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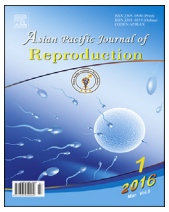

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Protective role of green tea on malathion-induced testicular oxidative damage in rats

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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

Table 1
Testis oxidative stress parameters in the control and experimental animals.

Groups	Catalase activity(U/mg)	Groups	Lipid peroxidation (μmol/mL)	Total antioxidant capacity (μmol/mL)	Total thiol molecules (mM)
Group 1	6.53 ± 0.70	Control rats	3.20 ± 0. 22	3.80 ± 0.50	0.38 ± 0.08
Group 2	2.89 ± 0.90	Green tea-treated control rats	2.68 ± 0.49	8.85 ± 0. 28[a]	0.53 ± 0.10
Group 3	7.90 ± 0.60[b]	Malathion-treated rats	4.59 ± 0.56[b]	3.07 ± 0.49[b]	0.28 ± 0. 04
Group 4	6.26 ± 0.30[b]	Green tea + Malathion-treated rats	3.35 ± 0. 46	3.41 ± 0.23[b]	0.34 ± 0.05

Group 1: control rats; Group 2: green tea-treated control rats; Group 3: malathion rats; Group 4: malathion-treated green tea rats. Values are expressed as Mean ± SEM (n = 5 per group); [a] Significantly different ($P < 0.05$) compared with control group; [b] Significantly different ($P < 0.05$) compared with green tea group, Values are group Mean ± SEM (n = 5 per group).

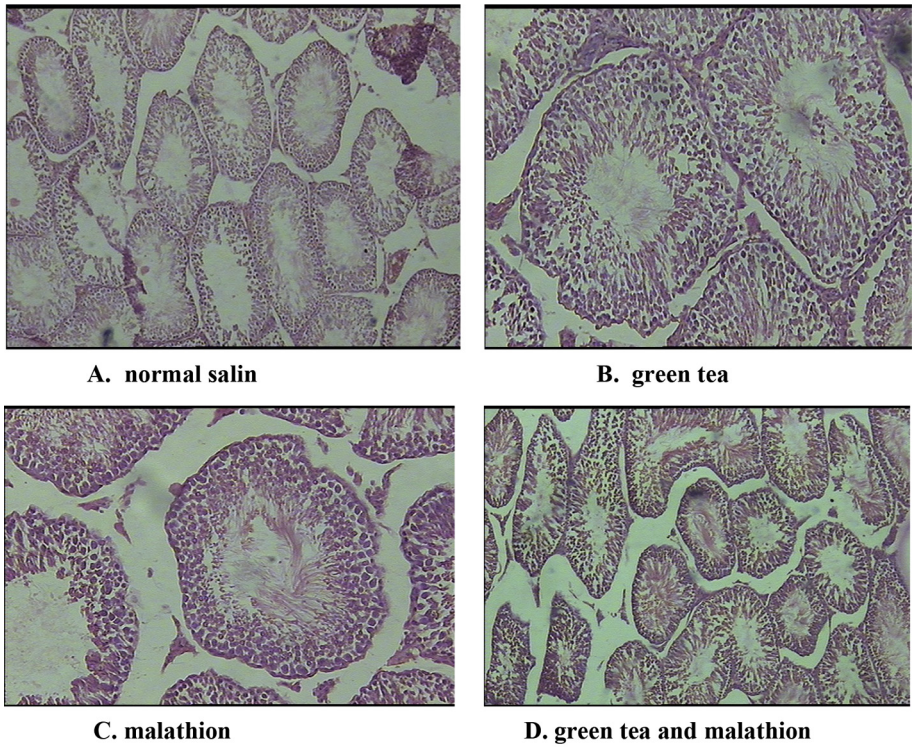

Figure 1. Light photomicrograph of seminiferous tubules.
(A) Seminiferous tubules in the control group showed normal appearance. (B) Light photomicrograph of seminiferous tubules in green tea-treated group. (C) Reduced height of germinal epithelium and disorganization of the tubules were noticeable. An increase intestinal tissue was observed. Primary spermatocytes were located distance from basal lamina. (D) Light photomicrograph of seminiferous tubules in malathion exposed and green tea extract-treated group.

normal (Figure 1A, B). 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 observed. Primary spermatocytes were located distance from basal lamina (Figure 1C). Seminiferous tubules in malathion exposed and green tea extract-treated are normal (Figure 1D).

4. Discussion

The present study demonstrated administration of total green tea extract (TGTE) improves the oxidative injuries such as lipid peroxidation, total antioxidant capacity and total thiol groups in testis tissue. Thus, TGTE may be involved in protecting against neuronal degenerative stress and in the increasing; reactive oxygen species (ROS). Malathion-based cholinesterase inhibition results in damage of different tissues such as kidney, liver and testes. Recently, it has attracted more attention owing to impairment in testicular function following the OPs poisoning [21]. Investigators reported that OPs-induced gonadal toxicity and oxidative stress [22]. The results of the present study indicated that malathion administration a significantly decrease in TAC and increase in CAT activity in the testicular tissue rats.

Reactive oxygen species (ROS) and oxidative damage to biomolecules as a mechanism of OPs toxicity [2] may contribute to male infertility by reducing sperm function [23]. TGTE are well known scavengers of ROS and RNS. In this study, organization of TGTE decreased the testis oxidative status. Antioxidative enzymes are activated by TGTE intake [24], and the antioxidative strength of human plasma increases with continual ingestion of green tea [25,26]. These antioxidative defense systems might also prevent oxidative damage in the brain. Long-term intake of GTE may be important because cells are constantly exposed to oxidative stress [27]. Activated microglia and astrocytes can release cytokines, ROS and nitric oxide (NO) that may contribute to the memory deficits

[28,29]. Also, in animal studies, tea polyphenol administration was shown to decrease serum LPO level due to its potential antioxidant activity [24]. Green tea can act as scavengers of free radicals caused by ROS and prevent radical damage. Additionally, degeneration, necrosis, interstitial edema, desquamative germinal cells and the deceleration of spermatogenesis were also seen in the malathion-treated rats. These results are in agreement with our earlier studies at which similar changes were observed in the histological structure of testis connected with oxidative stress after treatment of some cytotoxic agent. The possible explanation for the protective effects of green tea against malathion-induced increase in LPO might be its ability to react with the oxygen metabolites. Our study showed a significant decrease in oxidative damage in testis issue. In recent years, several antioxidant agents have been used to prevent I/R-induced tissue damage in experimental testicular torsion such as superoxide dismutase, catalase, calcium channel blockers, oxypurinol, allopurinol and melatonin [23,30].

Conflict of interest statement

There is no conflict of interests.

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