Original Article

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The Effect of Tricyclazole on Testosterone Changes and Testicular Structure in Mice

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ABSTRACT

BACKGROUND AND OBJECTIVE: Tricyclazole is a systemic fungicide, used for eradicating rice blast disease. This substance has replaced Hinozan pesticides and appears to have adversly effects on reproductive tissues and hormone levels. Due to its widespread use in agriculture, the effects of this toxin on testosterone changes and the testicular structure of mice were evaluated in this study.

METHODS: In this experimental study, 30 adult male NMRI mice, weighing 33±3g, were selected and divided to equal three groups: control, experimental 1, and experimental 2groups. The mice in experimental groups 1 and 2 orally recieved 20 and 40 mg/kg of tricyclazole, respectively for two weeks (5 consecutive days per week), while the control group received no toxins. Twenty-four hours after the last injection, the mice were killed with ether and then samples were taken from all groups. After providing testicular tissue sections, different lines of spermatogenic cells, Leydig cells, and the diameter of seminiferous tubules were measured, using a eye piece. Testosterone level was measured via radioimmunoassay, and finally, the obtained data were analyzed.

FINDINGS: Testosterone level was 1.26±0.44 ng/ml in experimental group 1, 1.12±0.46 ng/ml in experimental group 2, and 0.16±0.059 ng/ml in the control group ($p<0.05$). The relative weight of the testis, the diameter of seminiferous tubules, the number of Leydig cells, and the number of blood vessels in experimental groups 1 and 2 significantly increased, compared to the control group ($p<0.05$). Furthermore, the diameter of the lumen area in group 2 (93.94±1.70 mm) showed a significant increase, compared to the control grouop (82.32±1.71 mm) ($p<0.05$).

CONCLUSION: The results indicated that tricyclazole toxin can impair testosterone secretion and the testicular structure, leaving a adversly effect on sperm production system.

KEY WORDS: Tricyclazole, Testis, Spermatogenesis, Testosterone.

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Introduction

Chemical pesticides are synthetic materials used for food production. These substances can have undeniable adverse effects on the health of producers, consumers, and the environment (1, 2). Fungicides are

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among the most important pollutants, used to eradicate diseases and pests and increase production (3, 4). Tricyclazole, a systemic fungicide from the triazole benzothiazyl family, is used to fight rice blast disease in Asian countries, especially Iran. This substance has a relatively high retention in soil and water, and contact with it can lead to health problems (5, 6). Tricyclazole belongs to a group of pesticides with moderate damage on the environment. Studies have shown that this toxin and its metabolites cause more damage in aquatic organisms, campared to mammals (7,8). Triazoles are quickly absorbed into the body and about 70% of them are decomposed in the liver by converting into active metabolites. They are excreted from the body through kidneys within 24 hours, although the toxin residues may cause damage to body tissues (9,10). Toxic damage on body tissues is dependent on dose, cell structure, metabolites, and contact duration (11).

Most pesticides with different ratios have poisonous and harmful side-effects on a number of organisms, organs, and biological processes. Today, the reproductive tract is known as one of the organs in humans and animals, influenced by environmental chemicals (12, 13). Although the exact mechanism of tricyclazole in the body is unknown, but,some researchers suggest the production of free radicals and lipid peroxidation in cell membranes as the main mechanism of damage to cells and various organs (14). Due to causing defects in cell function and gene expression, these compounds are classified as genetic and celluar toxins (15).

Studies have shown that tricyclazole causes skeletal defects such as craniofacial deformity, hydrocephalus, and changes in cell growth and differentiation by the stimulation and inhibition of gene expression (16). These toxins increase the testosterone level, anogenital distance, and testicular weight and lead to hepatomegaly and fertility reduction (17, 18). Some researchers believe that tricyclazole has no effects on the testicular weight, although it causes liver hypertrophy and inhibits cytochrome gene expression (such as cytochrome P450) in the liver and testis (19). Tricyclazole toxin interferes with the production of some compounds in the body and sometimes causes an increase in triglyceride, cholesterol, glucose, and blood lactate. In addition, elevated levels of alanine aminotransferase and aspartate aminotransferase and the reduction of alkaline phosphatase are amongst the severe side-effects (20). Some believe that long-term use of tricyclazole reduces the testicular weight, the diameter of seminiferous tubules, and finally, the number and motility of sperms (21).

Considering the abovementioned findings, the increasing use of synthetic chemicals in the environment, and the reduced sperm count in men over the last five decades, which is due to the vulnerability of active and sensitive tissues (such as the testis and reproductive system) to external factors, we performed this study to determine the effect of tricyclazole on blood testosterone level and testicular structure in mice.

Methods

In this experimental study, 30 NMRI white mice, with an average weight of 33±3g and 10-12 weeks of age, were purchased from Pasteur Institute of Iran in Amol. The mice were kept in standard cages at 22±2°C. All of them were kept in a 12:12 hr light-dark cycle. The animals were randomly divided into three groups: control group, experimental group 1, and experimental group 2. Tricyclazole 95% was purchased from Golsam Gorgan chemical Company. Then, tricyclazole solution with different concentrations was prepared in distilled water and orally used on a daily basis at a specific time in 2 weeks (5 days a week).

The mice in groups 1 and 2 received 20 and 40 mg/kg of tricyclazole, respectively; the control group did not receive any toxins. All animals were kept in an optimal environment, and after 24 hours of the last gavage, they were anesthetized. The blood samples were obtained from the heart, using an insulin syringe. The collected blood samples were centrifuged at 3000 rpm for 15 min and the serum was separated. Serum samples were stored for further investigation in a

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freezer. In the later stages, testosterone level was determined via radioimmunoassay, using DIAsource kit, made by Ariafarmed Company. After blood collection, the mice were dissected and their testes were removed. Testicular weight was measured by a Japanese digital scale (trademark AND) with an accuracy of 0.001 g. After washing and drying the samples, they were placed in formalin fixative solution 10%. After the routine procedures of tissues sampling, sections with 5 micron width were prepared and stained with hematoxylin and eosin. Afterwards, different cell lines including spermatogonia, spermatocytes, and spermatids, as well as Leydig cells and the diameter of seminiferous tubules were measured, using eyepiece graticule (per unit area with an interval of three). Testicular diameter was measured by a micrometer. For data analysis, one-way ANOVA and Duncan tests were performed using SPSS version 16. $p<0.05$ was considered statistically signifcant.

Results

The relative weight and diameter of the testes: The relative weight of testis in the experimental groups receiving 20 and 40 mg/kg of tricyclazole significantly increased, compared to the control group ($P<0.05$). The diameter of the testis in the experimental groups, compared to the control group, decreased, although it was insignificant (table 1).

The diameter of seminiferous tubules and lumen area: The diameter of seminiferous tubules in groups 1 and 2 significantly increased, compared to the control group ($p<0.05$). The diameter of seminiferous tubules in group 2 showed a more signifcant increase, compared to the experimental group 1. Furthermore, the findings revealed that the diameter of the lumen area in group 2 significantly increased, compared to the control group ($p<0.05$). Although the diameter of the lumen area increased in group 1, compared to the control group, the difference was not statistically significant (table 1).

The number of spermatogonia, spermatocytes, and spermatids: The number of germ cells in groups 1 and 2 insignifcantly decreased, compared to the control group. The spermatocytes and spermatids decreased in experimental groups 1 and 2, compared to the control group, but the difference was not statistically significant (table 2).

Leydig cells and blood vessels: Microscopic examination of testicular tissue slices showed that the number of Leydig cells in experimental groups 1 and 2, compared to the control group, had a significant increase ($p<0.05$).

The samples of group 2 exhibited a more significant increase, compared to group 1. Also, the number of blood vessels showed a more significant increase in groups 1 and 2, compared to the control group ($p<0.05$) (table 2).

Testosterone measurement: By measuring blood testosterone, it was found that the mean value significantly increased in experimental groups 1 and 2, compared to the control group ($p<0.05$), while the level of this hormone was not significantly different between groups 1 and 2 (table 2).

Table 1. Comparison of the mean relative testicular weight, testicular diameter, the diameter of seminiferous tubular, and lumen area in the three groups

Groups / Parameters	Control group	Experimental group 1 (20 mg/kg)	Experimental group 2 (40 mg/kg)
Relative testicular weight (g)	0.258±.0348 [b]	0.2812±0.0694 [a]	0.2832±0.0663 [a]
Testicular diameter (mm)	6.375±0.124 [a]	6.365±0.151 [a]	6.256±0.228 [a]
Seminiferous tubule diameter (mm)	198.96±2.697 [c]	2.3.29±1.78 [b]	216.73±1.32 [a]
Lumen area diameter (mm)	82.32±1.71 [b]	85.06±1.75 [b]	93.94±1.703 [a]

In each row, there are mean values which have at least one letter in common; these mean values are not significantly different at 5% in Duncan test.

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Table 2. Comparison of the mean values of germinal cells, Leydig cells, testosterone level, and vessel number per unit area (micrometer) in the three groups

Groups / Parameters	Control group	Experimental group 1 (20 mg/kg)	Experimental group 2 (40 mg/kg)
The number of spermatogonia	[a]9.45±0.257	[a]8.86±0.319	[a]9.12±0.273
The number of spermatocytes	19.29±0.77[a]	17.63±0.697[a]	16.78±0.804[a]
The number of spermatids	6.55±0.4[a]	6.42±0.293[a]	6.33±70.355[a]
Leydig cells	45.42±2.617[c]	61.29±1.717[b]	63.64±2.675[a]
Testosterone level (Ng/ml)	0.16±0.059[b]	1.26±0.442[a]	1.12±0.469[a]
The number of blood vessels	0.7399±0.034[b]	1.7±0.272[a]	1.6±0.14[a]

In each row, there are some mean values that have at least one letter in common; these values are not significantly different at 5% in Duncan test

Discussion

The results of the present study manifested a significant increase in the number of Leydig cells, blood vessels, the diameter of seminiferous tubules, luminal area, the relative testicular weight, and testosterone level in the experimental groups, compared to the control group. Moreover, the findings of this study showed a reduction in the number of spermatogonia, spermatocytes, and spermatids, as well as testicular diamater, though statistcially insignificant. Also, the diameter of testicular exhibited an insignifcant decrease. The decline in testicular diameter appears to be related to the reduced number of spermatogenic cells due to the effect of toxin in the present study.

These findings are in agreement with other studies, in which researchers found that triazole intake was the reason for testicular atrophy (22). It seems that in addition to testicular tissues, these types of toxins influence and change the weight of other body organs such as the ovaries, liver, and kidneys (13,22,23). Studies have shown that some tricyclazole toxins produce free radicals in body tissues that can react with macromolecules and micromolecules; this may lead to genetic changes caused by molecular changes. These changes end in the breaking of chemical bonds, which can cause biological damage.

Moreover, these toxins cause membrane lipid peroxidation and DNA damage. In this study, free radicals formed during the gavage affected the testicular tissues in the body, inducing cell death in spermatogenic cells (14, 18, 24). The significant increase in the number of Leydig cells after the administration of tricyclazole is among other findings of this study. Some researchers have shown that some triazoles increase mitotic divisions and Leydig cell hyperplasia (21). It seems that Leydig cell growth is also associated with increased mitotic division. The study of blood serum testosterone level showed a significantly increased rate, compared to the control group. Leydig cells are responsible for the secretion of testosterone hormones.

Therefore, after the number of leydig cells increases, the rate of this hormone in blood serum is subsequently expected to increase. These chemicals can affect the production, transfer, and metabolism of androgen (20, 25). Also, it is possible for tricyclazole to inhibit aromatase enzyme (26). This enzyme is responsible for the conversion of testosterone to estradiol. Therefore, it seems that similar to tricyclazole, this toxin prevents the conversion of testosterone to estradiol and ultimately increases the level of this hormone in the blood. If we examine this issue from different aspects, we see that many factors are involved in increasing testosterone level. By increasing the synthesis of steroids, increased number of Leydig cells, and inhibition of aromatase enzyme, testosterone hormone level increases.

According to the results of our study, the increase in testosterone level was directly correlated with the increased number of Leydig cells. Another finding of this study was the increased diameter of seminiferous tubules. Due to a slight decrease in the number of

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spermatogenic cells in this study, the diameter of the tubes was expected to decrease; however, the results showed otherwise. In fact, various toxins have different effects on testicular tissues and the diameter of seminiferous tubules. Some combinations increase the diameter of the tubules (27), while others have the opposite effect (12, 13, 28).

However, by loosening the connective tissues and smooth muscles surrounding the seminiferous tubules, some pesticides change the diameter of tubules (2). Our results also indicated an increased diameter, which can be inffered as a sign of the rupture of tubules (25). Such disorders are associated with the increased dose of toxin and duration of exposure to pesticides. In our study, the increased dose of tricyclazole caused more testicular disorders. The destruction of cell lines can suggest that similar to tricyclazole, the effect of triazole is dose-dependent. If these substances are overused, they can be a source of damage to the reproductive system. In fact, the elevated testosterone level increases the possiblity of these injuries.

The increased number of blood vessels is another finding of the present study. The increase or decrease of blood vessels occurs when some part of the body suffers from certain injuries, since the physiological system of the body transfers blood to the damaged area to repair the tissues. However, damages caused by drugs and toxins are completely different. When various toxins enter the body, they may be absored by the organs and tissues, leading to their destruction. Therefore, the rush of blood vessels may be necessary for repairing the tissues. However, reagarding the fact that tricyclazole did not cause a signifcant decrease in testicular cell lines in this study, it seems that the increased dose caused more damage to germinal cells and sperm production.

The mechanisms by which pesticides cause injury and damage to the reproductive system in animals are very complex. One of the mechanisms may be hormonal disorders. Considering the hormonal balance and its role in reproductive function, it can be deduced that by changing hormonal balance, especially testosterone, these toxins can have devastating effects on the reproductive system. Another mechanism by which these toxins can affect sperm production is their direct effect on testicular tissues, induction of apoptosis, and decreased cell lines, which ultimately lead to unwanted infertility.

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