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Original article

Ameliorating effects of green tea extract on cadmium induced reproductive injury in male Wistar rats with respect to androgen receptors and caspase- 3

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ABSTRACT

Cadmium is one of the potent environmental endocrine disruptors that has adverse effects on male fertility. This study was conducted to investigate the protective role of green tea extract (GTE) on cadmium chloride ($CdCl_2$) induced reproductive toxicities in male Wistar rats. Thirty-six male Wistar rats were divided to 4 groups, each one contained 9 rats. Control group, they were gavaged distilled water, meanwhile the treated groups gavaged $CdCl_2$ (3 mg/kg), GTE (70 mg/kg) and $CdCl_2$ + GTE (3 mg/kg and 70 mg/kg) for 63 days. In GTE+ $CdCl_2$ treated rats, the final body weight, relative testicular weight, epididymal weight, seminal and prostate glands weights were significantly ($P < 0.05$) increased compared to $CdCl_2$ intoxicated rats. Sperm cell concentration was significantly ($P < 0.05$) increased in $CdCl_2$ + GTE group, while sperm morphological abnormalities were significantly ($P < 0.05$) decreased than $CdCl_2$ group. The levels of superoxide dismutase (SOD) and reduced glutathione (GSH) were elevated significantly ($P < 0.05$) in $CdCl_2$ + GTE group, meanwhile catalase activity was significantly ($P < 0.05$) declined than $CdCl_2$ group. Hypercholesterolemia was observed in $CdCl_2$ group than control one while GTE+$CdCl_2$ group revealed significant ($P < 0.05$) reduction in cholesterol level than $CdCl_2$ treated group. Testicular androgen receptors and caspase-3 protein expression showed marked reduction and increase ($P < 0.05$) in $CdCl_2$ group than control, respectively. Treatment with GTE with $CdCl_2$ significantly ($P < 0.05$) increased androgen receptors, while decreased caspase-3 protein than $CdCl_2$ group. In conclusion, GTE exhibited protective effect on testes by inhibiting $CdCl_2$ induced oxidative damage and cellular apoptosis.

1. Introduction

The incidence of decreased fertility has been increased over the last 50 years [1]. In addition, infertility is a serious problem that affects about 15% of couples seeking conception. Male infertility is contributed to about the half of these individual cases [2]. Exposure to environmental endocrine disruptors has been supposed to be one of casual factors for male infertility [3]. Endocrine disruptors, either natural or man-made, are wide range chemicals that may alter endocrine system and adversely affect human, animals and wildlife [4,5].

Cadmium (Cd) is known as endocrine disruptor due to its ability to disturb hormonal production involved in the regulation of reproductive processes [6]. Cd has been used widely in several industries such as; manufacture of batteries, pigments, stabilizers for plastics, electroplating, coating, and alloys [7]. The industrial use of a large amount of Cd and the disposal of waste containing Cd led to gradual increase in Cd concentration in water, soil, and food [8]. Cd discharged in water and soil is accumulated in plants, especially cereals. Moreover, a tobacco plant can concentrate Cd

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from contaminated soils, a cigarette contains from 1 to 2 μg of cadmium [9]. Therefore, human and animals are usually exposed to such toxicant through eating food and drinking water plus inhalation due to smoking and manufacturing activities [10]. In addition, the biological half-life of Cd may reach 20–40 years. It accumulated particularly in the kidneys and liver [11]. Moreover, Othman et al. [12] added that testis is considered a suitable organ for the bio monitoring of cadmium accumulation.

Testis has been demonstrated to be a primary target organ to Cd [13]. Numerous studies demonstrated that Cd can cause severe testicular injury through reduction of testicular weight, testicular haemorrhage, oedema, necrosis, and atrophy, as well as reduce sperm cell count, sperm motility, and testosterone hormone concentrations [14,15]. These effects primarily due to the ability of Cd to pass blood testes barrier [10], alter hypothalamic-pituitary-testicular axis [16], induce oxidative stress [17], inflammatory cytokines as interleukin-6, interleukin-1β and tumour necrosis factor-α (TNF-α) [18], and DNA damage and apoptosis of germ cells [15,19].

Androgen receptor (AR) plays a crucial role in the pathogenesis of infertility, where it has post-meiotic role during male germ cell differentiation [20]. Moreover, oxidative stress is believed to play a major role in the pathogenesis of infertility and testicular dysfunction induced by cadmium. It is well known that oxidative stress leads to activation of nuclear factor- κ B signalling pathway which is crucial for regulation of many genes involved in inflammatory responses, as TNF-α, inducible nitric oxide synthase, cyclooxygenase-2 and caspase family of proteases leading to eventual germ cell death or apoptosis [21]. Also, previous studies demonstrated the effectiveness of several antioxidant and anti-inflammatory agents or plants against cadmium-induced testicular toxicity [17].

Green tea (Camellia sinensis) is one of the most consumed herbal beverages all over the world. It contains many compounds that are beneficial for health, including caffeine and characteristic polyphenolic compounds, including (2)-epicatechin (EC), (2)-epicatechin-3-gallate (ECG), (2)-epigallocatechin (EGC), (1)-gallocatechin, (2)-epigallocatechin-3-gallate (EGCG), and (1)-catechin (C) [22]. There is an increasing interest about the green tea role in maintaining health and treating disease. Many of the putative health benefits of green tea are presumed to be caused by its antioxidant effects [23]. Another potential benefit of green tea is as an anti-inflammatory agent that was proven by several animal studies [24]. Moreover, green tea plays protective role on male reproduction by reducing the oxidative stress, improving semen quality and the testicular function [25]. Therefore, current study was performed to investigate the protective effect of green tea extract (GTE) on testicular function of $CdCl_2$ exposed male Wistar rats. Besides, clarification of the possible mechanisms underlying this effect such as determination of serum cholesterol, testicular oxidative stress, androgen receptors and caspase-3.

2. Material and methods

This experiment was approved by Ethics Committee for Animal Use of the Suez Canal University (protocol #2016-073).

2.1. Material preparation

$CdCl_2$ was obtained from Oxford Lab. Co., India (CAS: 35658-65-2). $CdCl_2$ was dissolved in distilled water in a rate of 1% solution and given daily by gavage by a dose of 3 mg/kg body weight.

Green tea was obtained from Royal Company, Egypt. Preparation of aqueous extract of green tea was performed according to the method described by Dahiru and Obidoa [26]. Each 1 gm extract contained EGCG, 55%, EGC, 22% ECG, and 5.5% EC as determined by HPLC in Toxicology Department, National Research Centre, El-Doki, Egypt. Green tea extract HPLC standards were purchased from Cayman Chemical Company, USA. The analysis was performed according to Friedman et al. [27]. Each rat received 70 mg/kg GTE by gavage daily [22].

2.2. Animals and housing

A total of 36 adult male Wistar rats weighing 145–160 g were obtained from lab animal house, Faculty of Veterinary Medicine, Suez Canal University, Egypt. They were kept in plastic cages with wood shavings with food and water supplementation ad libitum. Rats were housed at controlled temperature around 26 ± 1 °C, 60% humidity and under natural day light rhythm. Rats were treated humanely with regard to alleviating animals suffering.

2.3. Experimental design

The experimental animals were divided into equal 4 groups. The first one (n = 9) was served as control and received distilled water only. The second group (n = 9) received $CdCl_2$ at concentration of 3 mg/kg body weight of 1% solution by gavage tube [28]. The third one (n = 9) was administrated GTE (70 mg/kg body weight) by gavage tube. The fourth one (n = 9) were administrated $CdCl_2$ at concentration of 3 mg/kg body weight of 1% solution and GTE (70 mg/kg body weight) by gavage tube. All groups received different treatments for 63 days.

2.4. Tissue and blood sampling

Retro orbital Blood samples were obtained from rats under effect of light diethyl ether anaesthesia in plain test tubes. Collected blood left 15 min to clot then kept in refrigerator for 3 h. They were centrifuged at 3000 rpm for 20 min. The obtained sera were stored at −20 °C for determination of total cholesterol.

Following the blood collection, the rats were sacrificed using over dose of chloroform and the following organs were dissected; testes, tail of the epididymis, seminal and prostate glands. Right testis for each rat per group was kept at −80 °C for assessments of antioxidant enzymes. Left testis was put in 10% neutral formalin buffer for histopathology and immunohistochemistry.

2.5. Body weight and organ relative weights

Final body weight for each rat per group was recorded before sacrificing Testes, tail of epididymis, prostate and seminal glands were weighed. Their relative weights were determined according to the following formula (organ weight/body weight × 100) for each experimental rat.

2.6. Sperm cell concentration (SPCC) and morphological assay

The contents of epididymis were obtained by cutting of the cauda epididymis then squeezing it in a sterile clean watch glass. These contents were diluted 5 times with warm 2.9% trisodium citrate dehydrate buffer followed by thorough mixing for sperm count [29]. Sperm viability and abnormalities were detected according to Okamura et al. [30].

2.7. Testicular reduced glutathione, superoxide dismutase (SOD) and catalase assay

Frozen testes were homogenized in phosphate buffer (pH 7.4). The testicular homogenates were subjected to cold (4 °C) centrifugation at 4000 rpm for 15 min. The supernatants were kept at −80 °C until analysis. SOD, GSH and catalase activity were