Original Article

Green Tea Protects Testes against Atrazine-induced Toxicity in Rat

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ABSTRACT

Background: Atrazine (ATZ) is a common herbicide in agriculture for control of grass and broad-leaved weeds. It persists in the environment and causes reproductive problems in both human and animals. The present study was aimed at protective effect of green tea against ATZ toxicity in the reproductive system of male rats.

Methods: The present study was performed in Veterinary School, Shahid Bahonar University of Kerman in 2016. ATZ and treatment groups received ATZ daily 200 mg/kg BW orally for 14 d. In addition, 0.2% methanolic green tea extract was administrated in the treatment group.

Results: In histopathologic investigation, number of germinal layers reduced in the most seminiferous tubules in the ATZ group and spermatids were absence. Necrotic spermatocytes, spermatids, and spermatozoa were evident in the testicular tubules. In the morphometric measurements, tubular diameter, germinal epithelium height, and meiosis index decreased significantly.

Conclusion: Green tea extract had reduced testicular toxicity of atrazine significantly. ATZ induces toxicity through oxidative damage and green tea extract can protect the testes due to antioxidant activity of its polyphenols especially flavonoids.

Keywords: Atrazine, Histopathology, Rat, Reproductive System, Testis.

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INTRODUCTION

Atrazine (ATZ, 2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is an herbicide commonly used for control of broadleaf and grassy weeds in corn, sorghum, sugarcane, cotton, and pineapple crops [1]. Atrazine and its metabolites persistent in water are found in soil especially in farming seasons [2, 3]. This herbicide inhibits electron transport and results in photosynthesis disruption and death of broad-leaf plants [4]. ATZ makes disturbances in endocrine system and induces noxious effects on the reproductive function in human and animal species [5, 6]. Induction oxidative stress is a common mechanism for endocrine disrupting chemicals that lead to producing free radicals. Because of plentiful polyunsaturated fatty acids in the membrane of spermatozoa, these cells are susceptible to reactive oxygen species [7]. Reduction in sperm number and motility [8, 9], hermaphroditism in frogs [10], change in sex ratio in fish [11], delay in sexual maturation [12] and loss of prostate and seminal vesicle weights [9] are reported adverse effect of atrazine in male health.

Antioxidants neutralize destructive damages of free radicals and could be useful for prevention or treatment of oxidative stress in various diseases [13, 14]. Green tea extract (GTE) has strong antioxidant activity and ability to scavenging free radicals [15].

In this study, protective effect of green tea against ATZ toxic damages on the reproductive system of male rats were evaluated by histopathological and morphometric investigations.

MATERIALS AND METHODS

Plant Extract Preparation

The present study was performed in Veterinary School, Shahid Bahonar University of Kerman in 2016. Green tea leaves were dried at room temperature (30 ± 5 °C) and then powdered by Moulinex food processor. The alcoholic extract

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was obtained by maceration of 100-gr powder with methanol. The extract was filtered using a sterile cloth sheet. The filtrate was evaporated under reduced pressure at temperature below 45 °C with a rotary evaporator until the extract obtained. The extract samples were stored in universal bottles and kept at 4 °C before to use.

Animals and Experimental Plan

Male Wistar rats (weighing 200-220 gr) were used. The animals were housed under a 12-h light-dark cycle with free access to the standard food and fresh water *ad libitum*. The experimental period was considered 14 d.

The study was approved by the Ethics Committee of School of Veterinary Medicine, Shahid Bahonar University of Kerman, Iran.

The rats were divided into four groups (n= 6) as follow:

Group I: control rats received 1 ml distilled water /day by gavage.

Group II: 0.2 % of green tea extract in daily water +1 ml distilled water /day by gavage,

Group III: 200 mg/kg BW/day atrazine diluted with corn oil in 1 ml volume by gavage,

Group IV: 200 mg/kg BW/day atrazine diluted with corn oil+ 0.2 % methanolic extract of green tea**.**

Histopathologic Investigation

Some samples (1 cm^3 thickness) from tests were taken and fixed in 10% neutral buffered formalin for histopathological examination. The tissue sections were processed using standard procedure. Sections in 5 µm thickness were provided from the embedded paraffin tissues and then stained with hematoxylin-eosin (HE). The tissue section studied by a light microscope for spermatogenesis percentage, meiosis index (the ratio between the numbers of round spermatids to the number of primary spermatocytes), high of germinal epithelium and tubular diameter. Spermatogenesis index (SI) was evaluated by Johnsen's score for each testicular section. In total, 100 tubules were grade on a level of 1-10 according to the no cells to complete spermatogenesis and the mean score [16].

RESULTS

In the normal control group, the tests were consisted of seminiferous tubules lined by the germinal epithelium that separated by interstitial connective tissue. All stages of spermatogenesis including spermatogonia, primary spermatocytes, round spermatids, and elongated spermatids arranged as columns. Cluster of young spermatozoa was observed in the lumens of the seminiferous tubules. Testicular sections did not show any histopathologic change. Spermatogonia placed on the basement membrane and next to these, spermatocytes occupied the middle part. In the next layer, spermatids were visible and then mature elongated spermatozoa were present in the lumen of seminiferous tubules (Figure 1).

Fourteenth day of experiment, in ATZ group, testes exhibited reduction in number of germinal layers and spermatids. The normal arrangements of germ cells were disrupted. Many tubules contained admixture of necrotic spermatocytes, spermatids, and spermatozoa. In this group, spermatids with nuclear fragments and pyknosis were present in the most tubules (Figure 2). In the morphometric measurements, tubular diameter, germinal epithelium height, and meiosis index decreased significantly (P<0.01). In addition, a significant reduction in the spermatogenic index was observed in the ATZ group (P<0.001). The Johnson score shifted from a level of 5.7 (no spermatozoa) in ATZ group to 8.9 (few spermatozoa) in the ATZ-GT group (Table 1).

In ATZ-GT group, lesions of the seminiferous tubules were considerably less severe due to treatment by green tea extract. Spermatogenesis was observed in some seminiferous tubules but the number of cells decreased in compared with the normal control group (Figure 3).

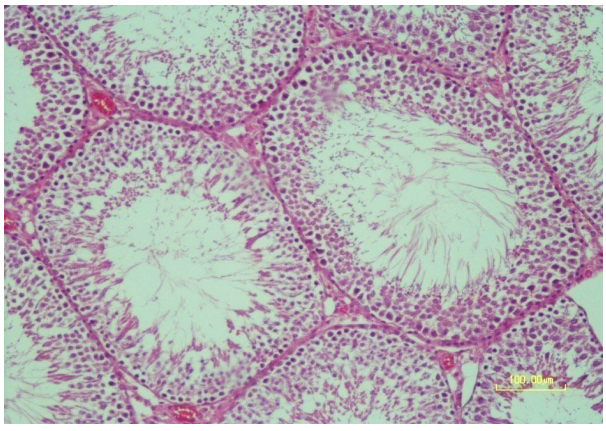

Figure 1. Normal control group. Seminiferous tubules lined by the germinal epithelium with all stages of spermatogenesis (HE).

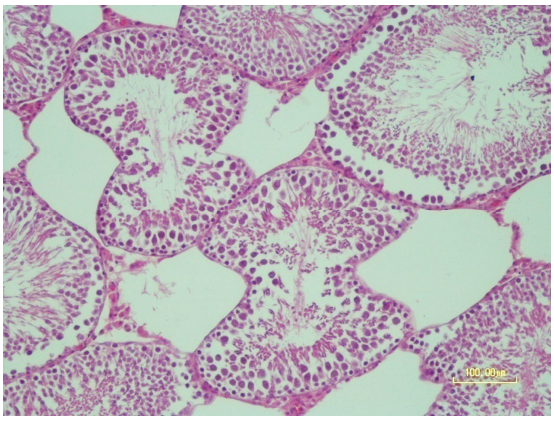

Figure 2. ATZ group. Shrinkaged seminiferous tubules and reduction of spermatogenesis (HE).

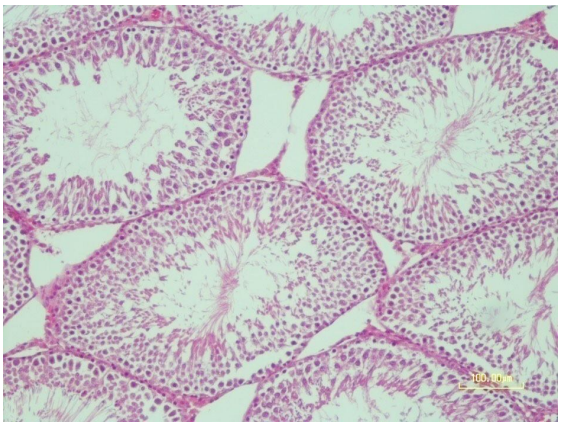

Figure 3. ATZ-GT group. Damaged seminiferous tubules (asterisk) adjacent to tubules with active spermatogenesis (HE).

Table 1. Effect of Atrazine and green tea on the morphometric parameters.

Spermatogenesis %	Germinal epithelium high	Seminiferous diameter	Meiosis index	Johnsen scoring	Groups
88±0.89[a]	59.14±0.64[a,b]	318.73±3.66[a]	2.43±0.16[a,b]	8. 8±0.17[a]	Normal control
52.14±2.67[b]	55.33±0.64[c]	285.53±4.57[b]	1.19±0.07[c]	5. 71±0.17[b]	ATZ
90.76±2.18[a]	61.06±0.54[a]	313.88±4.03[a]	2.79±0.13[a]	8. 97±0.14[a]	ATZ-Green tea
83.71±1.74[a]	58.48±0.45[b]	307.33±3.65[a]	2.18±0.1[b]	8. 47±0.18[a]	Green tea

DISCUSSION

In humans, reproductive abnormalities and infertility are increasing because of exposure to contaminated foodstuffs, drinking water and air with different environmental toxins such as organochlorine pesticides, polychlorinated biphenyls, dioxins, phytoestrogens, chlorotriazines and other xenoestrogens [17]. Long-term uses of these chemicals cause hypospadias, cryptorchidism and testicular degeneration [18]. ATZ is an important chlorotriazine that uses widely as spray over various crops and vegetables for control weeds. ATZ has considered great attention due to its extensive use and high persistence in ecosystem. Human and animals are directly or indirectly at the risk of exposure to this herbicide [10].

ATZ and its metabolites have been reported to be toxic for male reproductive system in animal models [19, 20]. In the present study, the role of green tea extract on atrazine (200 mg/kg BW for 14 d) was evaluated in male rat. In the histopathologic examination, ATZ had caused decreased germinal cells in the seminiferous tubules, increasing the abnormal sperms, absence of spermatozoa and presence of necrotic spermatids in the seminiferous tubules lumen. Treated rats showed reduction of testicular lesions following administration of green tea extract. In the recent years, the use of the green tea extract products is considered. Tea polyphenols especially flavonoids have antioxidant properties. The antioxidant activity of polyphenols is primarily related to the aromatic rings and hydroxyl groups that neutralize free radicals. Polyphenols and tea catechins are electron transferor and effective scavengers for reactive oxygen species in vitro [21, 22]. These components form 25%–35% of the dry weight of green tea leaves [23]. Extract of green tea prevents lipid oxidation, therefore, uses in foods containing fats and oils.

The present results are in accordance with the previous studies on ATZ -induced damages and effects of different traditional treatments. The protective effect was investigated of date fruit extract as an antioxidant-rich nutraceutical on atrazine-induced testicular toxicity. ATZ (200 mg/kg bw for 16 d) significantly decreased the testicular weight, sperm count and motility, level of GPx, SOD, CAT and the testicular lipid peroxidation in the control group. Treatment with date fruit extract reduced lipid peroxidation of tests and preserved the level of SOD, CAT, and GPx. They suggested treatment with Date fruit

extract could protect the testis from oxidative damage of Atrazine [24].

Medical plants improved Atrazine toxicity in other organs. Protective effect was studied of *Andrographis paniculata* (AP) against renotoxicity of ATZ in albino rats. AP is a medicinal plant containing diterpenoids, flavonoids, and polyphenols. Ethanolic extract of AP (150 mg/kg bw) was found to protect the rats from renotoxic action of Atrazine by decreased in the level of lipid peroxidation and increased the activities of antioxidant enzymes in the kidney. The effect of ginger (120 mg/kg bw, each alternative day) was evaluated on oxidative stress of ATZ (78.25 mg/kg bw) in liver and kidney of mice during 14 d. Atrazine decreased activities of the various antioxidant enzymes and antioxidant power [25].

ATZ influences androgen secretion and decrease testosterone level. The exposed animals suffer from decline of sperm motility, number, and viability of sperm, and increasing abnormal sperms that can lead to infertility [24, 26]. ATZ induces toxicity in different organs through produce of free radicals. This herbicide passes blood-testis barrier and disturb the junction between Sertoli cells and germ cells. Furthermore, these impairments might be related to reduction of testosterone production of Leydig cells [27]. The risk of herbicides for health shows specially occurrence of some neoplastic lesions like lymphomas, breast cancer, testicular cancer and lymphohematopoietic [28, 29].

CONCLUSION

Despite different studies about reproductive toxicity of Atrazine, little information is present on its molecular mechanisms. In according to prevalent and persistent of this herbicide in the environment and ecosystems, further investigations are needs to determine the exact pathogenesis of Atrazine.

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