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Toxicity of crude rhizome extract of *Kaempferia galanga* L. (Proh Hom)

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Abstract

The ethanolic rhizome extract of *Kaempferia galanga* L. (Zingiberaceae) was studied by conventional pharmacological methods including the Hippocratic screening test, and acute and subacute toxicities in rats. The hexane fraction was tested for dermal irritation in rabbits. The ethanolic extract, when tested by the Hippocratic screening test, demonstrated signs that indicated CNS depression such as a decrease in motor activity and respiratory rate, and a loss of screen grip and analgesia. In the acute toxicity test, oral administration of 5 g/kg of *Kaempferia galanga* produced neither mortality nor significant differences in the body and organ weights between controls and treated animals. Moreover, both gross abnormalities and histopathological changes were not comparatively detectable between all controls and treated animals of both sexes. In subacute toxicity studies, no mortality was observed when varying doses of 25, 50 or 100 mg/kg of ethanolic *Kaempferia galanga* extract were administered orally per day for a period of 28 days. There were no significant differences in the body and organ weights between controls and treated animals of both sexes. Hematological analysis showed no differences in any of the parameters examined (WBC count, platelet, hematocrit and hemoglobin estimation) in either the control or treated groups of both sexes. However, the differential leukocyte counts showed a slight but significant decrease of lymphocyte count in the 50 and 100 mg/kg male rat groups. In the blood chemistry analysis, no significant change occurred in the blood chemistry parameters, including glucose, creatinine, blood urea nitrogen (BUN), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (Alk-P), total protein and albumin of both sexes. Pathologically, neither gross abnormalities nor histopathological changes were observed. No sign of irritation was observed during the dermal irritation test of the hexane fraction of *Kaempferia galanga*.

Keywords: *Kaempferia galanga*; The Hippocratic screening test; Acute and subacute toxicities in rats; Dermal irritation test in rabbits

1. Introduction

Kaempferia galanga (“Proh hom” in Thai; Zingiberaceae) is an acaulescent perennial that grows in Southern China, Indochina, Malaysia and India. The rhizomes of the plant, which contains essential oils, have been used in a decoction or powder for indigestion, cold, pectoral and abdominal pains, headache and toothache. Its alcoholic maceration has also been applied as liniment for rheumatism (Keys, 1976; Lieu, 1990). In Chinese medicine, *Kaempferia galanga* rhizomes have been used as an aromatic stomachic, and also as incense. The constituents of this rhizome, hitherto reported, have included cineol, borneol, 3-carene, camphene, kaempferol, kaempferide, cinnamaldehyde, *p*-methoxycinnaamic acid, ethyl cinnamate, and ethyl *p*-methoxycinnamate (Nakao and Shibu, 1924). Ethyl *p*-methoxycinnamate was reported to inhibit monoamine oxidase (Noro et al., 1983). The methanolic extract of *Kaempferia galanga*, which identified as ethyl cinnamate, ethyl *p*-methoxycinnamate and *p*-methoxycinnamic acid, showed larvicidal activity against the second stage larva of dog roundworm, *Toxocara canis* (Kiuchi et al., 1988). Evaluation for amebicidal activity in vitro against three species of *Acanthamoeba*: *Acanthamoeba culbertsoni*, *Acanthamoeba castellanii*, and *Acanthamoeba polyphaga*; the causative agents of granulomatous amebic encephalitis and amebic keratitis, found that the *Kaempferia galanga* extract possessed an effective amebicidal for all three species (Chu et al., 1998). Vimala et al. (1999) found that the rhizome extract of *Kaempferia galanga* exhibited Epstein-Barr virus (EBV) activiation inhibitory activity

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when screened for anti-tumour promoter activity using the short-term assay of inhibition of 12-*O*-tetradecanoyl phorbol-13-acetate (TPA)-induced EBV early antigen in Raji cells.

Recently, Pitasawat et al. (1998) demonstrated significant larvicidal activity against *Culex quinquefasciatus* in three of ten plant extracts, including those from *Kaempferia galanga*, *Illicium vernum* and *Spilanthes acmella*, which had LC_{50}values of 50.54, 54.11 and 61.43 ppm, respectively. Subsequently, larvicidal and repellent activities of *Kaempferia galanga* fractions, i.e. the hexane fraction, dichloromethane fraction 1, dichloromethane fraction 2, and methanolic fraction have been affirmed by Choochote et al. (1999). They declared that the hexane fraction possessed larvicidal potency against *Culex quinquefasciatus* (LC_{50} = 42.33 ppm), repelled *Aedes aegypti* (ED_{50} = 30.73 ug/cm^2), and provided biting protection for 3 h in the laboratory. It could also protect in the field against *Armigeres subalbatus*, *Anopheles barbirostris*, *Anopheles aconitus*, *Mansonia uniformis*, *Culex quinquefascitus*, *Culex gelidus*, *Culex tritaeniorhynchus* and *Aedes aegypti*. Additionally, it did not cause dermal irritation when applied on human skin. Before applying the effective formula of *Kaempferia galanga* for mosquito control, it is important to evaluate the toxic effects of the extract, which is essential when a safe dose has to be selected. Therefore, the present study was initiated for analysing the acute and subacute toxicites and dermal irritation of the extract in laboratory animals.

2. Materials and methods

2.1. Laboratory animals

Adult Sprague–Dawley rats of either sex, aged 6–8 weeks with a weight of 250–300 g were purchased from the National Laboratory Animal Center, Salaya Mahidol University, Nakorn Pathom, Thailand. Adult albino rabbits of either sex, weighing between 4 and 5 kg were obtained from the Animal Unit of the Faculty of Medicine, Chiang Mai University, Thailand. The animals were kept in an animal room where the temperature was maintained at 22 ± 3 °C under a 12 h light–dark cycle. They were provided with food and water ad libitum for 1 week to acclimatize them before starting the experiment.

2.2. Preparation of plant extract

The rhizomes of *Kaempferia galanga* were purchased in Chiang Mai province, Thailand. Voucher specimen (PHCO-CM-012) was deposited at the Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Thailand. The extraction was performed by macerating 1.5 kg of dried and powdered *Kaempferia galanga* with 5 l of hexane or 95% ethanol at room temperature for 2 days. After suction filtering through a Buchner funnel, the hexane or ethanolic filtrates were evaporated by a rotary evaporator at 40–60 °C, and then lyophilized to yield hexane or ethanolic extracts. Extracts isolated from *Kaempferia galanga* were kept at −20 °C until testing.

2.3. Hippocratic screening test

The effect of the ethanolic extract of *Kaempferia galanga* on the general behavior of conscious animals was evaluated in rats, as described by Malone and Robichaud (1962). Intraperitoneal administration of five dose levels of test substance suspended in 5% Tween 80 were given to groups of nonfasted rats. A control group received an equal volume of this vehicle. Two females and two males from a total of 24 animals were used for each dosage level. Signs and symptoms induced by the test substance were recorded prior to administration. Then again at 5, 15, and 30 min, and 1, 2, and 24 h after administration, and once daily thereafter for 7 days. The rats that died within 24 h were autopsied and recorded for pathological changes. On the seventh day after treatment, the live animals were sacrificed for the examination of their internal organs (heart, lungs, liver, spleen, kidneys, adrenals, testes, ovaries, uterus, thymus, brain, eyes, stomach, intestines, etc.) for abnormal signs. Any changes in their vital organs compared with those of the control animals were recorded.

2.4. Acute toxicity studies

Ethanolic extract of *Kaempferia galanga* suspended in 5% Tween 80 was administered to the groups of rats in a single oral dose by gavage using a feeding needle (at least three doses). The control group received an equal volume of the 5% Tween 80 vehicle. Ten females and ten males were used for each dosage level. They were deprived of food, but not water 16–18 h prior to the administration of the test suspension. Observations of toxic symptoms were made and recorded systematically at one, two, four and six hours after administration. Finally, the number of survivors was noted after 24 h and these animals were then maintained for a further 13 days with observations made daily. At the conclusion of the experiment, all surviving animals were sacrificed with an injection of pentobarbital and their organs such as liver, lungs, heart, spleen, adrenals, kidneys, testes and ovaries were excised and weighed. The pathological observations of these tissues were performed on gross and microscopic bases. Histological examinations were performed on the preserved tissues with particular emphasis on those which showed gross pathological changes.

The toxicological effect was assessed on the basis of mortality, which was expressed as LD_{50}. If a test at one dose level of at least 5 g/kg body weight produced no compound-related mortality, then a full study using three dose levels might not be necessary.

2.5. Subacute toxicity

The animals were divided into five groups of eight females and eight males totaling 80 rats. Ethanolic extract of *Kaempferia galanga* suspended in 5% Tween 80 was administered orally by gavaging at three dose levels of the extract daily to three groups of rats for a period of 28 days. The control group received an equal volume of the 5% Tween 80 vehicle. In order to assess reversibility, an alcoholic extract of *Kaempferia galanga* was administered to a group of rats at 100 mg/kg daily for 28 days, with no treatment for the following 14 days. All rats were weighed and observed daily for physiological and behavioral responses. Any rats that died during the test period were tested pathologically, and all animals were examined at the end of the test period.

2.6. Parameters

2.6.1. Blood analysis

All surviving animals fasted overnight and were anesthetized afterwards for blood collection from a common carotid artery. Blood samples were collected into heparinized and dry non-heparinized centrifuge tubes. A blood analysis (both hematology and chemistry) was carried out. The heparinized blood was used for a hematological study which included WBC and differential leukocyte counts, platelet, hematocrit and hemoglobin estimation. The non-heparinized blood was allowed to coagulate before being centrifuged and the serum was separated. The serum was assayed for glucose, creatinine, blood urea nitrogen (BUN), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatases (Alk-P), total protein and albumin.

2.6.2. Tissue analysis

Immediately after the blood collection, the animals were sacrificed for tissue studies. The organs such as liver, lungs, heart, spleen, adrenals, kidneys, testes and ovaries were removed, blotted free of blood and weighed immediately on a Precisa electronic balance for subsequent analysis. Eyes, brain, thymus, intestines, uterus, epididymis, seminal vesicles, prostate glands, and thoracic spine and muscle with the sciatic nerve were also observed. Histological examinations were performed on the preserved tissues with particular emphasis placed on those that showed gross pathological changes.

2.7. Dermal irritation test

In the present study, determination of the effect of the hexane fraction of *Kaempferia galanga* on the skin was conducted to examine the potential for producing extensive tissue damage or corrosions. The method was performed according to the slightly modified procedure of OECD Test Guidelines (OECD, 1981). Six adult albino rabbits (three males, three females) with healthy, intact skin were used. Approximately 24 h before the test, their fur was shaved off the dorsal area of their trunk. Care had to be taken to avoid abrading the skin.

A 0.5 ml dose of 250 mg/ml hexane fraction of *Kaempferia galanga* dissolved in absolute ethanol was applied to a small area (2 cm × 3 cm) of the test site, which was then covered with a gauze patch. The patch was held in place with non-irritating tape. The animal accessability to the patch with resulting ingestion/inhalation of the test substance had to be prevented. A symmetrical test site area for each animal served as a control and was applied in an equal volume of absolute ethanol. Croton oil was used as a positive control. Exposure duration was 4 h. At the end of the exposure period, the residual test substance was removed, where practicable, using absolute ethanol or an appropriate solvent without altering the existing response or the integrity of the epidermis. The animals were examined for signs of erythema, edema and the responses were scored at 30–60 min, and then at 24, 48 and 72 h after patch removal. Further observations might be needed, as necessary, to establish reversibility.

2.8. Statistical analysis

Either the analysis of variance (ANOVA) or Student's *t*-test (SPSS/PC computer program) was employed to analyze the results statistically. A statistical comparison was carried out using the Duncan Multiple Range Test. All values were expressed as back transformed mean ± S.D. Differences below the probability level of 0.05 were considered statistically significant.

3. Results

3.1. Hippocratic screening test

The general behavioral changes of the rats were observed following intraperitoneal injections of the ethanolic extract of *Kaempferia galanga* at 25, 100, 250, 800 and 2000 mg/kg doses, which were graded through time. Doses of 25, 100 and 250 mg/kg did not cause any detectable changes, whereas, a dose of 2000 mg/kg seemed to be lethal and caused two out of four rat deaths within 24 h. Signs and symptoms, which occurred in response to *Kaempferia galanga* extract, decreased in motor activity and respiration. A loss of screen grip was observed when high doses (800 and 2000 mg/kg) were given. A 2000 mg/kg dose of *Kaempferia galanga* extract caused analgesia. The intensity of responses grew with increasing doses, and the effects of *Kaempferia galanga* extract persisted more for than 2 h. The rats that died from a high dose (2000 mg/kg) of *Kaempferia galanga* extract showed signs of respiratory failure (decreased respiratory rate and irregular breathing) before death. The internal organs of both controlled and treated groups did not show any unusual signs and were found to be normal in both size and color.

Table 1
Body and organ weights (g) of rats treated with ethanolic extract of *Kaempferia galanga* in an acute toxicity

	Control (5% Tween 80)	*Kaempferia galanga* (5 g/kg)
Body weight		
Male		
Initial	303 ± 24	287 ± 28
Final	369 ± 20	347 ± 27
Increased (%)	22.10 ± 6.29	21.46 ± 9.66
Female		
Initial	237 ± 24	231 ± 15
Final	260 ± 26	239 ± 13
Increased (%)	9.65 ± 4.48	3.68 ± 6.49
Organ weight		
Male		
Lung	1.314 ± 0.263	1.276 ± 0.218
Heart	1.32 ± 0.084	1.283 ± 0.078
Liver	10.541 ± 0.813	10.627 ± 1.821
Spleen	0.779 ± 0.105	0.744 ± 0.067
Adrenal	0.026 ± 0.003	0.025 ± 0.003
Kidney	1.269 ± 0.085	1.227 ± 0.106
Testis	1.978 ± 0.173	1.820 ± 0.137
Female		
Lung	1.090 ± 0.114	1.212 ± 0.191
Heart	1.029 ± 0.057	0.965 ± 0.051
Liver	6.563 ± 1.084	6.090 ± 0.984
Spleen	0.627 ± 0.052	0.560 ± 0.032
Adrenal	0.028 ± 0.004	0.032 ± 0.004
Kidney	0.874 ± 0.071	0.862 ± 0.038
Ovary	0.046 ± 0.010	0.038 ± 0.009

Data are expressed as mean ± S.D., $n = 10$. No statistical difference between control and *Kaempferia* group ($P > 0.05$).

3.2. Acute toxicity

No death was recorded during the treatment period in either the control or treated groups given 5 g/kg of ethanolic extract of *Kaempferia galanga* orally. The animals did not show any changes in general behavior or other physiological activities. There were no significant differences between the control and treated groups in the body and organ weights of male and female rats (Table 1). Moreover, there was no significant difference in the testicular weight of male rats and, similarly, there was no significant difference in the ovarian weight of female rats. Pathological examinations of the tissues on a gross and microscopic basis indicated that there were no detectable abnormalities. No pathological alterations were grossly detected. The organs of both control and treated groups were unremarkable and comparable to each sex. No further evidence of histopathological changes were seen. The architectures of the internal organs examined and their cellular appearances were comparatively unremarkable in both groups and sexes.

3.3. Subacute toxicity

No death was recorded during the treatment period in either the control or treated groups. The animals did not show any changes in general behavior or other physiological activities. The body and organ weights of the male and female rats, which were administered an alcoholic extract of *Kaempferia galanga* at 25, 50 or 100 mg/kg doses daily for 28 days, are given in Table 2. There were no significant differences in the body and organ weights between control and treated animals of both sexes. Similarly, a withdrawal from *Kaempferia galanga* treatment produced no significant changes in both male and female rats.

3.4. Hematological and biochemical observations

The hematological analysis (Table 3) showed no significant differences in any of the parameters examined in either the control or treated groups of both sexes. However, the differential leukocyte counts (Table 4) showed a slight but significant decrease of lymphocyte count in the 50 and 100 mg/kg male rat groups with no significant differences in neutrophils, eosinophils and monocytes in all male rat groups. In contrast, there were no significant changes in any of the differential leukocyte counts in female rats. Blood chemistry analysis (Table 5) revealed no significant changes in any of the parameters examined in either the control or treated groups of both sexes.

In the separated groups, the ethanolic extract of *Kaempferia galanga* was administered at 100 mg/kg daily for 28 days followed by a treatment-free period of 14 days, when a slight but significant decrease in hemoglobin (HGB) was observed in male rats (Table 3). In accordance with the previous 28 days treatment, the decrease of lymphocyte count was still significant in the male rats that had been withdrawn from treatment. Moreover, a significant increase in neutrophil count, with no marked changes in the other parameters, was observed. However, there was no significant change in any of the leukocyte counts in female rats (Table 4). Surprisingly, the rats that had been withdrawn from treatment displayed a significant decrease in BUN, protein, and Alk-P in males and a significant fall of AST in females (Table 5). However, no gross abnormalities and no further evidence of histopathological changes were seen in all control and treated rats of both sexes.

3.5. Dermal irritation test

Dermal application of 250 mg/ml hexane fraction of *Kaempferia galanga* dissolved in absolute ethanol produced no irritation in both male and female rabbits. No signs of erythema, eschar and edema were observed. No other reactions were seen during the test. In the case of the positive controls that had been treated with croton oil, well-defined erythema and moderate edema were observed in both male and female rabbits from 24 to 72 h after application. No differences in the degree of skin irritation were seen when male and female rabbits were compared.

Table 2
Body and organ weights (g) of rats treated with ethanolic extract of *Kaempferia galanga* in a subacute toxicity

	Control (5% Tween 80)	*Kaempferia galanga* (mg/kg)			
		25	50	100	100[a]
Body weight					
Male					
Initial	242 ± 12	233 ± 8	227 ± 12	228 ± 9	238 ± 9
Final	279 ± 19	268 ± 18	263 ± 13	271 ± 20	343 ± 11
Increased (%)	18 ± 6	18 ± 8	19 ± 9	19 ± 7	–
Female					
Initial	211 ± 13	216 ± 8	210 ± 16	212 ± 8	211 ± 8
Final	224 ± 13	237 ± 17	234 ± 12	238 ± 12	251 ± 14
Increased (%)	8 ± 6	10 ± 6	12 ± 10	12 ± 8	–
Organ weight					
Male					
Lung	1.070 ± 0.116	1.238 ± 0.453	0.919 ± 0.206	1.016 ± 0.046	1.299 ± 0.440
Heart	1.058 ± 0.128	1.042 ± 0.117	0.982 ± 0.107	1.069 ± 0.104	1.228 ± 0.099
Liver	8.672 ± 1.899	8.066 ± 1.458	8.167 ± 2.292	8.320 ± 1.900	9.632 ± 0.705
Spleen	0.599 ± 0.084	0.554 ± 0.049	0.592 ± 0.149	0.587 ± 0.086	0.772 ± 0.083
Adrenal	0.024 ± 0.003	0.023 ± 0.002	0.025 ± 0.004	0.024 ± 0.002	0.024 ± 0.004
Kidney	1.056 ± 0.078	0.994 ± 0.062	1.014 ± 0.055	1.020 ± 0.100	1.274 ± 0.146
Testis	1.742 ± 0.113	1.777 ± 0.094	1.722 ± 0.080	1.744 ± 0.112	1.831 ± 0.135
Female					
Lung	0.993 ± 0.061	1.004 ± 0.087	1.015 ± 0.131	1.174 ± 0.300	0.998 ± 0.092
Heart	0.909 ± 0.081	0.857 ± 0.062	0.897 ± 0.102	0.929 ± 0.094	1.006 ± 0.096
Liver	5.399 ± 2.458	6.203 ± 1.130	6.328 ± 2.178	6.171 ± 0.993	7.130 ± 1.715
Spleen	0.531 ± 0.061	0.511 ± 0.037	0.543 ± 0.028	0.534 ± 0.099	0.574 ± 0.046
Adrenal	0.033 ± 0.007	0.028 ± 0.004	0.031 ± 0.004	0.028 ± 0.004	0.029 ± 0.003
Kidney	0.792 ± 0.055	0.849 ± 0.052	0.801 ± 0.069	0.851 ± 0.073	0.899 ± 0.095
Ovary	0.056 ± 0.006	0.060 ± 0.010	0.058 ± 0.014	0.067 ± 0.011	0.046 ± 0.010

Data are expressed as mean ± S.D., $n = 8$. No statistical difference between the control and *Kaempferia* group ($P > 0.05$).
[a] A separate group was administered at 100 mg/kg daily for 28 days followed by no treatment for 14 days.

Table 3
Hematological values of rats treated with ethanolic extract of *Kaempferia galanga* in a subacute toxicity

	Control (5% Tween 80)	*Kaempferia galanga* (mg/kg)			
		25	50	100	100[a]
Male					
WBC ($\times 10^6$/ml)	4.99 ± 1.38	4.30 ± 0.88	4.94 ± 1.97	4.70 ± 1.09	6.14 ± 1.02
HGB (g/dl)	16.30 ± 0.78	16.27 ± 0.63	16.10 ± 0.55	16.40 ± 0.83	15.20 ± 0.60*
HCT (%)	49.12 ± 2.75	48.57 ± 2.22	47.75 ± 2.05	48.38 ± 3.89	46 ± 2
PLT ($\times 10^6$/ml)	971 ± 72	1102 ± 495	878 ± 116	948 ± 128	980 ± 158.70
Female					
WBC ($\times 10^6$/ml)	3.26 ± 1.12	3.58 ± 0.91	3.99 ± 1.22	4.24 ± 1.68	3.01 ± 1.01
HGB (g/dl)	15.26 ± 0.62	15.25 ± 0.73	15.71 ± 0.73	15.50 ± 0.49	14.78 ± 0.69
HCT (%)	44.88 ± 1.25	44.75 ± 2.71	45.38 ± 1.85	45.43 ± 1.72	44.50 ± 2.14
PLT ($\times 10^6$/ml)	905 ± 153	957 ± 139	939 ± 308	881 ± 177	895.38± 129.61

Data are expressed as mean ± S.D., $n = 8$.
[a] A separate group was administered at 100 mg/kg daily for 28 days followed by no treatment for 14 days.
* Significantly different from the control ($P < 0.05$).

4. Discussion

In toxicity studies, including the Hippocratic screening test, acute, subacute and dermal toxicities were elucidated in small laboratory animals. Ethanolic extract of *Kaempferia galanga* was used in most of these tests with the exception of the dermal irritation test, since this form was convenient to prepare, could be easily applied, and its larvicidal potency ($LD_{50} = 50.54$ ppm) was comparable to that of the hexane fraction ($LD_{50} = 42.33$ ppm) (Choochote et al., 1999). In the dermal irritation test, however, it was necessary to use the same form (hexane fraction) as that used for skin application in both laboratory and field repellent tests (Choochote et al., 1999).

The Hippocratic screening test is commonly used in the preliminary screening of medicinal plants to detect inter-

Table 4
Differential white blood cell count of rats treated with ethanolic extract of *Kaempferia galanga* in a subacute toxicity

	Control (5% Tween 80)	*Kaempferia galanga* (mg/kg)			
		25	50	100	100[a]
Male					
Neutrophil	3 ± 2	3 ± 2	5 ± 3	4 ± 3	8 ± 3*
Eosinophil	1 ± 1	1 ± 2	2 ± 2	1 ± 1	1 ± 1
Lymphocyte	95 ± 3	95 ± 3	89 ± 4*	91 ± 5*	89 ± 6*
Monocyte	1 ± 1	1 ± 1	2 ± 1	2 ± 1	1 ± 1
Female					
Neutrophil	6 ± 4	6 ± 3	8 ± 5	4 ± 2	7 ± 3
Eosinophil	1 ± 1	2 ± 2	3 ± 6	2 ± 4	1 ± 1
Lymphocyte	90 ± 6	89 ± 6	85 ± 10	91 ± 6	90 ± 6
Monocyte	2 ± 1	3 ± 2	2 ± 1	2 ± 2	2 ± 1

Data are expressed as mean ± S.D., $n = 8$.
[a] A separate group was administered at 100 mg/kg daily for 28 days followed by no treatment for 14 days.
* Significantly different from the control ($P < 0.05$).

esting pharmacological activities (Malone and Robichaud, 1962). The test has revealed some pharmacological activities of *Kaempferia galanga*, which are observed from drug-induced signs and symptoms. According to the screening study, the ethanolic extract of *Kaempferia galanga* has been found to cause a dose-related decrease in motor activity and respiration, and a loss of screen grip and analgesia. These effects, therefore, suggest a CNS depressant activity. However, a loss of the righting reflex has not been observed. This indicates that the extract possesses some selective CNS depressant or sedative activity. The loss of screen grip can be taken as an indication for skeletal muscle relaxant activity, of which, the site of action can be peripheral (at neuromuscular junction) or central. The extract tested shows an analgesic activity at the highest dose (2000 mg/kg). It is possible that the CNS depression and paralysis of skeletal muscle, which are caused by the extract, tend to modify the response to pain stimulation.

Following a dose of 2000 mg/kg of ethanolic extract, given intraperitoneally, two of four rats died within 24 h. Marked respiratory depression (decrease respiratory rate and irregular breathing) was found to occur before death. This respiratory failure could be due to central or peripheral action. Centrally, the failure could be due to a CNS depression, whereas, peripherally the failure could possibly be due to an inhibitory action at the neuromuscular junction.

Based on the results in an acute toxicity study, it was concluded that a dose of 5 g/kg of ethanolic extract of

Table 5
Blood chemistry values of rats treated with ethanolic extract of *Kaempferia galanga* in a subacute toxicity

	Control (5% Tween 80)	*Kaempferia galanga* (mg/kg)			
		25	50	100	100[a]
Male					
Glucose (mg/dl)	144 ± 19	145 ± 19	133 ± 30	157 ± 68	160 ± 20
BUN (mg/dl)	21 ± 4	21 ± 4	22 ± 4	22 ± 4	15 ± 2*
Creatinine (mg/dl)	0.54 ± 0.07	0.49 ± 0.08	0.51 ± 0.14	0.56 ± 0.13	0.42 ± 0.12
Protein (g/dl)	5.5 ± 0.5	5.4 ± 0.2	5.3 ± 0.4	5.5 ± 0.5	4.9 ± 0.3*
Albumin (g/dl)	2.7 ± 0.5	2.6 ± 0.5	2.4 ± 0.2	2.6 ± 0.4	2.6 ± 0.5
AST (S.F. unit)	120 ± 14	116 ± 15	118 ± 12	138 ± 28	99 ± 27
ALT (S.F. unit)	42 ± 6	47 ± 12	45 ± 14	43 ± 23	35 ± 6
Alk-P (B.L.B. unit/l)	233 ± 84	242 ± 51	215 ± 54	196 ± 95	135 ± 25*
Female					
Glucose (mg/dl)	140 ± 42	128 ± 39	141 ± 28	137 ± 35	168 ± 28
BUN (mg/dl)	19 ± 5	19 ± 4	19 ± 6	20 ± 6	19 ± 6
Creatinine (mg/dl)	0.50 ± 0.15	0.51 ± 0.06	0.51 ± 0.14	0.53 ± 0.12	0.55 ± 0.09
Protein (g/dl)	5.4 ± 0.4	5.4 ± 0.6	5.5 ± 0.5	5.6 ± 0.4	5.6 ± 0.5
Albumin (g/dl)	2.7 ± 0.6	2.5 ± 0.2	2.8 ± 0.6	2.5 ± 0.99	2.5 ± 0.8
AST (S.F. unit)	123 ± 17	118 ± 20	107 ± 16	112 ± 6	78 ± 7*
ALT (S.F. unit)	41 ± 20	28 ± 6	29 ± 12	31 ± 7	27 ± 7
Alk-P (B.L.B. unit/l)	168 ± 123	124 ± 80	136 ± 81	105 ± 38	135 ± 75

Data are expressed as mean ± S.D., $n = 8$.
[a] A separate group was administered at 100 mg/kg daily for 28 days followed by no treatment for 14 days.
* Significantly different from the control ($P < 0.05$).

Kaempferia galanga, given orally, appeared to be preferably non-toxic. This is in accordance with the study of Mokkhasmit et al. (1971), who administered 50% ethanolic extract of *Kaempferia galanga* to groups of Yenken Denken Tokyo mice. They reported that 10 g/kg of *Kaempferia galanga* extract, which was administered by oral and subcutaneous routes, did not produce any toxic symptoms. There must be a point, however, at which it can be concluded that a test substance is practically non-toxic or non-lethal after an acute exposure. This test limit for acute oral toxicity is generally considered to be 5.0 g/kg body weight. If no mortality is observed at this dose level, a higher dosage is generally not necessary (Hayes, 1989).

In a subacute toxicity study, it appeared that the ethanolic extract of *Kaempferia galanga* at a dose of 25 mg/kg did not produce any marked changes in both male and female rats, as evidenced by the parameter examined. A dose of 50 and 100 mg/kg, which was administered for 28 days, caused a decrease in the lymphocyte count. Although the decrease was statistically significant, it might not have had clinical relevance. The decrease in lymphocyte count was still evident after 14 days in males that were maintained without treatment, thus suggesting a lack of reversibility in this parameter. It is difficult to explain why, upon the removal of *K galanga* extract administration from rats, the neutrophil count was increased in the male ones only. However, bacterial infection may have occurred during the experimental period. In the same way, the significant changes in some of the blood chemistry parameters in both male (BUN, protein and Alk-P) and female rats (AST), which had been withdrawn from treatment for 14 days, were very doubtful. Even though the changes noted were slight, they were statistically different from the control. It is important to stress that the significant changes seen were mild in nature, but it should be borne in mind that these changes did occur. However, at present the clinical relevance of the findings noted upon the removal of *Kaempferia galanga* extract are not known and warrants a more extensive study.

The dermal irritation test must be performed for further human safety in case of substance exposure. Animals have been used to assess dermal irritation by the observation of visible changes ranging from erythema and edema to corrosion and ulceration. Information that derives from tests for dermal irritation serves to identify the possible risk to the population who use and are exposed to substances such as mosquito repellents. In the present study, the treatment of hexane fraction produced no signs of irritation. It also produced no irritation in human volunteers in both laboratory and field repellent studies (Choochote et al., 1999). This finding indicates that hexane fraction of *Kaempferia galanga* can be categorized as a ‘non-irritant’.

It can be concluded that *Kaempferia galanga* demonstrates less toxicity, but it is considered as an effective botanical insecticide with high larvicidal activity and a protective effect against mosquitoes (Choochote et al., 1999). Chu et al. (1998) found that *Kaempferia galanga* extract exhibited amebicidal activity in vitro against three species of *Acanthomoeba*: *Acanthamoeba culbertsoni*, *Acanthamoeba castellanii*, and *Acanthamoeba polyphaga*, that were not lytic for normal macrophage cultures. Similary, the rhizome extract of *Kaempferia galanga* exhibited Epstein-Barr virus activiation inhibitory activity that had no cytotoxicity effect in Raji cells (Vimala et al., 1999). However, a subchronic toxicity test should have been conducted to establish the adverse effects of a repeated response to *Kaempferia galanga* extract. Moreover, it is conceivable that humans and animals may receive or be exposed to this substance through applied use or by chance. More research in the production and use of this substance should be encouraged in order to provide an additional weapon in the overall strategy of vector and disease control.

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