Analysis of Testicular Toxicity of Solanine in Mice

Yubin Ji[1, 2], Jingchao Sun[1,2], Langlang[1, 2]

(1*The Center for Life Sciences and Environmental Sciences, Harbin University of Commerce. 138 Tongda Street Daoli District, Harbin 150076, Heilongjiang Province, China.* 2 *Engineering Research Center of Natural Anticancer Drugs, Ministry of Education. Harbin 150076, Heilongjiang Province, China.* 9619181@qq.com, nmlpoi@yeah.net)

Abstract: Solanine is one of chemicalcomponents in the tuber and the sprout of the potato which is toxic to human. Some studies on the toxicity of solanine on humans and animals have been reported, little is known about the mechanism of its testicular toxicity. In present study, the toxicity of solanine on male reproductive function was investigated in adult male Kunming mice. Compared with the control group, there was an obvious pathological change in testis, and the expression levels of 3β-HSD and vimentin decreased when the test concentration of solanine was at 21 mg/kg/day. Meanwhile, there was a significant dose- and duration-dependent reduction in the testicular weight and organ coefficient. However, no changes have been detected about the level of testosterone and there was a dramatic increase in the expression of LH in Leydig cells. Results of this study suggested that solanine leaded to male reproductive toxicity influencing the functions of Sertoli cells and Leydig cells.

Key words: solanine; testicular toxicity; Sertoli cell; Leydig cell

1 Introduction

Solanine is found in the tuber and the sprout of the potato (Solanum tuberosum L.)[2], and its negative effects have been reported in central toxicity[3,4], gastrointestinal toxicity[5], and teratogenicity to the mouse embryo[6]. Also, it was shown that solanine could damage testis by inducing sperm malformation in mice[7]. However, currently, very limited information is known about toxic mechanism of solanine on the testis.

The mammalian testis provides suitable and necessary environment for spermatogenesis and maturation of spermatozoa[8]. The testis is constituted by seminiferous tubules which contain Sertoli cells and spermatogenic cells, and the gaps of seminiferous tubules are filled with Leydig cells[9]. Vimentin has been described in Sertoli cells, where it plays important roles in the modifications of Sertoli cell morphology, and involves in the junctional processes and cytoplasmic organization occurring during spermatogenesis. In addition, it is one of the components of the blood-testis barrier. Spermatogenic cells in the seminiferous epithelium are protected from most toxicants by blood-testis barrier, formed by tight junctions between Sertoli cells at their basolateral surfaces[10]. Leydig cell is another kind of functional cell which excretes plenty of androgenic hormones (most of them is testosterone). 3β-HSD in the Leydig cell is an important rate limiting enzymes in the synthesis process of testosterone and controlling testicular androgenesis[11]. Also the testosterone is regulated by hypothalamic-pituitary- gonadal axis[12].

In this study, the testicular toxicity of solanine was analyzed by investigating the functional changes of Sertoli cells, Leydig cells and hypothalamic-pituitary- gonadal axis in mice testis. We found that solanine induced the testicular toxicity by damaging the Sertoli cells and the Leydig cells.

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2 Methods and materials

2.1 Experimental animals

Forty adult male Kunming mice were selected for this experiment. Animals were maintained under standard laboratory conditions for seven days.

Animals were divided equally into four groups. Group A (control group) were injected normal saline equivalent for 14 days. Group B, C, D were respectively treated with 5.25, 10.5 21 mg/kg/day through intraperitoneal injection for 14 days.

2.2 Drugs and reagent

Solanine (99 % purity) was extracted from the nightshade, and was provided by Heilongjiang Institute for Drug Control; Rabbit anti mouse 3β-HSD, Rabbit anti mouse Vimentin, Rabbit anti mouse LH, and SABC kit were bought from Wuhan Boster Biotechnologies (Wuhan, China); Fluorescein-Conjugated AffiniPure Goat Anti-Rabbit lgG was product of Beijing Zhongshan Biotechnology Co., Ltd. (Beijing, China); Collagenase type Ⅰ was from Amersco (USA); ELISA testosterone kit was from Rapid Bio Co (USA) and Triton X-100 was from Watson Biotechnologies, Inc., (Shanghai, China).

2.3 Apparatuses

Invert microscope (CKX-41-32, OLYMPUS, Japan); flow cytometer (EPICS XL-MCL, Beckman-Coulter, US); microplate reader (680, Bio-Rad, US).

2.4 Histology

For histological studies, the testis was fixed overnight in Davidson fluid, dehydrated in ethanol, transparentized in xylene and embedded in paraffin. Tissue sections (5 μm) were mounted on glass slide and dried at 37 ℃ for 3 h. The sections were then deparafinized with xylene, rehydrated with alcohol and water. The rehydrated sections were stained with haematoxylin and eosin, dehydrated in ethanol transparentized in xylene, coated with neutral gum, examined under a light microscope.

2.5 Organ coefficient

Organ Coefficient % = weight of testis (g) / weight of mouse (g) × 100 %

2.6 ELISA

According to the kit instructions (Rapid BioCo US).

2.7 Immunohistochemistry

The 4μm sections were dewaxing to water; put the sections in 3% H_2O_2 at room temperature in dark for 10 min then immersed in boiling citrate buffer for 3 times, each time should cool down 20-30 min. After cooling, the samples were washed 1-2 times by PBS; the washed sections were blocked by goat serum fluid for 10 min, then thrown the surplus water, but washing; put the appropriate dilution of first antibody on the sections at 4 ℃ overnight. The next day biotinylated secondary antibody working solution was put on the sections at room temperature for 30 min ; then dropwised SABC, 37 ℃, 30min; plused DAB and controlled reaction time under the Microscope; then the sections were washed by distilled water; hematoxylin counter stained the sections about 10s then hydrochloric acid alcohol differentiation for 6s; dehydrated, transparent, seal sheet. Observed the sections under the Microscope[13].

2.8 Flow cytometry

The samples prepared were analyzed on an Epics XL-MCL fluorescence activated flow-cytometer. Briefly, the Leydig cells were tightly gated by volume and complexity on a forward, and side light scattering mode and by protein (LH, 3β-HSD) expression. At least 105 cells were analyzed in each case. The percentage of one-color positive cells was measured. The following rabbit anti-mouse antibodies were used for labeling protein (LH, 3β-HSD) in Leydig cells; Fluorescein-Conjugated AffiniPure Goat Anti-Rabbit IgG conjugated the first antibody in the dark at 37℃ for 30 min[14]. Subsequent analysis was performed with Cell Quest software.

2.9 Statistical analysis

All the values are expressed as mean ± SD in each group. Significant differences between the groups were determined with SPSS 15.0 software using one-way analysis of variance (ANOVA). Differences were considered significant when $p < 0.05$.

3 Results

3.1 The results of testicular histological observation

Histological sections of group A showed that: the seminiferous tubules were arranged in dense, structural integrity, Sertoli cells and spermatogenic cells were arranged in order, the sperm can be seen in the seminiferous tubules. **(Fig. 1(1)).** Compared with group A, group B revealed a less mature luminal spermatozoa in the seminiferous tubules. **(Fig. 1(2)).** Morphological alterations of group C indicated the reduction of the size of the seminiferous tubule, the decrease of the number of the seminiferous tubules, and the degeneration and vacuolation in spermatogonia, spermatocytes and spermatids. **(Fig. 1(3)).** The highest dose of solanine (21mg/kg/day) produced histological alterations that included severe degenerative changes. Seminiferous tubules exhibited disintegration of germinal epithelium, detachment and degenerative changes of lining cells, reduction in the number of round spermatids, defoliation of spermatozoa from Sertoli cells, significant failure of their maturation to mature spermatids and therefore almost absence of mature spermatozoa. **(Fig. 1(4)).**

Table 1 The effect of solanine on the weight and organ coefficient of testis (mean ± sd, n=10)

Group	Solanine dose (mg/kg/day)	Weight (g)	Organ coefficient (%)
A	—	0.106±0.018	0.416±0.065
B	5.25	0.100±0.020	0.420±0.046
C	10.5	0.084±0.013	0.346±0.020*
D	21	0.063±0.016*	0.222±0.050**

*Significantly different from control groups, $P < 0.05$. ** Significantly different from control groups, $P < 0.01$.

3.2 The analysis of organ coefficient

At necropsy after termination of treatment, significant decreases in organ weight and organ coefficient were observed in the testes, in the group D (treated with 21mg/kg/day solanine group). This might demonstrate that solanine has an obvious influence on the development of testis. **(Table 1).**

Table 2 The effect of solanine on the serum testosterone (mean ± sd, n= 10)

Group	Solanine dose (mg/kg/day)	serum testosterone (ng/L)
A	—	921.17±36.39
B	5.25	885.19±11.12
C	10.5	859.26±26.34
D	21	792.45±64.15

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3.3 ELISA detection of testosterone

There was no difference of testosterone between the control and the other sampling points, but only a downward trend. The reason for this result was complicated, and we thought that one possible explanation was the function change of hypothalamic- pituitary-gonadal axis. **(Table 2).**

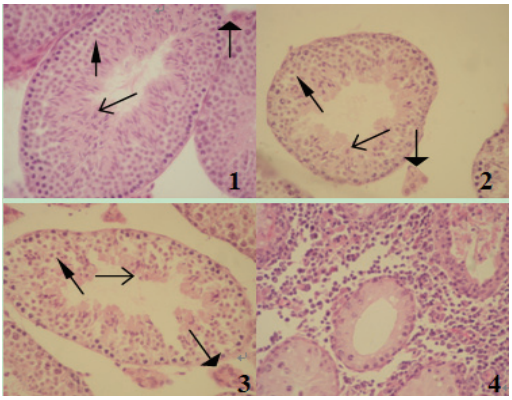

FIG.1 (1) The control group. Showing normal arrangement and distribution of sperms and germ cells**;** (2) Treated with solanine at 5.25 mg/kg for 2 weeks. It revealed a fewer mature luminal spermatozoa in the seminiferous tubules. Sertoli cells and Leydig cells appeared normal. (3) Treated with solanine at 10.5 mg/kg for 2 weeks. It showed such as reduction in number of the seminiferous tubules, degeneration and vacuolation in spermatogonia, spermatocytes and spermatids; (4) Treated with solanine at 21 mg/kg for 2 weeks. Seminiferous tubules exhibited disintegration of germinal epithelium, reduction in the number of round spermatids. (×100, ×400).

↑ Sertoli cells ↑ Spermatogenic cells ↑ Leydig cells

3.4 Immunohistochemistry analysis

Vimentin was visualized by immunohistochemical staining. In Sertoli cells from control mice, vimentin was seen surrounding the nucleus and extending to apical extensions in a ‘‘spoke-like’’ pattern. **(Fig. 2(1)).** After 14 days’ treatment (5.25 mg/kg/day), there was a loss in the staining of the apical vimentin extensions with a concomitant increase in the perinuclear staining intensity. **(Fig. 2(2)).** In group C, a there was a dramatic loss in vimentin staining in the testis, and also, there was a dramatic vacuolization. **(Fig. 2(3)).** In group D, vimentin had collapsed toward the Sertoli cell nucleus, and there was no ‘‘spoke-like’’ pattern in the testis. In addition some Sertoli cells even defluxioed into the lumens. **(Fig. 2(4)).**

3.5 Flow cytometry assay

The flow cytometry was used to investigate the expressions of LH and 3β-HSD in the Leydig cells.

In the FACS, 10000 events were analyzed and this was one of three affinity results. The result showed an obvious increase of expression of LH in Leydig cells. Compare with that of control group. **(Fig. 3).** The percentage

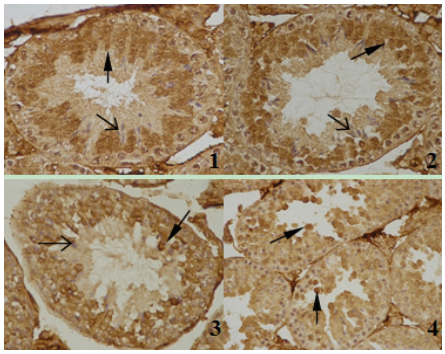

FIG. 2. (1) The control group. Vimentin staining is seen radiating from the Sertoli cell perinuclear region with apical ‘‘spoke-like’’ extensions. (2) Many of the Sertoli cell vimentin apical extensions have lost. A dense vimentin filament staining is observed surrounding the nucleus. (3) Vimentin staining was seen a dramatic loss in the testis, and there happened a significant vacuolization. (4) Vimentin had collapsed toward the Sertoli cell nucleus and none ‘‘spoke-like’’ pattern in the testis, there was a increase in sloughing of the seminiferous epithelium is evident by detached germ cells (×400). ↑ Sertoli cells ↑ Spermatogenic cells

of LH at 21 mg/kg dose was 83.4 %, and the value is 50.1 % in the control group. The result of 3β-HSD assay indicated that the expression of 3β-HSD was significantly inhibited by solanine **(Fig. 4).**As we can see, the percentage of 3β-HSD in Leydig cells which treated with 21 mg/kg solanine was 15.2 % , which was much less expressed than the control group.

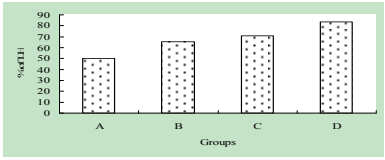

FIG. 3. It has showed a damatic increase of LH. (A) The control group; (B) Treated with 5.25 mg/kg solanine for 14 days; (C) Treated with 10.5 mg/kg solanine for 14 days; (D) Treated with 21 mg/kg solanine for 14 days.

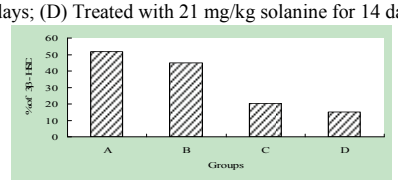

FIG. 4. It has showed a inhibition of 3β-HSD. (A) The control group; (B) Treated with 5.25 mg/kg solanine for 14 days; (C) Treated with 10.5 mg/kg solanine for 14 days; (D) Treated with 21 mg/kg solanine for 14 days.

4 Discussion

Previous study showed that solanine was a testicular toxicant, with the most sensitive spermatic end-points being sperm count and motility, followed by progressive motility, viability, and presence of abnormal sperm with, finally, the fertility index and tailless sperm meiosis of secondary spermatocytes being suppressed[7]. From the results of testicular histological observation in this study, the degeneration and decrease of germ cells, especially spermatocytes and spermatids, were found. This is a rea-

sonable relationship between organ coefficient and the weight of the testis, because the weight and organ coefficient of the testis are largely depended on the mass of the differentiated spermatogenic cells. A reduction in the organ weight should due to the decreased sperm production[15].

The results of immunohistochemistry from our study demonstrated that the Sertoli cell was one of target cells of solanine, as the vimentin in treated groups had obvious histopathologic changes. As we know, vimentin plays an important role in the Sertoli cells, therefore, the disintegration of vimentin may induce the toxicant passing through blood-testis barrier to damage other cells. That is to say Leydig cell which is another kind of functional cell in the testis may be damaged by solanine , and the low expressions level of 3β-HSD in the Leydig cells from present study had confirmed this point. The mechanism of inhibition of 3β-HSD has related two reasons: the Leydig cells are damaged by the toxicant; and/or the toxicant injury the hypothalamic-pituitary- gonadal axis and make a decrease of LH. Several evidences have pointed to the regulation of 3β-HSD by LH[16]. Pioneer studies performed by Makoto [12] conclusively identified the central poison could influence male reproductive function by damaging the hypothalamic-pituitary-gonadal axis, the primary function of which is to produce LH. Solanine is considered to be a kind of central toxicity by inhibiting the activity of cholinesterase[17]. However, high abundance of LH by solanine treatment in our study contradicted the results of Makoto. It might imply that Leydig cell is another target cell of solanine and the impaired Leydig cells tend to cause a concomitant compensatory change of hypothalamic-pituitary- gonadal axis which makes an increase of LH in the testis.

3β-HSD is an important rate-limiting enzyme in synthesis the process of testosterone, so the low level of 3β-HSD may induce a decrease of serum testosterone in general. As testosterone facilitates maturation process of spermatocytes, its depletion may compromise the development of germ cells. Leydig cells are the supplier of testosterone in the testis and sometimes subjected to be the targets of chemical injury as we discussed above, which resulting in the depletion of testosterone secretion and loss of spermatocytes, especially those of pachytene phase. When Leydig cells are damaged and the testosterone level in the testis is decreased, LH secretion from the pituitary will be enhanced to promote the recovery of Leydig cells[18]. The toxic effect of solanine was not obviously shown in the level change of serum testosterone might due to the high level of LH. However, the results of histopathological observation and immunohistochemistry indicated the severe damage of spermatogenic process and the remarkable decrease of spermatogenic cell. There have two possible reasons: solanine breached blood-testis barrier and damaged spermatogenic cells directly; and/or solanine injuried Sertoli cells induced inhibition of ABP (Androgen-binding Protein), as biological function played by testosterone should be combined with ABP[19]. We have not investigated this mechanism in our study, which might have some relationship with the damage of Sertoli cells

5 Conclusion

Based on the results from the present study we concluded that solanine induced the testicular toxicity by damaging the Sertoli cells and the Leydig cells. Solanine disrupted the function of hypothalamic-pituitary-gonadal axis, but didn't impair hypothalamic and pituitary directly. Our findings indicated that in the future studies on the mechanism of the testicular toxicity of solanine we should pay more attention on the functional changes of Sertoli cell and the spermatogenic cell.

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