ORIGINAL ARTICLE

Chronic toxicity study of curcuminoids in rats

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Abstract

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A six-month chronic toxicity study of curcuminoids extracted from the powdered dried rhizome of *Curcuma longa* L. was performed in six groups of 15 Wistar rats of each sex. Water control group received 5 ml of water/kg BW/day, while tragacanth control group received 5 ml of 0.5% tragacanth suspension/ kg BW/day orally. Three treatment groups were given the suspension of curcuminoids powder at the doses of 10, 50 and 250 mg/kg BW/day, which were 1, 5 and 25 times of the proposed therapeutic dose. The fourth treatment group, or the recovery group, also received 250 mg/kg BW/day of curcuminoids for six months, but two weeks of no curcuminoids treatment elapsed before the time of sacrifice. It was found that the growth rate of male rats receiving curcuminoids 50 mg/kg BW/day was significantly higher than that of the tragacanth control group. Curcuminoids did not produce any significant dose-related changes of hematological parameters. In the group of male animals receiving 250 mg/kg BW/day of curcuminoids, actual and relative liver weights and the level of alkaline phosphatase (ALP) were significantly higher than those of the two controls, but the ALP level was still within a normal range. There appeared to be a higher incidence of mild

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degree of liver fatty degeneration and adrenocortical fatty degeneration in this group of animals; however, the incidence was not significantly different from that of the two controls. The results indicated that long-term administration of curcuminoids at therapeutic dose (10 mg/kg BW/day) did not produce any toxicity in rats. However, at higher doses, it may affect the function and morphology of the liver in a reversible manner.

Key words : curcuminoids, toxicity

บทคัดย่อ

ปราณี ชวลิตธำรง ทรงพล ชีวะพัฒน์ สดุดี รัตนจรัสโรจน์ สมเกียรติ ปัญญามัง
อัญชลี จูฑะพุทธิ และ ชฎา พิศาลพงศ์

การศึกษาพิษเรื้อรังของเคอร์คิวมินอยด์ในหนูขาว

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ผู้วิจัยได้ศึกษาพิษเรื้อรังนาน 6 เดือนของเคอร์คิวมินอยด์ในหนูขาวพันธุ์วิสตาร์ 6 กลุ่ม ๆ ละ 15 ตัวต่อเพศ หนูกลุ่มควบคุมกลุ่มแรกได้รับน้ำ 5 มล./น้ำหนักตัว 1 กก./วัน (มล./กก./วัน) ทางปาก ส่วนกลุ่มควบคุมกลุ่มที่สองได้รับทรากาค้าน 5 มล./กก./วัน หนูกลุ่มทดลอง 3 กลุ่มแรกได้รับน้ำยาแขวนตะกอนของเคอร์คิวมินอยด์ในทรากาค้าน ในขนาด 10, 50 และ 250 มก./น้ำหนักตัว 1 กก./วัน (มก./กก./วัน) หรือเทียบเท่ากับ 1, 5 และ 25 เท่าของขนาดใช้ในคนต่อวัน ส่วนหนูกลุ่มทดลองกลุ่มที่ 4 เป็นกลุ่มที่ใช้ศึกษาว่าอาการพิษที่เกิดจากเคอร์คิวมินอยด์สามารถหายเป็นปกติได้หรือไม่หากไม่ได้รับสารนั้นแล้ว หนูกลุ่มนี้ได้รับเคอร์คิวมินอยด์ 250 มก./กก./วัน เช่นกัน แต่หลังจากได้รับสารครบ 6 เดือน ได้หยุดให้สารเป็นเวลา 2 สัปดาห์ก่อนทำการผ่าชาก พบว่าอัตราการเจริญเติบโตของหนูเพศผู้ที่ได้รับเคอร์คิวมินอยด์ 50 มก./กก./วัน สูงกว่ากลุ่มควบคุมที่ได้รับทรากาค้านอย่างมีนัยสำคัญ เคอร์คิวมินอยด์ไม่ทำให้เกิดการเปลี่ยนแปลงของค่าทางโลหิตวิทยาใด ๆ ที่มีความสัมพันธ์กับขนาดของสารที่ให้ ในหนูเพศผู้ที่ได้รับเคอร์คิว-มินอยด์ 250 มก./กก./วัน พบว่าน้ำหนักจริงและน้ำหนักสัมพัทธ์ของตับ และระดับของอัลคาไลน์ ฟอสฟาเตส สูงกว่ากลุ่มควบคุมทั้งสองกลุ่ม แต่ระดับของอัลคาไลน์ ฟอสฟาเตสยังอยู่ในช่วงของค่าปกติ แม้ว่าหนูกลุ่มนี้ดูเหมือนจะมีอุบัติการณ์ของไขมันสะสมในตับและชั้นคอร์เท็กซ์ของต่อหมวกไตสูง แต่อุบัติการณ์ดังกล่าวก็ไม่ได้แตกต่างจากกลุ่มควบคุมทั้งสองอย่างมีนัยสำคัญ ผลการศึกษาทั้งหมดชี้ให้เห็นว่าการให้เคอร์คิวมินอยด์ในขนาดที่ใช้ในคน (10 มก./กก./วัน) ติดต่อกันเป็นเวลานาน ไม่ทำให้เกิดความเป็นพิษในหนูขาว อย่างไรก็ตาม เคอร์คิวมินอยด์ในขนาดสูงอาจมีผลต่อการทำงานและโครงสร้างของตับได้ แต่เป็นการเปลี่ยนแปลงที่กลับเป็นปกติใหม่ได้เมื่อหยุดใช้เคอร์คิวมินอยด์

สถาบันวิจัยสมุนไพร กรมวิทยาศาสตร์การแพทย์ กระทรวงสาธารณสุข ถนนติวานนท์ อำเภอเมือง จังหวัดนนทบุรี 11000

Turmeric is the dried rhizome of *Curcuma longa* L. of the family Zingiberaceae. It has long been used as a food-coloring agent and spice all over the world, especially in Asia. Based on the clinical study conducted in Thailand, turmeric is recommended by WHO and Thailand's Essential Drug List as an herbal medicine for the treatment of dyspepsia (Thamlikitkul, *et al.*, 1989, World Health Organization, 1999, National Drug Committee, 2000).

Chemically, turmeric contains curcuminoids, volatile oil, starch and resin. Curcuminoids refer to a group of compounds present in turmeric, which are chemically related to its principal constituent, curcumin (diferuloylmethane). Three main curcuminoids that can be isolated from turmeric are curcumin, desmethoxycurcumin and bisdemethoxycurcumin. These curcuminoids are responsible for the yellow color of the herb (Department of Medical Sciences, 1998).

There are many reports on pharmacological and clinical studies of curcuminoids and the doses of curcuminoids used in those clinical studies varied from 500 mg to 1.2 g per day (Majeed, *et al.*, 1995, Soni and Kuttan, 1992). Recently it has been discovered that curcuminoids are potent antioxidant and possess chemopreventive activity (Selvam, *et al.*, 1995, Grinberg, 1996, Ramsewak, *et al.*, 2000, Huang, *et al.*, 1994, Limtrakul, *et al.*, 1997, Limtrakul, *et al.*, 2001). However, there appears to be only a few published articles on the toxicity of turmeric but none on the toxicity of curcuminiods (Bhavani, *et al.*, 1980, Sittisomwong, *et al.*, 1990, Qureshi, *et al.*, 1992). The present study was therefore conducted to determine toxicity of curcuminoids extract in rats in order to obtain scientific evidence about the safety of this group of compounds upon long-term consumption. The result of this study can be used to promote the safe use of curcuminoids in Thailand.

Materials and Methods

Preparation of curcuminoids

Dried rhizomes of *Curcuma longa* L. were collected from local market and ground into powder. The turmeric powder was extracted with ethanol and then evaporated at low pressure to obtain ethanolic extract in the form of semisolid residue containing oil and curcuminoids. The oil part was then removed to give curcuminoid extract. The curcuminoid contents of the extract used in the experiment were 58-67% and the ratio of curcumin : demethoxycurcumin : bisdemethoxycurcumin was 1 : 0.4-0.5 :0.2-0.3. The curcuminoid extract was suspended to the desired concentrations with 0.5% tragacanth suspension.

Treatment of the animals

Ninety male Wistar rats weighing 290-320 g and 90 female rats weighing 200-230 g from the National Laboratory Animal Center, Mahidol University, Nakhon Pathom province, were used. The animals were housed in the animal facility of the Department of Medical Sciences. The temperature in the animal room was kept at 25 $\pm$ 1 $^{\circ}$C with 60% relative humidity. The animals were allowed to have free access to food and clean water.

Six-months toxicity study

Ninety Wistar rats of each sex were randomly divided into 6 groups of 15 animals per sex. Group 1 (water control) received water 5 ml/kg BW/day orally and Group 2 (tragacanth control) received 0.5% tragacanth suspension 5 ml/kg BW/day. Groups 3-6 were given the curcuminoids suspended in 0.5% tragacanth suspension at the doses of 10, 50, 250 or 250 mg/kg BW/day, respectively. Body weight and food intake was measured weekly and the animals were observed for signs of abnormalities throughout the study. At the end of 6-month treatment period, the 1st-5th groups of rats were fasted for 18 hours, then anesthetized with ether and sacrificed by drawing blood samples from the inferior vena cava for hematological and biochemical examinations. The 6th group of rats, the recovery group, was allowed to have free access to food and water without curcuminoids administration for another 14 days before being sacrificed.

Hematological analysis was performed using an automatic hematological analyzer (Cell dyne 3500, Abbott). Hematological parameters measured were white blood cell (WBC), %neutrophil, %lymphocyte, %monocyte, %basophil, %eosinophil, red blood cell (RBC), hemoglobin, hematocrit (Hct), platelet, plateletcrit (PCT), %reticulocyte, and reticulocyte.

Biochemical analysis of serum samples was performed using an automatic chemistry analyzer (Hitachi model 912). Biochemical parameters measured were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), p-amylase, bilirubin, creatinine, blood urea nitrogen (BUN), cholesterol, triglyceride, total protein, albumin, uric acid, glucose, sodium, potassium, and chloride.

The positions, shapes, sizes and colors of internal organs, namely, brain, heart, both kidneys and lungs, trachea, esophagus, stomach, liver,

pancreas, intestine, spleen, bladder, and testis in male rats or ovary and uterus in female rats were visually observed for any signs of gross lesions. These organs were then collected, weighed to determine actual and relative organ weights, and preserved in 10% buffered formalin solution. Tissue slides were later prepared and stained with hematoxylin and eosin, and histopathological examinations were performed by a veterinary pathologist.

Statistical analysis

Data were statistically analyzed using SPSS/PC program and statistically significant difference was set at $p< 0.05$. Food consumption, body weight, hematology, serum biochemistry and organ weight (absolute and relative) data analyzed by one-way ANOVA followed by Bonferoni's test or Tamhane 's test. Histopathological results were evaluated by Fisher exact test at $p<0.05$.

Results

Effects of the curcuminoids on body weight and food intake

The body weights of male rats receiving 50 mg/kg/day of curcuminoids were significantly higher than those of the tragacanth control group from the first week until the end of the study (Figure 1). The body weights of male rats receiving curcuminoids 10 mg/kg/day were significantly higher than those of the tragacanth control during the 3rd-5th weeks.

In female rats, the body weights of the group receiving curcuminoids 50 mg/kg/day were significantly higher than those of the water control during the 5th until the 8th weeks. The body weights of the group receiving curcuminoids 250 mg/kg/day were significantly higher than those of the water control between the 1st and16th weeks. The body weights of tragacanth group were significantly higher than those of the water control group during the 1st-8th weeks (Figure 1).

The food intakes of both male and female rats receiving curcuminoids were significantly higher than those of the tragacanth controls on some weeks during the study (Figure 2).

Effect of curcuminoids on actual organ weight and relative organ weight

Male rats treated with curcuminoids at the dose of 250 mg/kg/day had a higher actual weight and relative weight of the liver than the water and tragacanth control groups, and had a higher actual weight of the left kidneys than the tragacanth

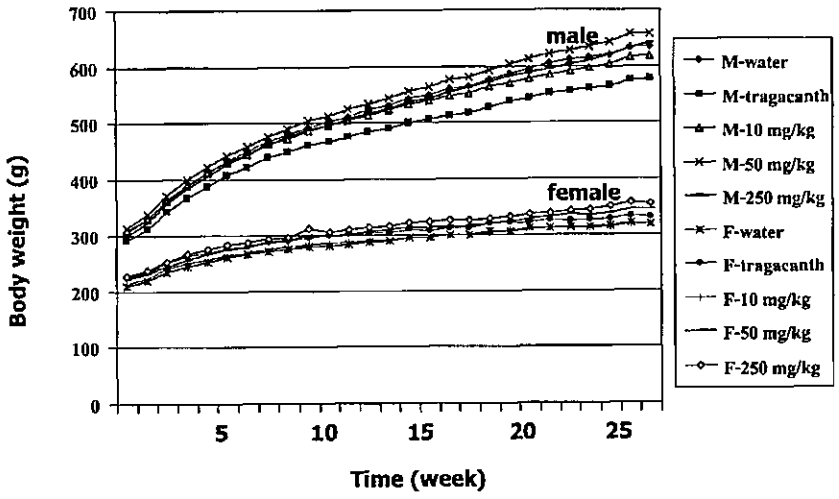

Figure 1. Growth curves of male and female rats receiving curcuminoids for 6 months.

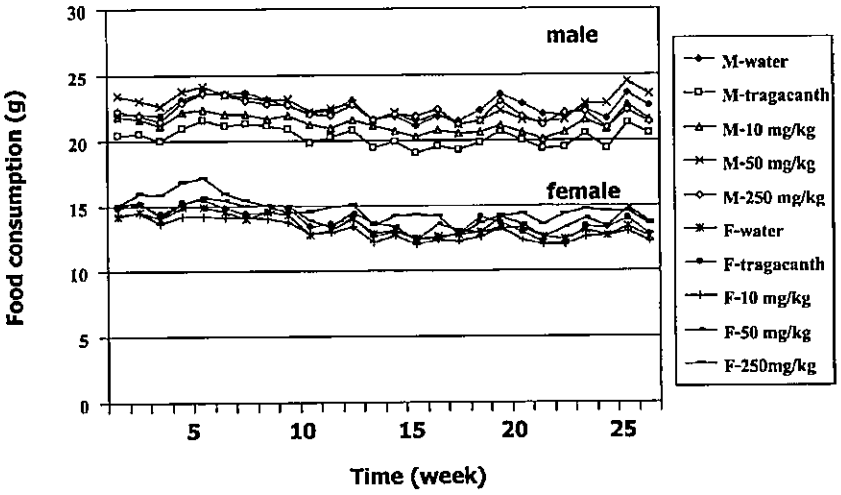

Figure 2. Food consumption of male and female rats receiving curcuminoids for 6 months.

control group. Male rats treated with curcuminoids at the dose of 50 mg/kg/day had a higher weight of the liver but a lower relative weight of the brain than the tragacanth control group. (Table 1 and Table 3).

Female rats treated with curcuminoids at the dose of 250 mg/kg had a higher actual weight of the liver than the water and tragacanth control groups. Female rats treated with curcuminoids at the dose of 50 mg/kg had a higher actual weight of the brain than the water control group. (Table 2).

Effect of curcuminoids on hematological parameters

Tables 5 and 6 showed that there was no difference of the number of white blood cells, %neutrophil, %lymphocyte, % monocyte, %basophil, %eosinophil, the number of red blood cells, hematocrit, platelet, PCT, or the number of reticulocytes between curcuminoids-treated groups and those of the water and tragacanth control groups of both male and female rats. The group of male rats receiving curcuminoids at the dose of 250 mg/kg/day had significantly lower hemoglobin level than the water control group. In the recovery group of male rats, the number of reticulocytes was significantly lower than that of the water control group, while %hematocrit was significantly lower than those of the two control groups.

Effect of the curcuminoids on blood chemistry

In male and female rats, no difference in the serum levels of AST, ALT, P-amylase, bilirubin, creatinine, BUN, triglyceride, total protein, uric acid, glucose, sodium, potassium or chloride was found between all curcuminoids-treated groups and the water and tragacanth control groups. The group of male rats receiving curcuminoids at the dose of 250 mg/kg/day had significantly higher ALP than the tragacanth control group and had significantly higher albumin than the water and tragacanth control groups. The group of female rats receiving curcuminoids at the dose of 250 mg/kg/day had significantly higher cholesterol than the tragacanth control (Table 7-8).

Effect of curcuminoids on histopathology of internal organs

Upon gross examinations of internal organs, no abnormal signs were observed. Histopathological results indicated that some lesions were found in some groups or all groups of animals in the lung, heart, liver, kidney, spleen, intestine,

Table 1. Actual organ weight and body weight of male rats given curcuminoids orally for 6 months.

Male	Group of animals					
	water	tragacanth	10 mg/kg/day	50 mg/kg/day	250 mg/kg/day	250-R mg/kg/day
	N= 15	N=15	N=15	N=14	N=15	N=15
Initial body weight	306±18	292±17	309±12	315±5**	300±15	301±24
Final body weight	635±63	576±48	618±53	657±44**	640±76	670±68**
Weight gain	329±51	285±35	309±51	342±42	340±67	368±59**
Brain	2.08±0.075	2.10±0.063	2.11±0.067	2.11±0.070	2.14±0.120	2.14±0.043
Heart	1.50±0.16	1.47±0.14	1.45±0.13	1.54±0.094	1.54±0.24	1.58±0.17
Right kidney	1.37±0.11	1.31±0.10	1.31±0.11	1.40±0.12	1.60±0.60	1.49±0.17
Left kidney	1.33±0.11	1.24±0.13	1.24±0.15	1.34±0.11	1.39±0.12**	1.42±0.18**
Urinary bladder	0.143±0.032	0.143±0.021	0.153±0.022	0.169±0.030	0.145±0.034	0.148±0.031
Liver	14.66±1.97	13.10±1.30	14.17±1.20	15.39±1.55**	17.05±2.03*,**	15.77±2.08**
Spleen	1.10±0.13	1.04±0.15	1.14±0.15	1.12±0.14	1.15±0.17	1.11±0.16
Stomach	2.19±0.20	2.04±0.22	2.18±0.20	2.23±0.17	2.23±0.21	2.29±0.25**
Lung	1.87±0.28	1.70±0.18	1.87±0.22	1.72±0.14	1.83±0.13	1.92±0.18**
R adrenal	0.035±0.066	0.033±0.054	0.030±0.051	0.036±0.055	0.034±0.068	0.034±0.055
L adrenal	0.040±0.071	0.037±0.047	0.035±0.055	0.039±0.061	0.039±0.067	0.038±0.054
Right testis	3.16±0.55	3.10±0.37	3.24±0.26	3.23±0.25	3.23±0.44	3.21±0.23
Left testis	3.09±0.38	3.15±0.42	3.24±0.31	3.54±1.08	3.22±0.41	3.20±0.25

Each value represents mean±SD.
*** Significantly different from water control group ($p < 0.05$).**
**** Significantly different from tragacanth control group ($p < 0.05$).**

thyroid gland, and testis (in male rats), or uterus and mammary gland (in female rats) (Table 9-10). Meanwhile, no lesion was found in the brain, pancreas, esophagus, and salivary gland in all groups of animals. The lesions found in all or some groups of both male and female animals were lymphoid proliferated peribronchioles, fatty degeneration of the liver, tubular cyst of the kidney, lymphoid hyperplasia of the spleen, and lymphoid aggregation in the submucosal layer of the intestine. However, the incidence of those changes in the controls and curcuminoids-treated groups was not significantly different (Table 9-10).

Other histopathological findings in some groups of male rats treated with curcuminoids were focal myocardiosis, testicular atrophy, follicular hyperplasia of the thyroid gland. The incidence of those abnormalities was, however, neither dose-related nor significantly different from that of the controls (Table 9). Adrenocortical fatty degeneration were found in all groups of male rats with the highest incidence (10/15) in the

Table 2. Actual organ weight and body weight of female rats given curcuminoids orally for 6 months.

Female	Group of animals					
	water	tragacanth	10 mg/kg/day	50 mg/kg/day	250 mg/kg/day	250-R mg/kg/day
	N= 15	N=15	N=15	N=15	N=15	N=15
Initial body weight	209±9	225±8*	214±14	221±11	227±17*	221±14
Final body weight	318±29	330±24	318±31	345±31	356±37	364±55*
Weight gain	110±25	105±19	104±24	124±27	130±31	143±47*,**
Brain	1.91±0.066	1.94±0.099	1.94±0.088	1.99±0.066*	1.96±0.055	1.93±0.071
Heart	0.91±0.090	0.95±0.099	0.91±0.049	0.97±0.059	1.00±0.095	0.98±0.117
Right kidney	0.82±0.082	0.81±0.043	0.80±0.106	0.87±0.217	0.89±0.112	0.81±0.241
Left kidney	0.78±0.072	0.78±0.058	0.76±0.092	0.76±0.119	0.85±0.087	0.80±0.092
Urinary bladder	0.078±0.011	0.086±0.012	0.082±0.095	0.086±0.011	0.085±0.012	0.080±0.096
Liver	6.94±0.74	6.98±0.55	6.78±0.73	7.44±0.65	8.47±1.55*,**	7.80±1.15
Spleen	0.65±0.052	0.74±0.14	0.68±0.12	0.69±0.10	0.74±0.10	0.77±0.19
Stomach	1.57±0.17	1.59±0.18	1.57±0.23	1.59±0.20	1.59±0.17	1.63±0.20
Lung	1.33±0.11	1.33±0.13	1.28±0.11	1.32±0.11	1.34±0.11	1.33±0.098
R adrenal	0.039±0.064	0.038±0.081	0.039±0.049	0.038±0.055	0.041±0.079	0.040±0.068
L adrenal	0.043±0.058	0.040±0.080	0.039±0.055	0.043±0.044	0.042±0.089	0.041±0.065
R ovary	0.065±0.014	0.062±0.017	0.062±0.013	0.060±0.014	0.062±0.016	0.084±0.116
L ovary	0.063±0.017	0.064±0.097	0.068±0.019	0.067±0.014	0.068±0.019	0.058±0.011
uterus	0.63±0.26	0.80±0.30	0.67±0.14	0.71±0.23	0.81±0.17	0.72±0.23

Each value represents mean±SD.
*** Significantly different from water control group ($p < 0.05$).**
**** Significantly different from tragacanth control group ($p < 0.05$).**

group treated with curcuminoids 250 mg/kg, yet the incidence was not significantly different from controls (Table 9). An isolated case of renal cell carcinoma was detected in one male rat receiving curcuminoids 250 mg/kg

In female rats, tubular cast was found in the kidneys of all groups of animals and glandular hyperplasia of the mammary glands was found in some groups of animals, but the incidence was not dose-related or significantly different between groups (Table 10). Glandular hyperplasia of the uterus and cervix was found in only one animal treated with 50 mg/kg curcuminoids, while congestion of the adrenal gland was found in only one animal in the water control group.

Discussion

Even though it was found that the body weights of some groups of curcuminoids-treated

Table 3. Organ weight relative to body weight @ (g/kg BW) of male rats given curcuminoids orally for 6 months.

Male	Group of animals					
	water	tragacanth	10 mg/kg/day	50 mg/kg/day	250 mg/kg/day	250-R mg/kg/day
	N= 15	N=15	N=15	N=14	N=15	N=15
Final body weight	635±63	576±48	618±53	657±44**	640±76	670±68**
Brain	3.36±0.27	3.72±0.30*	3.51±0.32	3.28±0.21**	3.49±0.52	3.24±0.33**
Heart	2.42±0.24	2.60±0.24	2.40±0.19	2.39±0.17	2.49±0.29	2.38±0.18
Right kidney	2.20±0.18	2.33±0.23	2.16±0.15	2.18±0.24	2.56±0.81	2.25±0.18
Left kidney	2.14±0.16	2.20±0.24	2.05±0.19	2.08±0.22	2.26±0.21	2.13±0.18
Urinary bladder	0.23±0.054	0.26±0.032	0.25±0.048	0.26±0.050	0.24±0.049	0.22±0.048
Liver	23.45±1.67	23.14±1.79	23.38±1.30	23.82±1.86	27.54±3.14*,**	23.66±1.76
Spleen	1.76±0.17	1.83±0.24	1.88±0.20	1.74±0.23	1.86±0.26	1.68±0.21
Stomach	3.53±0.32	3.60±0.26	3.60±0.33	3.46±0.33	3.61±0.44	3.46±0.34
Lung	3.00±0.31	3.01±0.25	3.09±0.41	2.67±0.23	2.97±0.39	2.90±0.32
Right adrenal	0.059±0.092	0.059±0.098	0.054±0.085	0.056±0.098	0.058±0.016	0.052±0.010
Left adrenal	0.064±0.091	0.067±0.013	0.059±0.010	0.062±0.011	0.066±0.020	0.056±0.071
Right testis	5.08±0.87	5.48±0.56	5.38±0.66	5.04±0.59	5.26±0.97	4.86±0.52
Left testis	5.00±0.74	5.56±0.67	5.37±0.68	5.53±1.90	5.23±0.88	4.84±0.48

@ Organ weight relative to body weight is expressed as (g organ weight/g body weight) × 1000
Each value represents mean ± SD.
* Significantly different from water control group ($p < 0.05$).
** Significantly different from tragacanth control group ($p < 0.05$).

rats were significantly higher than those of the controls on some weeks during the experimental period, this may in part be due to the initial body weights which were significantly higher than those of the controls from the beginning of the study. There was no difference of the hematological parameters between curcuminoids-treated groups and those of the control groups, except for male rats treated with high dose of curcuminoids that had significantly lower hemoglobin level than the water control group, but the higher hemoglobin level was still within the normal range (Gad, 1992)

Biochemical examinations of the serum showed that male rats receiving high dose of curcuminoids had a significantly higher ALP level than the tragacanth control, and a significantly higher albumin level than the two control groups (Table 7). However, both the ALP and albumin levels of this group of animals were still within the normal range (Gad 1992). In addition, these changes appeared to be reversible since the levels of both ALP and albumin in the recovery group were not different from those of the two controls. Female rats receiving high dose of cur-

Table 4. Organ weight relative to body weight [@] (g/kg BW) of female rats given curcuminoids orally for 6 months.

Female	Group of animals					
	water	tragacanth	10 mg/kg/day	50 mg/kg/day	250 mg/kg/day	250-R mg/kg/day
	N= 15	N=15	N=15	N=15	N=15	N=15
Final body weight	318±29	330±24	318±31	345±31	356±37	364±55*
Brain	6.14±0.53	6.03±0.51	6.25±0.45	5.94±0.55	5.65±0.53	5.48±0.79*
Heart	2.94±0.28	2.96±0.32	2.92±0.16	2.87±0.20	2.88±0.23	2.75±0.34
Right kidney	2.62±0.31	2.53±0.25	2.59±0.28	2.60±0.74	2.55±0.34	2.27±0.69
Left kidney	2.50±0.29	2.44±0.27	2.45±0.22	2.26±0.35	2.45±0.26	2.25±0.30
Urinary bladder	0.25±0.032	0.27±0.040	0.27±0.028	0.26±0.034	0.24±0.047	0.23±0.041**
Liver	22.35±3.10	21.61±1.30	21.85±2.25	22.09±2.16	24.23±3.54	21.73±2.04
Spleen	2.11±0.29	2.30±0.44	2.18±0.31	2.05±0.35	2.13±0.27	2.14±0.44
Stomach	5.07±0.80	4.94±0.59	5.06±0.85	4.73±0.67	4.57±0.60	4.60±0.77
Lung	4.27±0.49	4.12±0.52	4.12±0.33	3.94±0.50	3.87±0.45	3.76±0.52
Right adrenal	0.13±0.019	0.12±0.029	0.13±0.020	0.12±0.017	0.12±0.020	0.11±0.016
Left adrenal	0.14±0.028	0.12±0.026	0.13±0.020	0.13±0.016	0.12±0.023	0.12±0.024
Right ovary	0.21±0.048	0.19±0.057	0.20±0.039	0.18±0.043	0.18±0.052	0.25±0.37
Left ovary	0.20±0.052	0.20±0.032	0.22±0.054	0.20±0.033	0.20±0.051	0.17±0.041
Uterus	2.01±0.81	2.50±0.92	2.19±0.55	2.13±0.70	2.35±0.58	2.04±0.73

[@] Organ weight relative to body weight is expressed as (g organ weight/g body weight) × 1000
Each value represents mean ± SD.
*** Significantly different from water control group ($p < 0.05$).**
**** Significantly different from tragacanth control group ($p < 0.05$).**

cuminoids had significantly higher cholesterol level than the tragacanth control; however, it appeared to be a reversible change because cholesterol level of the recovery group was not different from that of the tragacanth control (Table 8).

Histopathological examination of the internal organs of male rats receiving high dose of curcuminoids showed an apparently dose-related incidence of mild degree of fatty degeneration in the liver and adrenocortical fatty degeneration than that of the two controls. The incidence of both histopathological findings in the recovery group, however, appeared to be lower than that of the high dose group and not significantly different from that of the controls suggesting that these observed pathological changes were reversible. Since there were no change of serum triglyceride or glucose levels in curcuminoids-treated male rats, the fatty degeneration of the two organs was not likely due to an increase of serum triglyceride or glucose levels. The reason for the fatty change in the two organs was not known.

Table 5. Hematological examination results of male rats given curcuminoids orally for 6 months.

Male	Group of animals					
	water	tragacanth	10 mg/kg/day	50 mg/kg/day	250 mg/kg/day	250-R mg/kg/day
	N= 15	N=15	N=15	N=14	N=15	N=15
WBC (K/uL)	6.73±2.14	5.33±1.27	5.76±1.32	5.92±1.27	5.20±1.51	6.37±1.26
%Neutrophil	17.88±5.76	17.46±5.66	18.70±4.89	18.56±5.34	15.75±4.36	16.78±3.85
%Lymphocyte	78.69±5.19	79.32±6.18	77.98±5.21	77.96±5.47	80.28±5.72	79.80±4.14
%Monocyte	1.32±1.43	1.22±1.66	1.04±1.27	1.06±1.07	1.78±1.91	0.99±0.89
%Basophil	0.64±0.24	0.55±0.26	0.56±0.29	0.64±0.47	0.66±0.27	0.70±0.31
%Eosinophil	1.48±0.29	1.44±0.49	1.71±0.65	1.79±0.53	1.52±0.33	1.73±0.73
RBC($\times 10^6$/uL)	9.01±0.39	8.79±0.32	8.77±0.39	8.90±0.39	8.74±0.45	8.43±0.74*
Hemoglobin (g/dL)	16.22±0.57	15.78±0.46	15.73±0.43	15.76±0.41	15.56±0.48*	15.87±0.55
%Hematocrit	46.65±2.11	45.58±1.90	44.47±2.69	45.55±1.29	44.65±1.99	42.69±4.38*,**
Platelet (K/uL)	929.87±111.73	869.10±115.16	908.23±58.07	905.07±73.06	900.43±103.50	906.80±125.08
PCT (%)	0.92±0.14	0.84±0.15	0.89±0.13	0.86±0.081	0.86± 0.099	0.92±0.17
%Reticulocyte	18.48±6.51	13.19±6.06	14.38±2.57	13.82±5.14	14.93±5.58	13.73±5.07
Reticulocyte (K/uL)	1676.53± 579.59	1158.93± 569.99	1260.00± 270.40	1223.21± 457.83	1312.86± 510.37	1133.07± 429.57*

Each value represents mean ± SD.
* Significantly different from water control group ($p < 0.05$).
** Significantly different from tragacanth control group ($p < 0.05$).

Therefore, if curcuminoids will be taken at a high dose for a long period of time, patients should be advised to observe themselves for any possible sign of liver toxicity, i.e. juandice or yellowing of the skin or the eye, brown urine, nausea, vomiting, abdominal pain, light-colored stool, unusual tiredness, and loss of appetite. In addition, liver function test should also be performed periodically.

Conclusion

Six-month chronic toxicity study of curcuminoids in Wistar rats indicated that curcuminoids at the doses of 10 and 50 mg/kg/day did not produce any significant dose-related changes of organ weights, hematological parameters, serum chemistry, or pathology of the internal organs. Both male and female rats receiving curcuminoids 250 mg/kg/day had higher actual weights of the liver than those of the two control groups. Fatty degeneration of the liver occurred in a dose-dependent manner in male rats, while it was observed in 2 out of 15 female rats receiving the highest dose of curcuminoids. In addition, a dose-related adrenocortical fatty degeneration was also observed in curcuminoids-treated male rats but not in female rats. However, the incidence of these pathological changes was not significantly different between curcuminoids-

Table 6. Hematological examination results of female rats given curcuminoids orally for 6 months

Female	Group of animals					
	water	tragacanth	10 mg/kg/day	50 mg/kg/day	250 mg/kg/day	250-R mg/kg/day
	N= 15	N=15	N=15	N=15	N=15	N=15
WBC (K/uL)	3.16±1.51	2.83±1.18	2.40±0.67	2.65±1.13	2.69±0.92	2.36±0.96
%Neutrophil	19.91±8.37	21.36±11.16	22.06±6.65	23.19±4.60	21.33±5.71	21.93±6.80
%Lymphocyte	75.56±9.62	75.49±11.12	73.54±7.70	72.31±5.55	73.40±6.31	73.22±8.05
%Monocyte	2.05±1.84	0.90±0.44	1.47±1.42	1.54±1.68	2.26±2.48	2.09±2.19
%Basophil	0.51±0.54	0.48±0.31	0.55±0.49	0.50±0.24	0.73±0.49	0.57±0.32
%Eosinophil	3.10±4.28	1.77±0.61	2.37±0.92	2.46±1.11	2.29±0.62	2.19±0.84
RBC($\times 10^6$/uL)	8.00±0.48	7.77±0.56	7.86± 0.50	7.82±0.49	7.75±0.40	7.63±0.49
Hemoglobin (g/dL)	15.54±0.49	15.18±0.75	15.31±0.55	15.31±0.74	15.20±0.46	15.10±0.46
%Hematocrit	44.38±2.59	43.16±1.75	43.74±2.62	43.79±2.26	43.24±1.93	42.40±2.10
Platelet (K/uL)	855.07±100.51	803.20±84.62	848.00±98.19	843.37±100.51	814.03±87.63	812.30±99.91
PCT (%)	0.84±0.12	0.76±0.073	0.80±0.11	0.81±0.11	0.76±0.069	0.78±0.09
%Reticulocyte	16.72±6.57	17.49±5.13	15.47±5.70	12.81±5.85	15.81±4.72	16.59±5.36
Reticulocyte (K/uL)	1340.07± 535.38	1343.80± 337.44	1234.50± 493.81	983.73± 423.91	1223.07± 400.45	1268.14± 444.68

Each value represents mean ± SD.
* Significantly different from water control group ($p < 0.05$).
** Significantly different from tragacanth control group ($p < 0.05$).

treated animals and the two controls, and was lower in the recovery group suggesting a reversible nature of these changes. Taken together, the results suggested that long term administration of curcuminoids at a high dose might affect the liver of the rat morphologically and functionally in a reversible manner.

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Table 7. Blood chemistry results of male rats given curcuminoids orally for 6 months

Male	Group of animals					
	water	tragacanth	10 mg/kg/day	50 mg/kg/day	250 mg/kg/day	250-R mg/kg/day
	N= 15	N=15	N=15	N=14	N=15	N=15
AST (U/L)	70.47±8.55	68.67±6.91	71.93±10.09	73.86±14.42	67.07±6.58	71.13±15.09
ALT (U/L)	39.60±9.17	36.20±4.89	35.60±6.95	39.07±10.48	35.73±7.98	42.13±16.01
ALP (U/L)	67.47±9.16	62.73±9.74	69.07±16.37	74.21±12.74	79.40±13.39**	68.13±12.67
p-amylase	1960.87± 260.43	1833.73± 139.52	1884.60± 205.10	2099.36± 207.49	1992.73± 311.97	2063.60± 271.07
Bilirubin (mg/dL)	0.066±0.039	0.073±0.037	0.070±0.028	0.080±0.025	0.069±0.042	0.052±0.036
Creatinine (mg/dL)	0.67±0.062	0.68±0.045	0.65±0.061	0.65±0.041	0.65±0.063	0.67±0.041
BUN (mg/dL)	18.64±1.55	19.19±3.16	18.28±2.38	17.64±2.05	17.80±2.04	17.63±1.83
Cholesterol (mg/dL)	89.31± 19.55	85.75± 16.37	91.71± 18.77	81.68± 15.80	104.47± 22.79	118.40± 24.20*,**
Triglyceride (mg/dL)	183.42± 72.53	139.42± 33.75	155.20± 30.13	149.90± 38.95	152.12± 54.28	253.94± 88.26**
Total protein (g/dL)	6.95±0.31	6.83±0.19	6.91±0.29	6.94±0.23	6.94±0.29	7.15±0.31**
Albumin (g/dL)	4.27±0.14	4.23±0.09	4.25±0.14	4.36±0.13	4.44±0.19*,**	4.24±0.17
Uric acid (mg/dL)	2.71±1.34	1.51±0.59	1.90±1.03	1.84±0.87	2.01±0.89	1.87±0.87
Glucose (mg/dL)	185.57±30.78	154.04±17.72*	165.93±21.81	169.66±22.36	171.07±28.88	172.28±19.04
Sodium (mmol/L)	146.47±2.26	146.80±1.82	147.33±1.84	147.86±1.66	148.20±1.90	147.13±1.25
Potassium (mmol/L)	6.54±1.37	5.69±0.81	5.65±0.37	5.44±0.48	5.65±0.43	5.93±0.75
Chloride (mmol/L)	109.80±2.43	111.27±1.87	111.40±1.55	111.57±1.83	111.87±2.26	112.73±2.31*

Each value represents mean±SD.
* Significantly different from water control group ($p < 0.05$).
** Significantly different from tragacanth control group ($p < 0.05$).

Table 8. Blood chemistry results of female rats given curcuminoids orally for 6 months

Female	Group of animals					
	water	tragacanth	10 mg/kg/day	50 mg/kg/day	250 mg/kg/day	250-R mg/kg/day
	N= 15	N=15	N=15	N=15	N=15	N=15
AST (U/L)	86.87±29.15	93.07±24.86	102.80±41.78	97.40±36.66	73.80±12.39	74.07±11.75
ALT (U/L)	43.60±17.44	40.93±19.58	47.33±17.77	47.00±18.35	36.80±11.10	35.40±9.83
ALP (U/L)	22.67±4.12	23.27±7.27	20.73±3.20	23.60±6.43	23.60±4.61	22.67±4.29
p-amylase	1217.53± 299.96	1143.27± 170.25	1146.67± 178.65	1082.53± 212.42	1230.60± 167.27	1298.60± 238.92
Bilirubin (mg/dL)	0.097±0.037	0.096±0.051	0.099±0.040	0.080±0.052	0.112±0.059	0.074±0.035
Creatinine (mg/dL)	0.75±0.079	0.76±0.084	0.76±0.070	0.77±0.090	0.75±0.065	0.74±0.061
BUN (mg/dL)	21.45±3.45	21.95±3.72	22.34±2.70	21.50±2.60	19.99±3.22	19.05±3.13
Cholesterol (mg/dL)	71.59±14.39	65.02±10.76	74.24±9.83	79.05±16.66	83.46±18.86**	72.53±16.38
Triglyceride (mg/dL)	109.93±30.98	112.82±40.87	122.29±39.66	127.74±39.82	134.81±41.25	143.35±71.07
Total protein (g/dL)	7.27±0.49	7.28±0.36	7.31±0.27	7.40±0.36	7.56±0.29	7.33±0.44
Albumin (g/dL)	5.00±0.31	5.00±0.35	5.09±0.21	5.12±0.25	5.28±0.22	5.05±0.27
Uric acid (mg/dL)	1.77±0.86	1.43±0.91	1.94±1.21	1.47±0.68	1.58±0.82	1.48±0.55
Glucose (mg/dL)	141.07±22.46	133.45±19.19	144.32±33.92	139.75±23.68	143.33±15.77	153.70±23.05
Sodium (mmol/L)	147.13±1.60	147.67±1.76	147.67±1.68	148.07±1.44	148.20±1.57	148.47±0.92
Potassium (mmol/L)	5.42±0.94	4.69±0.96	5.15±1.06	4.88±0.95	5.05±0.95	4.63±0.67
Chloride (mmol/L)	113.20±1.66	113.07±1.58	113.40±1.40	113.33±1.84	113.73±1.94	116.93± 1.87*,**

Each value represents mean±SD.
* Significantly different from water control group ($p < 0.05$).
** Significantly different from tragacanth control group ($p < 0.05$).

Table 9. Histopathological results of visceral organs in male rats given curcuminoids orally for 6 months

Organs	Microscopic findings	Group of animals					
		water	tragacanth	10 mg/kg/day	50 mg/kg/day	250 mg/kg/day	250-R mg/kg/day
Lung	Lymphoid proliferated peribronchioles	4/14	6/14	8/15	4/14	3/15	8/15
Heart	Focal myocardiosis	3/14	1/14	1/15	1/14	0/15	0/15
Liver	Fatty degeneration	4/14	3/14	2/15	5/14	8/15	4/15
Kidney	Tubular cyst	2/14	2/14	0/15	2/14	0/15	2/15
	Renal cell carcinoma	0/14	0/14	0/15	0/14	1/15	0/15
Spleen	Lymphoid hyperplasia	0/14	0/14	0/15	1/14	0/15	0/15
Intestine	Lymphoid aggregated submucosal layer	2/14	2/14	4/15	2/14	0/15	2/15
Testis	Atrophy	1/14	2/14	0/15	0/15	1/15	0/15
Adrenal gland	Cortical fatty degeneration	5/14	5/14	2/15	5/14	10/15	6/15
Thyroid gland	Follicular hyperplasia	0/14	0/14	0/15	2/14	0/15	0/15

The results were expressed as the number of rats with pathological findings per total number of rats treated

Table 10. Histopathological results of visceral organs in female rats given curcuminoids orally for 6 months

Organs	Microscopic findings	Group of animals					
		water	tragacanth	10 mg/kg/day	50 mg/kg/day	250 mg/kg/day	250-R mg/kg/day
Lung	Lymphoid proliferated peribronchioles	4/15	5/14	6/14	2/15	2/15	6/15
Heart	Myocardial calcification	1/15	0/14	0/14	0/15	0/15	0/15
Liver	Fatty degeneration	0/15	0/14	0/15	0/14	2/15	0/15
Kidney	Tubular cast	5/15	3/14	4/14	7/15	6/15	5/15
	Tubular cyst	0/15	0/14	0/14	1/15	0/15	0/15
Spleen	Lymphoid hyperplasia	0/14	0/14	0/15	1/14	0/15	0/15
Intestine	Lymphoid aggregated submucosal layer	2/15	2/14	1/14	0/15	0/15	3/15
Uteus and cervix	Glandular hyperplasia	0/15	0/14	0/14	1/15	0/15	3/15
Mammary gland	Glandular hyperplasia	0/15	2/14	2/14	0/15	0/15	3/15
Adrenal gland	Congestion	1/15	0/14	0/14	0/15	0/15	0/15

The results were expressed as the number of rats with pathological findings per total number of rats treated

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