological evaluation. One testis was fixed in Bouvins fluid and processed for histopathological studies The levels of lipid peroxides (LPO)[7]and glutathione (GSH)[8] & superoxide dismutase (SOD)[9] were detected in the tissue homogenates of other testis.

Statistical Analysis

Values were expressed in mean ± SEM. SPSS version II was used for statistical analysis. Differences between groups were assessed by one-way analysis of variance .The Post Hoc (LSD) test was used for intergroup comparison. P <0.05 was taken as significant.

RESULTS

In the present study, the level of lipid peroxidation (MDA) (58.166 ± 2.466) was significantly high (P<0.001) &GSH(3.414 ± 0.176) & SOD(5.078 ± 0.191) were low in cadmium treated rats compared to normal control. Pre-treatment with vitamin E & C in combination showed a protective effect by decreasing MDA (20.832±1.513,P<0.001) & increasing GSH(4.509±0.142;P<0.001 & SOD(10.512 ± 0.530; P<0.001) level(Table-1). The testes of normal control rats showed the normal architecture of the testes (Fig-1). No detectable histological alterations showed in the testes of rats pretreated with vitamins in combination (Fig-2). But,the testes of male albino rats intoxicated with cadmium chloride alone showed decreased spermatogenesis(less than 10% of tubule) & atrophy of the tubules(Fig-3). Testes of the rats pretreated with vitamin E & C in combination prior to cadmium administration were normal and showed spermatogenesis in more than 50% of the tubule & also interstitial cells were normal(Fig-4)

Table 1: Effects of pretreatment with vitamin E & C in combination for 30days prior to cadmium administration on rat testis. The values are expressed as mean± SEM. In each group eight animals were used. NS= not significant versus Gr.I.. ***p<0.001 versus Gr.I & Gr.II. p<0.001 versus Gr.III

Groups	GSH(nmol/mg)	SOD(units/gm protein)	MDA(nmol/gm wet tissue)
Gr.I	5.951±0.379	12.451±0.655	5.113±0.277
Gr.II	5.873±0.345^{NS}	12.523±0.732^{NS}	5.230±0.212^{NS}
Gr.III	3.414 ± 0.176***	5.078±0.191***	58.166±2.466***
Gr.IV	4.509±0.142§§§	10.512±0.530§§§	20.832±1.513§§§

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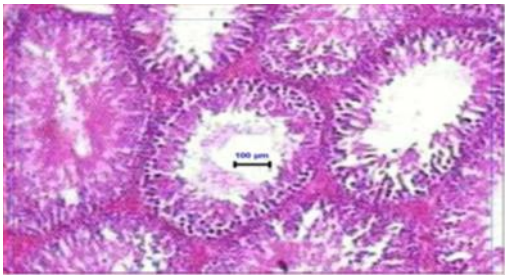

Figure 1: Testes of male albino rat treated with 0.9% saline showing normal structure of seminiferous tubules(H&E ;10X). Scale bar 100µm(Gr.I)

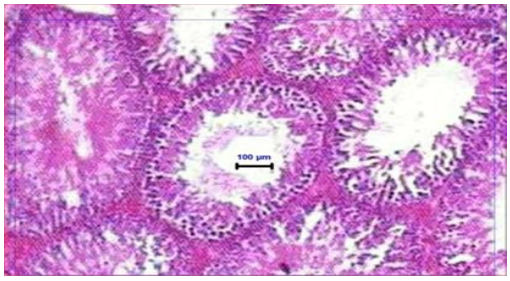

Figure 2: Testes of male albino rat treated with Vitamin E and C in combination showing normal structure of seminiferous tubules (H&E ;10X. Scale bar 100µm(Gr.II)

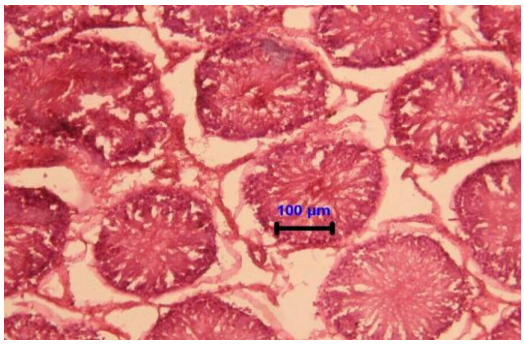

Figure 3: Testes of male albino rat intoxicated with cadmium chloride alone showing atrophy of the tubules. (H& E;10X). Scale bar 100µm(Gr.III)

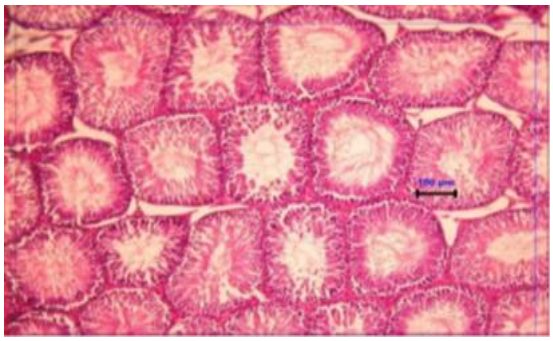

Figure 4: Testes of male albino rats pre-treated with vitamin E & C &cadmium chloride showing normal seminiferous tubules. (H& E; 10X). Scale bar 100µm(Gr.IV)

DISCUSSION

Cadmium is widely distributed in the environment because of its many industrial applications. The health risk to humans from acute and chronic cadmium exposure has been well documented. Previously, Mueller (1986) reported that single-dose cadmium administration increased lipid peroxidation and decreased GSH in the liver [10]. Many investigators reported that the reduction of GSH levels leads to elevation of LPO [11,12]. The present study demonstrated that the levels of GSH & SOD in the tissues homogenates of testes were significantly declined in cadmium-group comparing with controls. Various mechanisms were suggested to be responsible for the cadmium toxicity. One of these mechanism includes cadmium binding to–SH groups from cell membrane proteins, cytoplasmic proteins, and enzymes. In addition, cadmium can reduce activities of several enzymes including enzymes antioxidants In addition, the authors showed that in vitro and in vivo cadmium administration in rats increased tissue lipid peroxidation [13]. In agreement with the previous results, the current study revealed that the levels of LPO were significantly higher in cadmium group than control group in the tissues homogenates of testes. Aruld has reported that oxidative stress by free radical toxicity caused by cadmium affected infertility[14]. Stajn et al. reported that different doses of cadmium increase organ lipid peroxidation (LPO) in many organs including male sex organs and brought about changes in the antioxidant defense system[15]. Blanco et al. claimed that even with low doses of cadmium chloride (1mg/kg for one month) induced lack of spermatogenesis and severe necrosis of the testes of rats[16]. The result of the present study showed histological changes like decreased spermatogenesis(less than 10% of tubule) and atrophy of the tubules

In the present study, pretreatment with vitamin E & Vitamin C in combination prior to cadmium administration showed a significant reduction in the levels of LPO compared to cadmium treated group. The levels of GSH & SOD in rats pretreated with vitamins prior to cadmium administration were significantly elevated in comparison with cadmium treated group. The present study also revealed the pretreatment with vitamin E & C in combination prior to cadmium administration showed the normal architecture of the testis.

Hence, the result of the present study demonstrated the protective role of vitamin E & C in combination on cadmium chloride induced testicular damage.

CONCLUSION

The exposure of rats to cadmium chloride induces biochemical & histopathological effects in the testes. The increased oxidative stress resulted from cadmium intoxication in testicular tissue might be responsible, at least in part, for histopathological changes.Vitamin E & C in combination had protective effect against cadmium toxicity evidenced by reduction of LPO & increase in GSH and SOD level as well as normal architecture of the testes.

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