*Original Article*

# Chronic Toxicity Study of *Kaempferia parviflora* Wall ex. Extract

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***Abstract***

*K. parviflora* is a medicinal plant possessing high potential for development of various health products. The objective of this chronic toxicity study was to investigate the safety of ethanolic extract of chronic *Kaempferia parviflora* Wall ex Bak in Wistar rats. The animals were randomly divided into five groups, twenty four rats each (12 males and 12 females). Three treatment groups were orally administered with *K. parviflora* extract at doses of 5, 50 and 500 mg/kg/day for six months respectively, which were equivalent to 1, 10 and 100 times of human use, while two control groups were orally given with distilled water and 1.0% tragacanth, respectively. The results showed that male rats receiving *K. parviflora* extract at dose of 500 mg/kg had significantly lower body weight than both control groups ($p$<0.05). The alterations of a few hematological parameters in the highest dose-treated groups were within the normal range. Male rats receiving the highest dose of *K. parviflora* extract had significantly lower triglyceride level than their two control groups ($p$<0.05) whereas female rats receiving the same dose had significantly higher glucose and cholesterol levels than their control groups ($p$<0.05). Histopathological study of visceral organs revealed no remarkable lesions related to the toxicity of *K. parviflora* extract.

**Keywords:** Chronic toxicity test, *K. parviflora* extract, rat

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# บทคัดย่อ

## การศึกษาพิษเรื้อรังของสารสกัดกระชายดำ

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กระชายดำ เป็นพืชสมุนไพรที่มีศักยภาพสูงในการพัฒนาเป็นผลิตภัณฑ์สุขภาพ การศึกษาพิษเรื้อรังครั้งนี้ มีวัตถุประสงค์เพื่อศึกษาความปลอดภัยของสารสกัดกระชายดำด้วยเอทานอล ในหนูแรทพันธุ์วิสตาร์จำนวน 120 ตัว แบ่งออกเป็น 5 กลุ่มๆ ละ 24 ตัว (เพศละ 12 ตัว) โดยกลุ่มทดลอง 3 กลุ่มได้รับสารสกัดกระชายดำ โดยการป้อนทางปากในขนาด 5, 50 และ 500 มก./กก./วัน เป็นเวลา 6 เดือน หรือเทียบเท่ากับ 1, 10 และ 100 เท่าของขนาดกระชายดำที่คนรับประทาน กลุ่มควบคุม 2 กลุ่ม ได้รับน้ำกลั่นและสารละลายทรากาแคนท์ความเข้มข้นร้อยละ 1 ผลการทดลองพบว่า สารสกัดกระชายดำขนาด 500 มก./กก./วัน ทำให้หนูเพศผู้มีน้ำหนักตัวต่ำกว่า กลุ่มควบคุมด้วยน้ำและทรากาแคนท์อย่างมีนัยสำคัญ ($p < 0.05$) การเปลี่ยนแปลงบางค่าของค่าทางโลหิตวิทยาในกลุ่มที่ได้รับสารสกัดขนาด 500 มก./กก./วันยังคงอยู่ในช่วงปกติ กลุ่มหนูเพศผู้ที่ได้รับสารสกัดกระชายดำขนาด 500 มก./กก./วัน พบค่าไตรกลีเซอไรด์ต่ำกว่ากลุ่มควบคุมทั้งสองอย่างมีนัยสำคัญ ($p < 0.05$) ในขณะที่หนูเพศเมียที่ได้รับสารสกัดขนาดเท่ากันมีค่ากลูโคสและโคเลสเตอรอลสูงกว่ากลุ่มควบคุมทั้งสองอย่างมีนัยสำคัญ ($p < 0.05$) ผลการตรวจจุลพยาธิวิทยาของอวัยวะต่างๆ ไม่พบความผิดปกติใดๆที่บ่งชี้การเป็นพิษเรื้อรังที่เกิดจากสารสกัดกระชายดำ

**คำสำคัญ:** การทดสอบพิษเรื้อรัง สารสกัดกระชายดำ หนูแรท

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## *Introduction*

*Kaempferia parviflora* Wall Ex. Baker or Krachaidam is a plant belonging to the family Zingiberaceae (Sirirugsa, 1992). The rhizomes of this plant have been traditionally used for leucorrhea, oral diseases, abdominal pain, health promotion and aphrodisiac (Wutythamawech, 1997). Phytochemical studies revealed that the rhizomes of *K. parviflora* contained volatile oil (Wongsinkongman et al., 2003), chalcones (Herunsalee, 1987), phenolic glycosides (Azuma et al., 2008) and many flavonoids such as 5-hydroxy-7-methoxyflavonone, 5, 7-dimethoxyflavone and 3, 5, 7-trimethoxyflavone (Jaipetch et al., 1983). The *K.parviflora* has been demonstrated to possess antifungal, antiplasmodial, antimycobacterial (Yenjai et al., 2004), anti HIV-1 protease (Sookkongwaree et al., 2006), anti-allergic (Tewtrakool et al., 2008) and anti-gastric ulcers (Rujjanawate et al., 2005). It has been reported that the ethanolic extract and 5-hydroxy-3,7,3',4',-tetramethoxyflavone of this plant exhibit appreciable inhibitory effects on nitric oxide and PGE2 release from murine macrophage cells (Tewtrakul and Subhadhirasakul, 2008). In addition, the ethanolic extract of Krachaidam has been shown to induce relaxation of both aortic rings and ileum precontracted with phenylephrine and acetylcholine (Wattanapitayakul et al., 2008). Although *K. parviflora* has been shown to possess high potentials for development of health products, chronic toxicity study of its extracts has never been reported. In this study, we investigated chronic toxicity of the *K. parviflora* ethanolic extract in Wistar rats to support the use of health products from *K. parviflora*.

## *Materials and Methods*

***Kaempferia parviflora extract:*** *K. parviflora* was cultivated in Chattrakarn District, Phitsanulok Province, Thailand and two- month aged rhizomes were harvested in January 2004. The plant materials were identified by Dr. Bungorn Sripanidkulchai and the voucher specimen (KP-CRD10D) was kept in Herbarium at the Center for Research and Development of Herbal Health Products, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. The 95% ethanolic extract of *K. parviflora* rhizome (KPE) was prepared by the Center for Research and Development of Herbal Health Products, Khon Kaen University, Thailand. The KPE powder was brown in color and had a yield of 4.09% of *K. parviflora* dried rhizome powder. The content of total flavonoids were 35.52 mg/g of dried powder and the amount of three major markers: 5,7-dimethoxyflavone, 5, 7, 4'-trimethoxyflavone and 3, 5, 7, 3' 4'-pentamethoxyflovone assayed by Gas

chromatography were 24.138 mg/g dried powder. The KPE was homogeneously suspended in 1.0% tragacanth solution and adjusted to the desired concentrations for chronic toxicity study.

***Animals:*** One hundred and twenty Wistar rats (60 male rats and 60 female rats weighing approximately 180-200 and 170-190 g, respectively) were purchased from The National Laboratory Animal Center, Mahidol University. Animals were housed in a hygienic conventional animal room of the laboratory animal center, Department of Medical Sciences, where the environment of the room was maintained at 25±1°C with 60% humidity and 12 hour-light-dark cycle. They were raised with commercial pellet diet and clean water *ad libitum*. Prior to the chronic toxicity study, the animals were acclimatized to the environment for two weeks. This study was approved by the Institutional Animal Care and Use Committee, Department of Medical Sciences as (permission No. 49-011).

***Experimental design:*** The Wistar rats were randomly divided to five groups, 12 males and 12 females in each group. Three experimental groups were orally administered with KPE at the doses of 5, 50 and 500 mg/kg /day, respectively for six months. These doses were approximately equivalent to 1, 10 and 100 times of dried Krachaidam in human use (5-10 g/day/person). The other two control groups orally received distilled water and 1.0% tragacanth at the volume of 10 ml/kg respectively. Body weight and food consumption were recorded weekly and the animals were closely observed for general appearance, behavior and signs of abnormalities. At the end of the six-month treatment period, the animals were fasted overnight, anesthesized with diethyl ether inhalation. Blood samples were collected from posterior vena cava for determining hematological and serum clinical chemistry values.

Hematological analysis was performed using automatic hematological analyzer Cell Dyn® 3500 (Abbott Laboratories Ltd., USA). Parameters examined were hematocrit (Hct), hemoglobin, total red blood cell count (RBC), mean corpuscular volume (MCV), mean concentration hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), total white blood cell count (WBC) including differential cell count (neutrophils, eosinophils, lymphocytes, monocytes and basophils) and total platelet count. Clinical chemistry values were measured by using automatic chemistry analyzer Hitachi®912 (Hitachi Ltd., Japan) and parameter assays were alkaline phosphatase (ALP), alanine transminase (ALT), aspartate transminase (AST), total protein, albumin, bilirubin, blood urea nitrogen (BUN), creatinine, glucose, uric acid, triglyceride, cholesterol, sodium, potassium and chloride ions.

A complete necropsy was performed to determine gross pathological alterations of various visceral organs. The brain, heart, lung, liver, kidney, stomach, spleen, testis, uterus and adrenal glands were weighed by using Mettler Toledo® PB 153 balance (Mettler Toledo Internaltional Inc., Switzerland). The visceral organ weights were calculated into relative organ weight (g/1000 g body weight). The visceral organs were fixed in 10% buffered formalin and subjected to conventional histological process. Histopathological examination of visceral organs was performed on the brain, heart, lung liver, stomach, spleen, kidney, pancreas, intestines, bladder, adrenal and thyroid gland, lacrimal gland, salivary gland including testis, prostate gland, seminal vesicle in the male, ovary, uterus and mammary glands in the female.

***Statistical analyses:*** The data was analyzed using one way ANOVA and Bonferroni test in multiple comparison. Histopathological result was analyzed using Fisher's exact. Differences between groups were considered significant at $p<0.05$.

## *Results*

***Effect of KPE on body weight, food consumption and relative organs weight***: All KPE-treated groups had no significant differences in body weight compared to both water and tragacanth control groups, whereas male rats receiving KPE at dose of 500 mg/kg/day had significantly lower body weight than both control groups at week 8 till the end of the experiment (Figure 1). Male rats receiving KPE at dose of 500 mg/kg/day had significantly less food consumption than the water control group during week 12 to 15 and week 20 till the end of the experiment. Moreover, this group had significantly less food consumption than their tragacanth control group in some weeks. All KPE-treated female groups had no significant reduction in food consumption throughout the experiment (Figure 2).

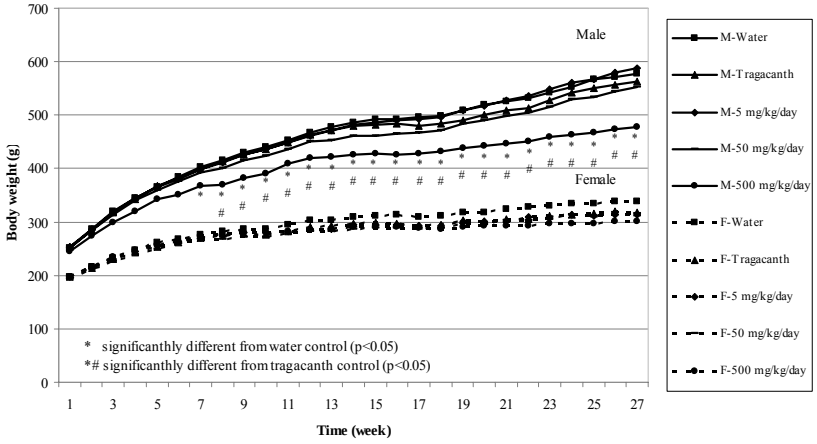

**Figure 1.** Growth curves of male and female rats receiving KPE for 6 months

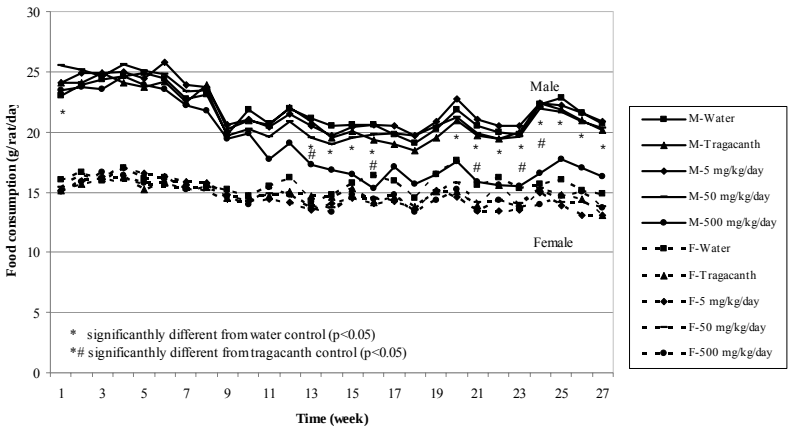


**Figure 2**. Food consumption of male and female rats receiving KPE for 6 months.

Only the highest dose-treated group, had significant increases in relative weight of some organs. Both male and female rats of this group had significantly higher relative weight of heart, liver, stomach, right kidney than both water and tragacanth control groups. The relative lung weight of the females was higher than that of the males and the tragacanth control groups and significantly higher than that of the water control group. The relative left kidney and bladder weight of the male rats was significantly higher than that of both water and tragacanth control groups (Table 1).

**Table 1** Body weight (g) and relative organ weight (g/1000g body weight) of male and female rats receiving *Kaempferia parviflora* extract (KPE) for 6 months

| Organs | Male rats | | | | | Female rats | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | | Dose of KPE (mg/kg/day) | | | Control | | Dose of KPE (mg/kg/day) | | |
| | Water n = 12 | Tragacanth N = 12 | 5 n = 12 | 50 n = 12 | 500 n = 11 | Water n = 12 | Tragacanth n = 12 | 5 n = 12 | 50 n = 12 | 500 n = 11 |
| Initial body weight | 252.08±19.86 | 253.93±20.46 | 252.79±17.19 | 252.30±23.11 | 244.31±27.31 | 197.17±10.84 | 197.82±8.95 | 197.57±14.08 | 193.23±8.93 | 198.15±13.68 |
| Final body weight | 558.87±54.49 | 544.86±31.06 | 567.71±36.35 | 509.53±114.01 | 454.34±58.59[a,b] | 327.35±40.58 | 305.95±25.43 | 302.11±12.80 | 302.86±26.25 | 279.70±34.99[a] |
| Brain | 3.87±0.36 | 3.95±0.22 | 3.74±0.31 | 3.93±0.39 | 4.61±0.66 | 6.17±0.78 | 6.51±0.47 | 6.49±0.25 | 6.51±0.48 | 7.01±0.78 |
| Heart | 2.55±0.29 | 2.53±0.25 | 2.50±0.20 | 2.61±0.18 | 3.05±0.59[a,b] | 2.99±0.26 | 3.14±0.21 | 3.14±0.16 | 3.13±0.20 | 3.45±0.29[a, b] |
| Lung | 3.08±0.29 | 3.23±0.23 | 2.97±0.38 | 3.17±0.42 | 3.56±0.54[a] | 4.24±0.45 | 4.25±0.29 | 4.38±0.31 | 4.41±0.29 | 4.76±0.30[a, b] |
| Liver | 23.62±2.16 | 25.24±2.22 | 23.56±1.95 | 25.16±2.87 | 31.33±4.20[a, b] | 25.24±3.06 | 26.46±3.71 | 25.52±3.36 | 25.87±2.04 | 39.57±5.47[a, b] |
| Stomach | 3.81±0.42 | 3.83±0.34 | 3.56±0.43 | 3.90±0.52 | 5.31±1.12[a, b] | 5.10±0.73 | 5.23±0.49 | 5.45±0.77 | 5.71±0.58 | 8.70±0.82[a, b] |
| Spleen | 1.57±0.13 | 1.54±0.19 | 1.46±0.13 | 1.55±0.16 | 1.54±0.54 | 2.10±0.38 | 2.26±0.39 | 2.16±0.31 | 2.08±0.15 | 2.31±0.27 |
| Right Kidney | 2.20±0.19 | 2.23±0.13 | 2.13±0.16 | 2.31±0.17 | 2.96±0.45[a, b] | 2.74±0.28 | 2.88±0.32 | 2.78±0.27 | 2.84±0.33 | 3.33±0.32[a, b] |
| Left Kidney | 2.10±0.24 | 2.14±0.17 | 2.03±0.12 | 2.23±0.16 | 2.91±0.64[a, b] | 2.61±0.30 | 2.73±0.22 | 2.66±0.27 | 2.70±0.18 | 2.84±0.84 |
| Right Testis | 5.34±0.59 | 5.16±0.99 | 5.10±0.51 | 5.48±0.71 | 5.86±1.59 | | | | | |
| Left Testis | 5.35±0.74 | 5.45±0.83 | 5.17±0.43 | 5.49±0.75 | 5.97±1.51 | | | | | |
| Right Adrenal | 0.06±0.02 | 0.07±0.01 | 0.05±0.01 | 0.06±0.02 | 0.07±0.02 | 0.15±0.04 | 0.13±0.02 | 0.12±0.02 | 0.12±0.02 | 0.13±0.03 |
| Left Adrenal | 0.07±0.01 | 0.07±0.01 | 0.06±0.01 | 0.07±0.02 | 0.08±0.02 | 0.15±0.04 | 0.14±0.02 | 0.14±0.02 | 0.12±0.01 | 0.14±0.03 |
| Bladder | 0.31±0.05 | 0.31±0.04 | 0.33±0.09 | 0.34±0.08 | 0.41±0.08[a, b] | 0.31±0.45 | 0.32±0.05 | 0.32±0.06 | 0.31±0.04 | 0.36±0.08 |
| Uterus | | | | | | 2.15±0.82 | 2.33±0.59 | 2.40±0.78 | 2.10±0.57 | 2.98±1.43 |
| Right Ovary | | | | | | 0.25±0.05 | 0.25±0.06 | 0.24±0.06 | 0.23±0.05 | 0.34±0.11 |
| Left Ovary | | | | | | 0.26±0.09 | 0.25±0.05 | 0.25±0.06 | 0.24±0.05 | 0.29±0.06 |

The values are expressed as mean ± SD.

[a] significantly different from water control group ($p$<0.05) [b] significantly different from tragacanth control group ($p$<0.05)

**Table 2** Hematological values of male and female rats receiving *Kaempferia parviflora* extract (KPE) for 6 months

| Parameters | Male rats | | | | | Female rats | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | | Dose of KPE (mg/kg/day) | | | Control | | Dose of KPE (mg/kg/day) | | |
| | Water n = 12 | Tragacanth n = 12 | 5 n = 12 | 50 n = 12 | 500 n = 11 | Water n = 12 | Tragacanth n = 12 | 5 n = 12 | 50 n = 12 | 500 n = 11 |
| Hematocrit (%) | 50.08±2.02 | 48.53±1.58 | 48.37±2.89 | 49.33±3.64 | 48.67±4.80 | 49.26±2.87 | 48.20±5.26 | 49.28±4.37 | 46.47±1.28 | 48.51±2.06 |
| Hemoglobin (g/dl) | 15.48±0.61 | 15.07±0.45 | 15.17±0.86 | 15.43±0.98 | 15.12±1.44 | 15.44±0.74 | 15.55±0.89 | 15.48±1.18 | 14.64±0.44 | 15.18±0.59 |
| RBC ($\times 10^6$/mm$^3$) | 9.20±0.44 | 8.94±0.27 | 8.88±0.54 | 9.06±0.56 | 8.76±0.61 | 8.47±0.57 | 8.38±0.50 | 8.43±0.49 | 8.00±0.35 | 8.35±0.48 |
| MCV (um$^3$/red cell) | 54.50±2.42 | 54.30±1.21 | 54.49±1.59 | 54.40±1.56 | 55.55±3.14 | 58.16±1.10 | 59.05±1.91 | 58.38±2.39 | 58.15±2.07 | 58.16±1.55 |
| MCH (pg/red cell) | 16.83±0.76 | 16.86±0.36 | 17.11±0.56 | 17.02±0.40 | 17.27±1.00 | 18.24±0.57 | 18.56±0.57 | 18.38±0.64 | 18.33±0.74 | 18.19±0.61 |
| MCHC (g/dl RBC) | 30.91±0.42 | 31.06±0.47 | 31.39±0.42 | 31.29±0.46 | 31.10±0.53 | 31.35±0.58 | 31.45±0.49 | 31.47±0.50 | 31.53±0.48 | 31.26±0.45 |
| WBC ($\times 10^3$/mm$^3$) | 3.71±0.81 | 3.70±1.15 | 3.28±0.71 | 4.38±1.35 | 3.96±1.10 | 1.98±0.38 | 2.32±0.89 | 2.06±0.57 | 2.51±0.89 | 2.70±0.76 |
| Neutrophil (%) | 26.90±5.12 | 27.60±6.18 | 25.13±5.69 | 26.48±4.83 | 28.07±5.19 | 22.06±4.79 | 20.93±6.02 | 19.34±5.14 | 22.82±6.54 | 14.82±3.98[a] |
| Eosinophil (%) | 1.61±0.50 | 1.80±0.66 | 2.04±0.93 | 1.76±0.55 | 0.83±0.29[a, b] | 1.55±0.75 | 1.20±0.46 | 1.28±0.79 | 1.07±0.39 | 0.61±0.40[a, b] |
| Lymphocyte (%) | 66.36±6.64 | 65.26±7.05 | 68.60±5.48 | 66.85±7.61 | 68.43±3.62 | 69.40±9.75 | 73.85±5.31 | 76.01±6.41 | 73.55±6.25 | 82.22±4.33[a, b] |
| Monocyte (%) | 3.92±3.99 | 4.01±3.55 | 3.28±2.76 | 3.72±4.49 | 1.79±2.48 | 3.42±2.99 | 3.09±2.95 | 2.49±2.11 | 1.51±0.99 | 1.38±0.70 |
| Basophil (%) | 1.21±1.02 | 1.33±1.08 | 0.96±0.87 | 1.18±0.75 | 0.89±0.61 | 1.08±0.56 | 0.95±0.65 | 0.96±0.60 | 1.03±0.50 | 0.98±0.51 |
| Platelet ($\times 10^3$/mm$^3$) | 864.13±138.32 | 877.88±121.06 | 842.96±88.76 | 903.54±99.18 | 820.15±147.05 | 932.08±114.32 | 865.46±104.46 | 880.54±56.13 | 825.67±42.71 | 914.63±100.76 |
| Hematocrit (%) | 50.08±2.02 | 48.53±1.58 | 48.37±2.89 | 49.33±3.64 | 48.67±4.80 | 49.26±2.87 | 48.20±5.26 | 49.28±4.37 | 46.47±1.28 | 48.51±2.06 |
| Hemoglobin (g/dl) | 15.48±0.61 | 15.07±0.45 | 15.17±0.86 | 15.43±0.98 | 15.12±1.44 | 15.44±0.74 | 15.55±0.89 | 15.48±1.18 | 14.64±0.44 | 15.18±0.59 |

The values are expressed as mean ± SD.

[a] significantly different from water control group ($p$<0.05) [b] significantly different from tragacanth control group ($p$<0.05)

***Effects of KPE on hematological and clinical chemistry values***: Eosinophils in both male and female rats treated with 500 mg/kg/day of KPE were significantly lower than those in both water and tragacanth control groups. Lymphocytes in the highest dose female group were significantly higher

than those in both water and tragacanth control groups whereas neutrophils were significantly lower than those in only the water control group (Table 2). In the male rats, ALP and triglyceride levels of the highest dose-treated group were significantly lower than those of the water control group and the latter's were also lower than those of the tragacanth control group. Creatinine value of the 50 mg/kg/day-treated males was significantly lower than that of both water and tragacanth control groups. In the female rats, uric acid in the group receiving KPE at dose of 50 mg/kg/day was significantly lower than that in the water control group. Glucose and triglyceride levels of the highest dose-treated group were significantly higher than those of both water and tragacanth control groups. Besides, BUN and sodium levels of the 50 mg/kg/day-treated females were significantly higher than those of the water control group (Table 3).

***Effects of KPE on histopathological alterations of the visceral organs:*** Necropsy revealed no remarkable gross lesions in any organs in all KPE-treated groups including both water and tragacanth control groups. Histopathological alterations found in all KPE-treated groups were not significantly different from those in both water and tragacanth control groups except male rats receiving KPE at doses of 50 and 500 mg/kg/day had significantly lower incidence of mild centrilobular fatty degeneration in the liver and GALT proliferation in large intestine than both of water and tragacanth control groups. In other organs, there was no remarkable histopathological lesions in KPE-treated groups and both control groups. Histopathological results are shown in table 4.

**Table 3** Biochemical values of male and female rats receiving *Kaempferia parviflora* extract (KPE) for 6 months

| Parameters | Male rats | | | | | Female rats | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | | Dose of KPE (mg/kg /day) | | | Control | | Dose of KPE (mg/kg /day) | | |
| | Water n = 12 | Tragacanth n = 12 | 5 n = 12 | 50 n = 12 | 500 n = 11 | Water n = 12 | Tragacanth n = 12 | 5 n = 12 | 50 n = 12 | 500 n = 11 |
| ALT(U/L) | 46.83±17.00 | 37.58±6.36 | 40.83±4.95 | 44.00±15.31 | 48.40±9.76 | 37.58±15.13 | 37.27±9.30 | 35.33±6.95 | 40.75±19.26 | 44.42±10.98 |
| AST(U/L) | 93.58±15.01 | 89.92±9.85 | 95.85±10.20 | 99.00±17.60 | 94.80±12.79 | 100.17±48.81 | 97.58±22.75 | 90.33±13.17 | 101.92±37.04 | 86.50±19.43 |
| ALP(U/L ) | 54.42±8.12 | 50.67±6.49 | 57.58±9.96 | 50.67±6.40 | 40.50±10.22[a] | 25.33±5.00 | 28.33±11.41 | 24.67±8.27 | 20.83±6.91 | 26.42±9.69 |
| BUN (mg/dl) | 19.89±1.90 | 19.38±1.67 | 18.94±1.73 | 19.58±2.34 | 20.63±3.86 | 20.06±3.47 | 21.69±1.53 | 24.66±4.37 | 24.63±5.55 | 25.83±4.73[a] |
| Creatinine (mg %) | 0.60±0.06 | 0.60±0.06 | 0.58±0.04 | 0.50±0.08[a,b] | 0.54±0.09 | 0.61±0.07 | 0.60±0.08 | 0.62±0.07 | 0.63±0.14 | 0.61±0.18 |
| Albumin (g/dl) | 4.20±0.15 | 4.19±0.17 | 4.25±0.19 | 4.20±0.16 | 4.24±0.42 | 6.93±0.33 | 6.84±0.37 | 6.91±0.28 | 6.80±0.23 | 6.91±0.30 |
| Bilirubin (mg/dl) | 0.10±0.02 | 0.10±0.03 | 0.09±0.04 | 0.10±0.04 | 0.09±0.04 | 4.77±0.19 | 4.76±0.25 | 4.76±0.17 | 4.69±0.18 | 4.82±0.25 |
| Total protein (g/dl) | 6.73±0.33 | 6.74±0.39 | 6.81±0.30 | 6.62±0.27 | 6.57±0.59 | 0.14±0.03 | 0.16±0.03 | 0.16±0.05 | 0.15±0.05 | 0.14±0.04 |
| Glucose (mg/dl) | 193.07±28.55 | 210.71±41.20 | 193.06±24.25 | 199.10±53.24 | 192.21±35.06 | 141.59±19.03 | 140.84±20.16 | 130.25±25.65 | 155.56±24.09 | 174.96±21.43[a, b] |
| Uricacid (mg/dl) | 2.45±0.97 | 3.36±1.71 | 2.93±1.25 | 3.30±2.20 | 3.14±1.02 | 2.76±1.29 | 2.52±1.42 | 2.11±0.92 | 1.44±0.58[a] | 2.52±0.87 |
| Triglyceride (mg/dl) | 108.78±50.52 | 127.13±42.80 | 100.73±35.55 | 114.42±37.02 | 41.90±16.24[a, b] | 46.69±12.75 | 46.00±13.65 | 37.20±7.93 | 43.39±8.44 | 64.36±28.31 |
| Cholesterol (mg/dl) | 82.31±22.07 | 79.65±15.68 | 77.83±13.73 | 81.99±22.88 | 95.87±34.00 | 68.93±21.49 | 62.67±21.37 | 68.86±13.93 | 81.85±16.98 | 116.18±20.30[a, b] |
| $Na^+$(mmol/l) | 144.58±1.08 | 145.83±1.27 | 146.25±1.71 | 146.17±1.03 | 146.40±2.32 | 144.92±1.51 | 145.67±1.44 | 145.75±1.48 | 146.25±1.36 | 147.17±1.40[a] |
| $K^+$(mmol/l) | 6.27±0.50 | 5.75±0.58 | 5.95±1.28 | 6.27±1.50 | 5.79±1.76 | 6.36±1.42 | 6.28±1.82 | 6.01±1.50 | 4.69±0.88[a] | 5.68±1.19 |
| $Cl^-$(mmol/l ) | 104.17±1.19 | 104.67±1.37 | 105.67±1.44 | 104.58±1.88 | 104.20±2.30 | 105.75±1.71 | 106.50±1.31 | 106.92±2.39 | 106.42±1.24 | 105.50±1.62 |

The values are expressed as mean±SD.

[a] significantly different from water control group ($p$<0.05); [b] significantly different from tragacanth control group ($p$<0.05)

**Table 4** Histopathological results of visceral organs in male and female rats receiving *Kaempferia parviflora* extract (KPE) for 6 months

| Organs | Microscopic findings | Male rats | | | | | Female rats | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Dose of KPE (mg/kg /day) | | | | | Dose of KPE (mg/kg /day) | | | | |
| | | control | Tragacanth | 5 | 50 | 500 | control | Tragacanth | 5 | 50 | 500 |
| Lung | BALT proliferated | 6/12 | 6/12 | 11/12 | 9/12 | 10/11 | 6/12 | 6/12 | 4/12 | 8/12 | 6/12 |
| Heart | Focal myocardiosis | 3/12 | 1/12 | 3/12 | 2/12 | 2/11 | | | | | |
| Liver | Centrilobular fatty degeneration | 4/12 | 9/12 | 7/12 | 1/12[b] | 0/11[b] | 3/12 | 2/12 | 0/12 | 2/12 | 0/12 |
| | Bile ductule proliferation | NRL | NRL | NRL | NRL | NRL | 0/12 | 0/12 | 0/12 | 0/12 | 1/12 |
| Kidney | Cystic nephrosis | NRL | NRL | NRL | NRL | NRL | 2/12 | 0/12 | 1/12 | 1/12 | 0/12 |
| | Chronic pyelonephritis | NRL | NRL | NRL | NRL | NRL | 0/12 | 2/12 | 1/12 | 1/12 | 0/12 |
| Small intestine | GALT proliferation in submucosa | 3/12 | 1/12 | 2/12 | 2/12 | 1/11 | 0/12 | 3/12 | 0/12 | 0/12 | 1/12 |
| Large intestine | GALT proliferation in submucosa | 2/12 | 5/12 | 1/12 | 0/12 | 0/11 | 1/12 | 0/12 | 2/12 | 0/12 | 1/12 |
| Testis | Testicular atrophy | 1/12 | 1/12 | 012 | 0/12 | 0/11 | | | | | |
| | Orchitis | 0/12 | 0/12 | 0/12 | 1/12 | 0/11 | | | | | |
| Adrenal gland | Cortical fatty infiltration | 3/12 | 4/12 | 8/12 | 5/12 | 4/11 | | | | | |

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

[b] significantly different from tragacanth control group ($p$<0.05)

(NRL: No remarkable lesions, BALT: Bronchial associated lymphoid tissue, GALT: Gut associated lymphoid tissue)

## *Discussion*

In the present study, KPE at the dose of 500 mg/kg/day affected the body weight in only male rats after receiving KPE for two months onwards. This finding is in accordance with previous finding in the male rats receiving *K. parviflora* rhizome powder at 2000 mg/kg/day in the sixth month of chronic toxicity study (Chivapat et al., 2004). The significantly lower body weight in the highest dose-treated male group may contribute to the decrease in their food consumption. These alterations indicated that the highest dose of KPE may affect some regulation signal of food intake and metabolism of the animals (Berdanier, 2004). However, there were no overt signs of toxicity and sign and ill health animal found in this group. The increase of relative heart, lung, liver stomach, kidney weights in the highest dose–treated male and female rats including urinary bladder in the male may be due to the decrease of body weight as histopathology of these organs did not show any associated abnormalities. Hematological results revealed a significant decrease in neutrophils and an increase in lymphocytes in the highest dose-treated female group including a decrease of eosinophils in both male and female treated with the highest dose, however these alterations were within the normal range (Gad, 1992). In addition, the decrease of eosinophils in both males and females treated with

the highest dose of KPE was consistent with our previous study (Chivapat et al., 2004). Although the decreases in ALP and triglyceride level in the male rats receiving the highest dose were observed, these findings did not indicate any clinically pathologic states (Stockham and Scott, 2002). The significant decreases in uric acid and potassium levels in the female rats receiving KPE at 50 mg/kg bw/day showed no dose dependency and thus might not be related to KPE. There were increases of BUN and sodium level in the highest dose female group; however these alterations were in the normal range (Gad, 1992). It was found that the highest dose-treated female group had higher glucose level, compared to their control groups whereas our previous report revealed no alterations in glucose level in the female group receiving the highest dose of *K. parviflora* rhizome powder (Chivapat et al., 2004). This discrepancy may be due to the difference in chemical constituents and their contents between KPE and the crude drug. The elevated level of cholesterol of only the highest dose of KPE-treated female rats was similar to that found in the six-month treatment of *K. parviflora* crude powder (Chivapat et al., 2004). The significant decrease of centrilobular fatty degeneration in the liver of the male groups receiving KPE at doses of 50 and 500 mg/kg/day compared to the tragacanth group may be due to KPE. Wu et al. (2006) demonstrated that total flavonoids from the aerial parts of *Laggera alata* possessed both in vitro and in vivo hepatoprotective effects and nine flavonoids such as 3', 4', 5-trihydroxyl-3, 7-dimethoxyflavone, 5-hydroxy-3,3',4',7- tetramethoxy-flavone were isolated. As there are various flavonoids in Krachaidam rhizomes (Sutthanut et al., 2007) and thus some of them might be responsible for hepatoprotective effect. The decrease of large intestinal lymphoid tissue proliferation in the male rats receiving KPE at the dose of 50 and 500 mg/kg/day compared to the tragacanth control group may contribute to anti-inflammatory effect of KPE. It is demonstrated that the ethanolic extract of *K. parviflora* and some of its methoxyflavones exhibit high activity against the release of inflammatory mediators i.e. nitric oxide and PGE2 from LPS-induced macrophage (Tewtrakul and Subhadhirasakul, 2008). Histopathological findings in some organs of all KPE-treated groups were not different from those of both control groups, therefore these may not contribute to KPE.

In conclusion, our chronic toxicity study of KPE in rats at doses of 5, 50 and 500 mg/kg/day indicated that KPE did not produce any overt pharmacotoxic signs and abnormality in hematolo-logical values including most of clinical chemistry values. Moreover, KPE did not cause any histopathological alterations in various studied organs. However, female rats receiving KPE at dose of 500 mg/kg or approximately 100 folds higher than the dose of dried Krachaidam in human use had significantly higher level of glucose and cholesterol whereas the male rats receiving this dose had less food consumption and body weight than their control groups. Hence, this information will be beneficial guidance to consider the appropriate dose level of KPE in further health product developments.

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