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Original Research Paper

Effects of Atrazine Toxin on Levels of LH, FSH and Testosterone Hormones in Adult Male Rat

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ABSTRACT

Atrazine is a widely used herbicide in agriculture. In the present study, the effects of this herbicide on the levels of pituitary-testis hormones in adult male rats were investigated. In total, 40 male Wister rats were used. These animals were randomly divided into five groups of eight: the control group, which was left untreated, the sham group receiving solvent, and three experimental groups receiving (100mg/kg), (200 mg/kg) and (400mg/kg) dosages of Atropine, respectively. Dosages of solvent and herbicide were injected intra peritoneally for 14 days, and afterward, blood samples were taken and serum levels of LH, FSH and testosterone were measured by RIA method. The body and testicular weights were also determined. The mean results were evaluated by ANOVA and Tukey test among the experimental and control groups. Mean body weight showed significant decrease in experimental groups relative to control group. Testes weights were decreased only in experimental groups receiving 200,400 (mg/kg) dosage of Atrazine. In addition, serum levels of LH showed a significant decrease in experimental groups receiving 200, 400 (mg/kg) dosages of Atrazine, compared to the control group. Mean concentration of FSH decreased significantly in the experimental groups receiving 400(mg/kg) dose, while testosterone level decreased significantly in all the experimental groups, compared to the control group. Atrazine can interfere with food absorption mechanisms and cause body and testicular weight loss by increasing estrogen and decreasing testosterone levels. According to the results of present study and other investigations, this herbicide probably decreases the secretion of LH, FSH and testosterone concentrations through reducing the pituitary weight and secretion of GnRH from hypothalamus, thereby, decreasing the activities of pituitary-testis axis and spermatogenesis processes.

INTRODUCTION

The synthetic chemicals are a group of compounds which are absent in nature, instead they are synthesized from simpler compounds or through decomposition of more complex materials. Some of these organic chemicals are toxic and are used against various pests, and usage of these compounds is an environmental and health hazard for both human and other organisms. There is an increasing concern that certain chemicals in the environment can cause endocrine disruption in exposed humans and wildlife. Investigations of potential effects on endocrine function have been limited mainly to interactions with hormone receptors (Sanderson et al. 2000).

Atrazine is widely used against long and broad leaf herbaceous plants in corn fields and gardens. This herbicide is most effective against weeds when applied prior to growth. In some areas, Atrazine is used for the selective control of weeds in restoration of pine forest, cultivation of Christmas trees, long leaf seed fields as well as in chemical fallow. In addition, it is used in dry areas as a nonselective herbicide. Atrazine is common name for 2-chloro-4-(ethylamino)-4-(isopropylamino)-s-triazine. Its chemical formula is $C_8H_{14}ClN_5$. This herbicide was first introduced in 1958. Its commercial name is Atrax and in Iran is marketed under the name of Gesaprim. This compound is a white crystalline solid with the solubility of 33 ppm in water at 27°C. Its molecular weight is 215.68 gr/M, and its half life in soil, pure and sea water and in vertebrates is about 4, 3 and 30 days and less than 72h, respectively. However, this time length may increase up to 385 days in sandy arid regions. Atrazine is absorbed through roots and transmitted via apoplast. It is also absorbed through leaves. The herbicide inhibits plant growth through interference with photosynthesis and leaf death. Other effects on the leaves include membrane and chloroplast destruction. It is known that all the plant organs are inhibited by Atrazine herbicides. In addition, it interferes with the metabolism of phytohormones, prevents stomatal opening in light and causes their closure in normal temperature. Studies show that Atrazine causes adverse effects on liver, kidney and cardiovascular system in animals exposed to it (Chan et al. 2006 and Li et al. 2006). Furthermore, it causes changes in sperm morphology and a reduction in sperm motility (Kniewald et al. 2000). Usage of Atrazine interferes with the reproduction of old rats and modifies the ovarian cyclic rhythm. It also changes the prolactin level in female rats which may be the result of its effect on the hypothalamus (Hafiez et al. 1971). In humans and other animals, Atrazine causes skin sensitivity, light sensitivity, respiration disor-

der, paralysis, weakening of the organs, specially arms and legs, chemical and structural modification in brain, heart, liver, lung, kidney and ovary, and delay in the growth of endocrine organs (Chan et al. 2006). Moreover, it has been observed that Atrazine brings about modifications in the levels of ovarian hormones and reproduction activities in animals. There is some information about its adverse effects and the embryonic growth in women and premature delivery. Exposure of pregnant animals to high levels of Atrazine reduces embryo survival (Rayner et al. 2004).

Evidence showed that Atrazine metabolites can postpone maturation of the male rats. This effect is probably due to the alteration in steroid secretion and changes in the growth of reproductive organs (Stoker et al. 2000). Other studies have demonstrated that Atrazine can affect sperm morphology and motility and modify serumic level of prolactine in female rats. According to some research findings, Atrazine can postpone maturation of female rats (Laws et al. 2000). The results of an investigation revealed that toxification of herbicides, such as Atrazine may cause cardiovascular disorders (Chan et al. 2006).

Long exposure of workers to Atrazine increases prostate cancer significantly. Other investigators have shown that 400 ppm (or 22.5 mg) treatment of Atrazine brings about irregularity in the old rat reproduction and alters the ovarian cyclic rhythm (Eldridge et al. 1999). In the present study, effects of this herbicide on the concentrations of LH, FSH and testosterone hormones and testicular functions in rat were investigated in order to determine its probable side effects on the fertility.

MATERIALS AND METHODS

The animals used in this study were 40 male Wister rats, each weighing about 200g and 2.5-3 months old. These animals were supplied by animal house of Islamic Abad University of Kazerun. Weight difference among the various groups was less than±10%. They were kept in special polycarbonate cages with laced roof made of steel. Cages were placed in a room at about 22±2°C and 12 hours (7 a.m. till 7 p.m.) light and 12 hours dark cycle for 14 days. All the ethical and animal rights principles were considered and followed in various steps of this study.

Animals were divided into five groups of eight as follows: A control group left untreated and all environmental conditions and food for this group were the same as those for other groups; a sham group which received 1 mL distilled water (as solvent); and three experimental groups which received 100mg/kg/d, 200mg/kg/d and 400mg/kg/d dosages of dissolved Atrazine, respectively. Dosages of solvent and herbicide were injected intraperitoneally for 14 days.

After two weeks, animals were weighed precisely, and then were anaesthetised by ether. The anaesthetised animals were laid on appropriate device and blood samples were taken from their hearts by 5 mL syringes. Blood samples were put in clean tubes lacking anticoagulant and left at 37°C incubator for 15 min. Coagulated samples were centrifuged for 15 min at 5000 rpm. Sera were put in the special tubes, covered by Para film, labelled and kept frozen for three days to be tested later for different hormones. The concentrations of LH, FSH and testosterone were measured by routine laboratory tests, i.e., radioimmunoassay (RIA) (Picard et al. 2008). Internal organs of the animals were carefully examined anatomically and testes were removed, washed with saline and precisely weighed. The mean results were evaluated by appropriate statistical methods including ANOVA and TUKEY tests among experimental and control groups and were plotted by Excel, and $P < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

According to the results, mean body weight decreased significantly in experimental groups receiving different dosages of Atrazine (Fig. 1). Similarly, the results of testicular weight are shown in Fig. 2. As seen, the mean weight of testes showed a significant decrease in the experimental groups receiving 200, 400 (mg/kg) dosages of herbicide relative to the control group.

The results obtained for LH concentration did not change significantly in the experimental group with 100 (mg/kg) dose, but it decreased significantly in 200, 400 (mg/kg) dosages of Atrazine. Fig. 3 shows the changes in the levels of LH among the various groups. Results for FSH indicate a significant decrease in the experimental group receiving 400 (mg/kg) dose of Atrazine (Fig. 4). Concentrations of testosterone in all the experimental groups decreased significantly in comparison of the control group (Fig. 5).

Based on the present findings, the injection of 100, 200 and 400 mg/kg of Atrazine for 14 days causes a significant decrease in mean body weight (Fig. 1). Findings of other investigators showed a 9% body weight reduction in the rats taking 100 mg/kg Atrazine per day; similarly, this reduction was 21% for the rats receiving 200 mg/kg Atrazine per day. Body weight drop is due to a reduction in the food consumption and absorption (Trentacoste et al. 2001). In addition, Stocker et al. (2000) showed that 53 days usage of Atrazine higher than 200 mg/kg brings about a significant body weight reduction in the rats (Hafiez et al. 1971). Other studies indicate that Atrazine interferes with food absorption mechanism in dogs, rats and birds, and can reduce food intake and growth. According to various researches, Atrazine is a powerful endocrine degradator and causes pituitary weight loss

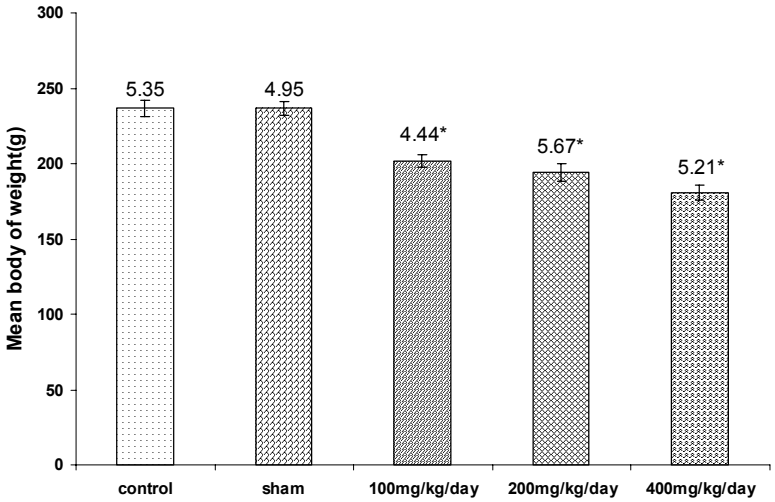

Fig. 1: Comparative body weight in the experimental groups receiving different doses of atrazine and control.

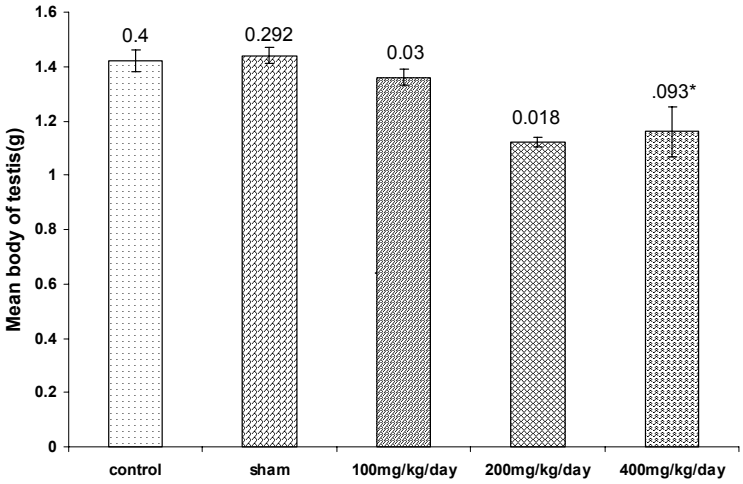

Fig. 2: Comparative mean body weight of testis in the experimental groups receiving different doses of atrazine and control.

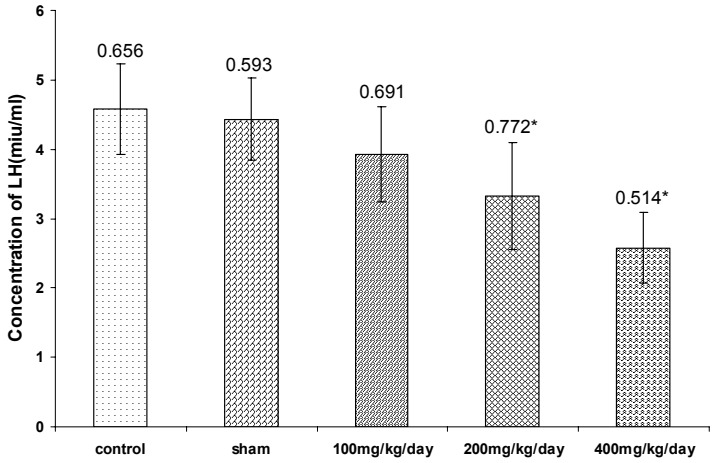

Fig. 3: Comparative concentration of LH in the experimental groups receiving different doses of atrazine and control.

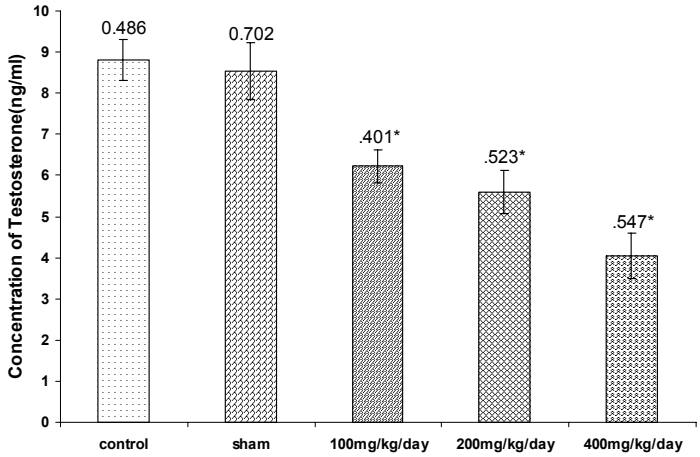

Fig. 5: Comparative concentration of testosterone in the experimental groups receiving different doses of atrazine and control.

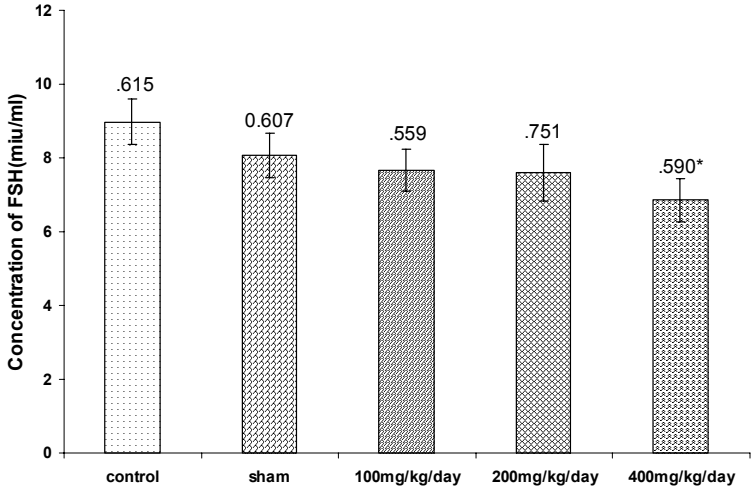

Fig. 4: Comparative concentration of FSH in the experimental groups receiving different doses of atrazine and control..

(Stoker et al. 1999 and Fan et al. 2007). Probably, endocrinal degradation and pituitary weight loss bring about reduction in pituitary hormones, including growth hormone, thereby decreasing the body weight.

Results of this study show a significant decrease in testis weight in the experimental groups receiving 200, 400 (mg/kg) dosages of Atrazine (Fig. 2). Findings of Wilhelms et al. (2005) indicate that the Atrazine usage causes testicular weight loss in recently hatched quail. The results of the present study are in agreement with the findings of Knewald et al. (1995) suggesting that usage of high levels of Atrazine can cause weight loss in testes and androgen producing organs. Findings of other investigators indicate that Atrazine reduces weight of the prostate, seminal vesicle and epididymis; similarly, it can decrease sperm counts and motility and causes essential changes in testicular tissues as well as reducing the testicular protein concentrations (Simic et al. 1994). Since Atrazine decreases food intake, absorption and growth, the observed testicular weight loss is expected (Reynolds et al. 1974). According to the present results, level of LH did not change significantly in the experimental group receiving 100 (mg/kg) doses; however, it decreased in the groups taking 200, 400 (mg/kg) dosages of Atrazine (Fig. 3). The findings about Atrazine reduction of LH and testosterone levels in premature male rats is inconsistent with the results of Trentacosts et al. (2001). As mentioned above, Atrazine causes pituitary weight loss and the observed decrease in LH level is expected (Fan et al. 2007). Studies of Stoker et al. (2000) have demonstrated that Atrazine usage prevents prolactin and LH rises in ovariectomised adult rats, and 200 mg/kg/d Atrazine reduces LH level. It has been shown that Atrazine lowers concentrations of LH in the rats and Japanese quail. Atrazine and its metabolite (DACT) lowers the sensitivity of GnRH and LH receptors (Stoker et al. 2000). Perhaps, Atrazine decreases GnRH secretion, followed by a reduction in LH level. As seen in Fig. 4, level of FSH in

the experimental group taking maximum dose of Atrazine drops considerably. Atrazine usage in the female rats lowers the pulsatile secretion of GnRH indirectly. Therefore, the decrease observed in FSH secretion is expected. Taking into account the present and previous findings, it seems that Atrazine decreases FSH secretion through pituitary gland weight loss and reduction in GnRH secretion. According to Connor et al. (1996) testicular weight is an indication of FSH secretion. Hence, based on testicular weight loss observed in the present study, FSH decrease is not unexpected. FSH drop leads to and interferes with spermatogenesis (Leonhardt et al. 1999).

As seen in Fig. 5, mean level of testosterone in the groups receiving 100, 200, 400 (mg/kg) dosages of herbicide decreased significantly. In a study about the effect of Atrazine on the sex hormones in adult male African frogs (*Xeneopus laews*), it was demonstrated that this herbicide reduces testosterone level in frogs similar to other vertebrates (male fish). This decrease could be the result of an increase in aromatase (Hecker et al. 2005 and Canton et al. 2005). Evidence suggests that Atrazine has an indirect estrogenic role. This compound exerts its estrogenic activities through promoting aromatase. *In vitro* studies indicate that Atrazine induces aromatase activity in the adrenocortical cells. This is supported by an increase in estradiol level and a decrease in testosterone level. In addition, steroidal and esterogen concentrations in the male rats are elevated after Atrazine usage. Also, activities of androgenic receptors are inhibited by this herbicide *in vitro*. Therefore, the observed decrease in testosterone level by Atrazine is probably due to the promotion of aromatase activity and conversion of testosterone to estradiol. Reduction in testosterone secretion is expected since the level of LH is also decreased in the present study. Furthermore, the activities of pituitary-testis axis are decreased by Atrazine through reducing secretion of CNS neurotransmitters such as norepinephrine, prolactine and LH and increasing dopamine. These neurotransmitters play a major role in the regulation of LH receptors on testicular leydig cells (Cooper et al. 2000). Body weight and brain activities are both involved in the maturation and reproductive processes. Metabolic changes associated with body weight loss or growth rate reduction prevent development of reproductive system and may be related to the materials involved in GnRH release. Such materials include insulin, essential fatty acids, and essential amino acids which are crucial for the synthesis of neurotransmitters. Thus, reduction in rats food uptake could lead to LH and testosterone decrease. Studies showed that Atrazine directly binds to SF-l and lowers testosterone secretion through its inhibition. Therefore, it appears that this herbicide decreases testosterone secretion by inhibition of SF-l receptors.

CONCLUSION

By reducing food uptake and increasing estrogen concentration, Atrazine may indirectly cause the conversion of testosterone to estrogen. The estrogen rise in male could bring about testicular weight loss. According to the present findings and other investigations, Atrazine usage could lead to a reduction in the secretion of LH, FSH and testosterone and a decrease in the activities of pituitary-testis axis and sperm production through pituitary weight loss and GnRH secretion from hypothalamus.

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