

Antioxidant Role of Beta Carotene: Protection against Cadmium Induced Testicular Toxicity

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ABSTRACT

Introduction: Cadmium (Cd) is an industrial pollutant that affects the male reproductive system. The purpose of present study was to investigate the protective role of Beta carotene on cadmium induced testicular damage. **Materials and Methods:** 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. 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 Beta carotene (10 mg/kg bw) orally for 30 days. Group III received a single dose of 1 mg/kg bw cadmium chloride and Group IV received Beta carotene for 30 days prior to cadmium administration. After the desired protocol, rats were sacrificed and both the testes were removed for biochemical and histopathological evaluation. One testis was fixed in Bouvins fluid and processed or histopathological studies. The levels of lipid peroxides (LPO) and glutathione (GSH) and superoxide dismutase (SOD) were detected in the tissue homogenates of other testis. **Results:** In the present study, the level of lipid peroxidation (LPO) was significantly high and GSH and SOD ($P < 0.001$) were low in cadmium treated rats compared to normal control. Pre-treatment with beta carotene showed a protective effect by decreasing LPO and increasing GSH and SOD level ($P < 0.001$). The morphological changes like atrophy of tubules, edema and decreased spermatogenesis in the testis of rats exposed to cadmium chloride. But, antioxidant showed the normal architecture of the testis. **Conclusion:** Results of the present study showed the antioxidative role of beta carotene in protecting the testis from cadmium induced toxicity.

Key words: Testis, Oxidative stress, Antioxidant, Lipid peroxidation, Superoxide demutase.

INTRODUCTION

Cadmium is a heavy metal which is extremely toxic. Buildup of cadmium levels in the water, air, and soil has been occurring particularly in industrial areas. Food and cigarettes are also a significant source of cadmium exposure.¹ Blood and kidney Cd levels are consistently higher in smokers than nonsmokers. Inhalation due to industrial exposure can be significant in occupational settings for example, welding or soldering, and can produce severe chemical pneumonitis.² Aside from tobacco smokers, people who live near hazardous waste or factories that release cadmium into the air have the potential for exposure to cadmium in air. Cadmium toxicity has been demonstrated in several organs. Cadmium induces tissue injury through creating oxidative stress.³⁻⁵ Cadmium acts as a catalyst in forming reactive oxygen species. It increases lipid peroxidation, in addition it depletes antioxidants, glutathione and protein-bound sulfhydryl groups⁶ Testes are known to be the target organs for cadmium toxicity. The mechanisms of cadmium toxic effects on the testes involve the damage of vascular endothelium, intracellular junctions, germ cells, and Leydig and Ser-

toli cells. This metal can reduce testosterone synthesis at various levels and deteriorate spermatogenesis.⁷ Antioxidants protect the tissue from oxidative stress. Several studies are aimed at antioxidant therapy to prevent cadmium induced testicular damage.^{8,9} However, studies regarding role of beta carotene on cadmium induced testicular toxicity are very few. Moreover, it is also seen that increasing environmental exposure to cadmium, currently existing occupational exposure and the prevalence of tobacco smoking has resulted in constant increase in the number of diagnosed fertility impairments. Hence present study was aimed to study whether pretreatment with beta carotene will be helpful in reducing cadmium induced testicular damage.

MATERIALS AND METHODS

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. Inbred adult male rats of wistar strain weighing 200-

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250g were used in the present study. Animals were housed in polypropylene cages (4-5 rats/cage) under standard laboratory conditions and fed *ad libitum* with commercial rodent chow (Hindustan Lever Limited) and water. Cadmium chloride (CdCl₂) (LobaChemie, India) was dissolved in normal saline. Beta carotene is dissolved in coconut oil and administered orally (10mg/kg bw).

Animals were divided into four groups of eight rats in each group. In the normal control group (Group I) rats were administered with the normal saline intraperitoneally. In Group-II animals received beta-carotene (10mg/kg bw) for 30 days orally. In untreated experimental control groups (cadmium treated group) rats were administered with single dose of 1 mg/kg bw (Group-III) cadmium chloride intraperitoneally. In pretreated groups rats were pretreated with beta carotene (10mg/kg bw) for 30 days orally and then injected with 1mg /kg bw (Group-IV) cadmium chloride intraperitoneally.

In all the groups, rats were sacrificed under anesthesia 15 days after the final cadmium administration. Following the completion of the experimental protocol animals in each group were anaesthetized by injecting sodium pentobarbitone (40mg/kg bw) intraperitoneally under aseptic conditions. Laparotomy was performed, and the reproductive organs were exposed. Both the testes were removed and cleaned of fat tissue and blood, then weighed. In each group, right testis was cut into small pieces and used for biochemical analysis and left testis was used for histo-pathological studies. Pieces of the right testis were transferred into a glass homogenizer containing 10ml of cold phosphate buffer saline solution of pH 7.4. The tissue was homogenized using a manual homogenizer. The unbroken cells and cell debris were removed by centrifugation at 3000 rpm for 10 mins by using Remi C 24 refrigerated centrifuge 40C. The obtained supernatant was used for the GSH, SOD and lipid peroxide estimation. Left testis was put into a bottle containing Bouins solution for the histo-pathological analysis.

Estimation of Testicular Lipid Peroxidation

Lipid peroxidation was estimated according to the method of Kartha and Krishnamurthy.¹⁰ This assay is based on the reaction of TBA with malondialdehyde (MDA), one of the aldehyde products of lipid peroxidation.

Estimation of Tissue Glutathione

Glutathione content in the tissue homogenate [10% w/v in 10mM potassium phosphate buffer (7.4pH)] was estimated by the method of Beutler *et al.*¹¹

Superoxide Dismutase Assay

Superoxide Dismutase (SOD) was estimated by original method of Beauchamp and Fridovich.¹²

Histological Slides Preparation

Specimens from testicular tissues were fixed in Bouins solution and dehydrated in ascending grades of ethanol alcohols, cleared in xylol, casted, blocked, cut at 2-5 μ m thickness and stained with hematoxylin-eosin for microscopic examination.¹³

Statistical Analysis

Values were expressed in mean \pm 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

Biochemical Results

The levels of LPO in the tissues homogenates of testes were significantly higher in cadmium-group than control group. Pre-treatment with beta carotene prior to cadmium administration showed significant decrease in the level of lipid peroxide. The levels of GSH and SOD in the tissues homogenates of testes were significantly declined in Cadmium-group comparing with controls. In the rats, pretreated beta carotene the levels of GSH and SOD were significantly elevated in comparison with cadmium-treated group (Table1).

Histopathological Results

The testes of normal control rats showed the normal architecture of the testes (Figure 1). No detectable histological alterations showed in the testes of rats pretreated with beta carotene (Figure 2). The testes of male albino rats intoxicated with cadmium chloride alone showed decreased spermatogenesis (less than 10% of tubule) and atrophy of the tubules (Figure 3). Testes of the rats pretreated with beta carotene prior to cadmium administration were normal and showed spermatogenesis in more than 50% of the tubule and interstitial cells were normal (Figure 4).

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.¹⁴ Many investigators reported that the reduction of GSH levels leads to elevation of LPO.^{15,16} The present study demonstrated that the levels of GSH and 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 mechanisms 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

Table 1: Effects of pretreatment with beta carotene for 30 days prior to cadmium administration on rat testis. The values are expressed as mean \pm SEM. In each group eight animals were used. NS= not significant versus Gr.I. * $p < 0.001$ versus Gr.I and Gr.II. www $p < 0.001$ versus Gr. III.**

variables	Group I	Group II	Group III	Group IV
MDA (nmol/gm wet tissue)	5.113 \pm 0.277	5.121 \pm 0.261 NS	26.687 \pm 1.229***	5.826 \pm 2.168www
GSH (nmol/mg protein)	5.951 \pm 0.379	5.405 \pm 0.218 NS.	3.570 \pm 0.08***	5.970 \pm 0.341www
SOD (units/gm protein)	12.451 \pm 0.51	12.498 \pm 0.428 NS	7.496 \pm 0.376***	12.548 \pm 0.624www

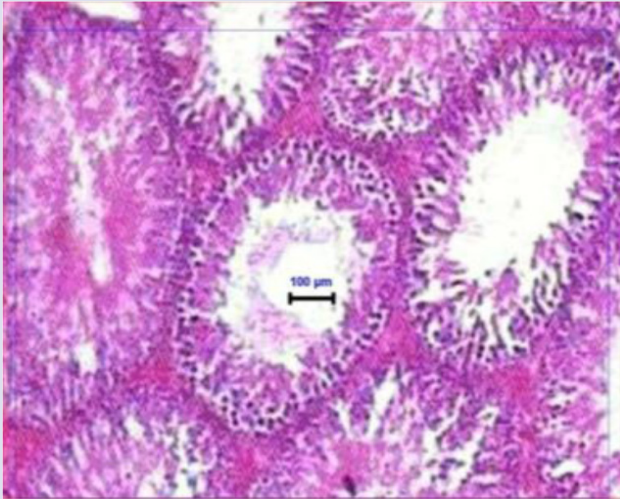


Figure 1: Testes of male albino rats treated with 0.9% saline showing normal structure of seminiferous tubules (H and E; 10X). Scale bar 100μm (Gr.I).



Figure 3: Testes of male albino rats with cadmium administration showing decreased spermatogenesis and atrophy of the tubules (H and E; 10X). Scale bar 100μm (Gr.IV).

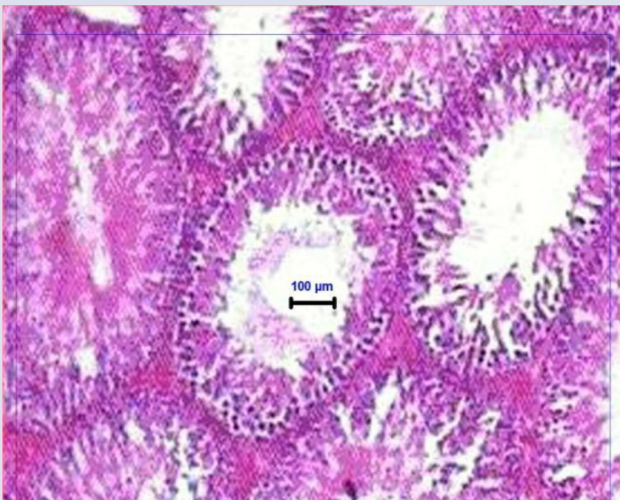


Figure 2: Testes of male albino rats treated with beta carotene showing normal structure of seminiferous tubules (H and E; 10X). Scale bar 100μm (Gr.I).



Figure 4: Testes of male albino rats treated with beta carotene prior to cadmium administration showing normal structure of seminiferous tubules (H and E; 10X). Scale bar 100μm (Gr.IV).

rats increased tissue lipid peroxidation.¹⁷ 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.¹⁸ 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.^{19,20} 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.²⁰ Moreover, Santos *et al.* reported that endothelial damage of the small blood vessels, edema and hemorrhage of the rat testes can be demonstrated by using just a single parenteral dose of cadmium chloride at 2-4 mg/kg.²¹ 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 beta carotene prior to cadmium administration showed a significant reduction in the levels of LPO compared to cadmium treated group. The levels of GSH and SOD in rats pretreated with beta carotene prior to cadmium administration were significantly elevated in comparison with cadmium treated group. The present study also revealed the pretreatment with beta carotene prior to cadmium administration showed the normal architecture of the testis.

CONCLUSION

Hence, the result of the present study demonstrated the protective role beta carotene on cadmium chloride induced testicular damage.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

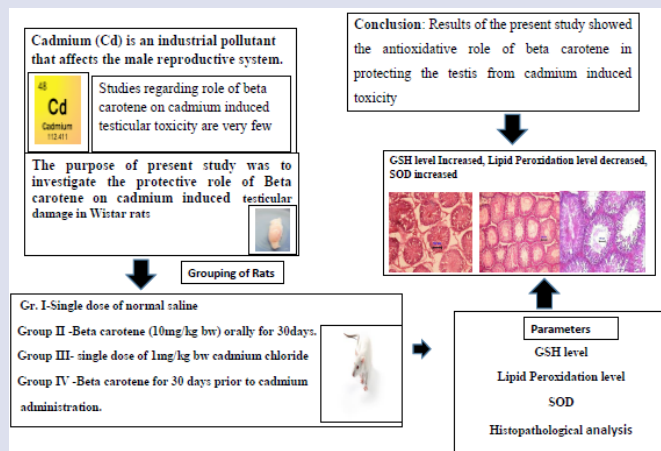
ABBREVIATIONS

Cd: Cadmium; **SOD:** Superoxide Desmutase; **GSH:** Reduced Glutathione; **LPO:** LipidPeroxidation.

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GRAPHICAL ABSTRACT



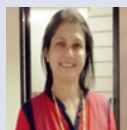
SUMMARY

Thus in summary the present study emphasizes the toxic effect of cadmium on testis and protective role of beta carotene on reducing the toxic effects of cadmium chloride.

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