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The Effects of Tannic Acid to the Cadmium on Mouse

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Tannic acid가 랫드의 카드뮴독성에 미치는 영향

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ABSTRACT — The tannic acid (0.5 mg/m*l*, 1.0 mg/m*l*, 2.0 mg/m*l*) and/or cadmium (20 mg/kg) were administered by oral administration. The results were as follows : 1. There were adverse effects on the weight changes and water consumption. But, the extent of adverse changes were decreased by tannic acid administration. 2. Also, there were some significant changes in organ weight, especially relative liver weight and relative brain weight by cadmium administration, but Ta1.0 group was significant changes in relative liver weight, relative lung weight and relative thymus weight compared with control group. 3. In the hematological patterns of administered mice, there were significant changes between cadmium treated groups and control group. Hemoglobin contents, packed cell volume, platelet count and neutrophill count were significantly change compared with control group. These changes were not shown in tannic acid treated group. 4. There were serological enzymatic changes in the cadmium treated mouse. In the tannic acid treated group 0.5, 1.0, 2.0 mg/m*l*, ALT, AST, BUN and creatinine were recovered to the extent of control group. From the above results, the tannic acid has some possible alleviative effects of cadmium toxicity upto the 2.0 mg/m*l*/day of oral dose for 4 weeks. But we need further study of mechanism for toxicty alleviating action of tannic acid to the heavy metals like cadmium.

Key words □ tannic acid, cadmium, mouse

Tannins occur naturally in relatively abundant amounts in fruits, herbal medicines and common beverages. Tannic acids (TA) has numerous chemical, food and pharmacological application. TA, a polyphenolic protein-denaturing agent, has been reported to reducer allergen levels in house dust and is marketed for that purpose as 1% and 3% solution in USA. Also, TA was shown to reduce oxygen to superoxide anion and has neither carcinogenicity potential in F344 rats nor modifying effects on spontaneous tumor development.[1-6]

Cadmium ranks close to lead and mercury as a metal of current toxicologic concern. It occurs in nature in association with zinc and lead. It quickly found application as an alloy, in electroplating of other metals, and as a pigment. Later it came to be used extensively in the manufacture of alkali storage batteries and plastics. Most early recorded cases of cadmiun poisoning were due to inhalation of cadmium fumes or dusts. The usual sources of cadmium for the general population are mainly food and inhaled tobacco smoke. Among foods, the usual concentration is less than 0.1 ug/g wet weight. Sources of cadmium in foods and other environmental media generated by man are not clearly defined.[7,8] Occupational or environmental exposure of cadmium induced deleterious effects to the various organs. Exposure to 1~5 mg/m^3 of cadmium for 8 hours may be fatal. Those who recover from high-level cadmium inhalation may have impaired lung function many years afterward, as may workers exposed to lower levels of cadmiun for a long period of time. Chronic inhalation and oral exposure to cadmium have the same target organ : the kidney. The kidneys are susceptible to cadmium toxicity be-

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cause cadmiun accumulates in the kidneys.[9]

Thus an understanding of how these polyphenols effect cadmium toxicity is of importance. Tannic acid have been used to study the potential adverse effects of tannins on rumen metabolism.[8]

Cadmium exposure by the industrial places and environmental pollutions induces toxicity in the various organs, especially respiratory exposure induces damages to the respiratory tracts, and gastro-intestinal exposure by the type of food or drinks induces vomiting and other type of GI damages above 15 mg/*l*.[9,10] ACGIH (American Conference of Governmental Industrial Hygienist) categorized cadmium to the A2 class and have TLV in the work places 0.01 mg/m^3.[11] Though the biochemical toxic mechanism of cadmium have not known clearly, we postulate the toxic mechanism of cadmium the inhibitory action of enzyme involving to the metabolism, the binding action to the sulphydryl (SH) of membranous protein. These actions make inhibit cell membrane transport or substance transport in biota.[12,13]

Preventive or alleviative effects to the cadmium toxicity were known as using the scavenger which was binding procedures on free oxygen radical released by lipidoxygenation to -SH radical, using facilitative procedures on metallothionein (MT) synthesis and using recovering procedures on cytotoxicity. Alleviative effects of cadmium toxicity using directive or indirective metallothionein synthetic materials L-ascorbic acid, Zn, pretreatment of cadmium were reported. Scavenging chemicals and progesterone could reduced the cadmium toxicity.[12,14-16] And, some components of gallic, diallyl disulfide, propylallyl disulfide, glutathione, thiolactic acid and vitaminieB1 have preventive effects of cadmium toxicity.[17]

Tannic acid was generally exposed by ingestion and be a expecting material for reducing the toxicity of heavy metals by screening of absorption in intestinal tracts and by forming insoluble salts be facilitate to excrete in the guts. The present study was conducted to determine the role of tannic acid pretreatment to the cadmium toxicity in mouse.

METHODS AND MATERIALS

Experimental animal

This experiments were accomplished in the laboratory of envirnonmental hygiene located in Yongin University, and experimental animal were supplyed by National Toxicology Institute. The experimental animal (ICR SPF mouse) used to the study after acclimatized to the laboratory environment for 1 week. The environmental condition of the laboraty were temperature 23±3°C, humidity 50±5%, ventilation 10~15 cycle/hr, light/dark cycle 12 hr/cycle, illumination 150~300 Lux. The experimental animals were housed in polycarbonate cage (280W×400L×1700Hmm), and autoclaved (121°C, 15 minute) bedding (CLEA Co.) were used.

Chemicals and treatments

Solutions of $CdCl_2$ (Sigma Co.) were prepared by dissolving the metal salt in distilled water and, the solutions of tannic acid were prepared according to the method of Kim.[7] Mice were administered with 0.5, 1.0, 2.0 mg/m*l* as tannic acid by bottled solution ad libitum. Also mice were administered with 20 mg/kg/day as cadmium by oral gavage for 4 weeks. All solution were made just prior to use. The administered solution by oral gavage was adjusted to 10 m*l*/kg of body weight. Mice were killed by cervical dislocation after adminstered for 4 weeks.

Body weight change and organ weight

Body weights change were recorded by 1 time/week, water cosumptions were recorded by 2 times/week. After administered during 4 weeks, the mice were killed and collected the the maternal blood (cardiac puncture), liver, kidneys and digestive organs, and placed in preweighed plastic dish for wet organ weight. Relative organ weights were calculated to the ratio to the final body weight.

Blood analysis

Mice were fasted for 24 hours before autopsied, and were killed by cervical dislocation and sampled whole blood in EDTA-Na treated bottle for analyzed WBC count, RBC count, HGB contents, PCV, platelet count, lymphocyte count and neutrophile count by using Technicon H1 system. Colleted blood placed in a refrigerator for 2 hours, and centrifuged 1,500 rpm for 15 minute on 4°C centrifuge. Supernatants (serum) were collected for biochemical analysis (Technicon RA-XT) alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, blood urea nitrogen (BUN).

Cadmium contents in organs

Total cadmium contents in whole blood, liver and kidneys was determined by digesting tissues in concentrated HCl (50 mg tissue/m*l* HCl) for 24 hours, centrifuged

for 10 minute at 300×g, followed by direct aspiration of supernatants using flame atomic absorption spectrophotometry (Sunil, ANALB9100A). Cd concentrations were calculated using certified AAS standard (Sigma Chemical Co., St. Louse, MO).

RESULTS

Body weight and water consumption changes

Body weight gain of cadmium treated group were significantly decreased ($p<0.05$) compared with control group on the day 7th, 14th, 21st and 28th of treatment. Body weight of tannic acid combined treated group except tannic acid 1.0 mg/m*l* combined treated group were recoverd to extent of the control group's.

Water consumption was showed in Table 2. Water consumption of all treated groups were significantly decreased ($p<0.05$) compared with control group. Administered tannic acid per mouse was calculated approximately, tannic acid 0.5 mg/m*l* combined group (Ta0.5) was 82 mg/kg/day, tannic acid 1.0 mg/m*l* combined group (Ta1.0) was 164 mg/kg/day, tannic acid 2.0 mg/m*l* combined group (Ta2.0) 328 mg/kg/day.

Organ weight changes

The absolute and relative organ weights of liver, kidney, spleen, heart, testis, brain, lung, thymus and stomach were calculated and presented on Table 3.

Relative weight of liver and brain of cadmium treated group were increased significantly compared with control

Table 1. Body weight change in male mouse orally administered with tannic acid and cadmium for 4 weeks

day / dose	0	7	14	21	28
			(days)		
Control	32.0±2.00	34.4±1.95	35.8±2.05	37.2±1.64	36.6±1.52
Cd	32.8±0.50	30.0±3.37*	30.0±2.00*	30.0±1.41*	30.5±1.29*
Ta0.5	32.8±2.63	34.5±1.73	33.5±2.38	34.3±1.71*	34.0±2.16
Ta1.0	33.0±3.94	32.0±2.65	31.2±5.26	32.4±4.28*	29.8±4.38*
Ta2.0	32.4±1.82	34.0±0.71	34.8±1.79	33.6±1.67*	36.2±0.45

Values (unit:gm) represent means±S.D. for each mouse
*: significantly different from control ($P<0.05$)
Cd: Cadmium ($CdCl_2$) administered 20 mg/kg/day for 4 weeks.
Ta0.5: Tannic acid 0.5 mg/m*l* and cadmium 20 mg/kg/day administeredfor 4 weeks.
Ta1.0: Tannic acid 1.0 mg/m*l* and cadmium 20 mg/kg/day administered for 4 weeks.
Ta2.0: Tannic acid 2.0 mg/m*l* and cadmium 20 mg/kg/day administered for 4 weeks.

Table 2. Water consumption in male mouse orally administered with tannic acid and cadmium for 4 weeks

(units : m*l*/day/mouse)

	Group				
	Control	Cd	Ta0.5	Ta1.0	Ta2.0
0~3 days	8.0	5.6	5.8	4.3	4.4
4~7 days	8.3	6.3	6.7	5.9	6.2
8~10 days	7.8	6.1	5.9	5.4	4.8
11~14 days	8.6	5.4	6.2	5.7	4.2
15~17 days	9.2	6.7	5.2	4.7	5.6
18~21 days	7.6	4.8	5.2	5.2	6.3
22~24 days	7.2	6.3	6.8	4.2	4.3
25~28 days	8.5	5.1	4.6	5.4	6.7
Total	7.8±0.59	5.8±0.66	5.8±0.77	5.1±0.63	5.3±1.01

*: Significantly different from control ($P<0.05$)
Cd: Cadmium ($CdCl_2$) administered 20 mg/kg/day for 4 weeks.
Ta0.5: Tannic acid 0.5 mg/m*l* and cadmium 20 mg/kg/day administered for 4 weeks.
Ta1.0: Tannic acid 1.0 mg/m*l* and cadmium 20 mg/kg/day administered for 4 weeks.
Ta2.0: Tannic acid 2.0 mg/m*l* and cadmium 20 mg/kg/day administered for 4 weeks.

Table 3. Absolute and relative organ weights in mouse orally administered with tannic acid and cadmium for 4 weeks

	Control	Cd	Ta0.5	Ta1.0	Ta2.0
Liver (gm)	2.25±0.22	2.14±0.23	2.16±0.31	2.13±0.34	2.08±0.41
Rel.wt (%b.w)	6.15±0.36	7.02±0.45*	6.35±0.59	7.15±0.75*	5.75±0.86
Kid.(L) (gm)	0.39±0.08	0.34±0.06	0.36±0.07	0.35±0.09	0.34±0.05
Rel.wt (%b.w)	1.07±0.14	1.11±0.12	1.06±0.15	1.17±0.18	0.94±0.13
Kid(R) (gm)	0.38±0.08	0.33±0.09	0.35±0.07	0.35±0.05	0.34±0.0
Rel.wt (%b.w)	1.04±0.14	1.08±0.11	1.03±0.08	1.17±0.15	0.94±0.2
Spl. (gm)	0.19±0.09	0.16±0.09	0.18±0.11	0.16±0.10	0.17±0.8
Rel.wt (%b.w)	0.52±0.17	0.52±0.15	0.53±0.32	0.54±0.18	0.47±0.17
Heart (gm)	0.18±0.05	0.19±0.07	0.19±0.04	0.18±0.06	0.19±0.04
Rel.wt (%b.w)	0.49±0.17	0.62±0.16	0.56±0.10	0.60±0.21	0.5 ±0.19
Tst(R) (gm)	0.16±0.07	0.14±0.10	0.14±0.06	0.14±0.04	0.14±0.05
Rel.wt (%b.w)	0.44±0.06	0.46±0.11	0.41±0.09	0.47±0.08	0.39±0.05
Tst(L) (gm)	0.15±0.04	0.14±0.06	0.13±0.04	0.14±0.05	0.15±0.03
Rel.wt (%b.w)	0.41±0.09	0.46±0.10	0.38±0.07	0.46±0.07	0.41±0.06
Brain (gm)	0.50±0.06	0.51±0.08	0.49±0.07	0.48±0.06	0.49±0.05
Rel.wt (%b.w)	1.37±0.22	1.67±0.21*	1.44±0.17	1.61±0.16	1.35±0.25
Lung (gm)	0.29±0.11	0.31±0.09	0.33±0.13	0.30±0.12	0.28±0.08
Rel.wt (gm)	0.79±0.23	1.02±0.28	0.97±0.17	1.01±0.31*	0.77±0.18
Thymus (gm)	0.11±0.06	0.10±0.05	0.09±0.04	0.10±0.07	0.09±0.05
Rel.wt (%b.w)	0.30±0.17	0.33±0.11	0.26±0.16	0.34±0.09*	0.25±0.11
Stomach (gm)	0.37±0.06	0.33±0.09	0.35±0.11	0.36±0.08	0.37±0.09
Rel.wt (%b.w)	1.01±0.17	1.08±0.24	1.03±0.19	1.21±0.15	1.02±0.22

a): Values are expressed as means±S.D. of each tissue.
*: significantly different from control (P<0.05)
Cd: Cadmium ($CdCl_2$) administered 20 mg/kg/day for 4 weeks.
Ta0.5: Tannic acid 0.5 mg/kg/day and cadmium 20 mg/kg/day administered for 4 weeks.
Ta1.0: Tannic acid 1.0 mg/kg/day and cadmium 20 mg/kg/day administered for 4 weeks.
Ta2.0: Tannic acid 2.0 mg/kg/day and cadmium 20 mg/kg/day administered for 4 weeks.

group (P<0.05). Relative weight of liver, lung and thymus of cadmium and tannic acid 1.0 mg/m*l* treated group were increased significantly compared with control (P<0.05). But, there were no significant change of organ weight on cadmium and tannic acid 0.5 or 2.0 mg/m*l* treated group.

Hematological changes

Table 4 shows hematological values of cadmium and tannic acid treated mouse. Hemoglobin contents, packed cell volume and platelet count of cadmium treated group were decreased significantly compared with control group (P<0.05). And the neutrophil count of cadmium treated group was incresed significantly compared with control group (P<0.05). But, there was no sigcificant change in the tannic acid treated groups.

Blood chemical changes

Table 5 shows blood chemical changes in the group of cadmium and tannic acid treated mouse. ALT and AST activity, BUN and creatinine contents were increased significantlyl in the cadmium treated mouse compared with control group. These changes were attenuated by the tannic acid administration.

Cadmium contents in organs

Table 6 shows cadmium contents in whole blood, liver and kidneys of micer treated with cadmium and tannic acid for 4 weeks. At the day of autopsy, the cadmium contents in whole blood were incresed significantly at cadmium control group compared with control group (P <0.05). In the tannic acid combined treated groups, the cadmium contents of whole blood were declined some extents, but there were no statistically significant compared with cadmium treated group at the level of α=0. 05. The cadmium contents of liver and kidneys were increased at the cadmium treated group compared with control group (P<0.05). Also, organ cadmium contents of tannic acid combined treated group's liver and kidneys were decresed some extents but there were no statistical significants compared with cadmium treated group.

Table 4. Hematological values in mouse orally administered with tannic acid and cadmium for 4 weeks

	Group				
	Control	Cd	Ta0.5	Ta1.0	Ta2.0
WBC $10^3/\mu l$	3.62±0.61	3.39±0.24	3.83±0.30	3.60±0.44	3.54±0.47
RBC $10^6/\mu l$	8.46±0.46	7.91±0.27	8.56±0.64	8.41±0.80	8.65±0.73
HGB g/dl	14.0±0.35	12.5±0.89*	13.8±0.59	13.4±0.74	13.4±0.79
PCV %	41.7±1.65	38.8±2.19*	40.4±2.19	40.8±2.69	40.6±1.69
PLT $10^3/\mu l$	774±64	699±33*	748±53	719±49	701±32
NEU $10^3/\mu l$	0.49±0.114	0.73±0.078*	0.57±0.192	0.59±0.158	0.55±0.211
LYM $10^3/\mu l$	2.88±0.300	2.57±0.801	2.54±0.526	2.57±0.580	2.55±0.570

*: Significantly different from control (P<0.05)
Cd: Cadmium ($CdCl_2$) administered 20 mg/kg/day for 4 weeks.
Ta0.5: Tannic acid 0.5 mg/kg/day and cadmium 20 mg/kg/day administered for 4 weeks.
Ta1.0: Tannic acid 1.0 mg/kg/day and cadmium 20 mg/kg/day administered for 4 weeks.
Ta2.0: Tannic acid 2.0 mg/kg/day and cadmium 20 mg/kg/day administered for 4 weeks.

Table 5. Blood chemistry values in mouse orally administeredd with tannic acids and cadmium for 4 weeks

	Group				
	Control	Cd	Ta0.5	Ta1.0	Ta2.0
ALT U/l	48±11.0	88±13.6	75±8.5	68±8.0	66±20.1
AST U/l	109±22.4	171±22.3	144±37.3	142±44.9	139±32.7
BUN mg/dl	18±2.9	24±5.3	23±5.9	21±5.6	19±4.7
CREAT mg/dl	0.8±0.11	1.0±0.27	0.9±0.16	0.9±0.18	0.9±0.16

*: Significantly different from control (P<0.05)
Cd: Cadmium ($CdCl_2$) administered 20 mg/kg/day for 4 weeks.
Ta0.5: Tannic acid 0.5 mg/kg/day and cadmium 20 mg/kg/day administered for 4 weeks.
Ta1.0: Tannic acid 1.0 mg/kg/day and cadmium 20 mg/kg/day administered for 4 weeks.
Ta2.0: Tannic acid 2.0 mg/kg/day and cadmium 20 mg/kg/day administered for 4 weeks.

Table 6. Cadmium contents in whole blood, liver and kidneys of mice treated with cadmium, tannic acid for 4 weeks

(unit : μg/g w.w.)

	Group				
	Control	Cd	Ta0.5	Ta1.0	Ta2.0
whole	0.011±0.008*	2.148±0.435	2.072±0.385	1.881±0.512	1.956±0.362
blood liver	0.109±0.022*	31.51±4.32	28.44±7.43	23.27±5.44	26.56±5.81
kidneys	0.372±0.052*	34.74±6.26	32.85±7.18	29.72±6.61	31.51±5.90

*: Significantly different from Cd group (P<0.05)
Cd: Cadmium ($CdCl_2$) administered 20 mg/kg/day for 4 weeks.
Ta0.5: Tannic acid 0.5 mg/kg/day and cadmium 20 mg/kg/day administered for 4 weeks.
Ta1.0: Tannic acid 1.0 mg/kg/day and cadmium 20 mg/kg/day administered for 4 weeks.
Ta2.0: Tannic acid 2.0 mg/kg/day and cadmium 20 mg/kg/day administered for 4 weeks.

DISCUSSION

Itai-itai (Japanese for 'hurt') disease in Japan is attribute to chronic cadmium poisoning. This disease is characterized mainly by osseous and renal lesions. Cadmium may be an etiological factor in various pathological processes including testicular tumors and necrosis, renal dysfunction, hypertension, arteriosclerosis, growth inhibition, chronic disease of old age, and damage to the central nervours system. Some of the toxic effects of Cd can be diminished or prevented by zinc, cobalt, selenium, and thio compounds. The mechanisms by which these compounds alleviate Cd toxicity are not understood clearly. The quentity of dietary Cd which is toxic to an-

imals is dependent on several variables, including animal species, method of administration, duration of exposre, and size of dose. While most acute poisoning occurs from inhaled Cd dust or fumes, most chronic cases arise from ingestion of feed contaminated by industrial sources. When high dietary Cd is fed, contents of blood are extremely low. Even intravenously injected Cd rapidly disappears from blood. Consequently, Cd data of blood have little diagnostic value. After absorption, most Cd is transported in plasma bound to gammaglobulin. However, some may be bound with hemoglobin or metallothionein in erythrocyte. Generally, highest cadmiuim concentrations are in kidney, followed by liver. However, initially, with low intake, liver may have more Cd than kidney. The liver, which is much larger than kidneys, contains more total Cd. Of the total body burden of radiocactive Cd, the kidney and liver contained 7.3% in goats and 42% in cows 14 days after oral dosing.[19] Itokawa Y. reported RBC counts $183\sim241\times10^4/mm^3$, hematocrit 19.7~31.7%, hemoglobin contents 10.9~12.8 g/d*l* after Cd treated 50 ppm on water. Also, alkaline phosphatase was 72.2~74.0 KA unit.[20] These results were similar to this study results. In mice, following oral administration of 2.0 to 4.6 g of tannic acid kg^{-1} bodyweight, periacinar coagulative and haemorrhagic necrosis occurred in the liver. In sheep given tannic acid intraperitoneally (0.1 g kg^{-1} bodyweight), liver necrosis occurred and plasma sodium and glucose level significantly ($P<0.05$) decreased while packed cell velume and plasma aspartante aminotransferase, alkaline phosphatase, creatinine and bilirubin significantly ($P<0.01$) rose.[21]

Tannic acid post-treatments enhance the antioxidant and antitumor-promoting effects of TA pretreatments. TAs inhibit the second rather than the first stange of tumor promotion. Plant TAs, therefore, may be valuable against tumor propagation but their efficacy may vary considerably depending on their origin.[22] A protein denaturing agents, TA, has been reported to reduce allergen levels in house dust and is marketed for that purpose as 1% and 3% solutions. Woodfolk et al reported after treatmtnt of dust samples in the laboratory with 3% TA, the apparent reductions in levels were 89%~96%. Similar effects were seen with dist samples from carpets treated with TA. TA to denature Fel d I demonstrated an 80% reduction in allergen, but only in samples with an initial concentration of less than 200 μg Fel d I/gm dust.[23] Ogasawara et al reported that provable maximum tolerable dose of TA in the drinking water would be more than 0.4%.[24] In consideration of the avoidance of drinking water, the maximum tolerable dose of tannic acid was determined to be 0.5%, when given in the drinking water. A combination study of the principle action mechanism to the cadmium induced metallothionein will be necessary, as each of them appears to exert alleviative effect of cadmium toxicity, one will also need to check for direct chemical action or indirect chemical action may be occurring.

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국문요약

Tannic acid(0.5 mg/m*l*, 1.0 mg/m*l*, 2.0 mg/m*l*)와 카드뮴(20 mg/kg)을 마우스에 경구투여한 결과는 다음과 같다. 1. Tannic acid와 카드뮴을 투여한 마우스의 증체량과 음수소비량에 변화가 있었으나, 카드뮴투여에 의한 변화는 tannic acid투여에 의하여 감소되었다. 2. 카드뮴투여에 의하여 간장의 상대중량과 뇌 상대중량이 대조군에 비하여 유의한 변화가 있었으며, tannic acid 1.0 mg/m*l* 투여군에서는 간장의 상대중량, 폐장의 상대중량, 흉선의 상대중량도 유의하게 변화하였다($P<0.05$). 3. Hemoglobin contents, packed cell volume, platelet count, neutrophill count 등의 혈액학적인 변화는 대조군에 비하여 카드뮴투여군에서 유의한 변화가 인정되었다. 그러나, 이러한 유의한 변화가 tannic acid를 동시 투여한 군에서는 나타나지 않았다. 4. 카드뮴을 투여한 군에서는 혈청학적 변화(ALT, AST, BUN와 creatinine)가 있었으나 tannic acid 0.5, 1.0, 2.0 mg/ml을 동시투여한 군에서는 회복되는 경향이 나타났다. 위의 결과로 미루어 카드뮴 투여에 의한 독성이 tannic acid 을 2.0 mg/m*l*/day 이상 4주간 투여하였을 때 경감효과가 나타날 수 있었다. 그러나, 카드뮴과 같은 중금속의 독성에 tannic acid가 어떻게 경감효과를 나타내는지에 관한 작용기전의 연구가 더 필요할 것으로 사료된다.

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