J. Biomed. Ther. Sci. 2014, 1(1), 41-47 Article

Journal of Biomedical & Therapeutic Sciences

Lycopene, a carotenoid antioxidant against Bisphenol A (BPA) motivated experimental male infertility

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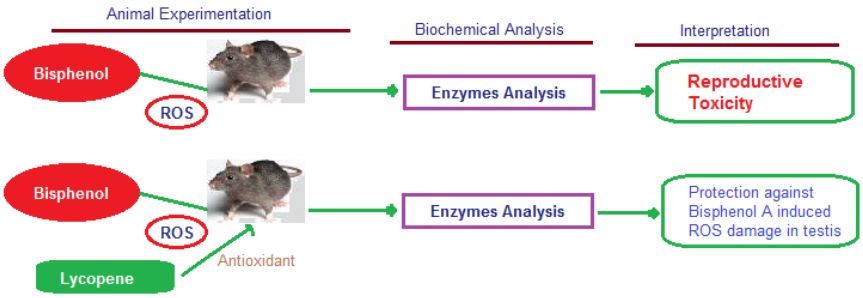
Received on: 4-Nov.-2014

ABSTRACT



Bisphenol A (BPA, 2,2-bis (4-hydroxyphenyl) propane) is a common environmental endocrine disruptors with estrogenic properties. It has been implicated to have death-defying effect on reproductive health in human and experimental rats because of its wide-ranging use in manufacturing of various consumer products. The present study was performed to evaluate the curative effect of lycopene on BPA induced testicular toxicity in rats. Toxicity on reproductive organ of adult male rats was induced by oral administered of Bisphenol A (200mg/kg body weight) dissolved in corn oil (1 ml) for 30 days. There was remarkable alteration in the levels of reproductive hormones, marker enzymes, ATPases and TCA cycle enzymes due to BPA-induced toxicity mediated by oxidative stress. Captivatingly, introduction of lycopene (10 mg/kg body weight given for 30 days orally) to BPA intoxicated group III rats, brought the biochemical modifications back to normal level. This result put forward that lycopene has an outstanding potential in reduction of testicular damage.

Keywords: Bisphenol A, Estrogenic Environmental Toxicant, Estrogenic Endocrine Disruptor, Marker enzymes, Testicular Toxicity

INTRODUCTION

Reproductive toxicity is the occurrence of biologically adverse effects on the reproductive system of male or females that may result from exposure to environmental agents.[1] The toxicity may be expressed as alterations to the male or female reproductive organs, the related endocrine system, or pregnancy outcomes. The manifestation of such toxicity may include adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behavior, fertility, gestation, parturition, lactation, developmental toxicity, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.[2] A variety of factors are associated with reproductive system disorders, including nutrition, environment, socioeconomic status, lifestyle, and stress.[3] Disorders of reproduction in humans include but are not limited to reduced fertility, impotence, menstrual disorders, spontaneous abortion, low birth weight and other developmental (including heritable)

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Cite as: *J. Biomed. Ther. Sci., 2014, 1(1), 41-47.*

defects, premature reproductive senescence, and various genetic diseases affecting the reproductive system and offspring.

Male infertility is difficult to diagnose, and about 60-75% of cases remain idiopathic. Global estimates suggest that nearly 72.4 million couples experience infertility problems.[4] Various environmental toxicants can cause severe organ toxicities through the metabolic activation to highly reactive free radicals.[5] Common environmental pollutants include pesticides and herbicides, volatile organic compounds, heavy metals, air contaminants and persistent organic pollutants, which may affect the fertility. Exposure to metals, mainly lead and cadmium has been long associated with low sperm motility and density, increased morphological anomalies and male infertility.[6]

Bisphenol A (2, 2-bis (4-hydroxyphenyl) propane) is one of the highest volume chemicals produced worldwide. BPA, an estrogenic endocrine disrupting chemical with two unsaturated phenol rings, is used in the production of polycarbonate plastics, epoxy resins used to line metal cans, and in many plastic consumer products. BPA has been shown to leach from food and beverage containers, and some dental sealants and composites under normal conditions of use.[7] A significant amount of BPA was detected in liquid from canned vegetables that are exposed to high temperature during autoclaving.[8] Because of its wide availability in the environment, and its estrogenic activity in specific responses *in vitro* and *in vivo*, adverse effects of BPA exposure on human health are possible.[8] It has been hypothesized that exposure during early development to xenoestrogens such as BPA may be the underlying cause of the increased incidence of infertility, genital tract abnormalities, and breast cancer observed in developed countries over the last 50 years.[9] BPA has been shown to alter endocrine function through multiple pathways, and a number of animal studies have reported adverse reproductive effects in males exposed to low levels of BPA in early life or in adulthood.[10] BPA even at a low dose affects spermatogenesis in the adult rat. A BPA dose as low as 20µg/kg tended to decrease testicular weight and significantly reduced both daily sperm production and the efficiency of spermatogenesis (DSP per gram testis) in the adult rat.[11]

Many plant derived substances collectively termed as ‘‘phytonutrients’’ or ‘‘phytochemicals’’ are becoming increasingly known for their beneficial effect on human health. These phytonutrients serve as protective agents against a wide variety of disease. The broad therapeutic effects of phytonutrients can be largely attributed to their antioxidant properties. The dietary antioxidant can augment cellular defenses and help to prevent oxidative damage.[12] The plants are mainly used as antioxidant due to its capability of scavenging free radicals. There is evidence in the literature on the beneficial effects of antioxidant supplementation of vitamins in male anti-infertility.[13] It is known that carotenoids play a major role in multiple pharmacological effects and it could act as spermatogenic activity and it also cure various disease.[14] The vegetable and fruits contains lycopene, as a dietary source of a carotenoid antioxidant, it has attracted considerable interest in recent years as an important phytochemical with a beneficial role in human health.[15]

Lycopene belongs to the family of carotenoid compounds found in fruits, vegetables and green plants. All carotenoids posses certain common chemical features consisting of a polyisoprenoid structure, a long conjugated chain of double bonds in the central position of the molecules, and a near bilateral symmetry around the central doublebond.[16] Lycopene is a noncyclic carotenoid having a molecular formula of $C_{40}H_{56}$ and it is a lipophylic compound that is insoluble in water.[17] Although red-colored fruits and vegetables are the most common sources of dietary lycopene, not all red-colored plants contain lycopene. Common food sources of lycopene are the tomatoes, processed tomato products, watermelons, pink grapefruits and papaya.[18] Recognition of lycopene as a potent antioxidant, and its preventive role in oxidative stress-mediated chronic diseases, researchers are beginning to investigate its role in protecting sperm from oxidative damage leading to infertility. Therefore, the present investigation attempts to appraise the defending outcome of lycopene on Bisphenol A influenced testicular toxicity in Sprague dawley rats.

MATERIALS AND METHODS

Chemicals and reagents

Bisphenol-A (BPA), Lycopene and 1-Diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). All other chemicals were of high purity analytical grade marketed by Sisco Research Laboratories Pvt, Ltd, Mumbai, India.

Animals

Healthy adult male Sprague Dawley rats at the age group of 45-48 days weighing between 140-160 g were procured from the Central Animal House Facility, Dr. ALM PG IBMS, University of Madras, Taramani, Chennai, Tamilnadu, India. The rats were handled as per the guidelines from the Institutional Animal Ethics Committee (IAEC No. 01/05/2012). The rats received a standard rat pellet diet and water *ad libitum*. The rats were housed under conditions of controlled temperature (26 ± 2ºC) with 12 h light and dark cycle throughout the experimental period.

Experimental protocol

The adult male Sprague Dawley rats were divided into four groups and each group consisting of six animals. Group I: Animals received 1 ml of emulsion of corn oil given orally for 30 days served as vehicle treated control. Group II: Animals exposed to Bisphenol A (200mg/kg bodyweight) dissolved in corn oil (1 ml) administered orally for 30 days.Group III: Animals exposed to Bisphenol A at the dose of 200mg/kg body weight dissolved in corn oil (1 ml) administered orally and from the 30 days followed by Lycopene (10 mg/kg body weight) given for 30 days orally.Group IV: Animals received lycopene alone at the concentration of 10 mg/kg body weight for 30 days orally.

Collection of samples

After the experimental period, the animals were fasted overnight and sacrificed by cervical dislocation under mild anesthesia. Blood samples were collected and the serum was separated by centrifugation at 2000 rpm at 4ºC for 10 minutes these samples was stored at -20ºC until assayed for further analysis. The abdominal region was wiped with normal saline, scrotum was dissected to expose the testes, epididymides and extraneous connective tissues were trimmed. The dissected organs (right and left) from each rat in the experimental groups were weighed. The right testes were fixed with buffered 6% formaldehyde solution for histological evaluations. The dissected organs were washed 2 to 3 times with saline and known weight of testis was homogenized in 0.1 M Tris-HCl buffer (pH 7.4). The homogenate was subjected to differential centrifugation and were used for the biochemical assays.

Biochemical Estimation

FSH and LH ELISA kit is a sandwich enzyme imunnoassay for the measurement of rat FSH in serum, plasma and other biological fluids. Enzyme Immunoassay kit used for the quantitative determination of serum testosterone. The activity of acid phosphatase was assayed by the modified reported method.[19,20] Activity of γ-Glutamyl transpeptidase was estimated.[21] Activity of Lactate Dehydrogenase was assayed.[22] Testicular 3β-HSD and 17β-HSD activity was determined.[19] Inorganic phosphate was estimated.[23] Na^+/ K^+- ATPase was estimated.[24] Activity of Ca^{2+}- ATPase was assayed.[25] Mg^{2+}ATPase were assayed.[26] The activity of Isocitrate dehydrogenase was assayed.[27] The activity of SDH was assayed.[28] The activity of Malate dehydrogenase was assayed.[29]

Statistical Analysis

Data are presented as the mean ± standard deviation (SD). One way analysis of variance (ANOVA) followed by Tukey's multiple comparison method was used to compare the means of different groups of by using SPSS 12.5 student's versions. Comparisons were made between group II and IV with group I and III for animal studies. P <0.05 was considerable statistically significant in all cases.

RESULTS

The effect of lycopene on hormonal levels in serum of control and experimental animals demonstrated in table 1. Due to Bisphenol A induced toxicity in group II animals the levels of hormones like FSH, LH and Testosterone were diminished when compared with group I animals. Treatment with lycopene these hormones levels were elevated in group III animals when compared with group II animals. However, there were no significant changes in this parameter observed in group IV animals when compared with group I control animals.

The activity of lycopene on marker enzymes in testes of control and experimental animals are represented in table 2. In group II bisphenol A toxicity induced experimental animals showed a remarkable elevated in the levels of marker enzymes such as ACP, γ-GT, LDH, 3β-HSD and 17β-HSD compare to group I control animals. These levels were significantly declined in group III due to management with drug when compared with group II. Group IV drug control did not show any significant difference in the activities of marker enzymes when compared with group I control animals.

Table 3 illustrates the activities of Na^+/K^+, Ca^{2+} and Mg^{2+} ATPases in testes of control and experimental animals. Bisphenol A induced toxicity group II animals shows a significant decline in the levels of Ca^{2+}, Na^+/K^+ and Mg^{2+}

Table 1. Efficacy of Lycopene on hormonal levels in serum of control and experimental animals

Parameters	Group I (Control)	Group II (BPA)	Group III (BPA + Lycopene)	Group IV (Lycopene)
FSH (pg/ml)	31.26±1.08	10.95±2.04[a]	18.22±1.70[a,b]	31.48±0.91[b,c]
LH (pg/ml)	21.23±0.28	9.20±1.05[a]	16.70±0.20[a,b]	21.11±1.37[b,c]
Testosterone (ng/ml)	4.01±0.99	1.00±2.02[a]	2.50±0.98[a,b]	4.05±1.00[b,c]

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

Table 2. Efficacy of Lycopene on marker enzyme in testes of control and experimental animals

Parameters	**Group I (Control)**	**Group II (BPA)**	**Group III (BPA + Lycopene)**	**Group IV (Lycopene)**
ACP µmoles of p-nitro phenol liberated/mg protein/min	120.15±1.22	151.07±0.88[a]	139.30±0.41[a,b]	119.78±1.03[b,c]
γ-GT µmoles of p-nitroaniline formed/mg protein/min	2.15±2.44	4.60±1.27[a]	3.00±0.93[a,b]	2.45±1.39[b,c]
LDH of pyruvate liberated/mg protein/ min	1.52±2.34	3.71±0.72[a]	2.12±0.09[a,b]	1.50±0.26[b,c]
3β-HSD nmoles of NAD^+ reduced/mg protein/min	1.10±0.58	2.00±0.94[a]	1.51±2.00[a,b]	1.10±0.80[b,c]
17β-HSD nmoles of NADPH oxidised/mg protein/min	0.3±1.22	0.9±0.76[a]	0.5±1.11[a,b]	0.3±0.53[b,c]

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

ATPases ($p<0.05$) when compared with control animals. These levels were found to be significantly increased on lycopene treatment ($p<0.05$) in group III animals when compared to group II animals. On the other hand, there was no significant variation in group IV drug treated animals when compared to group I control animals.

Table 3. Activities of Lycopene on membrane bound ATPase in testes of control and experimental animals

Parameters µ mol of inorganic phosphate liberated/mg protein/min	**Group I (Control)**	**Group II (BPA)**	**Group III (BPA + Lycopene)**	**Group IV (Lycopene)**
Na^+/K^+ ATPase	1.80±0.33	0.92±1.06[a]	1.46±1.37[a,b]	1.81±0.19[b,c]
Ca^{2+} ATPase	36.14±0.28	21.70±1.50[a]	28.11±3.21[a,b]	36.46±1.89[b,c]
Mg^{2+} ATPase	0.55±1.87	0.18±1.23[a]	0.37±2.22[a,b]	0.55±0.71[b,c]

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 $p<0.05$

Table 4. Effect of Lycopene on mitochondrial TCA cycle enzymes in testes of control and experimental animals

Parameters	**Group I (Control)**	**Group II (BPA)**	**Group III (BPA + Lycopene)**	**Group IV (Lycopene)**
Isocitrate Dehydrogenase n mol of α – ketoglutarate formed/mg protein/min	30.45±2.08	17.11±0.77[a]	24.54±1.33[a,b]	30.15±2.41[b,c]
Succinate Dehydrogenase n mol of succinate oxidized/mg protein/min	25.56±0.79	10.11±5.46[a]	18.44±0.99[a,b]	26.01±0.82[b,c]
Malate Dehydrogenase n mol of NADH oxidized/mg protein/min	39.77±0.01	21.63±0.66[a]	34.08±1.76[a,b]	39.10±3.12[b,c]

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 $p<0.05$

The effect of lycopene on the levels of Isocitrate dehydrogenase (ICDH), Succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) in the testes of control and experimental animals are represented in table 4. A significant decrease in the levels of mitochondrial TCA cycle enzymes were observed in group II animals when compared to group I control animals ($p<0.05$). In this connection, the levels of TCA cycle enzymes are significantly increased in group III drug treated animals ($p<0.05$) when compared to group II animals. No alterations in group IV drug administered animals when compared with group I control animals.

DISCUSSION

Reproductive toxicity can be defined as a dysfunction of the reproductive system induced by chemical agents. The toxic effects of drugs and environmental chemicals on the human reproductive system are of major health concern, and incidents of chemically induced germ-cell damage, sterility and infertility appear to be increase in recent years.[30] Further they also reported that the unfavorable effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring. In this aspect, various reports reviled that the effect of chemicals that would interfere with reproductive ability or capacity may influence the infertility.[31] In developed countries, humans are exposed to a wide spectrum of man-made chemicals. Besides occupational exposure scenarios, exposures can also occur through air, dust, water, food and using consumer and personal-care products. From all these sources, chemicals end up in the human body primarily via ingestion, inhalation or dermal absorption. Phthalates and BPA are long-standing man-made chemicals produced worldwide in more than 1 million tonnes each year. Phthalates and BPA have received considerable attention because of the various facets of their proven toxicity in animal studies and because of their ubiquitous presence in the environment and in humans.[32] In this connection, Bisphenol A is a global environmental contaminant; it enters into humans as it leaches from the lining of tin cans into foods, from dental sealants into saliva and from polycarbonate bottles into their contents. Which are widely distributed across the globe It is reported that Bisphenol A has adverse effects on the reproductive system as well as the hormonal system.[33]

Leutinizing hormone (LH) is a glycoprotein, Follicle stimulating hormone (FSH) and Testosterone the main male gonadal hormone essential for sperm production and maintenance are required for normal spermatogenesis.[34] In the testes, Luteinizing hormone binds to receptors on Leydig cells, stimulating synthesis and secretion of testosterone.[35] Hormones from the pituitary as well as the testes play an imperative role in spermatogenesis. The LH, FSH and Testosterone are the main hormones involved in male reproduction. Assessment of male fertility is based principally on semen analysis, but supplemented with physical examination and laboratory testing of hormones where appropriate.[36] FSH and testosterone support meiosis, exhibit an anti-apoptotic action on spermatocytes and spermatids, and act co-operatively to promote spermatid maturation and sperm release. Dysfunction of Leydig cells will disturb the normal testosterone levels.[37] It was reported that LH concentration increased and Testosterone levels were decreased during the complete failure of leydig cells.[38] However, hormonal production may be reduced in rats due to the seminiferous tubular damage. An elevation in circulating levels

of inhibin, a glycoprotein of primarily sertoli cell origin which inhibits FSH synthesis and secretion by the pituitary, could account for the observed decrease in serum FSH level.[39] In the present investigation the administration of Bisphenol A decreases the FSH and LH levels, due to the influences of environmental toxicant on sertoli cells, leydig cells and seminiferous tubule. The toxicity induced rats with treatment of lycopene significantly decreased the toxic effects of BPA and the level of FSH, LH and testosterone were greater than before. This might be due to the presence of the active constituents such as carotenoids which directly or indirectly scavenge the oxidative damage to various cells and organs while normalizing their functions. It has been reported that carotenoids and antioxidants remarkably reduce oxidative damages in the cells.[40]

Marker enzymes are very sensitive and they are released to the circulation when damage occurs to the tissues. It is an indicator used to evaluate the manipulation of diseases and varies according to the degree of manifestation occurs in the biological system.[41] Phosphatases are a group of non-specific phosphomonoesterases enzymes that hydrolase esters of orthophosphates in alkaline or in acid condintion. Acid phosphatase is a lysosomal enzyme which is widely distributed in many body fluids and tissues.[42] ACP located in lysosome of leydig cells is involved in the protein synthesis by abduction of sex hormones. Changes in the activity of ACP may be used as an indicator of spermatogenesis function. Lactate dehydrogenase (LDH) is a bi-directional cytoplasmic enzyme capable of reversible formation of pyruvate and lactate in cells. LDH, widely present in sertoli and spermatogenic cells, plays an important role in testis energy production and biotransformation. The activities of LDH in testicular tissue are associated with the maturation of the germinal epithelial layer of seminiferous tubule.[43] γ-GT is considered to be the marker enzyme of sertoli cells function of testes and the activities of this enzyme vary inversely with the number of spermatozoa and its maturation. It is a useful marker enzyme which regulates intra testicular levels of androgen and it has been reported that the reduced activity of this enzymes reduces androgen production.[44] Increase in γ-GT activity is characteristic of testicular atrophy associated with damage to germ cells and sertoli cells by many xenobiotics.[45] 3β-Hydroxysteroid dehydrogenase and 17β-Hydroxysteroid dehydrogenase are the key regulatory enzymes for testicular androgen biosynthesis and also a marker for the leydig cells.[46] The steroidogenesis in the testes is mainly controlled by two rate-limiting enzymes, namely 3β-HSD and 17β-HSD. Both the enzymes are directly involved in the biosynthesis of testosterone. Any alteration in the activity of these enzymes reflects in the androgen production.[47] In the present study, Bisphenol A was found to have effects on testis enzyme activities. From the results, it is infered that the effect of BPA on steroidogenic enzyme activities might be due to ROS and the result of an elevation in testicular conjugated MDA. Contrarily, the administration of lycopene increased the activity of this enzyme. This may be due to the various phytochemical constituents present in the carotenoids. Therefore species of lycopene is hightly effective in reducing free radicals including lipid peroxides and improved metabolic activity of mitochondria.

Major changes in the seminal fluid components appear to be related to abnormal spermatozoal function and fertilizing capacity.[48] Activities of ATPase pathway is responsible for the active transport of Ca^{2+}, Na^{+}/K^{+}, and Mg^{2+} across the cell membrane at the expense of ATP. The most important property of ATP in spermatozoa is fuelled motility.[49] ATPases can harness the energy from a proton gradient, using the flux of ions across the membrane via the ATPase proton channel to drive the synthesis of ATP.[50] The Na^{+}, K^{+}-ATPase commonly known as sodium pump, is responsible for coupled extrusion and uptake of Na^{+} and K^{+} ions across the plasma membranes of most eukaryotic organisms. Na^{+}, K^{+}-ATPase is a member of the P2c family of P-type ATPases superfamily.[51] It is reported that lipid peroxidation was also associated with the inhibition of Na^{+} K^{+} ATPase activity in proximal tubule cell lysate and this occurs secondary to mitochondrial injury.[52] Moreover, the mitochondrial DNA mutations related to oxidative phosphorylation have reduced sperm motility.[53] Ca^{2+} is considered a prime regulator of sperm motility, capacitation and in the initiation of the acrosome reaction processes. Calcium plays a role in cell growth and differentiation during spermatogenesis. Ca^{2+} gradients across the plasma membrane required for Ca^{2+} homeostasis and signaling are maintained in part by plasma membrane Ca^{2+}- ATPase activity. In somatic cells, Ca^{2+}-ATPase activity was found to be affected by Bisphenol A due to its toxicity.[54] Mg^{2+} ATPase plays a role in endogenic process in addition to ion transport. The ion sensitive Mg^{2+} ATPase utilizes a part of ATP that is not directly related to the change in free energy for sodium transport. In the present investigation, it was observed that the level of ATPases was inhibited in erythrocyte membrane in Bisphenol A administered animals and this may be due to the peroxidation of the membrane lipids by the toxicant which initiates the loss of membrane integrity and enzyme activity. However, lycopene treatment showed an augment in activities of ATPases which may be due to the protective role of membrane integrity of the carotenoid through its antioxidant activity. Since these membranes bound enzymes are thiol group containing enzymes, that are lipid dependant and hence the restoration of the activities of ATPase enzymes suggest the ability of lycopene to protect the thiol group from oxidative damage through inhibition of lipid peroxidation.

Spermatogenesis is a complex process, hormonally regulated, that involves mitotic cell division, meiosis and the process of spermiogenesis. Besides other events, germ cell differentiation is characterized by a gradual structural modification of many organelles, including mitochondria, which play a unique role in all stages of testis development. The morphological and functional maturity of germ cell mitochondria are a reflection of the permanent change in the testicular microenvironment. Each of these steps represents a key element in the spermatogenic process. Defects which occur in any of them can result in the failure of the entire process and lead to the production of defective spermatozoa and reduction or absence of sperm production.[55] Oxidative metabolism; energy production and free

radical generation are the principal biological reactions occurring inside mitochondria. In addition to the above, mitochondria participates in an important process of apoptosis. Mitochondrial causes of infertility have triggered interest because of its presence in the tail of sperm and immense need of energy for sperm motility.[56] Thus, mitochondria accumulate oxidative damage more rapidly than the rest of the cell, contributing to mitochondrial dysfunction.[57] Normally associated with generation of ATP through oxidative phosphorylation mitochondria lose their membrane integrity following increase in free radical activities of membrane bound enzymes and ultimately death.[58] The reducing equivalents for the oxidative phosphorylation are supplied by TCA cycle, which lodges various dehydrogenases, which are also capable of producing ROS.[59] Under normal physiological conditions, Acetyl coA that enters TCA cycle combines with oxaloacetate to form citrate which spontaneously loses the inherent energy either in terms of NADH or FADH. Accordingly, reduced activities of Isocitrate dehydrogenase (ICDH), Succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) observed in testis mitochondria in Bisphenol A induced rats suggests a downward shift in the efficiency of TCA cycle and electron transfer reactions.[60] On the contrary, in the present investigation treatment with lycopene significantly increased the TCA cycle enzymes and this may be due to the antioxidant activity exhibited by the carotenoids.

CONCLUSION

In conclusion, lycopene exhibited a remarkable antioxidant bustle. By the virtue of its efficacy and non-toxicity it can be considered as a potential compound for further pharmaceutical development in the treatment of male infertility.

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