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Lycopene abrogates di-(2-ethylhexyl) phthalate induced testicular injury by modulating oxidative, endocrine and inflammatory changes in mice

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ABSTRACT

Di (2-ethylhexyl) phthalate (DEHP) is one of the environmental pollutants that causes testicular damage. Lycopene (LYCO), the main active carotenoid in red fruits and vegetables, has well-known antiinflammatory and antioxidant activities. The present study aimed to investigate the effects of LYCO on DEHP-induced testicular injury in male mice. DEHP (2 g/kg, p.o.) was given for two weeks to mice. LYCO was given at 4 mg/kg, p.o., for two weeks, starting the same day of DEHP insult. Serum testosterone, luteinizing hormone and follicle stimulating hormone and testicular total antioxidant status, malondialdehyde, nitric oxide, glutathione, superoxide dismutase, glutathione peroxidase, catalase, tumor necrosis factor-α, interleukin-1β were measured. Also, testicular histopathological examination and sperm analysis were evaluated. Results showed that administration of LYCO significantly attenuated the DEHP-induced gonadotoxicity. Also, the gonadoprotective effects of LYCO were confirmed by the histopathological examination of the testes. Our results suggested that LYCO has produced attenuation of inflammatory, oxidative stress and hormonal parameters against DEHP-induced gonadotoxicity.

1. Introduction

Di (2-ethylhexyl) phthalate (DEHP) is one of the most widely used phthalate esters [1]. DEHP is plasticizer used in several plastic formulations such as polyvinyl chloride (PVC) [2]. PVC products that contain DEHP are used in medical or non-medical devices such as bags for blood or parenteral nutrition, tubings, catheters, plastic bags and food packaging [3]. Moreover, DEHP is not covalently bound to plastics, and therefore it can leach into the surrounding media such as air and water. DEHP ingested on a daily basis through oral ingestion and inhalation [4]. The Agency for Toxic Substances and Disease Registry (ATSDR) reports that the acceptable range of exposure to DEHP is 3–30 μg/kg/day [5]. One of the most critical concerns about DEHP exposure is the hazardous effect on human reproductive organs [6]. It is well-established that in males, DEHP exhibits antiandrogenic effects by inhibiting testicular steroidogenesis. On the other hand, DEHP induced the tubular atrophy and testicular degeneration [7]. DEHP causes testicular damage by increasing the production of reactive oxygen species (ROS) and lipoperoxidation [1, 8]. Furthermore, DEHP decreases the total antioxidant capacity level and depletes the protective antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) in the testes [8, 9]. In this regard, some antioxidant compounds such as resveratrol, quercetin, and curcumin have previously been used in preventing the DEHP testicular toxicity by neutralizing the effect of ROS and enhancing the activity of the antioxidant enzymes [8, 9].

Lycopene (LYCO) belongs to the family of carotenoids, which is present in red fruits and vegetables such as ripened tomatoes, watermelons and papayas [10]. LYCO is a powerful antioxidant which is 47 times stronger than vitamin E [11]. Indeed, LYCO exerts the antioxidant effect by scavenging free radicals [12]. It is well established that LYCO has several biological properties such as neuroprotective [13], anticancer [14] and hypocholesterolemic agent [15]. Moreover, it has been demonstrated that LYCO reduces proinflammatory cytokine such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) [16].

With this background, the present study was undertaken to investigate the effects of LYCO supplementation on modulation of oxidative stress and inflammation in DEHP-induced testicular injury in mice.

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2. Materials and methods

2.1. Animals

Thirty-five male mice (mean age: 4–6 weeks) were obtained from animal house of Ahvaz Jundishapur University of Medical Sciences. Animals were maintained at 22 ± 2 °C with a 12 hour light: dark cycle and with access to food and water ad libitum during the course of the study [17]. Animal care and experiments were conducted in accordance with the US National Institute of Health (NIH Publication No. 85.23, revised 1985) guides for the care of laboratory animals and approved by the Institutional Animal Ethics Committee Guidelines for the use of experimental animals (Ethic code: IR.DUMS.REC.1396.14).

2.2. Drugs and treatment

Di (2-ethylhexyl) phthalate (DEHP; CAS Number: 117-81-7) was purchased from Sigma–Aldrich Chemical Company, Osaka, Japan. Lycopene (LYCO; CAS Number: 502–65-8) were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). All other chemicals and reagents used were analytical grade and prepared from Merck Company (Darmstadt, Germany).

Animals were randomly divided into five groups (seven mice in each group). All drugs and vehicle were administered through oral gavage for two weeks.

Group I received corn oil (5 ml/kg).
Group II received DEHP (2 g/kg, dissolved in corn oil).
Group III received DEHP (2 g/kg, dissolved in corn oil) and LYCO (4 mg/kg, dissolved in corn oil).
Group IV received LYCO (4 mg/kg, dissolved in corn oil).

At the end of the experimental durations, the animals were anesthetized with the combination of ketamine/xylazine (60/6 mg/kg, i.p.) and blood samples were collected through cardiac puncture from the left ventricle. The resulting serum was then transferred to polypropylene

tubes and stored at −20 °C for biochemical investigations. Then animals were sacrificed by decapitation. For epididymal sperm analysis, both the cauda epididymides were removed. For histological studies, the left testis was fixed in 10% phosphate buffered formalin. For biochemical estimations, the right testis was homogenized (1/10 w/v) in ice-cold Tris-HCl buffer (0.1 M, pH 7.4) and kept in −20 °C for biochemical assays. The protein content of all homogenates samples was determined by the method of Bradford [18] using BSA (bovine serum albumin) as standard.

2.3. Epididymal sperm analysis

Epididymal sperm analysis assessment was performed for all the animals in different experimental groups. Both the cauda epididymides were excised and dissected in 1 ml of T6 medium (contained 4 mg/ml BSA), incubated for 1 h at 37 °C and 5% CO2 in a petri dish to disperse sperm from the tubules. All the procedures were performed at 37 °C, and all equipment and reagents that came into contact with the sperm were prewarmed to and maintained at 37 °C. For evaluating the percent of sperm motility, progressive and vitality (motile living sperm, immotile living sperm and dead sperm), we used the method described by Zare et al. [19].

2.4. Biochemical investigations

2.4.1. Serum analysis

Mice testosterone ELISA Kit (MyBioSource Co, cat num: MBS843463), mice Luteinizing hormone (LH) ELISA Kit (MyBioSource Co, cat num: MBS041300), and mice Follicle Stimulating Hormone (FSH) ELISA Kit (MyBioSource Co, cat num: MBS2507988) were used to determine serum testosterone, LH, and FSH levels.

The detection limits of the assay were 0.066 ng/ml for testosterone, 0.15 mIU/ml for LH and 1.406 mIU/ml for FSH. The detection range of the assay were 0.1–25.0 ng/ml for testosterone, 0.3–60 mlU/ml for LH and 2.344–150 mIU/ml for FSH.

2.4.2. Assay for redox status in testes homogenates

The total antioxidant capacity (TAC) level was measured by the method described by Benzie and Strain [20]. For enzymatic antioxidant status of testes, supernatants were used for the determination of SOD, CAT and, GPx activity as well as GSH level following the method as described by the earlier study [21–23]. For testis lipid peroxidation, we used the method described by Buege and Aust [24], which estimates the malondialdehyde (MDA) formation and for nitric oxide (NO) levels, we used the Griess diazotization reaction [25].

2.4.3. Measurement of proinflammatory response

To determine testicular TNF-α and IL-1β levels, the supernatants were thawed and then analyzed by TNF-α Assay Kit (IBL company, cat num: IB49688) and IL-1β Assay Kit (IBL company, cat num: IB99538). Cytokine concentrations in the samples were expressed as pg/mg of protein.

The detection limits of the assay were 2.535 pg/ml for TNF-α and 20.0 pg/ml for IL-1β. The standard range of the assay were 7.81–500 pg/ml for TNF-α and 12.5–800 pg/ml for IL-1β.

2.4.4. Histopathological examination

For this purpose, testes were fixed in 10% phosphate buffered formalin, embedded in paraffin, sectioned at 5 μm; and stained with hematoxylin and eosin (H&E) for light microscopic examinations. The staging of seminiferous tubules was carried out according to the Hess et al., article [26]. Twelve cross-sections of stage VII-VIII seminiferous tubules from each animal were analyzed by Motic Images Plus 3.0 software for tubular diameter (from basal lamina to the other side basal lamina), epithelial height (basal lamina to neck of elongated spermatids) and luminal diameter.

2.5. Statistical analysis

All the data were expressed as mean ± standard deviations (SD) and all comparisons were made by one-way ANOVA test followed by Tukey's post hoc analysis and differences were considered significant at $p < 0.05$.

3. Results

3.1. Effects of LYCO on DEHP-induced changes of serum hormone levels (Fig. 1)

Administration of DEHP resulted in a significant decrease in serum testosterone when compared with the oil group ($p < 0.001$). Administration of LYCO to DEHP-treated mice increased the level of serum testosterone when compared with the DEHP group ($p < 0.05$).

Our results showed that administration of DEHP significantly increased the serum LH and FSH in comparison with the oil group (all $p < 0.001$). Moreover, LYCO treatment in DEHP-treated mice only decreased the level of serum LH when compared with the DEHP group ($p < 0.05$). Furthermore, administration of LYCO to normal mice did not significantly change the levels of this serum hormone in comparison with oil group.

3.2. Effects of LYCO on DEHP-induced changes of testicular TAC (Fig. 2)

Administration of DEHP resulted in a significant increase in testicular TAC when compared with the oil group ($p < 0.001$).