PROTECTIVE ROLE OF LYCOPENE ON BISPHENOL A INDUCED CHANGES IN SPERM CHARACTERISTICS, TESTICULAR DAMAGE AND OXIDATIVE STRESS IN RATS

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ABSTRACT

Bisphenol A (BPA) is an estrogenic environmental toxicant, a synthetic monomer widely used to polymerize polycarbonate plastics and resins. BPA has been implicated to have hazardous effect on reproductive health in human and experimental rats. Lycopene is a powerful antioxidant and potential high-value compound for the prevention of many diseases. The present study has been designed to investigate the protective role of lycopene on BPA induced body and organ weight, sperm count, sperm motility, antioxidant enzymes levels, oxidative stress levels and histopathological studies in rats. The results revealed that administration of BPA significantly induce the testicular damage which was evident from the body and organ weight, sperm count, sperm motility, antioxidants enzymes level were significantly decreased and increased level of oxidative stress in rat testes. Interestingly, administration of lycopene with BPA intoxicated rats were found the body and organ weight, sperm count, sperm motility, antioxidant enzymes were significantly increased and decreased levels of oxidative stress parameters. Histopathological examination revealed that lycopene reduced testicular damages induced by BPA. This inevitably confirms that lycopene has a prominent role in preventing the testicular damage during treatment. In conclusion, BPA induced antioxidant and oxidative stress alteration lead to male infertility because of antioxidant potential of lycopene, ameliorates the changes which are induced by BPA.

KEYWORDS: Bisphenol A; Lycopene; Oxidative stress; Reproductive organs; Sperm characters.
INTRODUCTION

There is an increasing concern that environmental contaminants disrupt male reproduction of wildlife and humans and play an important role in the decline of quality and quantity of human semen. These compounds include bisphenol A [BPA, 2,2-bis (hydroxyphenyl) propane] one of the environmental contaminants used extensively in industry and commerce to manufacture polycarbonate plastics (which can be used in baby bottles, water storage tanks, or supply pipes) and components of food packaging (for example, in the lining of food cans), among other applications. Previous studies reported that BPA involves reproductive toxicities in rats.

![Structure of Bisphenol A (BPA)](image)

Oral administration of BPA even at much lower doses resulted in reduced daily sperm production. This susceptibility of neonatal animals to BPA versus adults may be related to the lower level of liver enzyme activity that can metabolize BPA to its non-toxic BPA-glucuronide form which is then excreted by the kidney. There are some reports suggesting testicular toxicity of bisphenol A in rats and mice. Instead, we focus on the destructive effects of bisphenol A on male reproductive functions, such as reducing sperm count in men and rodents and by perturbing the blood testes barrier (BTB) integrity in immature rats. Free radicals are often generated by various environmental contaminants when exposed to living systems. Polycyclic aromatic hydrocarbons (PAHs) are one of the environmental contaminant and well recognized for its capacity to produce free radicals and the products are formed by incomplete combustion of organic matter. Environmental contaminants have been shown to induce ROS generation in both intra- and extracellular spaces of cells or individuals leading to cell death and tissue injury. ROS have been shown to play an important role in the defense mechanisms against pathological conditions but excessive generation of free oxygen radicals may damage tissues. ROS generation can be from the mitochondria and a variety of enzymes including the xanthenes- and NADPH-oxidases, and the cytochrome P450s. These enzymes specialize in the professional generation of ROS or produce these toxic metabolites as an inadvertent consequence of their biochemical activity. In order to address this risk, the testes have developed a sophisticated array of antioxidant systems. Antioxidant enzymes form the first line of defense against free radicals in organisms. Various natural and synthetic substances possessing antioxidant properties should be investigated as to their possible protective effects on BPA induced tissue or cellular toxicity. Antioxidant therapy could be important to reduce the BPA induced toxicity. Antioxidant therapy could be important to reduce the BPA induced toxicity. Concomitantly, numerous medicinal plants, and their formulations are used for several health disorders in ethnomedical practices as well as a traditional system of medicine in India. The exposure of toxicants to adult rats could induce testicular damage and it can be prevented by the administration of tomato products such as sauce, salsas, soup, etc. The important active compound present in tomato and tomato products is lycopene. Lycopene,
an aliphatic hydrocarbon, is one of the 600 known naturally occurring carotenoids. Recently, lycopene, a naturally occurring carotenoid in tomatoes, it has been attracted considerable attention as a potential chemopreventive agent. In addition, lycopene has received particular attention in recent years as a result of studies indicating that it is a highly efficient antioxidant and has a singlet-oxygen and free radical scavenging capacity. The unique characteristics of lycopene are less toxic with more antioxidant potency. Based on this background the hypothesized that lycopene could protect testes morphology and its function by scavenging reactive oxygen species. The aims of this study was to investigate the effects of BPA on sperm characteristics, biochemical changes related to oxidative stress in testes tissue and histopathology of testes and to highlight the protective effect of lycopene on these parameters.

**MATERIALS AND METHODS**

(i) **Chemicals**
Bisphenol A was purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Lycopene was obtained as a gift from Phytoremedies Biolabs Pvt. Ltd., Pune, India. All the other chemicals used in this study were of analytical grade available commercially.

(ii) **Animals**
24 healthy adult male Sprague–Dawley rats (170±10g) were procured from the Central Animal House Facility, Dr.ALM PG IBMS, University of Madras, Taramani for this study. The animals were housed in well ventilated large spacious polypropylene cages and had 12 hrs light and dark cycle and 25±2°C throughout the experimental period. The animals received a balanced diet of commercially available pellet rat feed and water *ad libitum*. The Guidelines for Breeding and Experiments on Animals, 1998 defined by the Ministry of Social Justice and Empowerment of India were followed and the protocol was approved by the Institutional Animal Ethics Committee (IAEC No. 01/05/2012).

(iii) **Experimental design**
The adult male Sprague Dawley rats were divided into four groups with six animals in each group. Group I served as control receiving saline following treatment with 0.5 ml corn oil through the experimental period. Group II animals exposed to Bisphenol A (200mg/kg bodyweight) dissolved in corn oil administered orally for 30 days. Group III animals exposed to Bisphenol A will be treated with lycopene (10mg/kg bodyweight) orally for 30 days. Group IV animals will be treated with lycopene (10mg/kg bodyweight) dissolved in corn oil for 30 days orally. The doses of BPA and lycopene in this study were selected on the basis of previous studies.

(iv) **Collection of tissues**
The testes and cuda epididymes were dissected out and washed with saline, blotted and then used to determine the weight. The tissue was homogenized in Tris-HcI buffer (0.1 M pH 7.4). This was stored at - 80° C, until its use for further analysis. Testes were fixed in Bouin’s fixative (85 ml of saturated picric acid added to 10 ml of 40% formaldehyde and made up to 100ml with glacial acetic acid) for histological evaluation.

(v) **Sperm Characteristics**
Epididymal sperms were counted with a hemocytometer using a modification of the method described by Latchoumycandane and Mathur (2009). The sperm motility was determined by the method of Yokoi et al (2003). Dead sperms Wyrobek et al (1983).

(vi) **Biochemical Analysis**

*Estimation of antioxidants enzyme levels*
The activity of superoxide dismutase (SOD) was determined by the method of Marklund and Marklund (1972). The catalase (CAT) activity was measured by the method of Sinha (1972). The activity of glutathione peroxidase (GPx) was assayed by the method of Rotruck et al (1973). The level of reduced glutathione (GR) was estimated by the method of Staal et al (1969).
Assay of oxidative stress levels
Lipid peroxidation (LPO) was assayed by the method of Hogberg et al (1974)\textsuperscript{37} and Hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) was estimated by the method of Devasagayam et al (1987)\textsuperscript{38}.

(Vii) Histopathological examination
Fixed testes tissue samples in Bouin’s were embedded in paraffin, sectioned at 5 mm, and were stained with hematoxylin and eosin (H&E). Light microscopy was used for the evaluations.

STATISTICAL ANALYSIS
The values are expressed as Mean±S.D for six rats in each group. Statistically, significance differences between the groups were calculated using One-way Analysis of Variance (ANOVA) followed by the Student’s Turkey’s for multiple comparisons using Statistical Package for Social Sciences (SPSS) computer package.

RESULTS
Previous studies reported that in toxicity conditions, the body weight is significantly reduced \textsuperscript{39}. In this present investigation, the effect of lycopene on mean value of body, organ weight of control and experimental animals are presented in (Table 1). The body weights were found to be significantly decreased in group II BPA exposed toxicity bearing animals when compared with group I control animals (p<0.05). On the contrary, the administration of lycopene increased the body weight in group III animals when compared to group II animals (p<0.05). However, no significant changes were observed in lycopene alone treated group IV animals when compared to group I control animals.

Table 1
The effect of Lycopene on body and organ weight of control and experimental animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (BPA)</th>
<th>Group III (BPA + Lycopene)</th>
<th>Group IV (Lycopene)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>161.41±1.15</td>
<td>145.64±1.07</td>
<td>157.34±1.16</td>
<td>160.43±1.23</td>
</tr>
<tr>
<td>Testis weight (g)</td>
<td>1.45±0.10</td>
<td>0.84±0.11</td>
<td>1.17±0.12</td>
<td>1.41±0.10</td>
</tr>
<tr>
<td>Epididymus weight (g)</td>
<td>1.93±0.14</td>
<td>1.42±0.11</td>
<td>1.74±0.13</td>
<td>2.04±0.14</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six animals in each group
a – Group I Vs Group II, III and IV
b – Group II Vs Group III and IV
c – Group III Vs Group IV
The significance at the level of p<0.05

On the other hand, significant decrease in testes and epididymal weight was observed in group II toxicity induced animals. In contrast, the testes and epididymal weights were found to be increased in group III lycopene treated animals (p<0.05) when compared to group II animals. However, no changes were observed in testes and epididymal of both group IV lycopene alone treated and group I control animals. Spermatozoa have been considered to be highly susceptible to lipid peroxidation in the presence of elevated ROS levels, due to the abundance of poly unsaturated fatty acids in their membrane. The sperm count and percentage of abnormal sperm have been associated with infertility in males\textsuperscript{40}. The sperm count and motility and morphology in epididymis sperm of control and experimental animals are presented in (Table 2). The sperm count and motility and morphology was decreased significantly in group II toxicity bearing animals when compared to group I control animals. On the other hand in group III animals the administration of lycopene showed a significant (p<0.05) increase in the sperm count, motility and dead sperms when compared to group II animals. In the present investigation there was no significant change were observed in group IV lycopene alone treated animals when compared to group I control animals.
Table 2
The level of Sperm characteristics in control and experimental animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (BPA)</th>
<th>Group III (BPA+ Lycopene)</th>
<th>Group IV (Lycopene)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm count (10^6/ml)</td>
<td>174.51±1.32</td>
<td>90.44±0.71^a</td>
<td>154.14±1.19^a,b</td>
<td>175.58±1.37^b,c</td>
</tr>
<tr>
<td>Sperm Motility (%)</td>
<td>79.46±0.65</td>
<td>44.10±3.86^a</td>
<td>69.74±5.36^a,b</td>
<td>78.52±6.06^b,c</td>
</tr>
<tr>
<td>Dead Sperms (%)</td>
<td>10.29±0.83</td>
<td>44.40±3.35^a</td>
<td>18.48±1.40^a,b,c</td>
<td>10.58±0.96^b,c</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six animals in each group
a – Group I Vs Group II, III and IV
b – Group II Vs Group III and IV
c – Group III Vs Group IV
The significance at the level of p<0.05

Reactive oxygen species are produced in normal cellular metabolism and in abundant in pro-oxidant states. ROS are degraded by the organized system of antioxidant enzymes. Superoxide dismutase (SOD) Catalyse the dismutation of superoxide anion radical to hydrogen peroxide, which is further metabolized by catalase and to a lower extent by glutathione peroxidase. The enzymic antioxidant such as SOD, GPx, GR and CAT levels in the testes of control and experimental animal’s level were studied and the results are presented in (Graph.1 & 2).

Graph 1
Effect of lycopene on SOD, GPx and GR activity in BPA-exposed adult SD rat testes

In group II toxicity bearing animals, the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase were significantly decreased when compared to group I control animals. These enzymes activities were significantly reverted back to
Lipid peroxidation is a well established mechanism of cellular injury in animals and is used as an indicator of oxidative stress in cells and tissue. Lipid peroxidation is unstable and decomposes to form a complex series of compounds including reactive carbonyl compounds. It is a type of oxidative degeneration of polyunsaturated lipids, has been implicated in a variety of pathogenic processes. Extensive lipid peroxidation in biological membranes causes decrease in membrane potential and eventual rupture leading to release of cell and organelle content. Cellular biomolecules like lipids are the most susceptible to oxidative damage. The effect of Lycopene protects on LPO, H$_2$O$_2$ in testes of control and experimental animals are presented in (Graph. 3). In testes the LPO (lipid peroxidation) and H$_2$O$_2$ (hydrogen peroxide) were found to be significantly increased in group II BPA toxicity bearing animals when compared with group I control animals. Conversely, the administration of lycopene reduces the peroxidation reaction in group III animals ($p<0.05$). No marked alteration of lipid peroxidation was observed in group IV lycopene alone treated animals when compared to group I control animals.
Graph 3

Efficacy of lycopene on LPO, \( H_2O_2 \) level in BPA-exposed adult SD rat testes

![Graph showing efficacy of lycopene on LPO, \( H_2O_2 \) level in BPA-exposed adult SD rat testes](image)

Values are expressed as mean ± SD for six animals in each group:
- a – Group I vs Group II, III and IV
- b – Group II vs Group III and IV
- c – Group III vs Group IV

Units are expressed as: LPO= µmol of MDA equivalent formed/mg protein; \( H_2O_2 \)= nmol of \( H_2O_2 \) generated/mg protein.

Histopathological studies on the testis sections were showed varying morphological changes in BPA-exposed rats, which include extensive seminiferous tubules damage, which was evidenced from the necrotic changes (Fig. 1). On the other hand lycopene treatment decreased the degree of damage to an appreciable extent and the testes showed an almost normal architecture.

Figure 1

Histopathological sections of testes of control and experimental animals

![Histopathological sections of testes of control and experimental animals](image)
Testicular section from the group I control rat shows the normal spermatogenesis and histological structure of the seminiferous tubules and intertubular spaces. In group II BPA exposed rat shows a decrease in the number of spermatogenic cells in the seminiferous tubules with intertubular spaces. In group III BPA exposed rat treated with lycopene shows improvement in the number of spermatogenic cells in the seminiferous tubules with intertubular spaces. In group IV lycopene alone treated rat shows no changes in spermatogenesis and histological structure of the seminiferous tubules and intertubular spaces.

DISCUSSION

Over the past fifty years evidence shown that the quality of semen has considerably declined due to the increase environmental pollution mainly in industrial factors. Bisphenol A is an industrial chemical that has been used in the production of polycarbonate plastics etc. Earlier studies indicate that BPA alterations in hypothalamic-pituitary-gonadal axis and reduction in Sertoli cell phagocytic function caused by BPA administration is probably responsible for the pathogenesis of testicular and spermatozoa toxicity. Spermatogenic cells are targeted by cytotoxic agents because of their high mitotic activity. Damages in spermatogonia result in prolonged sterility or oligozoospermia. The chance of recovery of spermatogenesis from cytotoxic insult, and also the extent and speed of recovery, are related to the antioxidant therapy. BPA exposed results in damage of different tissues, such as heart, kidney, liver, and testes. Recently, it has attracted more attention owing to impairment in testicular function following chemotherapy. The toxicity of BPA has been investigated in detail and well documented. BPA induced testicular toxicity in animal models has been reported by many investigators. In the present study, the degree of BPA-induced damage in the reproductive organs with the determination of body, testes and epididymal weights of rats was assessed, epididymal sperm concentration, sperm motility, dead sperms, antioxidant enzyme activities, lipid peroxidation, hydrogen peroxide and histopathological alteration this will ameliorates by administration of lycopene. Previous studies reported that administration of Bisphenol A significantly decreased the weights of testes and epididymis, which may be due to the inhibition of spermatogenesis, decreased elongated spermatids and steroidogenic enzyme activity. However, in this present study, testes and epididymis weights of the group treated with BPA alone were significantly lower than the control group. It is thought that severe parenchymal atrophy in the ST of rats after BPA administration causes this reduction. Interestingly, lycopene treatment in BPA-administered rats showed normal testes, epididymis weights. The results of the present study indicated that BPA administration at the dose of 200 mg/kg resulted in a significantly decrease in sperm count, motility and increase in dead sperm in the rats. Several investigators have suggested that BPA treatment may adversely affect the quality and quantity of sperm, which is responsible for male fertility. It is widely accepted that oxidative stress and the production of free radicals are involved in BPA action, in terms of both antitumor effects and other organ toxicity. Increased oxidative stress damages the sperm membranes, proteins, and DNA associated with male fertility. Excessive production of semen reactive oxygen species (ROS), which causes abnormality in spermatozoa, could be an indicator for male infertility. High concentrations of hydrogen peroxide induce lipid peroxidation can cause cell death. The consequences of such oxidative stress are a loss in motility and fertilizing ability of sperm and the induction of DNA damage in sperm nucleus. The loss of sperm function is due to peroxidation of unsaturated fatty acids in the sperm plasma membrane, as a consequence of which the latter loses its fluidity and the cells lose their function. To protect spermatogenesis from toxicant exposure, many clinical and experimental trials of antioxidant agents have been attempted. Antioxidant therapy could be important to reduce the BPA induced toxicity. The natural chemotherapeutic agents have low side effects, toxicity and are
involved in the carcinogenic potential by modulating carcinogen detoxification, inhibiting lipid peroxidation, or by improving in vivo antioxidants defense mechanism\textsuperscript{53}, so we select carotenoids are naturally occurring chemotherapeutic agents. Carotenoids, as potential antioxidants, are well known as highly efficient scavengers of singlet oxygen (\textsuperscript{1}O\textsubscript{2}) and other excited species. During \textsuperscript{1}O\textsubscript{2} quenching, energy is transferred from \textsuperscript{1}O\textsubscript{2} to the lycopene molecule, converting it to the energy-rich triplet state. Trapping of other ROS, such as OH, NO\textsuperscript{2} or peroxynitrite, in contrast, leads to oxidative breakdown of the lycopene molecule. Thus, lycopene may protect in vivo against oxidation of lipids, proteins, and DNA\textsuperscript{54}. Lycopene has been shown to have the highest antioxidant activity among the carotenoids in cell protection against hydrogen peroxide and nitrogen dioxide radical components. In addition, lycopene has been reported to attenuate oxidative stress and exert anticancer effects both in vitro and in vivo\textsuperscript{55}. Previous studies reported that oral lycopene therapy in men with idiopathic infertility provided an improvement in male infertility, especially in sperm characteristics\textsuperscript{56}. Spermatozoa have been considered to be highly susceptible to the damage induced by ROS because of their high content of polyunsaturated fatty acids. To counteract the effects of ROS, spermatozoa are equipped with antioxidant defense systems, which prevent cellular damage\textsuperscript{57}. In our study, significant decrease in sperm motility and increase in abnormal sperm rates was observed in rats treated with BPA alone, but normalization of these parameters was observed in the group III treated with lycopene. A rational mechanism for potential anticarcinogenic and antimutagenic effects of \(\beta\)-carotene and other carotenoids is their ability to scavenge free radicals that cause oxidative DNA damage\textsuperscript{58}. These findings are in agreement with the data of the present study. The protective effects of lycopene against BPA-induced abnormal sperm rates may be attributed to the antioxidant properties of lycopene. These observations might also indicate that lycopene has protective role on BPA induced oxidative stress. Naturally there is a dynamic balance between the amount of free radicals generated in the body and antioxidant defense system that scavenge them and protect the body against their deleterious effects\textsuperscript{59}. Antioxidant such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) glutathione reductase (GR) protect cells against free radical destruction. In the present study, the bisphenol A exposed rats showed decreased activities of antioxidant enzymes SOD, CAT, GPx, GR, and increased levels of LPO, H\textsubscript{2}O\textsubscript{2} in the testes of rats. Oxidative stress, which include Reactive oxygen metabolites such as superoxide, hydroxyl radical, singlet oxygen are the responses to stress. H\textsubscript{2}O\textsubscript{2} are generally considered cytotoxic agents because of their ability to induce lipid peroxidation in tissues and cell membrane\textsuperscript{60}. In spermatozoa several antioxidant defense systems, namely, glutathione peroxidase, superoxide dismutase and catalase are known to operate. Superoxide dismutase generally dismutases the superoxide anion radical into H\textsubscript{2}O\textsubscript{2} which is degraded by catalase and glutathione peroxidase/reductase system using reduced glutathione. The reduction in the activity of catalase reflects the inability of epididymal sperm to eliminate H\textsubscript{2}O\textsubscript{2} generated after the exposure to bisphenol A. Glutathione peroxidase/reductase directly acts as antioxidant enzymes to inhibit sperm lipid peroxidation\textsuperscript{61}. Increased lipid peroxidation may indicate an increased oxygen free radical generation and has been associated with mid-piece abnormalities and decreased sperm counts\textsuperscript{62}. In our present study the BPA exposed rats showed decreased activities of antioxidant enzymes superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase, and increased levels of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) and lipid peroxidation (LPO) in the testes of rats. In contrast the lycopene exhibited a protective effect which may be due to anti-lipid peroxidative and free radical scavenging properties that establishes the antioxidant potential. Examination of paraffin sections stained with haematoxylin eosin failed to reveal consistent differences in the histology of the testes of lycopene alone treated animals.
when compared with that of the section of control animals. The histological changes in testes of rats treated with BPA showed histological perturbation including severe damage within the seminiferous tubules and vascular degeneration on the spermatogenic and sertoli cells cytoplasm. The germinal epithelium of the seminiferous tubules was thinner in places and spermatids were almost absent; sperm numbers was low and there were no sperm in the lumen. It is reported that marked atrophy of the seminiferous tubules and irreversible lesion were observed in BPA exposed rats. On the contrary the administration of lycopene significantly reverted back the altered structures to near normal this might be due to the free radical scavenging activity.

**CONCLUSION**

The data of this experiment suggest that, lycopene positively modulated the antioxidant activity and reduce the lipid peroxidation by quenching and detoxifying the free radicals induced by BPA against male infertility.

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