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Testicular Toxicity of 2-Bromopropane in Male Sprague-Dawley Rats

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2-Bromopropane의 수컷 랫트를 이용한 고환 독성시험

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요 약

택트 스위치 제조공정 침지액의 주성분인 2-bromopropane 독성에 대한 연구로 최단기의 폭로로 농도를 달리하여 3주간 반복 투여 시험을 시행하여, 흰쥐의 혈액 및 세정관의 변화를 관찰하기 위해 투여기간 동안의 체중의 변화, 고환, 간, 신장 등의 장기무게, 혈액화학과 혈액학적 분석 및 고환의 병리조직의 변화 등을 관찰하여 2-bromopropane의 급성투여 조건에서의 중독현상을 비교·분석하였다.

농도를 달리하여 투여에 따른 체중변동은 통계적으로 유의한($P<0.05$) 체중의 감소를 나타내었다. 1,000 mg/kg 투여군에서 백혈구수, 적혈구수, 혈구용적과 혈색소 농도에서 유의한 변화($p<0.05$)를 보였다. 조직병리학적 소견으로 정소에서는 세정관의 정조세포와 정모세포의 괴사를 볼 수 있었고, 기저막의 비후, 세정관의 Sertoli 세포는 광범위하게 세포질성 공포현상을 보여주고 있다. 또한 간질조직에서는 Leydig 세포의 증식을 볼 수 있었다.

2-bromopropane의 손상부위는 조혈과 생식계가 표적으로 생각되며, 고농도 투여가 저농도 투여에 비해 독성이 심하며 독성물질의 양-반응 관계를 보여주고 있다.

Key words : 2-Bromopropane, intraperitoneal injection, testicular toxicity

INTRODUCTION

A sudden happening of reproductive and hematopoietic disorders occurred in workers exposed to solvents containing 2-bromopropane (2-BP) as a substitute solvent for 1,1,2-trichloro-1,1,2-trifluroethane (freon 113) in an electronic factory of South Korea in 1995.[1,2]

2-BP has a severe toxic effect on the female and male reproductive organs and hematopoietic organs on the recent data of some animal experiments.[3-8]

Ichihara *et al.*[9] confirmed the testicular toxicity of 2-BP in animals by inhalation exposure ranging between 0~3,000 ppm. The testes are the main target organs tested for 2-BP toxicity, and also effect the hematopoietic system in another laboratory.[6,7]

In this report, we describe our recent findings on the histopathological changes of the seminiferous tubules and protein pattern by electrophoresis caused by a repeative intraperitoneal injection of 2-BP with rats.

MATERIALS AND METHODS

1. Chemicals

2-BP (purity, over 99%) was purchased from Tokyo Kasei Co. (Japan). Others chemicals used were purchased from Wako Chemicals (Osaka, Japan).

2. Animals

Six-weeks-old, specific pathogen-free Sprague-Dawley rats were purchased from the Daehan experimental animal center (Korea). The animals were acclimatized for 2 weeks before the start of the experiment. The rats were housed in stainless steel cages with regulated within limits of $23 \pm 2°C$ and $55 \pm 7\%$ relative humidity. The rats were maintained in a 12 hr light-dark cycle, food and filtered water were provided *ad libitum*. Set of 3~4 rats were divided into cages. Then, the rats were put into four groups; the lose levels per body weight 0 (control), 250, 500, and 1,000 mg/kg.[6] 2-BP dissolved in vehicle olive oil was injected into the intraperitoneum 6 times per week for 3 weeks but 1,000 mg/kg dose group was from 2 weeks after the start, because all the rats seemed to be seriously ill. Four rats were killed and the left six one were not injected into the 2-BP, similarly to the control group.

3. Biochemistry and hematology

The animals nearly 11 weeks old were made to fast 1 day before the necropsy. The rats were weighed and briefly anesthetized with ether, and blood was collected from the abdominal aorta with 10 cc syringe. The following hematologic parameters were determined: white blood cell, percentage of lymphocytes, monocytes and granulocytes, red blood cell, hemoglobin, hematocrit, mean corpuscular vloume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell volume distribution width (with the Model Cell-DYN 1600. Abbott Co. USA).

The following blood chemistry parameters were determined: Aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, protein, glucose, urea nitrogen, creatinine, and cholesterol in blood (with the model Au-560, olympus Co. Japan).

4. Organ weight and histopatholgy of the testes

The testes, kidney, liver and spleen were dissected, and weighed. Samples from the testes were fixed in 10% neutral buffered formalin, then processed routinely, sectioned at 5 microns, stained with hematoxylin and eosin, and examined by light microscope.

5. Protein pattern analysis of testes

The total testes extracted from each group were homogenized and centrifuged (3,000 rpm, 4°C, 20 min) and the suspense of them were used as the protein determination and the sample of electrophoresis.

Protein determination was determined by the BSA method[10] to make use of protein assay Kit. 1% BSA (bovine serum albumin) was used as standard protein, and its amount of protein was measured by comparing its standard curve acquired by observing its spectrophotometer with the sample's spectrophotometer.

The spectrophotometer was measured at 570 nm by automatic ELISA Reader (Flow Laboratories Co., USA). 10% SDS-PAGE (SDS-polyacrylamide gel electrophoresis) by the method of Laemmli[11] was used to observe the aspect of testes total protein band in each group. Sample cooking was made at 100°C for 4 minutes in the determinated sample mixed with 5 × sample loading buffer. And each well was loaded with 22.39 mg/ml on the basis of 3.34 mg/ml, the result of protein determination.

Electrophoresis was run to 1 cm of gel for about 4 hours on the basis of stacking gel 15 mA and separating gel 25 mA within a tris-glycine buffer (0.025 M tris-base, 0.192 M glycine, 0.1% SD and pH 8.3).

The used protein marker was a product of Bio-Rad Co. Banding scanning was made by Bio-Capt program and Bio ID program.

6. Statistical analysis

A 2-way analysis of variance and Duncan's multiple range test were used to compare the body, organ weight, and blood biochemistry and hematology values of the control with the other 3 groups, the results were interpreted as significant below a level of 0.05.

RESULTS

1. Acute intraperitoneal injection toxicity test time course of body weight

Body weight gain of rats treated with 2-BP was suppressed in a dose-dependent manner (Fig. 1). Mean body weight in the 1,000 mg/kg dose group fell throughout the injection period than other group, but it recovered after the injection ended because of seriously ill.

2. Terminal organ weight

At 250 mg/kg and over, absolute weight of testes decreased in comparison with the control ($P<0.01$). Besides the testes, no other organs showed any significant organ weight loss (Table 1).

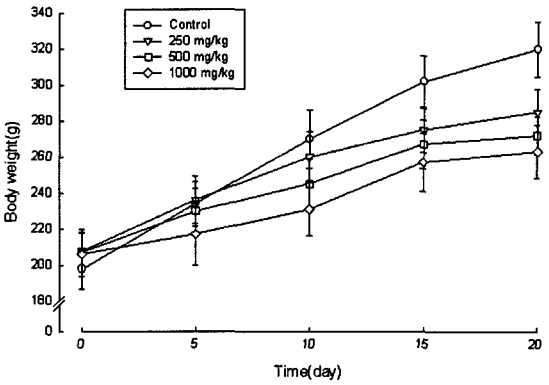

Fig. 1. Body weight changes of male rats treated with 2-bromopropane. Male rats in 9 weeks were treated with 0, 250, 500 mg/kg of 2-bromopropane for 21 days and 1,000 mg/kg of 2-bromopropane for 14 days. 10 rats were assigned to each treatment group but 1,000 mg/kg dose group was 6 rats.

3. Blood chemistry and hematological examination

The hematological examination of the 2-BP-treated animals did show any significant decreases in white blood cell count, percentege of monocytes, red blood cell count, and hemoglobin in the 500 mg/kg and 1,000 mg/kg group (Table 2). The blood chemi-

Table 1. Body weight and organ weights of male rats treated with 2-bromopropane

Group	Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Number of rats	10	10	10	6
Terminal body weight (g)	320±15.4	285±12.7	272±10.3*	263±14.7*
Relative organ weight				
Testis, right (mg/g body)	5.51±0.23	5.12±0.13**	4.85±0.29**	4.86±0.27**
Testis, left (mg/g body)	5.38±0.18	5.19±0.11**	4.85±0.34**	4.87±0.22**
Kidney, right (mg/g body)	3.68±0.25	4.11±0.14	4.15±0.19	3.72±0.16
Kidney, left (mg/g body)	3.63±0.17	4.12±0.27	4.04±0.31	3.87±0.25
Liver (mg/g body)	25.56±0.77	26.52±1.32	28.12±1.78	27.60±0.58
Spleen (mg/g body)	2.22±0.15	2.38±0.09	2.75±0.19	2.74±0.32

Each values represents the mean±S.D. of rats.
The dose levels per body weight were 0 (control), 250, 500, 1,000 mg/kg.
2-BP dissolved in vehicle olive oil was injected into the intraperitoneum 6 times per week for 3 weeks but 1,000 mg/kg dose group was 2 weeks.
* indicates $P<0.05$ versus control
** indicates $P<0.01$ versus control

Table 2. Hematological data for male rats treated with 2-bromopropane

Group	Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Number of rats	10	10	10	6
White blood cell count ($10^3/\mu l$)	7.44±0.54	6.36±1.00	5.36±1.41*	5.78±0.62*
Percentage of lymphocytes	87.04±6.72	84.62±4.93	92.9 ±7.59	91.98±5.49
Percentage of monocytes	7.48±3.29	7.92±4.31	4.62±3.97*	4.24±2.04*
Percentage of granulocytes	5.48±1.51	7.46±2.77	2.48±0.73*	3.78±1.48
Red blood cell count ($10^6/\mu l$)	6.82±0.51	7.40±0.29	6.32±1.21*	6.65±0.18*
Hemoglobin concentration (g/dl)	14.18±0.98	15.08±0.59	13.82±2.36*	13.65±0.44*
Hematocrit concentration (%)	38.36±3.60	41.32±1.82	39.4 ±6.39	42.38±0.75*
Mean corpuscular volume (fl)	56.2 ±1.30	55.8 ±0.84	55.9 ±0.85	55.0 ±0.82
Mean corpuscular hemoglobin (pg)	20.82±0.40	20.38±0.19	20.6 ±0.35	20.33±0.22
Mean corpuscular hemoglobin concentration (%)	37.06±1.29	36.5 ±0.34	36.96±0.46	36.95±0.51
Red cell volume distribution width (%)	15.14±0.64	15.16±0.62	18.78±0.36*	19.2 ±0.96*

Each value represents the mean±S.D. of rats.
The other conditions are same as described in the Table 1.
* indicates $P<0.05$ versus control
** indicates $P<0.01$ versus control

Table 3. Blood chemistry data for the male treated with 2-bromopropane

Group	Control	250 mg/kg	500 mg/kg	1,000 mg/kg
Number of rats	10	10	10	6
Aspartate aminotransferase (μ/l)	123.0 ±18.03	112.2 ±13.57*	108.75±10.95*	111.7 ±17.45*
Alanine aminotransferase (μ/l)	31.4 ±4.22	24.0 ±1.87	27.8 ±7.60	29.5 ±2.52
Alkaline phosphatase (μ/l)	96.6 ±7.86	69.2 ±4.97**	66.75±18.21**	52.5 ±12.33**
Protein (mg/dl)	6.26±0.17	6.22±0.08	5.98±0.36	6.05±0.25
Glucose (mg/dl)	78.4 ±6.27	82.4 ±11.67	80.4 ±7.30	91.5 ±14.66*
Urea nitrogen in blood (mg/dl)	18.4 ±2.96	17.24±1.50	13.9 ±3.71**	12.68±1.25**
Creatinine (mg/dl)	0.48±0.05	0.35±0.04	0.38±0.06	0.31±0.05
Cholesterol (mmol/l)	34.6 ±5.68	37.6 ±18.85*	31.6 ±11.58	37.6 ±8.62*

Each value represents the mean±S.D. of rats.
The other conditions are same as described in the Table 1.
* indicates $P<0.05$ versus control
** indicates $P<0.01$ versus control

cal analysis of the 2-BP-teated animals did not show any significant increase in aspartate aminotransferase, alkaline phosphatase, or urea nitrogen in blood in a dose-dependent manner (Table 3).

4. Histopathological examination

Histopathological findings of the testes treated with 2-BP showed a germ cells necrosis of spermatogonia and spermatocytes was exhibited in the seminiferous tubules (Fig. 2, B, C and D, Fig. 3, C and D), exfoliation of spermatid and spermatocytes, vacuolization of Sertoli cells (Fig. 2, B, C and D, Fig. 3, B, C and D) and hyperplasia of Leydig cells (Fig. 2, C and D). Disruption of basal lamina surrounding seminiferous tubules and Sertoli cells vacuolization was much more marked (Fig. 3, D).

5. Protein pattern analysis of testes

The bands and band peak densitometry resulted from each group are Table 4 and Fig. 4. Main protein forms protein band between 113,000 dalton (β-galactosidase) and 53,900 dalton (ovalbumin), and below 28,800 dalton (soybean trypsin inhibitor).

Particularly, the protein band density between

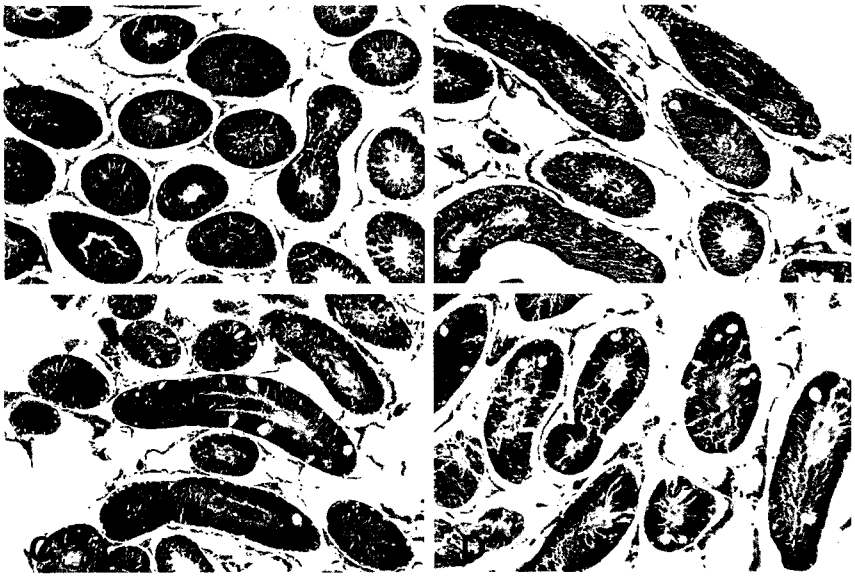

Fig. 2. Histopathology of testes of rats treated with 2-BP. A: Vehicle control. B: 2-BP at 250 mg/kg, shows early vacuolization of Sertoli cells. C: 2-BP at 500 mg/kg, shows tubular atrophy. D: 2-BP at 1,000 mg/kg, Sertoli cells vacuolization was much more marked. Note the atrophic seminiferous tubules, Sertoli cells vacuolization and hyperplasia of Leydig cells in C and D in comparison with A. (Hematoxylin-Eosin staining; original magnification × 40.)

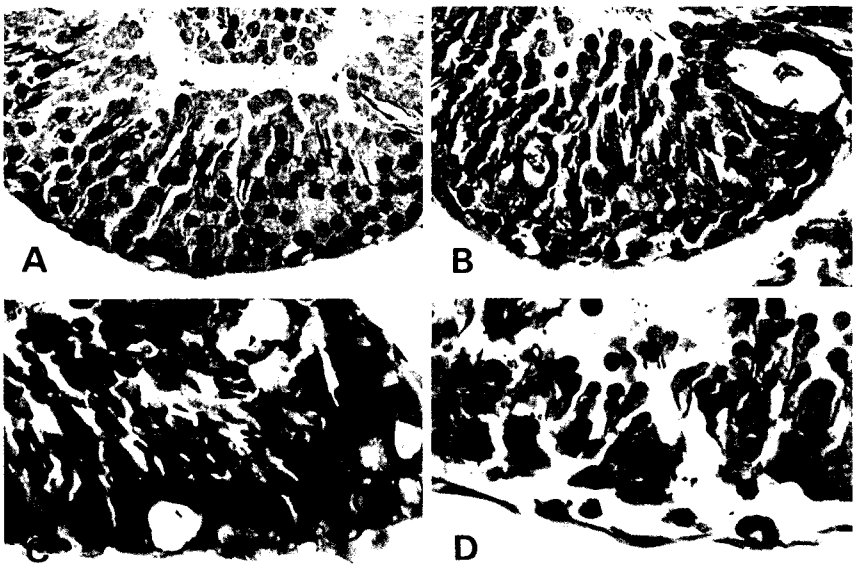

Fig. 3. Histopathology of testes of rats treated with 2-BP. A: Vehicle control, shows normal appearance of the seminiferous tubules and interstitial tissue membrane. B: 2-BP at 250 mg/kg, shows slightly changes of the atrophic seminiferous tubules and the Sertoli cells vacuolization. C: 2-BP at 500 mg/kg, shows moderately atrophied the seminiferous tubules and number of all types of germ cells decreased. D: 2-BP at 1,000 mg/kg, shows disruption of basal lamina surrounding seminiferous tubules, decrease of spermatogenic cells, exfoliation of spermatid and spermatocyte. Sertoli cells vacuolization was much more marked (Hematoxylin-Eosin staining; original magnification × 400).

Table 4. Band density variation of testes protein in 2-BP treated rats by densitometer

Band	Band density per % volume			
	control	250 mg/kg	500 mg/kg	1,000 mg/kg
1	100	93.20	99.34	102.66
2	100	86.74	84.81	87.91
3	100	83.60	83.62	83.85
4	100	80.46	92.24	93.67
5	100	80.01	83.06	77.67
6	100	91.03	102.63	91.80
7	100	93.15	102.19	95.25

* Band density was normalized to percentage of control group.

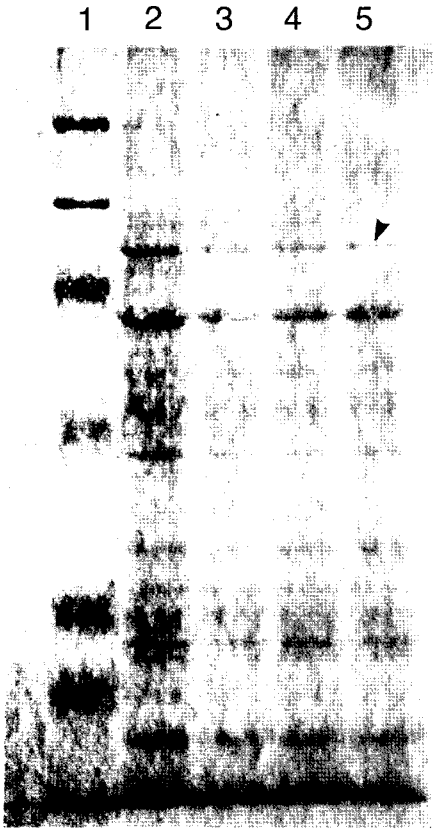

Fig. 4. SDS-PAGE protein banding patterns of testes treated with 2-BP. Lane 1, marker protein (205, 113, 75, 53.9, 34.9, and 28.8 kD, from top to bottom). Male rats in 9 weeks were treated with 0 (control, lane 2), 250 (lane 3), 500 (lane 4) mg/kg 2-BP for 21 days and 1,000 (lane 5) mg/kg of 2-BP for 14 days. Galactosidase was visualized by 10% SDS-PAGE (arrow head)

113,000 dalton (β-galactosidase) and 53,900dalton (ovalbumin) has decreased in 250 mg/kg dose group, but it has gradually increased to the higher density in 1,000 mg/kg dose group than in control group.

DISSCUSSION

According to the epidemiological survey by KISCO,[12, 13] of workers exposed to solvents containing 2-BP in the process of assembling tactile switches, these finding suggested that 2-BP might be the agent causing reproductive and hematopoietic hazards in a Korean electronic factory in 1995. After that several study revealed that 2-BP has a severe toxic effect on the female and male reproductive organs and hematopoietic organs in the animal experiments.[4-9, 13, 14]

Our study indicates that reproductive and hematopoietic toxicity were induced by 21 days of 2-BP treatment. Body weight gain was suppressed dose-dependently by 2-BP injection.[9] Weight loss of the testes should be noted that testicular change was caused by injection to 250 mg/kg or higher concentrations of 2-BP, but liver and kidneys showed little change in their weights suggested that 2-BP did not affect. Yu *et al.*[6] reported that the toxicity of 2-BP was dose-dependent in that the higher doses exhibited more severe toxicity than the lower doses in 28 -day repeated dose experiments. Lim *et al.*[14] indicated that 2-BP treatment induced a delay of the estrous cycle of female Sprague-Dawley rats. The anemia noted in the workers was macrocytic anemia, which exhibited an increase in the red cell distribution width. These symptoms were usually noted in aplastic anemia patients or patients treated with cytotoxic drugs.[15] The 2-BP treatment showed necrosis of germ cells, exfoliation of germ cells, vacuolation of Sertoli cells and hyperplasia of Leydig cells similar to that of dibromochloropropane (DBCP) treatment.[16] A high dose of 2-BP can decrease spermatogenesis by adversely affecting spermtogonia followed by depletion of spermatocytes, spermatids and spermatozoa.[17] The number of spermatogonia

decreased further by the repetition of 2-BP injection and spermatogonia are the target cells of 2-BP in rats.[18] Many chemicals (e.g., DBCP, monoethylhexylphthalate) affecting spermatogenesis act indirectly through their effect on the Sertoli cell rather than directly on the germ cells.[19] Sertoli cell vacuolation may be an early change accompanying progressive degeneration and exfoliation of germ cells, because this finding was much more significant in the rats in 1,000 mg/kg. The Sertoli cell vacuolations were also reported as an early change following the administration of other agents to rats.[9,20-22]

Hyperplasia of Leydig cell is generally observed as a compensatory reaction to disruption of seminiferous epithelium.[9] Wu *et al.*[23] suggest that 2-BP may exert its cytotoxic effects on Leydig cells in vitro. Impairment of Leydig cell function is a late sequela of the toxic effect of DBCP on human testicular function and signifies a state of irreversibility.[24]

Protein pattern by electrophoresis, the protein band density between 113,000 dalton (β-galactosidase) and 53,900 dalton (ovalbumin) has decreased in 250 mg/kg dose group, but it has gradually increased to the higher density in 1,000 mg/kg dose group than in control group. The specfic toxicity of 2-BP on germ cells suggest that meiosis may be the most susceptible stage for chemical insult.[25] Spermatids complete their development into sperm by undergoing a period of transformation (spermiogenesis) that involves extensive nuclear and cytoplasmic reorganization.[19] The change of protein band density is identical to metabolism reaction for the galactose formation to make androgen binding globulin in the Sertoli cell to control spermatogenesis condition.[26] Therefore, it can be identified that the Sertoli cells of seminiferous tubule makes an important role in the formation and movement process of spermatozoa by supplying the chemical substances including nutrients and hormone with them.

In conclusion, from histopathological changes and protein band of electrophoresis of the testes after three-week or two-week intraperitoneal injection of 2-BP, the results of this study estimated that spermatogenesis were effected significantly of 2-BP-induced in the testes in rats.

ABSTRACT

2-Bromopropane, important industrial chemical, specially in electronic industry at Yangsan in Korea has been reported to cause amenorrhea for female and azoospermia, oligozoospermia or reduced sperm motility for male. 2-BP was investigated through 21 days of repeated dose in male Sprague-Dawley rats. The dose levels per body weight were 0 (control), 250, 500 and 1,000 mg/kg. 2-BP dissolved in vehicle olive oil was injected into the intraperitoneum 6 times per week for 3 weeks, but 1,000 mg/kg dose group was 2 weeks because of serious illness.

Male rats showed significant decreases in body weight and right and left testis showed typical weight losses depending on the 2-BP. The number of white blood cell and red blood cell, percentage of monocytes, and hemoglobin decreased significantly in high dose ($P<0.05$). Red cell volume distribution width increased significantly in the high dose ($P<0.05$).

Histopathological findings of testes showed a decrease of spermatogenic cells, exfoliation of spermatid and spermatocyte, vacuolization of Sertoli cells and hyperplasia of Leydig cells. Protein band density between 113,000 dalton (β-galactosidase) and 53,900 dalton (ovalbumin) has decreased in 250 mg/kg dose group, but it has gradually increased to the higher density in 1,000 mg/kg dose group than in control group.

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