Original Research Article

Beta Carotene as an Antioxidant in Cadmium Induced Testicular Toxicity


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A b s t r a c t

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-III A (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-III A 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-III A. Photomicrograph of rat testis in Group-III A showed almost normal testicular architecture. Results of the present study showed the antioxidative role of beta carotene in ameliorating cadmium induced testicular damage.

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
usage o
and strict internationally accepted guidelines, for the
The present study was conducted following
Materials and methods
reducing cadmium induced testicular damage.
Hence present study was aimed to study whether
in the number of diagnosed fertility impairments.
Moreover,
existing occupational exposure and the prevalence
even.
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
fod ad libitum with commercial rodent chow
(Hindusthan 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)
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
intra-peritoneally. In pretreated groups rats were
pretreated with beta carotene (10mg/kg bw) for 30
days orally and then injected with 1mg /kg bw
(Group-III/A) cadmium chloride intra-peritoneally. 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)
intra-peritoneally 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
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
carcinogen. Cadmium acts as a catalyst in forming
reactive oxygen species. It increases lipid
peroxidation, in addition it depletes antioxidants,
glutathione and protein-bound sulphydryl 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.

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
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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
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 testsis and tubules had hardly any sperm 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](image)

**Table 1.** Effect of pre-treatment with beta carotene on cadmium chloride induced damage on epididymal weight (g) and sperm count ($\times 10^6$). Values given are Mean±SD, n=8 in each group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Beta carotene treated</th>
<th>Cadmium treated</th>
<th>Beta carotene and cadmium treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epididymal weight (g)</td>
<td>0.502±0.06</td>
<td>0.498±0.02</td>
<td>0.312±0.06***</td>
<td>0.492±0.09***</td>
</tr>
<tr>
<td>Sperm count ($\times 10^6$)</td>
<td>892.83±8.90</td>
<td>887.36±8.14</td>
<td>242.83±8.71***</td>
<td>801±5.12***</td>
</tr>
</tbody>
</table>

***$p<0.001$ compared to control and beta carotene treated group; $\dddot{p}<0.001$ compared to cadmium treated group;

![Table](image)

**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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Standard Tubular Diameter (µm)</th>
<th>Standard Epithelial Height (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>517.42±22.6</td>
<td>68.24±4.36</td>
</tr>
<tr>
<td>Beta carotene treated</td>
<td>511.68±31.6</td>
<td>63.81±3.87</td>
</tr>
<tr>
<td>Cadmium treated</td>
<td>414.26±23.4***</td>
<td>31.56±8.13***</td>
</tr>
<tr>
<td>Beta carotene and cadmium treated</td>
<td>508.36±23.70. ***</td>
<td>63.84±7.84***</td>
</tr>
</tbody>
</table>

**$p< 0.001$, compared to control and beta carotene treated group; $\dddot{p}<0.001$ 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-III A).

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 epidydimal 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.

References

