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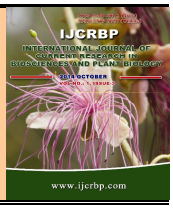

Original Research Article

Beta Carotene as an Antioxidant in Cadmium Induced Testicular Toxicity

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Abstract

Aim of present study was evaluate the anti-oxidative role of beta carotene against cadmium chloride induced oxidative stress on rat testis. Rats were divided into Group-I (0.9% Saline treated), Group-II (Beta carotene treated for 30 days), Group-III (injected with single dose of 1 mg/kg body weight cadmium chloride) and Group-IIIA (Beta carotene for 30 days+ cadmium administration). In all the groups, rats were sacrificed 15 days after the final cadmium chloride or saline administration. Exposure to cadmium chloride lead to significant (p<0.001) decrease in the epididymal weight and sperm count compared to Group-I and Group-II. Standard Tubular Diameter (STD) and Standard Epithelial Height (SEH) was also low in Group-III animals compared to Group-I and Group-II. Photomicrograph of rats in Group-I and Group-II showed normal architecture of testis and rats treated with cadmium chloride showed the destruction of the seminiferous tubules. But, rats of Group-IIIA showed significant (p<0.001) increase in epididymal weight and sperm count compared to Group-III. STD and SEH was also significantly (p<0.001) high in Group-IIIA. Photomicrograph of rat testis in Group-IIIA showed almost normal testicular architecture. Results of the present study showed the antioxidative role of beta carotene in ameliorating cadmium induced testicular damage.

Keywords

Antioxidant

Cadmium

Beta carotene

Sperm count

Standard tubular diameter

Introduction

Cadmium is an extremely toxic metal commonly found in industrial workplaces. Due to its low permissible exposure limit, overexposures may occur even in situations where trace quantities of cadmium are found. Buildup of cadmium levels in the water, air, and soil has been occurring particularly in industrial areas. In the industry, cadmium is hazardous both by inhalation and ingestion and can cause acute and chronic intoxications. Food is the source of cadmium. Cigarettes are also a significant source of cadmium exposure (Jarup et al., 2014). Various regulatory bodies have concluded that there is sufficient evidence to classify cadmium as a human

carcinogen. Cadmium acts as a catalyst in forming reactive oxygen species. It increases lipid peroxidation, in addition it depletes antioxidants, glutathione and protein-bound sulfhydryl groups (Flora et al., 2008). 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, Leydig and Sertoli cells. This metal can reduce testosterone synthesis at various levels and deteriorate spermatogenesis (Martynowiez et al., 2005).

An antioxidant is a molecule that inhibits the oxidation of other molecules. Plants and animals maintain complex system of multiple types of antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E, beta carotene etc. (Sies, 1997). Being an important flavonoid compound, beta carotene has powerful antioxidant functions, helps the body scavenge free radicals, thereby limiting the damage to cell membranes, DNA and protein structures in the cell. Research studies suggest that dietary intake of foods high in β-carotene has positive association with decreased risk of cardio-vascular disease as well as oral cavity, and lung cancers. When converted to vitamin A in the intestines it has all the functions of vitamin A such as visual cycle, reproduction (sperm production), maintenance of epithelial functions, growth and development. Several studies are aimed at antioxidant therapy to prevent cadmium induced testicular damage (Yan-Li et al., 2013; Rekha et al., 2011).

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 200-250g 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 (Hindusthan Lever Limited) and water**.** Cadmium chloride ($CdCl_2$) (LobaChemie, India) was dissolved in normal saline. Beta carotene is dissolved in coconut oil and administered orally (10mg/kg bw)

Experimental protocol and drugs

Animals were divided into five groups of eight rats in each group. In the normal control group (Group I) rats were administered with the normal saline intra peritoneally. 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-IIIA) 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 pentabarbitone (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 and immersed in Bouvins solution. Epididymis was carefully separated from the testis. Both the epididymis were removed and cleaned of fat tissue and blood and kept in cold buffered saline (0.9%). The epididymis were blotted dry and weighed.

Sperm count

The epididymis was minced in 1ml of phosphate buffered saline (pH 7.2) to obtain a suspension. The suspension was filtered through a nylon mesh. The sperm count was conducted in the filtrate as per the standard method in Neubauer's chamber (Kaplan and Pesce, 1987; Narayana et al., 2002). Briefly, an aliquot from the suspension (up to 0.5ml) was taken in leukocyte hemocytometer and diluted with phosphate buffered saline up to the mark 11. The

suspension was well-mixed and charged into Neubauer's counting chamber. The total sperm count in 8 squares (except the central erythrocyte area) of 1mm each was determined and multiplied by 5×10^4 to express the number of spermatozoa/epididymis.

To obtain the quantitative analysis of testicular damage, the tissues were processed and paraffin blocks were prepared as per standard protocols11. Five micron-thick sections were obtained and stained with haematoxylin and eosin for light microscopic analysis. To measure the standard tubular diameter (STD) five transversely cut seminiferous tubules from each testis were selected randomly and measured per cross-section using a stage micrometer that was calibrated with an eyepiece micrometer. The two diameters of the tubules, one perpendicular to the other, were measured. The average of the transverse and perpendicular diameters was taken for each animal. To measure the standard epithelial height (SEH), five transversely cut seminiferous tubules were randomly selected and measured per cross-section tubules according to Canan et al's method (Canan et al., 2002).

Statistical analysis

The data was expressed as Mean±SD. The difference between groups was compared for statistical significance by student '*t*' test with the level of significance set at $p<0.05$.

Results

In the present study, epididymal weight and sperm count was significantly low ($p<0.001$) in cadmium treated group compared to normal control group& beta carotene treated group (Table 1). Pretreatment with beta carotene prior to cadmium administration showed a significant increase in the epididymal weight as well as sperm count compared to cadmium treated rats.

Rats administered with 1mg/kg bw cadmium chloride (Group-III) showed a significant decrease in the STD and SHE compared to normal control (Group-I) as well as beta carotene treated group (Group-II). However, in the rats that were pretreated with beta carotene prior to cadmium administration showed significant increase in STD and SHE compared to untreated control group (Group-III). The normal architecture of the seminiferous tubules for Group-I and -II is shown in Figs. 1 and 2, respectively. The rats that were administered with cadmium chloride (Group-III) showed severe destruction of the tubular cells (Fig. 3). The majority of the cells showed degeneration testis and tubules had hardly any sperms when compared to Group-I and Group-II. The section of rat testis pretreated with beta carotene showed lesser atrophic and degenerative changes of tubular epithelium when compared to the untreated control group (Group-III). The seminiferous tubules in many of the sections showed near-normal architecture.

Table 1. Effect of pre-treatment with beta carotene on cadmium chloride induced damage on epididymal weight (g) and sperm count ($\times10^6$). Values given are Mean±SD, n=8 in each group.

Parameters	Control	Beta carotene treated	Cadmium treated	Beta carotene and cadmium treated
Epididymal weight (g)	0.502±0.06	0.498±0.02	0.312±0.06***	0.492±0.09♦♦♦
Sperm count ($\times10^6$)	892.83±8.90	887.36± 8.14	242.83±8.71***	801±5.12♦♦♦

***$p<0.001$ compared to control and beta carotene treated group; ♦♦♦$p<0.001$ compared to cadmium treated group;

Table 2. Effect of beta carotene pretreatment on cadmium induced testicular toxicity on standard tubular diameter and standard epithelial height. Values given are Mean±SD, n=8 in each group.

Groups	Standard Tubular Diameter (µm)	Standard Epithelial Height (µm)
Control	517.42±22.6	68.24±4.36
Beta carotene treated	511.68±31.6	63.81±3.87
Cadmium treated	414.26±23.4***	31.56±8.13***
Beta carotene and cadmium treated	508.36± 23.70. ♦♦♦	63.84±7.84♦♦♦

***$p< 0.001$, compared to control and beta carotene treated group; ♦♦♦P<0.001 compared to cadmium treated group;

Fig. 1: Testes of male albino rat treated with 0.9% saline showing normal structure of seminiferous tubules (H&E; 10X). (Group-I).

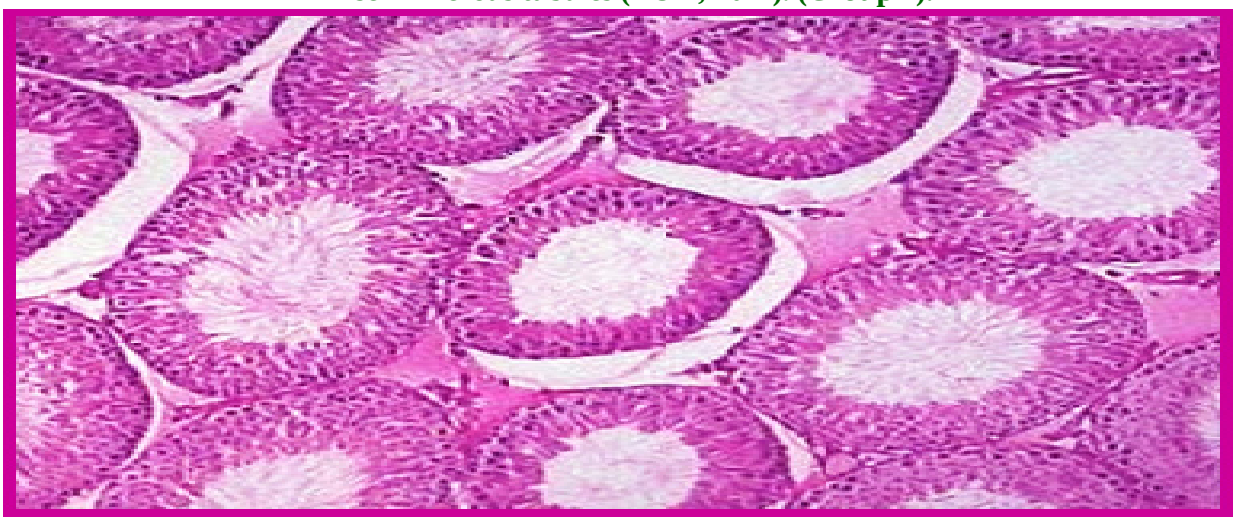


Fig. 2: Testes of male albino rat treated with beta carotene showing normal structure of seminiferous tubules (H&E; 10X) (Group-II).

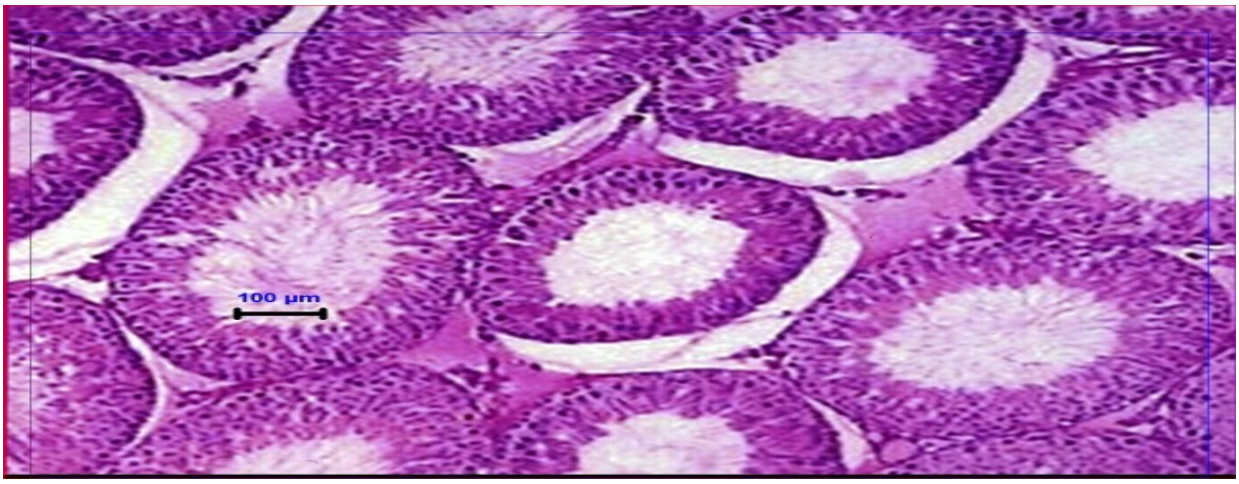


Fig. 3: Testes of male albino rat intoxicated with cadmium chloride alone showing atrophy of the tubules. (H&E; 10X) (Group-III).

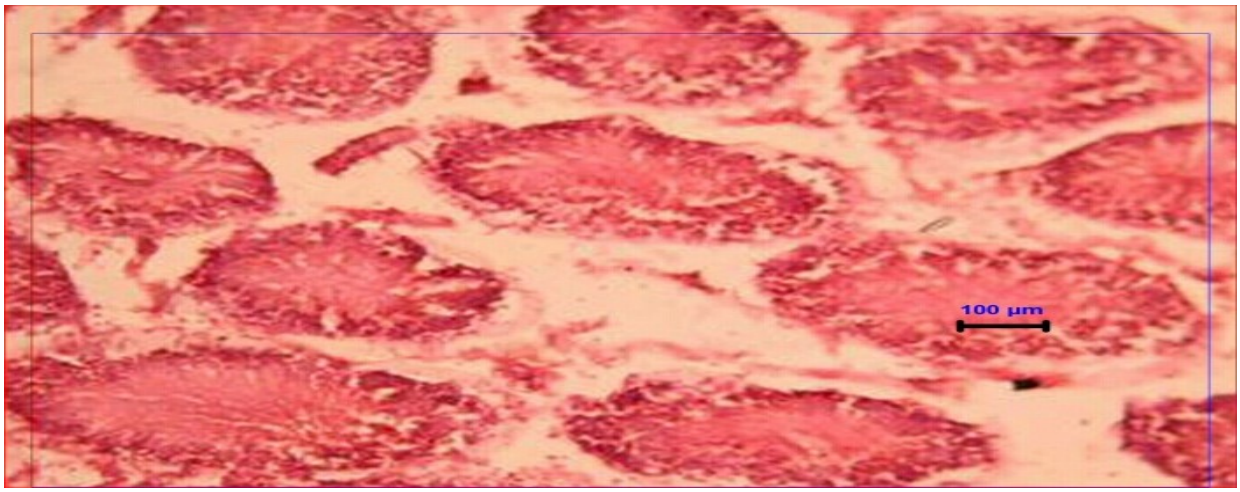
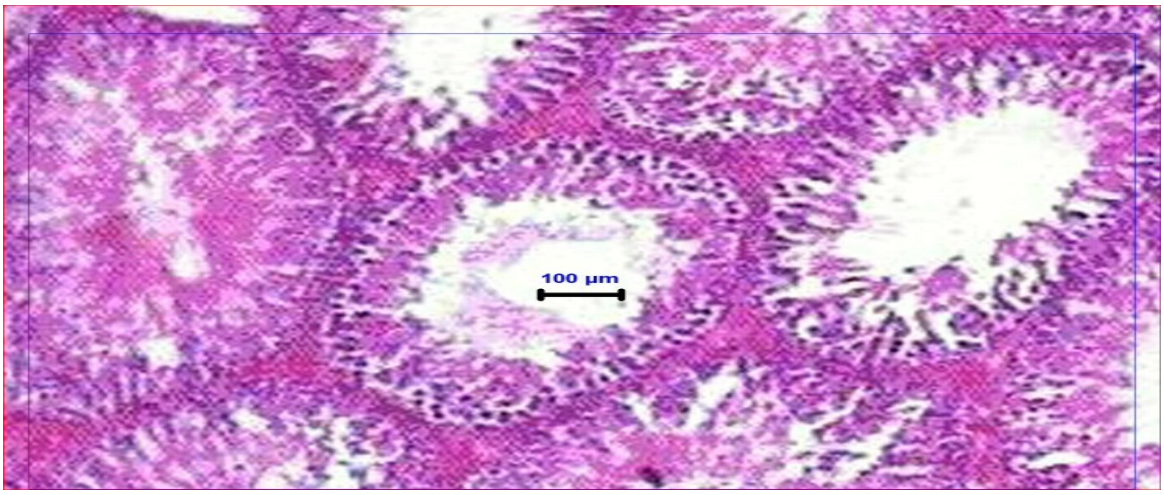


Fig. 4: Testes of male albino rats pre-treated with beta carotene and cadmium chloride showing normal seminiferous tubules (H&E; 10X) (Group-IIIA).



Discussion

Cadmium is a heavy metal and a major environmental toxicant. It has been suggested that Cd is involved in carcinogenesis in multiple organs including kidney, prostate, liver and pancreas (Waalkes et al., 1997; Goyer et al., 2004). The testis is extremely sensitive to cadmium toxicity. Since the 1950s, studies have shown that *in vivo* acute exposure to cadmium caused, germ cell loss, testicular edema, hemorrhage, necrosis, and sterility in several mammalian species (e.g., rodents, rabbit, dog, calf, stallion) (Acharya et al., 2008). Recent studies have also associated reduced male fertility, such as reduced sperm count and poor semen quality, in men exposed to cadmium and/or other environmental toxicants (Adejuwon et al., 1996; Chung and Cheng, 2001). In accordance with the previous studies, the results of the present study also showed a significant decrease in the epididymal weight and sperm count in rats exposed to cadmium chloride. Initial studies that recognized cadmium could induce profound and irreversible injury to mammalian testes described the disruption of endothelial cells of micro vessels, edema and hemorrhage by morphological analysis, apparently as the result of a primary disruption in the vascular system. This, in turn, would have affected the seminiferous epithelium, causing testicular ischemia and necrosis. In the present study administration of 1mg/kg bw cadmium chloride showed a decrease in STD and SEH of seminiferous tubules, and destruction of the tubular cells. Thus the result of the present study showed that oxidative stress is induced by cadmium on rat testis. Oxidative stress results from the production of oxygen radicals in excess of the antioxidant capacity of the stressed tissue. Studies have shown that antioxidant play role in reducing cadmium induced testicular toxicity. The present study suggest that pretreatment with beta carotene may control Cadmium induced testicular toxicity.

Testicular oxidative stress appears to be a common feature in much of what underlies male infertility, which suggests that there may be benefits to developing better antioxidant therapies for relevant cases of hypospermatogenesis. Thus present study throws a light on beta carotene therapy on testicular damage induced by cadmium chloride.

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