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Original article

Efficiency of lycopene against reproductive and developmental toxicity of Bisphenol A in male Sprague Dawley rats

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ABSTRACT

Bisphenol A (BPA) is an estrogenic environmental toxicant and it is a globally used endocrine disruptor that is incorporated in many plastic industries. The revelation BPA has been implicated to have perilous consequences on reproductive healthiness in human and experimental animals. Present examination endeavor to appraise a powerful antioxidant lycopene against an estrogenic compound BPA. Healthy adult male Sprague Dawley rats were subjected to Bisphenol A (200 mg/kg body weight) dissolved in corn oil (1 mL) administered orally for 30 days. The pathological alterations due to BPA encouraged oxidative stress were evaluated in testis and epidydimis tissues. Simultaneously, adjustments in testicular hormones, sperm characteristic, biological enzymes like antioxidant enzymes, elevated peroxide reactions and enhanced ROS formations were measured in reproductive toxicity rats. Captivatingly, oral administration of lycopene (10 mg/kg body weight) with BPA intoxicated rats reduced the testicular toxic condition, biochemical and morphological changes were brought back to normal. In termination, antioxidant potential of lycopene, ameliorates the changes that are induced by BPA.

1. Introduction

Infertility is a catastrophic condition. It is defined by the World Health Organization (WHO) as the inability to conceive naturally after at least one year of unprotected intercourse [1]. Infertility has been attributed to a number of factors such as infections of the genital tract, varicocele, developmental and anatomical abnormalities, endocrinopathies, immunological factors, environmental exposures, and genetic abnormalities. Global estimates suggest that nearly 72.4 million couples experience fertility problems [2]. More than 90% of male infertility cases are due to low sperm counts, poor sperm quality, or both. Sperm abnormalities can be caused by a range of factors including environmental pollutants, exposure to high heat for prolonged periods, genetic abnormalities, intake of alcohol, marijuana or cocaine, smoking, hormone deficiency or taking too much of a hormone [3]. The various environmental toxicants can cause severe organ toxicities through the metabolic activation to highly reactive free radicals [4]. Common environmental pollutants include pesticides and herbicides, volatile organic compounds, heavy metals, air contaminants and persistent organic pollutants, which may affect the fertility.

Endocrine disrupting chemicals (EDCs) are an important class of chemicals that interfere with the production, release, transport, metabolism, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis and the regulation of developmental processes [5]. Endocrine disruptor is defined by the US Environmental Protection Agency as an exogenous substance that changes endocrine function and causes adverse effects at the level of the organism, its progeny, and of populations or organisms. These chemicals in the environment have estrogenic activity. Some of the common EDCs include Bisphenol A (BPA), phthalates, and certain pesticides [6].

Bisphenol A (2, 2-bis (4-hydroxyphenyl) propane) is one of the highest volume chemicals produced worldwide, with over six billion pounds produced each year [7]. BPA, an estrogenic endocrine disrupting chemical with two unsaturated phenol rings, is used in the production of polycarbonate plastics, epoxy resins used to

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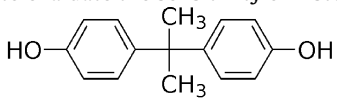
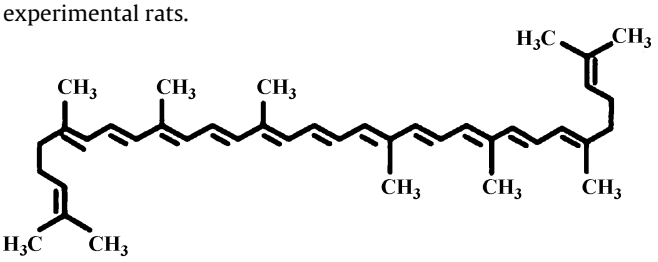
line metal cans, and in many plastic consumer products including toys, water pipes, drinking containers, eyeglass lenses, sports safety equipment, dental monomers, medical equipment and tubing, and consumer electronics [8]. BPA has been shown to leach from food and beverage containers, and some dental sealants and composites under normal conditions of use. 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 [9]. 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 [10]. Therefore, it is of interest to assess the effects of Bisphenol A on fertility of male rats and particularly, to evaluate the sensitivity of histological studies.



Chemical structure of Bisphenol A (BPA)

Fruits and vegetables are a rich source of phytochemicals, such as carotenoids, flavonoids, and other phenolic compounds. Studies have indicated that these phytochemicals, especially polyphenols, have high free radical scavenging activity, which help to reduce the risk of chronic diseases, such as cardiovascular disease, cancer and age related neuronal degeneration [11]. Vegetables like carrots, tomatoes, drumstick, and potatoes are an excellent source of nutrients and form an essential part of a well balanced diet. It must be incorporated into the diet for men facing infertility problems. These findings are consistent with a higher intake of antioxidants and micronutrients, which would have a positive influence in maintaining or improving semen quality [12].

Lycopene, as a dietary source of a carotenoid antioxidant, has been attracting considerable interest in recent years as an important phytochemical with a beneficial role in human health [13]. It is a non-cyclic carotenoid and lipophylic compound that is insoluble in water found in fruits, vegetables, and green plants. Lycopene is one of the most potent antioxidants, with a singlet oxygen quenching ability twice as high as that of β-carotene and ten times higher than that of α-tocopherol [14]. The 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 [15]. Thus, the overall data suggest that lycopene can play an important role as an energy and phytochemical source in human nutrition and animal feeding. Therefore, it is of interest to evaluate the efficacy of lycopene on Bisphenol A induced infertility in experimental rats.



Structure of Lycopene

2. Materials and methods

2.1. 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.

2.2. 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 ± 2 °C) with 12 h light and dark cycle throughout the experimental period.

2.3. Experimental protocol

The adult male Sprague Dawley rats were divided into four groups and each group consisted 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 (200 mg/kg bodyweight) dissolved in corn oil (1 mL) administered orally for 30 days. Group III: animals exposed to Bisphenol A could be treated with lycopene (10 mg/kg bodyweight) orally for 30 days. Group IV: animals received lycopene alone at the concentration of 10 mg/kg body weight for 30 days orally.

2.4. 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 4 °C for 10 minutes these samples was stored at –20 °C 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.

2.5. Biochemical estimation

Epididymal sperm were counted with a hemocytometer using a modification of the method described [16], Sperm morphology was scored [17], FSH, LH and testosterone by using ELISA kit, The level of lipid peroxides was assayed [18], Peroxide induced tissue lipid peroxidation was assayed [19], Ascorbate induced tissue lipid peroxidation was assayed [19], The activity of superoxide dismutase was determined [20], The activity of catalase was assayed [21], The activity of glutathione peroxidase was assayed [22], Glutathione was estimated [23], The level of ascorbic acid was estimated [24], The level of vitamin E was estimated [25], generation of ROS was determined [26].

2.6. Histological studies

Testis was fixed with buffered 6% formaldehyde solution, embedded in paraffin, sectioned and stained routinely with hematoxylin and eosin, and was observed microscopically. The appropriate fixative, embedment and stain for testis are Bouin's, glycol methacrylate and periodic acid Schiff/hematoxylin, but