EFFECT OF *KAEMPFERIA PARVIFLORA* WALL. EX. BAKER ON SEXUAL ACTIVITY OF MALE RATS AND ITS TOXICITY

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Abstract. *Kaempferia parviflora* Wall. Ex. Baker (Krachaidum) has long been used among Thai men for sexual enhancement. The aim of this study was to determine the effect of *K. parviflora* ethanolic extract on the sexual behavior of male rats and its toxicity. The experiment was divided into three groups of rats given *K. parviflora* extract at doses of 60, 120, and 240 mg/kg BW for 60 days, whilst a control group received distilled water at 1 ml/day per oral. The results showed that all groups of male rats had significantly higher courtship behavior during the first 10-minute period of observation than in the 2nd and 3rd 10-minute periods, except those receiving the highest dose of *K. parviflora*. They revealed the same amount of courtship behavior throughout a whole 30-minute period, which was significantly lower than the control group. There was no significant difference between treated and control groups in other sexual behaviors; mount frequency (MF), intromission frequency (IF), mount latency (ML), or intromission latency (IL). Toxicological study revealed no significant difference of hemoglobin, WBC or differential cell count. All dosages had no effect on kidney and liver function, according to the normal values of blood urea nitrogen (BUN), creatinine (Crea), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Nevertheless, the histopathological study showed a morphological change in the liver. It was concluded that *K. parviflora* extract at 240 mg/kg BW reduced the time in the first 10 mintues of rat courtship behavior and the use of high and chronic doses of *K. parviflora* in humans should be considered inadvisable.

INTRODUCTION

Kaempferia parviflora Wall. Ex. Baker (Krachaidum) is believed to have sexual enhancing activities (Churdboonchart, 2000; Wutythamawech, 2000) and is sometimes referred to as Thai ginseng. Its rhizomes have been used for health promotion, as an antiflatulent, for stomach discomfort, leucorrhea, as a diuretic and antidysenteric, and for the treatment of oral diseases (Chomchalow *et al*, 2003). In an *in vitro* study, the nine flavonoids isolated from the rhizomes of the *K. parviflora* exhibited antiplasmodial, antifungal, and antimycobacterial activities, but no cytotoxicity against KB, BC or NCI-H187 cell lines (Yenjai *et al,* 2004). Although, *K. parviflora* has shown positive effects on the seminal vesicle, spermatogenesis and health safety at doses of 60 and 120 mg/kg for 30 days (Jitjaingam *et al,* 2005), its sex-enhancing properties were challenged. While *K. parviflora* is promoted commercially as having aphrodisiac properties and has long been used among Thai men for sexual enhancement, there are no laboratory results to confirm or support its potential for consumer use.

In this research, we investigated the effects of *K. parviflora* on sexual behavior by video-recording and its toxicology by hematological values, blood chemical tests, and histopathological study, in male albino rats.

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MATERIALS AND METHODS

Preparation of animals

Thirty-one male and 20 female Wistar rats, approximately 200-240 grams body weight and 6 weeks of age were purchased from the National Laboratory Animal Center, Salaya, Nakhon Pathom. Animals were housed in groups (two rats per cage) under an inverted 12:12 hour LD cycle at 25±2 ˚C and with free access to food and water at the Faculty of Science, Chiang Mai University, in a facility for the use and care of laboratory animals.

Preparation of *K. parviflora* extract

K. parviflora was collected from Loei Province, Thailand, and identified by Dr Ratchada Pongsattayapipat, of the Botany section, Queen Sirikit Botanic Garden, Ministry of Natural Resources and Environment, Mae Rim, Chiang Mai. The rhizomes were sliced, dried at 60°C, ground into a fine powder and extracted with 50% ethanol in a Soxhlet apparatus and evaporated by rotary evaporation. The crude extract was diluted in distilled water to the desired concentrations.

Experiment design

The experiment consisted of three groups of rats

administered with *K. parviflora* extract at doses of 60, 120, and 240 mg/kg BW for 60 days, whilst a control group received distilled water at 1 ml/day per oral. The body weights of the rats were recorded weekly during the entire experiment. After 30 and 60 days of the application of each dose, animals were tested for male-rat sexual behavior. At the end of the treatment period, we collected blood and serum for hematological and blood chemistry testing and removed the livers and kidneys for histopathological study.

Female screening

Every morning, between 0800 and 0900 hour, the estrous cycle phases of each female were determined. Vaginal secretions were collected and placed on glass slides. Unstained material was observed under a light microscope, with 10 and 40 x objective lenses. The procedure followed by Norris (1997) and Marcones *et al* (2002) was followed. Each female had an estrous smear mainly consisting of anucleated cornified cells and exhibited good sexual receptivity and no rejection behavior, which was confirmed by Cicero *et al* (2001).

Sexual behavior testing procedure

The male rats' sexual behavior was tested in a rectangular glass observatory cage and a 10-minute adaptation period was allowed. Then, an estrous female was introduced and sexual behavior was recorded during 30 minutes' observation. Video recordings were made throughout the whole period for the following behavioral parameters: courtship behavior, mount latency (ML) and intromission latency (IL), mount frequency (MF) and intromission frequency (IF). Courtship behavior and latencies were expressed in seconds and frequencies displayed the numbers recorded as mean $\pm$ SE.

Blood chemistry and hematological tests

The blood chemistry; aspartate aminotransferase (AST) and alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine (Crea) and hematological tests; complete blood cell count (CBC): hemoglobin, hematocrit, total white blood cell (WBC) and differential cell count were evaluated with the cooperation of the AMS clinical service center, Faculty of Associated Medical Science, Chiang Mai University.

Histopathological study

Small blocks of liver and kidney were fixed in Bouin's solution for paraffin work and sectioned at 6 μm. Hematoxylin and eosin, periodic acid Schiff (PAS) and diastase digestion were used for tissue staining (Gridley, 1960; Prophet *et al,* 1992). All slides were evaluated by a medical pathologist under a light microscope.

Data analysis

To determine statistical significant differences among treatments, the sexual-behavior data were analyzed by one-way ANOVA followed by LSD. SPSS was employed for all statistical analyses.

RESULTS

Sexual behavior

There was no significant change in overall courtship behavior, mount frequency (MF), intromission frequency (IF), mount latency (ML) and intromission latency (IL) in the male rats (Table 1). The sexual behaviors were also observed for three 10-minute periods. All groups of male rats had significantly higher courtship behavior during the first 10 minutes of observation than in the 2nd and 3rd 10 minutes, except those receiving the highest dose of *K. parviflora* (Table 2). It was found that the courtship behavior in the first 10 minutes of observation of the male rats receiving 240 mg/kg BW was significantly lower than the control group, and there was no significant difference in courtship behavior throughout the whole 30-minute period. Male rats treated with 240 mg/kg for 60 days of *K. parviflora* root extract showed a significant reduction in courtship behavior without affecting general body metabolism.

Toxicological study

The results showed no significant difference between body weight gain of the male rats receiving *K. parviflora* extract at doses of 60, 120, and 240 mg/kg BW, and the controls (Fig 1). All groups had a significant increase in body weight. The CBC in all treated groups were not different from the controls (Table 3). Although the hemoglobin (Hb) of male rats receiving doses of 60 mg/kg were significantly lower than the control and the highest dose groups, there was no correlation with the extract dose in the male rats. The male rats in all groups receiving *K. parviflora* extract had no significant difference in ALT, BUN and Crea, whereas the levels of AST of the male rats receiving *K. parviflora* extract at doses of 60 and 240 mg/kg were significantly lower than the control group (Table 4). All groups were at a normal level (Sharp and La Regina, 1998). Gross or microscopic findings of kidneys revealed no association with *K. parviflora* treatment, while the male rats receiving *K. parviflora* extract had an incidence of vacuolar cell hypertrophy in the liver (Figure not shown).

Table 1
Sexual behavior parameters of male rats treated with *K. parviflora* extract.

Groups	Parameters				
	Courtship (mean± SE)	MF (mean± SE)	IF (mean± SE)	ML (mean± SE)	IL (mean± SE)
1 month					
Control (n=7)	550.00±69.91[a]	8.00±2.34[a]	4.83±2.90[a]	144.00±71.41[a]	834.71±342.16[a]
60 mg/kg (n=8)	522.12±44.12[a]	10.00±3.10[a]	19.62±9.11[a]	324.88±180.40[a]	805.75±303.51[a]
120 mg/kg (n=8)	487.38±78.81[a]	10.00±4.80[a]	11.25±6.20[a]	702.00±321.49[a]	1092.00±304.39[a]
240 mg/kg (n=8)	460.88±53.27[a]	2.75±1.06[a]	2.75±2.21[a]	187.62±82.24[a]	1271.88±261.96[a]
2 month					
Control	505.71±57.50[a]	7.14±2.39[a]	11.14±7.31[a]	340.71±247.26[a]	1287.71±330.68[a]
60 mg/kg	429.00±30.10[a]	8.62±2.56[a]	11.62±5.67[a]	563.75±275.43[a]	1011.75±306.20[a]
120 mg/kg	462.25±42.58[a]	12.25±5.14[a]	15.12±9.41[a]	252.12±138.19[a]	955.25±319.60[a]
240 mg/kg	353.62±45.26[a]	8.00±7.58[a]	12.12±7.53[a]	580.00±251.30[a]	1154.75±315.00[a]

There were no significant differences. MF = mounting frequency, IF = intromission frequency, ML = mount latency, IL = intromission latency. ANOVA followed by LSD ($p<0.05$).

Table 2
Time of courtship (seconds) of male rat after 30 days and 60 days, observed during the 1st, 2nd, and 3rd 10-minute observations over a 30-minute period. ANOVA followed by LSD ($p<0.05$).

	Groups			
	Control (mean ± SE)	60 mg/kg (mean ± SE)	120 mg/kg (mean ± SE)	240 mg/kg (mean ± SE)
1 month				
Courtship 1st	303.28±31.91[a]	236.38±24.40[a]	246.88±39.27[a]	223.50±28.90[a]
Courtship 2nd	165.83±49.40[b]	150.12±21.05[b]	133.88±27.79[b]	149.12±11.29[b]
Courtship 3rd	120.00±76.83[b]	136.00±23.37[b]	103.05±20.55[b]	88.25±21.86[b]
2 month				
Courtship 1st	288.00±33.04[a]	212.62±12.69[a]	231.50±20.88[a]	158.12±18.85[b]
Courtship 2nd	104.71±19.63[b]	113.75±14.35[b]	125.75±19.37[b]	105.00±26.63[b]
Courtship 3rd	113.00±19.23[b]	102.62±20.07[b]	105.00±18.07[b]	90.50±29.72[b]

[a,b] The mean differences are significant at the 0.05 level.

DISCUSSION

In the present study, *K. parviflora* was administered to male rats to determine its effects on rat sexual behavior, and its toxicity. No dose of *K. parviflora* produced any significant change in courtship behavior, mount frequency (MF), intromission frequency (IF), mount latency (ML) or intromission latency (IL) in the male rats. The courtship behavior in the first 10-minutes' observation of the male rats receiving 240 mg/kg BW was significantly lower than the control group. These results showed that *K. parviflora* extract did not enhance the sexual behaviors (courtship behavior, MF, IF, ML and IL) of male rats. In addition, it reduced courtship behavior, which involves complex sequencing of motor patterns and multisensory stimulation; without this pre-copulatory behavior, copulation may not occur because of inadequate stimulation received by the performance of the female (Knobil and Neill, 1998). However, this plant may affect the rats in other conditions, as reported in experiments with other plant species in non-copulating male rats (Ang and Ngai, 2001), the rats with impotent symptoms (Carro-Jua´rez *et al,* 2004), or different fractions of substance in differently aged

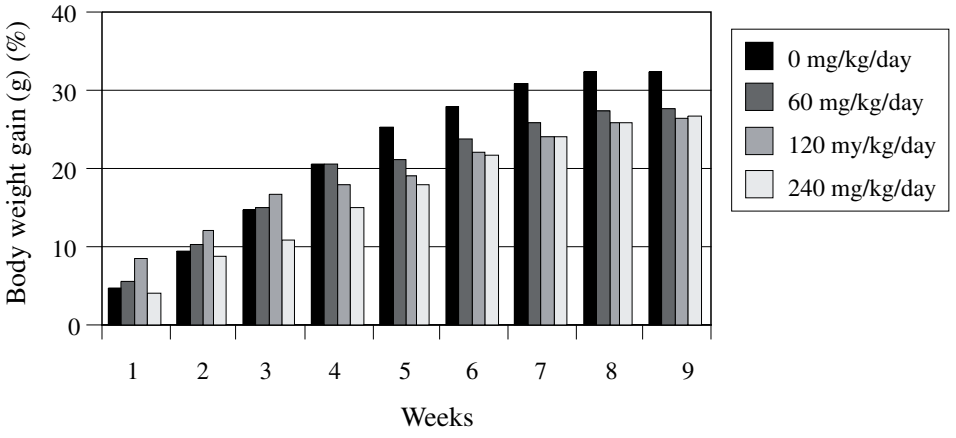

Fig 1- Average body weight gain (%) of male rats treated with *K. parviflora* for 60 days. Each point represents average body weight gain (%) at the end of each week.

Table 3
Hematological examinations of male rats treated with *K. parviflora* extract during 60 days, compared with control. ANOVA followed by LSD ($p<0.05$).

Parameters	Group of male rats			
	Control (mean ± SE)	60 mg/kg (mean ± SE)	120 mg/kg (mean ± SE)	240 mg/kg (mean ± SE)
Hemoglobin (g/dl)	15.88±0.22[ab]	14.92±0.23[c]	15.54±0.25[ac]	16.48±0.34[b]
Hematocrit (%)	44.40±1.29[a]	42.00±0.55[a]	42.40±1.08[a]	45.40±1.57[a]
WBC (mm^3)	3,240.00±963.64[a]	4,740.00±1,013.21[a]	4,600.00±1,176.44[a]	3,780.00±1,071.17[a]
Neutrophil (%)	25.00±8.41[ab]	23.20±7.00[ab]	8.20±2.03[a]	48.60±13.31[b]
Eosinophil (%)	0.00+0.00[a]	0.80+0.37[a]	0.40+0.24[a]	0.20+0.20[a]
Basophil (%)	0.00+0.00[a]	0.00+0.00[a]	0.00+0.00[a]	0.80+0.50[a]
Lymphocyte (%)	73.78+8.83[ab]	73.40+7.30[ab]	89.00+2.32[b]	48.00+13.23[a]
Monocyte (%)	1.20+0.58[a]	2.60+0.51[a]	2.20+0.73[a]	2.40+0.68[a]

[a,b,c] The mean differences are significant at the 0.05 level.

Table 4
Blood chemistry of male rats treated with *K. parviflora* extract during 60 days, compared with control. ANOVA followed by LSD ($p<0.05$).

Parameters	Group of male rats			
	Control (mean ± SE)	60 mg/kg (mean ± SE)	120 mg/kg (mean ± SE)	240 mg/kg (mean ± SE)
BUN (mg/dl)	27.40±2.23[a]	31.81±0.73[a]	27.40±1.17[a]	29.20±0.58[a]
Creatinine (mg/dl)	0.66±0.04[a]	0.78±0.06[a]	0.76±0.05[a]	2.92±2.27[a]
AST (IL/l)	176.20±28.96[a]	95.39±9.57[b]	132.60±16.70[ab]	97.20±11.26[b]
ALT (IL/l)	41.00±3.16[a]	36.60±2.66[a]	45.20±9.80[a]	36.60±2.48[a]

[a,b] The mean difference is significant at the 0.05 level.

animals (Ang *et al,* 2003).

Although *K. parviflora* has been known as a health-promoting herb (Wutythamawech, 2000; Chomchalow *et al*, 2003), there was no significant variation in food consumption between control and treatment groups (data not shown), which indicated that *K. parviflora* extract in these conditions did not affect overall metabolism or any of the enzymatic processes occurring within the cells of the body (Norris, 1997). CBC, ALT, BUN and Crea in all treated groups were not different from the controls. Although the hemoglobin (Hb) of male rats receiving doses of 60 mg/kg were significantly lower than the control and the highest-dose groups, the AST of the male rats receiving *K. parviflora* extract at doses of 60 and 240 mg/kg were lower than the control group. There was no correlation with dose of extract in the male rats, and the *K. parviflora* extract was not likely cause the change in Hb and AST. All parameters were within the normal range (Sharp and La Regina, 1998). The above indicate that liver and kidney function were not affected.

Gross or microscopic findings of the kidney had no association with *K. parviflora* treatment, but the male rats receiving *K. parviflora* extract had an incidence of hyalinized intracytoplasmic vacuole of the liver cells, which can be drawn upon to maintain normal glucose concentration in the blood (Fawcett, 1994). Nevertheless, periodic acid Schiff (PAS) and diastase digestion methods revealed the resistance of diastase digestion material, indicating that the substance accumulated in the vacuole was not glycogen. This pathologic finding was the only effect on liver morphology. All animals survived with normal growth throughout the experimental period. Due to a lower dose given in a sub-chronic time, the results may not have had an opportunity to make themselves fully apparent, both physically and morphologically. Some studies found high toxicity, such as in *Pueraria mirifica* (Sanchanta *et al*, 2005) and gallic acid (Niho *et al*, 2001), which were used at a higher dosage and for a longer duration. These findings revealed that *K. parviflora* did not have sex-enhancing properties in male rats. Moreover, the histopathological study showed a tendency towards hepatotoxicity. Further investigation involving chronic toxicity with high doses of *K. parviflora* is necessary.

In conclusion, ethanolic extract of *K. parviflora* did not enhance the sexual behavior of male rats, and may cause hepatotoxicity at the morphological level. However, hematological parameters and blood chemistry examination showed no significant differences between the treated and control groups. The use of high and chronic doses of KP in humans should be considered.

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