STUDIES ON THYROID FUNCTION IN RATS SUBJECTED TO REPEATED ORAL ADMINISTRATION WITH KOJIC ACID

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ABSTRACT — To elucidate the effects of kojic acid on thyroid function, the compound was given orally to male rats for 4 weeks at 0, 4, 15, 62.5, 250 and 1,000 mg/kg.

In 1,000 mg/kg treatment of kojic acid, the rats showed a slight decrease in motility, inhibition of body weight gain, and a decrease in food consumption. An increase in thyroid weight and a morphological change, *i.e.*, hypertrophy of epithelial cells of the thyroid gland follicles, were observed after 1 week of administration. In addition, the uptake of radioactive iodine from blood into the thyroid gland was enhanced and the TCA-precipitable radioactive iodine in the thyroid gland increased in those rats. However, the rates of the iodination in the thyroid gland did not change during the experiment period. Although serum T4 concentration was low in the rats treated with 1,000 mg/kg kojic acid, it was not observed in any changes in TSH concentration. None of these changes were found in the other groups. These observations suggest that massive administration of kojic acid may decrease blood T4 concentration and that thyroid function may be enhanced compensatorily.

On the other hand, the absorption of kojic acid was rapid as manifested by the T_{max} of blood concentrations of radioactivity, which was as short as 1.0 ± 0.0 hr, and the $t_{1/2}$ was 4.8 ± 0.3 hr. Blood concentrations of radioactivity disappeared nearly completely at 24 hr after administration. This result indicates that the toxic effect observed on the thyroid gland treated with only the largest dosage of kojic acid may depend on a fast decrease following a transient increase of concentration of the compound in the blood.

KEY WORDS: Kojic acid, Thyroid function, Uptake of radioactive iodine, Organified radioactive iodine, Repeated oral administration

INTRODUCTION

Kojic acid [5-hydroxy-2-(hydroxymethyl)-4-pyrone] is known to inhibit the biosynthesis of melanin in the epidermis of the skin by inhibition of tyrosinase, which is involved in the biosynthesis of melanin (Nakayama *et al.*, 1982; Higa, 1983). Therefore, the compound is used as an active ingredient for skin whitening in the field of quasi-drugs (Ohyama and Mishima, 1990) and as a browning-preventing agent in the field of foods, *i.e.*, perishables, *e.g.*, lobster, crab,

vegetable, and meat (Uchino, 1986a and 1986b).

It was reported that a decrease in blood T3 concentration, an increase in blood TSH concentration, and tumoral changes of the thyroid gland were observed in a 19-month mixed feeding study of kojic acid in mice (Fujimoto *et al.*, 1998). Furthermore, kojic acid lowered the uptake of iodine from blood into the thyroid gland, decreased blood T3 and T4 concentrations, and increased the secretion of TSH from the pituitary gland in a 4-week mixed feeding study of kojic acid in rats (Fujimoto *et al.*, 1999). We attempted to elucidate the

effects on thyroid function using rats treated with kojic acid orally.

MATERIALS AND METHODS

Effects of kojic acid on thyroid function

1. Animals and housing conditions

Male F344/Du Crj rats were purchased from Charles River Japan, Inc., (Kanagawa, Japan) at the age of 5 weeks. They were subjected to 6-day acclimatization before use. Housing was maintained at $24 \pm 2^\circ\text{C}$ temperature, $55 \pm 10\%$ humidity, and 12 hr lighting. The animals were allowed to freely take commercial pellet food (MF, Oriental Yeast Co., Ltd., Chiba, Japan) and water.

2. Test compound and administration

The structural formula of kojic acid is shown in Fig. 1. Kojic acid was obtained by fermenting a mutant strain of *Aspergillus sp.* at Sansho Seiyaku Co., Ltd. (Fukuoka, Japan). The compound was suspended in 0.5% carboxymethylcellulose (Wako Pure Chemical Industries Ltd., Osaka, Japan) solution as 0.8, 3, 12.5, 50 and 200 mg/mL solutions for 4, 15, 62.5, 250 and 1,000 mg/kg treatments, respectively. The largest dosage of 1,000 mg/kg was calculated for nearly corresponding to the highest concentration of 2% kojic acid in a mixed feeding study as reported previously (Fujimoto *et al.*, 1999). The dosing volume was 5 mL/kg. Animals were given the solution by gavage oral administration, and a plastic gastric tube was used to conduct once-a-day, 28-day (maximum), consecutive administration of the dosing solution. Control rats were given a 0.5% carboxymethylcellulose solution.

In the 1st, 2nd, 3rd and 4th weeks, necropsy groups were included in each group (24 groups in total). Furthermore, one preadministration necropsy group was included. The number of animals in each

group had to be ten. The animals were grouped on the basis of body weight on the day before the onset of administration according to the stratified serial randomization method.

3. General observation

Clinical signs of animals were checked twice a day by examination. Body weight was measured twice a week and also during necropsy. Food consumption and water consumption were determined twice a week.

The thyroid gland was weighed in all necropsied animals to calculate the weight of thyroid gland per body weight on the basis of body weight on the day of necropsy.

4. Determination of uptake and the iodination in thyroid gland

The uptake of iodine and the iodination were determined before the onset of administration, and at weeks 1, 2, 3, and 4 of administration. The number of animals subject to study at each time point had to be five in each group.

The rats were given ^{125}I -NaI (8×10^6 cpm/0.4 mL/head, 17 Ci/mg, NEN Life Science Products Inc., Tokyo, Japan) intraperitoneally after 24 hr of the last treatment with kojic acid at 1, 2, 3 and 4 weeks.

For measurement of radioactivity, blood of rats treated with kojic acid was collected at 24 hr after the administration of ^{125}I -NaI. Then the animals were subjected to fatal exsanguination to remove the thyroid gland. After weighing, the thyroid gland was homogenized in 0.5 mL cold 0.15 M NaCl-1 mM KI at 4°C , and 0.1 mL of the homogenates for measurement of radioactivity. Furthermore, a part of the homogenate was fractionated to determine the protein contents by the method of Lowry (Lowry *et al.*, 1974) using bovine serum albumin (Sigma, Tokyo, Japan) as the standard substance. To a given volume of the remaining homogenate was added the equivalence of 10% trichloro acetic acid (TCA, Wako Pure Chemical Industries Ltd., Osaka, Japan), and the homogenate was centrifuged ($1,300 \times g$, 10 min). After centrifugation, 5% TCA was added into the pellet to re-suspend the homogenate, which was then centrifuged for determination of the radioactivity in pellets. The radioactivity was measured using a gamma counter (auto gamma counter system, MINAXI 5530, Packard, USA).

5. Determination of serum concentrations of hormones

Blood was collected to prevent any influence by

Fig. 1. Chemical structure of kojic acid (5-Hydroxy-2-(hydroxymethyl)-4-pyrone).

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the compound during the procedure at 24 hr after the final administration by decapitation without anesthesia. The sample stood for about 1 hr at room temperature and was centrifuged (3,000 rpm, 10 min); the serum thus obtained was used in the study. To reduce stress, furthermore, the animals subject to study were handled from 5 days before sampling.

The serum collected was used to determine the following hormones: T3 according to the RIA tube solid phase assay (Diagnostic Products Corporation); T4 according to the RIA tube solid phase assay (Diagnostic Products Corporation); and TSH according to the RIA double antibody assay (Amersham).

6. Histopathological examination

After weighing, the thyroid gland was fixed in a 10% solution of neutral buffer formalin and was embedded in paraffin according to the routine procedure. Paraffinized sections were prepared for the control group, as well as the 250 and 1,000 mg/kg administration groups, and were then treated by hematoxylin and eosin stain before conducting microscopic examination.

Disappearance of radioactive kojic acid in the blood

Radioactive kojic acid was obtained by fermenting a mutant strain of *Aspergillus sp.* using ^{14}C -U-glucose as the raw material. The specific activity of ^{14}C -U-kojic acid obtained by purification was 2.157×10^6 dpm/mg. Three hundreds and fifty mg of ^{14}C -kojic acid was dissolved in 3.5 mL of a mixture solution of propyreneglycol and purified water (2:1) to prepare the $10 \mu\text{Ci}/0.1$ mL. The solution at a concentration of $10 \mu\text{Ci}/100$ g of body weight was orally administered to the male Wistar

rats (Clea Japan, Inc., Osaka) at the age of 5 weeks.

Blood was collected at 10 and 30 min and 1, 3, 6, and 24 hr after administration using a me'langeur to obtain 0.02 mL blood from the tip of the tail. Collected blood was determined by liquid scintillation counter (Model 654, Aloka, Japan).

Statistical analysis

The homogeneity of variance was first tested by Bartlett's test (Bartlett, 1937). In the case of homogeneous variance, Dunnett's multiple comparison test (Dunnett, 1955) was used to make a comparison with the control group. In the case of unhomogeneous variance, Steel's multiple comparison test (Steel, 1959) was used to make the comparison with the control group. The p value was set at 0.05 in all cases.

RESULTS

Effects of kojic acid on thyroid function

1. General observation

The control group, as well as the 4, 15, 62.5 and 250 mg/kg administration groups, showed no abnormalities in clinical signs throughout the administration period. Several animals in the kojic acid 1,000 mg/kg administration group showed a transient and slight decrease in motility from about 30 min to about 1 hr after dosing on days 18 to 28 of administration.

Body weight during the experiment is shown in Fig. 2. The kojic acid 1,000 mg/kg administration group showed significant inhibition of body weight as compared with the control group. However, the body weights in other groups treated with kojic acid were not

Fig. 2. Body weight change in rats treated orally with kojic acid for 4 weeks.

different from that in the control group.

The food consumption in each group was dependent on their body weight gain, and the kojic acid 1,000 mg/kg given to the rats induced significant inhibition of food consumption. (data not shown)

On the absolute and relative weights of thyroid gland, the kojic acid 4, 15, 62.5 and 250 mg/kg administration groups were nearly comparable to those in the control group at all of the 1 to 4 weeks of administration (Table 1). However, the kojic acid 1,000 mg/kg administration group showed absolute and relative weights of thyroid gland which were about 1.2-fold and 1.3-fold greater than those in the control group, respectively. However, the magnitude of increase from week

2 showed little difference as compared with week 1.

2. Uptake of iodine

As shown in Table 2, the rates of radioactive iodine uptake in the control group were 12.1 to 14.2% at weeks 1 to 4 after administration, and followed time courses at nearly constant levels. At all of weeks 1 to 4 of administration, the kojic acid 4, 15, 62.5 and 250 mg/kg administration groups showed rates of radioactive iodine uptake which were nearly comparable to those in the control group. At week 1, the kojic acid 1,000 mg/kg administration group showed a rate of radioactive iodine uptake (21.3%) which was about twofold higher than that in the control group; thereafter,

Table 1. Absolute and relative thyroid weight in rats treated orally with kojic acid.

Groups and dose	Before		1 week		2 weeks		3 weeks		4 weeks	
	Weight (mg)	Weight (mg/100 g B.W.)	Weight (mg)	Weight (mg/100 g B.W.)	Weight (mg)	Weight (mg/100 g B.W.)	Weight (mg)	Weight (mg/100 g B.W.)	Weight (mg)	Weight (mg/100 g B.W.)
Control	6.5 ± 0.4	5.4 ± 0.2	7.4 ± 0.8	4.9 ± 0.5	7.7 ± 0.8	4.3 ± 0.4	7.4 ± 0.9	3.6 ± 0.4	7.4 ± 0.7	3.3 ± 0.4
Kojic acid										
4 mg/kg	—	—	7.5 ± 0.9	4.9 ± 0.5	8.0 ± 1.0	4.4 ± 0.5	7.5 ± 1.1	3.6 ± 0.5	7.9 ± 0.8	3.5 ± 0.3
15 mg/kg	—	—	7.1 ± 0.7	4.7 ± 0.4	8.2 ± 0.7	4.5 ± 0.5	7.5 ± 0.7	3.6 ± 0.5	7.5 ± 0.8	3.3 ± 0.4
62.5 mg/kg	—	—	7.8 ± 0.5	5.1 ± 0.5	7.3 ± 0.9	4.1 ± 0.5	7.5 ± 1.2	3.6 ± 0.5	8.2 ± 0.7	3.6 ± 0.3
250 mg/kg	—	—	7.5 ± 0.8	5.0 ± 0.3	8.4 ± 1.3	4.8 ± 0.8	8.3 ± 1.3	4.0 ± 0.6	8.0 ± 0.8	3.7 ± 0.3
1000 mg/kg	—	—	9.1 ± 0.9**	6.4 ± 0.8**	11.3 ± 2.3 [#]	6.8 ± 1.4 ^{##}	10.4 ± 1.5**	5.6 ± 0.8**	11.2 ± 1.4**	5.7 ± 0.7 ^{##}

Each value represents the mean ± S.D. of ten rats.

** : p < 0.01 (Significantly different from control by Dunnett's multiple test)

[#] : p < 0.05, ^{##} : p < 0.01 (Significantly different from control by Steel's multiple test)

Table 2. Thyroidal uptake of Na¹²⁵I in rats treated orally with kojic acid.

Groups and dose	Uptake ^a (%)					Uptake/tissue ^b (%/mg)				
	Before	1 week	2 weeks	3 weeks	4 weeks	Before	1 week	2 weeks	3 weeks	4 weeks
Control	13.4 ± 1.1	12.1 ± 1.4	12.3 ± 2.2	12.2 ± 2.0	14.2 ± 1.7	2.1 ± 0.1	1.6 ± 0.2	1.6 ± 0.2	1.7 ± 0.3	1.9 ± 0.2
Kojic acid										
4 mg/kg	•	11.8 ± 0.1	12.6 ± 2.5	10.9 ± 2.1	12.7 ± 0.8	•	1.6 ± 0.1	1.7 ± 0.1	1.5 ± 0.0	1.6 ± 0.1
15 mg/kg	•	11.3 ± 1.3	13.7 ± 1.5	12.6 ± 2.0	14.0 ± 2.3	•	1.6 ± 0.2	1.8 ± 0.2	1.6 ± 0.2	1.9 ± 0.4
62.5 mg/kg	•	12.9 ± 2.0	11.2 ± 1.4	13.8 ± 2.1	15.4 ± 3.1	•	1.7 ± 0.2	1.5 ± 0.0	1.9 ± 0.2	1.8 ± 0.3
250 mg/kg	•	13.0 ± 1.1	14.5 ± 1.5	14.5 ± 1.6	16.5 ± 1.7	•	1.7 ± 0.2	1.8 ± 0.2	1.9 ± 0.2	2.1 ± 0.2
1000 mg/kg	•	21.3 ± 2.7 [#]	21.6 ± 4.8**	24.0 ± 3.1**	27.2 ± 3.8**	•	2.4 ± 0.3**	2.2 ± 0.2**	2.5 ± 0.3**	2.2 ± 0.2

Each value represents the mean ± S.D. of five rats.

a: Thyroidal ¹²⁵I uptake represents as % ¹²⁵I dose.

b: Thyroidal ¹²⁵I uptake/mg thyroid.

** : p < 0.01 (Significantly different from control by Dunnett's multiple test)

[#] : p < 0.05 (Significantly different from control by Steel's multiple test)

up to week 4 after administration, the group followed a time course at nearly constant and high levels. Regarding the uptake of iodine to the radioactivity administered, which was converted per gram of thyroid gland, the kojic acid 1,000 mg/kg administration group showed only significant increases in the converted value at weeks 1 to 3 as compared with the control group.

3. The iodination

On the TCA-precipitable radioactive iodine (iodinated compound) in thyroid gland, the kojic acid 4, 15, 62.5 and 250 mg/kg administration groups were nearly comparable to those in the control group as shown in Table 3. At weeks of 1 to 4 after administration, the kojic acid 1,000 mg/kg administration group showed significant increases or tended towards an increase as compared with the control group. The rates of the iodination in thyroid gland were different between the kojic acid administration groups and the control group.

4. T3, T4, and TSH concentrations in the serum

In the serum T3 concentration, the kojic acid 250 mg/kg administration group showed only a significant decrease at week 1 compared with the control rats, while the other groups showed no significant difference compared with the control rats at weeks 2 to 4 (Table 4).

The serum T4 concentration in the 1,000 mg/kg kojic acid-treated rat was significantly lower at week 4. However, no dosage of kojic acid affected the serum TSH concentration significantly.

5. Histopathological observation

Results of histopathological examination of the thyroid glands treated with kojic acid details are shown in Table 5.

The kojic acid 1,000 mg/kg administered group showed hypertrophy on epithelial cells of the thyroid gland at weeks 1 to 4. However, kojic acid 250 mg/kg administration did not produce a hypertrophy compared with the control rats.

Disappearance of radioactive kojic acid in the blood

The time course of blood concentrations of radioactivity after single oral administration of ^{14}C -kojic acid is shown in Fig. 3, and the pharmacokinetic parameters in Table 6.

The absorption of kojic acid was rapid as manifested by the $T_{\max}$ of blood concentrations of radioactivity which was as short as 1.0 ± 0.0 hr, with the $C_{\max}$ of $25.07 \pm 4.56 \mu\text{g eq./mL}$. The $t_{1/2}$ was 4.8 ± 0.3 hr.

Blood concentrations of radioactivity disappeared nearly completely at 24 hr after administration. Furthermore, the $\text{AUC}_{0-24\text{hr}}$ was calculated to be $101.54 \pm 19.35 \mu\text{g eq./mL}$.

DISCUSSION

The effects of kojic acid on thyroid function have been studied to detect any change in the function of the gland. The compound was given to male rats for 4 weeks, with repeated gavage oral administration at levels of 0, 4, 15, 62.5, 250 and 1,000 mg/kg.

The rats treated with kojic acid 1,000 mg/kg showed an increase in weight of thyroid gland, uptake of iodine from blood into the gland and the iodination in the gland. Although these changes were observed from week 1 after administration, the severity of the changes thereafter up to week 4 showed little difference as compared with that in week 1. The rats treated with kojic acid at 1,000 mg/kg showed no changes in the iodination ratio in the thyroid gland throughout the administration period. This evidence suggests that the thyroid function on iodinated compound might be enhanced to maintain a normal level according to an increase in uptake of iodine.

Furthermore, the rats treated with kojic acid at 1,000 mg/kg showed a decrease in serum T4 concentration and a morphological change, *i.e.*, hypertrophy of epithelial cells of the thyroid gland follicles. However, the other administration groups showed no effects on

Fig. 3. Concentration of radioactivity in blood after single oral administration of kojic acid at 100 mg/kg.

Table 3. The iodination in rats treated orally with kojic acid.

Groups and dose	TCA - precipitable ^{125}I (cpm/mg)					TPB ^a (%)				
	Before	1 week	2 weeks	3 weeks	4 weeks	Before	1 week	2 weeks	3 weeks	4 weeks
Control	133486 ± 10478	105447 ± 18815	114549 ± 12845	120001 ± 24695	135513 ± 13007	84.6 ± 4.2	85.2 ± 3.6	89.4 ± 3.1	90.1 ± 3.8	86.6 ± 5.0
Kojic acid										
4 mg/kg	•	112134 ± 5619	111234 ± 13365	104598 ± 5879	114342 ± 11083	–	90.1 ± 3.4	82.8 ± 4.7	88.1 ± 6.2	90.5 ± 3.2
15 mg/kg	•	108664 ± 18919	125636 ± 14811	115403 ± 26475	131277 ± 30665	–	86.0 ± 6.0	89.5 ± 2.1	88.0 ± 8.3	86.8 ± 7.3
62.5 mg/kg	•	124526 ± 9065	107648 ± 2278	132582 ± 18385	135575 ± 30752	–	93.5 ± 17.5	89.7 ± 1.7	88.3 ± 4.7	90.9 ± 6.7
250 mg/kg	•	114781 ± 15382	120942 ± 8605	133120 ± 17369	149497 ± 16777	–	85.1 ± 8.5	82.5 ± 7.0*	86.5 ± 2.5	82.3 ± 3.9
1000 mg/kg	•	174349 ± 21324**	158197 ± 16880**	189583 ± 22469**	161217 ± 18229	–	93.5 ± 16.1	90.7 ± 2.7	93.3 ± 3.2	90.3 ± 4.8

Each value represents the mean ± S.D. of five rats.

a: Thyroid protein binding of ^{125}I (TCA precipitable ^{125}I /Total thyroidal $^{125}\text{I} \times 100$)

*, p < 0.05, **, p < 0.01 (Significantly different from control by Dunnett's multiple test)

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Table 4. Serum levels of T3, T4 or TSH in rats treated orally with kojic acid.

Groups and dose	Serum levels																		
	T3 (ng/dl)						T4 (μg/dl)									TSH (ng/ml)			
	Before	1 week	2 week	3 week	4 week	Before	1 week	2 weeks	3 weeks	4 weeks	Before	1 week	2 weeks	3 weeks	4 weeks				
Control	233.7 ± 23.0	215.2 ± 28.2	213.0 ± 32.0	178.1 ± 12.4	195.7 ± 22.3	6.7 ± 0.8	5.1 ± 0.7	4.9 ± 0.7	4.5 ± 0.5	4.2 ± 0.3	8.6 ± 1.3	12.4 ± 5.0	12.5 ± 2.5	12.4 ± 4.7	9.1 ± 1.4				
Kojic acid 4 mg/kg		207.3 ± 12.0	186.8 ± 20.9	151.2 ± 13.8	210.1 ± 36.7		5.9 ± 0.4	4.8 ± 0.4	4.5 ± 0.5	4.6 ± 0.5		9.1 ± 1.8	19.0 ± 7.0	11.0 ± 2.4	10.1 ± 1.7				
15 mg/kg		211.9 ± 28.8	226.3 ± 39.2	181.1 ± 17.0	200.0 ± 6.5		5.7 ± 1.4	5.6 ± 0.6	4.8 ± 0.3	4.2 ± 0.3		9.9 ± 2.5	18.9 ± 4.2	10.9 ± 4.5	11.9 ± 5.0				
62.5 mg/kg		215.6 ± 11.6	240.7 ± 20.8	174.5 ± 26.1	201.3 ± 36.3		5.1 ± 0.2	5.1 ± 0.9	4.4 ± 0.6	4.5 ± 0.3		14.4 ± 9.9	18.2 ± 2.4	12.2 ± 5.4	8.4 ± 2.2				
250 mg/kg		171.4** ± 11.5	200.3 ± 20.7	172.3 ± 8.2	199.9 ± 17.4		4.9 ± 0.3	4.9 ± 0.9	4.3 ± 0.4	4.2 ± 0.3		11.8 ± 4.2	14.0 ± 7.8	14.5 ± 7.1	9.4 ± 1.4				
1000 mg/kg		190.3 ± 13.9	203.0 ± 31.2	153.4 ± 22.7	170.5 ± 18.3		4.0 ± 0.5	4.3 ± 0.7	3.8 ± 0.5	3.1** ± 0.5		16.3 ± 8.5	16.0 ± 5.8	19.2 ± 2.6	11.4 ± 3.8				

Each value represents the mean $\pm$ S.D. of five rats.** : $p < 0.01$ (Significantly different from control by Dunnett's multiple test)

the gland. It is well known that thyroid function is regulated by TSH, which is secreted from the pituitary gland. The secretion of TSH also depends on blood T4 concentrations maintained at constant levels by the negative feedback system. On the other hand, it is reported that the thyroid gland enlarges when blood T4 concentrations decrease and TSH is secreted excessively (Daichi 1995; Klaassen 1996; Hardman *et al.*, 1996; Imura 1988). In this study, the massive administration of kojic acid showed a decrease in blood T4 concentration. Therefore, thyroid hypertrophy may be a compensatory change secondary to a decrease in blood T4 concentration.

In a mixed feeding study using the rats for 4 weeks, kojic acid reduced rat body weight, decreased uptake of iodine from blood into the thyroid gland, decreased blood T3 and T4 concentrations, and

increased blood concentration of TSH secreted from the pituitary gland (Fujimoto *et al.*, 1999). Furthermore, in the mixed feeding study, 0.03% administration (corresponding to 15 mg/kg orally continual administration with feed) and higher dose level groups showed the above effects on the thyroid gland. As detailed above, these results differ from those of the present study.

The absorption of kojic acid was rapid as manifested by the $T_{\max}$ of blood concentrations of radioactivity which was as short as 1.0 ± 0.0 hr. The $t_{1/2}$ was 4.8 ± 0.3 hr. Blood concentrations of radioactivity disappeared nearly completely at 24 hr after administration. This suggests that kojic acid absorbed from the gastrointestinal system should be rapid in elimination from blood and gland. Although kojic acid concentration in blood in a mixed feeding study is unclear, kojic

Table 5. Histopathological findings in rats treated orally with kojic acid.

Organ and finding	Groups and dose	Pretreatment	1 week	
		Control	Control	Kojic acid 250 mg/kg 1000 mg/kg
Thyroid Hypertrophy, follicular		0/5	0/5	0/5 3/5

Organ and finding	Groups and dose	2 weeks		
		Control	Kojic acid	
			250 mg/kg	1000 mg/kg
Thyroid Hypertrophy, follicular		0/5	0/5	4/5

Organ and finding	Groups and dose	3 weeks		
		Control	Kojic acid	
			250 mg/kg	1000 mg/kg
Thyroid Hypertrophy, follicular		0/5	0/5	2/5

Organ and finding	Groups and dose	4 weeks		
		Control	Kojic acid	
			250 mg/kg	1000 mg/kg
Thyroid Hypertrophy, follicular		0/5	0/5	3/5

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Table 6. Pharmacokinetic parameter in blood after single oral administration of kojic acid at 100 mg/kg.

T _{max}	C _{max}	t _{1/2}	AUC _{0-24hr}
(hr)	(μ g eq./ml)	(hr)	(μ g eq./ml)
1.00 $\pm$ 0.0	25.07 $\pm$ 4.56	4.8 $\pm$ 0.3	101.54 $\pm$ 19.35

Each value represents the mean $\pm$ S.D. of three rats.

acid in mixed feeding may help to keep a certain level of the compound in the blood and may produce a toxic effect on the thyroid gland (Fujimoto *et al.*, 1999).

Therefore, the present data may explain a lesser toxic effect on a thyroid gland orally treated with kojic acid once a day. Further study on the actual or exact mechanism of kojic acid on thyroid function is proceeding.

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