Original Article

Toxic Effects of Atrazine on Reproductive System of Male Rats*

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Abstract

Objective This study was designed to evaluate the toxic effects of Atrazine (ATZ) on the reproductive system of male rats.

Methods Male Sprague-Dawley rats were exposed to ATZ by gavage at dosages of 0, 38.5, 77, and 154 mg/kg bw/day for 30 d. The toxic effects of ATZ to rats were assessed through histopathologcal observation, spermatozoa quality evaluation, testicular marker enzyme indicators, antioxidant capacity and reproductive hormone levels.

Results Significant adverse effects on reproductive system were observed in rats exposed to ATZ at different dosages compared with 0 mg/kg group, including an irregular and disordered arrangement of the seminiferous epithelium in 154 mg/kg group; a decreased spermatozoa number and an increased spermatozoa abnormality rate in 77 and 154 mg/kg groups; decreased levels of acid phosphatase (ACP), alkaline phosphatase (AKP), lactic dehydrogenase (LDH), and succinate dehydrogenase (SDH) with the increasing of ATZ concentration; a decreased level of total antioxidant capacity (TAC) in a dose-dependent manner, and a decreased reduced glutathione (GSH) level and an increased malondialdehyde (MDA) content in 154 mg/kg group; and decreased serum levels of testosterone (T) and inhibin-B (INH-B) and an increased serum level of follicle stimulating hormone (FSH) in 77 and 154 mg/kg groups, and an increased serum level of luteinizing hormone (LH) in 154 mg/kg group.

Conclusion These results suggested that relatively high doses of ATZ could exert reproductive toxicity of male rats.

Key words: Atrazine; Reproductive toxicity; Oxidative stress; Endocrine disrupter

Biomed Environ Sci, 2014; 27(4): 281-288 doi: 10.3967/bes2014.050 ISSN: 0895-3988

www.besjournal.com (full text) CN: 11-2816/Q

INTRODUCTION

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine, ATZ), a chloro-s-trazine herbicide, is used extensively worldwide for broadleaf and grassy weed control in corn, sorghum, sugarcane, cotton, and pineapple crops and landscape vegetation[1], so that ATZ and its metabolites are widely persistent in water and are mostly found in soil especially in farming seasons[2-4]. Despite ATZ has been banned in European Union and been restricted in other countries, it is still being used in large quantities worldwide up to now. It is one of the most widely used agricultural pesticides in United States[5], and its application in Asian countries has been growing. Therefore, humans and wildlife are at risk for exposure to ATZ.

In recent decades, there has been an increasing

*This work was supported by National Natural Science Foundation of China (81030053) and National High-technology R esearch and Development Program ('863' program) of China (2010AA023001).

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Received: February 28, 2013; Accepted: June 20, 2013

concern on clarifying the toxicological mechanisms of environmental chemicals to cause alterations in the reproductive system of humans and mammals. A number of reports have suggested that ATZ might have adverse effects on the reproductive function[6-13]. It has been shown that ATZ could reduce pituitary, prostate and seminal vesicle weights in rats, associating with decreased spermatozoa count, viability and motility[8-10], and cause infertility in men living in agricultural areas[11]. It has also been reported that ATZ could induce endocrine disruption and consequently interfere with physiological functions of various hormones. It was demonstrated that ATZ exposure from postnatal day (pnd) 21 to 53 had caused a significant decrease in serum and testicular T levels after administered at doses of 100-200 mg/kg bw/day in male Wistar rats[9]. Consistent with these observations, Friedmann demonstrated that ATZ in the dose of 50 mg/kg bw/day had significantly reduced the serum and testicular T levels, both in acute toxicity test (from pnd 46 to 48) and chronic toxicity test (from pnd 22 to 48), in juvenile Sprague-Dawley male rats by gavage[12]. A recent study demonstrated that the serum levels of testosterone (T), follicle stimulating hormone (FSH), luteinizing hormone (LH), and inhibin-B (INH-B) had decreased by 85% after 48 d exposure to high dose (300 mg/kg bw/day) of ATZ in adult male Wistar rats[13]. However, there is still little knowledge on the mechanisms of ATZ-induced detrimental impacts on spermatogenesis cells, and the effects of ATZ on serum levels of T, FSH, LT, and INH-B, especially the intrinsic correlation among these hormones, are not yet well clear.

In this study, the toxic effects of a 30-d exposure to ATZ on the reproductive system of male Sprague-Dawley rats were evaluated by a panel of assays, including histopathological observation of testes, measurements of spermatozoa count and spermatozoa abnormality rate, and detections of testicular marker enzymes, antioxidative capacity and the reproductive hormone levels in serum, to further investigate the mechanisms of the reproductive toxicity of ATZ in male rats and provide insights and perspectives for the potential harm of ATZ to human health.

MATERIALS AND METHODS

Chemicals

ATZ ($C_8H_{14}ClN_5$, CAS: 1912-24-9, ≥98% purity) was from Kesai Chemical Limited Company (Jinan, China). T, FSH, LH, and INH-B enzyme linked immune sorbent assay (ELISA) kits were from R&D systems (USA). Reagent kits of total protein (TP), alkaline phosphatase (AKP), acid phosphatase (ACP), lactic dehydrogenase (LDH), succinate dehydrogenase (SDH), total antioxidant capacity (TAC), reduced glutathione (GSH), and malondialdehyde (MDA) were from Nanjing Jiancheng Bioengineering Institute (China).

Animals and Treatments

Forty male Sprague-Dawley rats of Specific Pathogen Free (SPF) grade (aged 4 weeks and weighted 80±10 g) were purchased from Dashuo Laboratory Animal Reproduction Center in Jianyang, China [Certificate number: SCNK (CHUAN) 2008-29]. Animals were housed in polycarbonate cages in an environmentally controlled room (temperature 20 to 24 °C and 12 h light/12 h dark). Food and water were provided *ad libitum*.

After one-week acclimation, the rats were randomly divided into 4 groups, i.e. a control group and 3 treatment groups. ATZ was dissolved in corn oil and given to the rats by gavage at doses of 0 mg/kg bw/day (corn oil control), 38.5 mg/kg bw/day (ATZ-L), 77 mg/kg bw/day (ATZ-M) and 154 mg/kg bw/day (ATZ-H) respectively. The gavage volume was 5 mL/kg bw and the amount of gavage was adjusted every three days according to the varied weight of each rat. The total exposure duration was 30 consecutive days, and the solution of ATZ in corn oil was prepared each week. At the end of the experiment, the rats were sacrificed, and their testes, epididymis and accessory glands (seminal vesicles and prostate) were carefully dissected out and weighed.

In this study, all the experiments conducted in animals were in accordance with the guidance of ethical committee for research on laboratory animals of Sichuan University.

Histopathological Evaluation of Testis

The removed testes were fixed in Bouin's fixative as described by Zhen et al.[14]. After 24 h, testes were washed for three times and maintained in 70% ethanol. Samples were then embedded in paraffin and sectioned with rotary microtome (GMbh, Germany). The tissue sections of the testes were then stained with hematoxylin and eosin (H&E), and observed with an optical microscope (AX70, Olympus, Japan) with blind manner.