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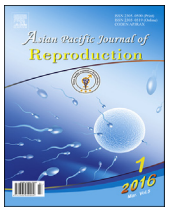

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Protective role of green tea on malathion-induced testicular oxidative damage in rats

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ABSTRACT

Objective: To examine effects of total green tea extract, a potent free radical scavenger on testicular tissue oxidative status.

Methods: 32 male albino rats of Wistar strain were divided into four groups, every group restricted 8 animals: (i) control rats; (ii) green tea-treated control rats; (iii) malathion rats; (iv) malathion-treated green tea rats. Animals received malathion 150 mg/kg and green tea 30 mg/kg for 24 h intraperitoneally. At the end of the treatment period, rat testis tissues were quickly removed and analyzed. Diameter of seminiferous tubules and germinal cell thickness, spermatogonia sertoli cells, primary spermatocytes, spermatids and leydig cell were evaluated. Also, oxidative stress evaluation was conducted based on total antioxidant capacity (TAC), lipid peroxidation (LPO) and total thiol molecules (TTM) in homogenate testis tissues.

Results: The results showed that total green tea extract improve oxidative damages against malathion group. Light photomicrograph of seminiferous tubules in malathion-treated group showed noticeable reduced height of germinal epithelium and disorganization of the tubules. An increased intestinal tissue was also observed. Primary spermatocytes were located distance from basal lamina indicating it induced damages to the intestinal tissue. While seminiferous tubules in malathion exposed and green tea extract-treated were normal.

Conclusion: This study demonstrated the effectiveness of TGTE on oxidative stress and testicular tissue damage induced in malathion in infertility disorders.

1. Introduction

Organophosphorus compounds (OPs) are cholinesterase inhibiting compounds and the main cause of pesticide poisonings [1]. Also inhibiting cholinesterase (ChE) activity, oxidative stress has been lately planned as a major toxicity mechanism for OPs both in acute and chronic poisoning cases [2,3]. Production of oxidative intermediates induced by different agents has been showed in cell death pathways principally mediated by the intracellular organelle mitochondria [4]. Testis, by producing steroids and possessing an unfortunate antioxidant group may possibly become a strong goal for the chronic oxidative stress produced during ageing [5]. It is recommended that testicular oxidative stress causing dysfunction of the organ may result in infertility [6]. Green tea (*Camellia sinensis* L.) is a beverage that is popular universal and have many pharmacological properties, such as anti-mutagenic, anti-proliferative, anticarcinogenic properties, and, more important for our aims, neuroprotective in models of degenerative disorders. These compounds are thought to be mediated by the green tea polyphenols (GTP), the four main mechanism of which are (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epicatechin (EC) [7–10]. Green tea consequent products are mainly extracts of green tea in liquid or powder type changeable in the proportion of polyphenols (45%–90%) and caffeine content (0.4%–10%) [11,12]. Various studies have indicated that polyphenolic compounds modern in the tea can diminish the risk of

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different illnesses, including diabetes, cancer and coronary heart disease [13,14]. Polyphenols in green tea have been shown to take effective antioxidant activity that is numerous folds more than that of Vitamin C and E [15]. Therefore, we found it appealing to investigate the *in vivo* effects of total green tea extract (TGTE) on testicular oxidative damage and testis tissue damage in malathion-induced toxicity rats.

2. Materials and methods

2.1. Plant materials

The leaves of *C. sinensis* L. (Theaceae) (*C. sinensis)* was purchased from the market in September 2013. Voucher specimen has been deposited at the Herbarium of the Faculty of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran.

2.2. Plant extraction

Dried and finely powdered aerial parts (1000 g) were extracted with methanol 80% (3 × 5 L) at room temperature for 4 weeks. Subsequent to elimination of the solvent in vacuuo at 50 °C, the remains (300 g, 30%, w/w) was stored at 4 °C in potted vials until usage.

2.3. Chemicals and drugs

Dithionitrobenzoic acid (DTNB), Tris base, 1,1,3,3′-tetraethoxypropane (MDA), 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), n-butanol, 2,4,6-tripyridyl-s-triazine (TPTZ), from Merck Chemical Co. (Tehran) and green tea were used in this study.

2.4. Animals and experimental design

In experimental study, male albino rats of Wistar strain weighing approximately 250–300 g obtained from the Pasteur Institute of Iran, were used all through this study. They were maintained at an ambient temperature of 25 ± 2 °C and 12/12 h of light–dark cycle. The experiments were conducted according to the ethical norms approved by Ethics Committee Guidelines. The experimental animals were divided into four groups, every group restricted 8 animals: (i) control rats; (ii) green tea-treated control rats; (iii) malathion rats; (iv) malathion-treated green tea rats. Animals received malathion 150 mg/kg and green tea 30 mg/kg for 24 h through intraperitoneal [16]. At the end of the treatment period, rat testis tissues were quickly removed and washed in ice-cold 0.9% NaCl solution and kept at 70 °C until they were analysed. Tissues were homogenized in ice-cold 0.15 M KCl (10%; wv 1/1). In addition, a portion of tissue homogenates were centrifuged at 600 g for 10 min at 4 °C to remove crude fractions and other testis immersed in bouin's fixative for histological study [17].

2.5. Evaluation of oxidative stress parameters

2.5.1. Assay of cellular lipid peroxidation (LPO)

For measuring the rate of lipid peroxidation, TBA was used which reacts with lipid peroxide molecules. The plasma samples were mixed with TCA (20%) and the precipitate was discrete in H_2SO_4 (0.05 M). TBA (0.2% in sodium sulfate 2M) was additional and heated for 30 min in boiling water bath. TBARS adducts were extracted by n-butanol and the absorbance was calculated at 532 nm. This reaction is shaped in acidic pH and high temperature and the maximum absorption is a pink complex in 532 nm [18].

2.5.2. Assay of total antioxidant capacity (TAC)

TAC was calculated by ferric reducing capacity of plasma (FRAP) method. This method is based on the ability of plasma in reducing Fe^{3+} to Fe^{2+} in the occurrence of TPTZ. The reaction of Fe^{2+} and TPTZ gives a complex with blue color and maximum absorbance in 593 nm [19].

2.5.3. Assay of total thiol molecules (TTM)

To estimate the plasma total thiol molecules, DTNB was used as a reagent. DTNB reacts with thiol molecules and create a yellow complex which has good absorbance at 412 nm in spectrophotometer [20].

2.6. Histological study

After fixation and tissue giving out, the 5 μ section were stained with Hematoxilin and Eosin. Germinal epithelium and seminiferous tubules were studied with light microscope. Histological assessment on testicular morphology was done under 40× magnification in five fields for each slide.

2.7. Statistical analysis

Results were expressed as the mean ± SE. For all animals in each group. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by post hoc Tokey test. Results were measured significantly different if $P < 0.05$.

3. Results

Table 1 shows the Mean ± SEM of variables related to either oxidative stress in animals test. A significant increase in TAC was observed in green tea *vs.* control. The values for green tea and control groups were (8.85 ± 0.28) and (3.80 ± 0.50) μmol/mL, respectively. Also, malathion reduced TAC significantly compared to green tea group (3.07 ± 0.49 *vs* 8.85 ± 0.28 μmol/mL). Combined treatment of green tea and malathion reduced TAC significantly compared with green tea group (3.41 ± 0.23 and 8.85 ± 0.28 μmol/mL). Green tea reduced catalase activity significantly compared with malathion group (2.89 ± 0.90 *vs.*7.90 ± 0.60 *vs.* U/mL). Catalase activity of the group treated with malathion and green tea were significantly higher than that treated by green tea alone (6.26 ± 0.30 *vs.* 2.89 ± 0.90 U/mL). LPO increased in malathion group compared with green tea group significantly (4.59 ± 0.56 *vs.* 2.68 ± 0.49 nmol/mL). No significant difference was observed in TTM between groups.

Light photomicrograph of seminiferous tubules in the control group showed normal appearance of the germinal epithelium and regular organization of seminiferous tubules were organized regularly. Spermatogonia sertoli cells, primary spermatocytes, spermatids and leydig cells of the control group are presented in Figure 1. Light photomicrograph of seminiferous tubules in both control and green tea-treated group germinal epithelium was