American Journal of Food and Nutrition, 2015, Vol. 3, No. 3, 64-68
Available online at http://pubs.sciepub.com/ajfn/3/3/1
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DOI:10.12691/ajfn-3-3-1

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Study of Antinociceptive Activity of *Kaempferia galanga* from Bangladesh in *Swiss albino* Mice

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Received March 23, 2015; Revised April 25, 2015; Accepted May 05, 2015

Abstract *Kaempferia galanga* (*K. galanga*) (Family: Zingiberaceae) has been used in the folk medicine for the treatment of inflammation, chest pain, cholera, headache, toothache, hypertension, and abdominal pain. Previous investigations on this plant suggested that the methanol extract of rhizome showed the analgesic activity. Therefore, the present study investigated the antinociceptive activity of different extracts of rhizome and leaves of *K. galanga*. The antinociceptive activity was evaluated by using acetic acid-induced writhing, hot plate and tail immersion tests in *Swiss albino* mice at the doses of 100 and 200 mg/kg body weight p.o. The acetone extract of rhizome (ACR), as well as petroether fraction (PEF), chloroform fraction (CHF), methanol fraction (MEF) and acetone extract of leaves (ACL) were examined for antinociceptive activity. In this study, all the extracts displayed significant ($p<0.05$ and $p<0.001$) antinociceptive action in a dose dependent manner. In acetic acid induced writhing method, chloroform and methanol extract of rhizome (200 mg/kg) showed 81.22% and 70.12% writhing inhibition, respectively whereas the standard drug Diclofenac-sodium (25 mg/kg) and Aspirin (100 mg/kg) exhibited 80.72% and 61.94% inhibition. In hot plate and tail immersion tests, the petroether extract of rhizome and acetone extract of leaves (200 mg/kg) produced maximum 69.41% and 81.69% nociception inhibition of thermal stimulus respectively. In this study Morphine (5 mg/kg) was used as standard. The present study revealed that the acetone extracts and fractions of rhizome and leaves of *K. galanga* possess an antinociceptive property which supports its use in traditional medicine and suggesting that the plant may be further investigated to discover its pharmacologically active natural products.

Keywords: *Kaempferia galanga, Zingiberaceae, writhing test, hot plate test, tail immersion test*

Cite This Article: Pritesh Ranjan Dash, Mahmuda Nasrin, Mahmud Tareq Ibn Morshed, and Mohammad Shawkat Ali, “Study of Antinociceptive Activity of *Kaempferia galanga* from Bangladesh in *Swiss albino* Mice.” *American Journal of Food and Nutrition*, vol. 3, no. 3 (2015): 64-68. doi: 10.12691/ajfn-3-3-1.

1. Introduction

K. galanga (Chandramulika in Bengali) belonging to the family of Zingiberaceae is a rhizomatous and small herb. The rhizomes of this plant are widely used in East Asia for a wide range of medicinal applications. The most common indications include rheumatism, asthma, headaches, cough, toothaches and use as a poultice for the application on bruises and wound [1]. The aromatic oil is used as condiment and as a folk medicine. In Bangladesh, rhizomes juices of *K. galanga* are used as a remedy for toothache or a wash for dandruff or scabs on the head. It is used as stimulant, stomachic and carminative and externally used to treat abdominal pain, swelling and rheumatism [2]. Literature survey revealed that extracts of *K. galanga* has anti-inflammatory and analgesic, nematicidal, mosquito repellent and larvicidal, vasorelaxant, sedative, antineoplastic, antimicrobial , anti-oxidant properties [3]. It also has antidiarrhoeal and cytotoxic properties [4,5]. Several compounds has been isolated from dicloromethane, hexane and methanol extracts of rhizome *K. galanga* i.e. ethyl-cinnamate and ethyl–*p*—methoxycinnamate [3]. Isolated ethyl-*p*-methoxycinnamate from *K. galanga* extracts responsible for various pharmacological actions including, nematicidal, mosquito repellent, anti-neoplastic and anti-microbial effects whereas ethyl cinnamate, a vital constituent of this plant, responsible for its vasorelaxant effects [6]. The major chemical constituents of the volatile oil from dried rhizome of *Kaempferia galanga* were ethyl-*p*-methoxycinnamate (31.77%), methylcinnamate (23.23%), carvone (11.13%), eucalyptol (9.59%) and pentadecane (6.41%), respectively. Other constituents of the rhizome include cineol, borneol, 3-carene, camphene, kaempferal, cinnamaldehyde, *p*-methoxycinnamic acid and ethyl cinnamate [7]. To date, different extracts of *K. galanga* have not been systemically studied. Therefore, as a part of our continuing studies [4,5,8] on natural products for their pharmacological properties we investigated acetone extracts of different parts of the plant of *K. galanga* for their antinociceptive activity.

2. Materials and Methods

2.1. Collection of the Plant Material

The plant of *K. galanga* was collected from the local area of Mauoa, Dhaka during December 2011. The collected plant was then identified by Bushra Khan, Principal Scientific Officer, Bangladesh National Herbarium, Mirpur, Dhaka and a voucher specimen has been deposited (DACB: 36,064) for further reference.

2.2. Extraction and Fractionation of the Plant Material

The identified plant's rhizome and leaves were cut into small pieces separately and then dried in the shade. After complete drying the rhizome and leaves were reduced to coarse powder separately with the help of a mechanical grinder and the powder was stored in a suitable container for extraction. The dried grinded powder weighed by rough balance. The plant parts were extracted by a cold extraction method. The rhizome (900 g) and leaves (200 g) powder were taken and soaked with 2700 ml and 600 ml of acetone, respectively, for 3 consecutive days at 25 °C. The extracts were filtered and evaporated on rotary evaporator under reduced pressure. Recovered solvent was again used for percolation for another 3 days. The process was repeated three times to obtain 58 g rhizome (yield 6.45%) and 4.14 g leaves (yield 2.07%) extracts of *K. galanga*. 8g of the rhizome extract was separated for experimental work and further 50g was used for fractionation. The rhizome extract was further partitioned using petroether, chloroform and methanol to obtain 18.72g, 18.42g and 2.86g extracts, respectively.

2.3. Chemicals

Acetic acid was product of Merck, Germany. Diclofenac sodium and aspirin were purchased from Square Pharmaceuticals Ltd., Bangladesh; morphine was purchased from Gonoshasthaya Pharmaceuticals Ltd., Bangladesh; 0.9% sodium chloride solution (Normal saline) was purchased from Orion Infusion Ltd., Bangladesh and other reagents were of analytical grade.

2.4. Experimental Animals

For the experiment *Swiss albino* mice of either sex, 4-5 weeks of age, weighing between 25-30 gm, were collected from the Animal Research Branch of the International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). Animals were maintained under standard environmental conditions (temperature: (24.0±1.0º), relative humidity: 55-65% and 12hrs light/12 hrs dark cycle) and had free access to fed and water *ad libitum*. The animals were acclimatized to laboratory condition for two weeks prior to experimentation. All the experimental animals were treated following the Ethical Principles and Guidelines for Scientific Experiments on Animals (1995) formulated by the Swiss academy of medical Sciences and the Swiss academy of Sciences. The University Animal Research Ethical Committee approved the experimental protocol.

2.5. Drugs and Treatment

After reconstituted into distilled water (1% DMSO was used to dissolved the extracts) all the extracts were administered to the mice at 100 and 200 mg/kg per orally by gavage. The water (5 ml/kg) was administered by gavage to the control group. All drugs, used as standard, were dissolved in 0.9% saline and administered intraperitoneally (i.p.) except aspirin. Diclofenac-sodium (25 mg/kg i.p.) and morphine (5 mg/kg i.p.) were used as standard peripheral and central antinociceptive agents, respectively.

2.6. Acute Toxicity Study

Mice were divided into control and test groups (n=6). The test groups received the extract per orally at the doses of 500, 1000, 1500 and 2000 mg/kg. Then the animals were kept in separate cages and were allowed to food and *ad libitum*. The control group received the water. The animals were observed for possible behavioral changes, allergic reactions and mortality for the next 72 h [9].

2.7. Antinociceptive Activity Study

2.7.1. Acetic Acid Induced Writhing Test

The method described by Dash *et al.,* (2011) [10] that was adopted to study the effect of the *K. galanga* extract on acetic acid induced writing test. Test samples and control (n=6) were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but Diclofenac-sodium (i.p) and aspirin (p.o) were administered 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed (abdominal contraction, elongation of the body and extension of the hind limb were referred as writhing) for the next 10 min. Percentage inhibition of writhing was calculated using the following formula:

$$\textbf{Writhing inhibition}\,(\%) = \left[\frac{\text{Mean no. of writhings (control)} - \text{mean no. of writhing (test)}}{\text{Mean no. of writhings (control)}}\right] \times 100.$$

2.7.2. Hot Plate Test

The hot plate test was performed according to the method described by Eddy and Leimbach (1953) [11]. The animals in the control group received water (5 ml/kg, p.o.) while the standard groups were treated with morphine sulphate (5 mg/kg, i.p.). The animals in the test groups were treated with 100 and 200 mg/kg, per oral of different extracts of *K. galanga.* Then the animals were placed on Eddy's hot plate kept at a temperature of 52±0.5 ^{0}C. A cut off period of 28s was observed to avoid damage to the paw. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60 and 90 min after the administration of the standard and test drugs (n=6). Percentage of elongation was calculated using the following formula:

$$\textbf{Elongation}\,(\%) = \frac{\text{Latency (test)} - \text{Latency (control)}}{\text{Latency (test)}} \times 100.$$

2.7.3. Tail Immersion Test

The procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice [12].The animals were treated as discussed above. 1 to 2 cm of the

tail of mice was immersed in warm water kept constant at 55±1°C. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latency period of 28s was defined as complete analgesia and the measurement was then stopped to avoid injury to mice. The latent period of the tail-immersion response was determined at 0, 30, 60 and 90 min after the administration of standard and test drugs (n=6). Percentage of elongation was calculated using the same formula used in hot plate test.

2.8. Statistical Analysis

The statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnett's multiple comparisons. The results obtained were compared with the control group. $P < 0.05$ and $P < 0.001$ were considered to be statistically significant.

3. Results

3.1. Acute Toxicity

Oral administration of different extracts of *K. galanga* at the doses of 500–2000 mg/kg did not produce any mortality or noticeable behavioral changes in mice within 72 hr observation period. Therefore, it can be suggested that *K. galanga* have low toxicity profile with LD_{50} greater than 2000 mg/kg.

Table 1. Effect of different extracts of *K. galanga* on acetic acid-induced writhing test in mice.

Group	Dose (mg/kg)	No. of writhings (Mean±SEM)	% of writhing	% of writhing inhibition
Control	5ml/kg	50.58±4.18**	100.00	--
Diclofenac-Na	25	9.75±0.77**	19.27	80.72
Aspirin	100	19.25±4.12**	38.06	61.94
ACR	100	17.17±3.19**	33.95	66.05
	200	15.08±2.50**	29.81	70.12
PEF	100	25.25±4.22**	43.65	50.07
	200	17.58±2.33**	34.76	65.24
CHF	100	22.08±3.53**	37.23	56.35
	200	9.5±1.73**	18.78	81.22
MEF	100	18.83±3.30**	37.23	62.77
	200	15.08±2.91**	29.81	70.12
ACL	100	22.08±2.55**	43.65	56.35
	200	20.08±3.33**	39.70	60.30

Control group received water 5ml/kg body weight (p.o.), standard groups received Diclofenac-Na 25mg/kg (i.p.) and Aspirin 100mg/kg body weight (p.o.).Standard drugs were administered 15 min before 0.7 % acetic acid administration. Writhing was counted for 15 min, starting after 5 min of acetic acid administration. Test groups ACR, PEF, CHF, MEF and ACL were treated with 100 and 200 mg/kg body weight of the extracts (p.o.) respectively. Values are mean ±SEM, (n=6); $^{**}p < 0.001$, *Dunnett t-test* as compared to control. **ACR**=Acetone extract of rhizome, **PEF**= Petroether fraction of rhizome, **CHF**=Chloroform fraction of rhizome, **MEF**=Methanol fraction of rhizome and **ACL**=Acetone extract of leaves.

Table 2. Effect of different extracts of *K. galanga* on hot plate test in mice.

Group	Dose (mg/kg)	Mean Reaction Time (s)			
		0 min	30 min	60 min	90 min
Control	5ml/kg	9.67±0.98	8.83±0.60	9.83±1.04	9.33±1.11
Morphine	5	10.83±1.76	17±2.09* **(48.06%)**	21.33±2.19* **(53.91%)**	16±1.80 **(41.68%)**
ACR	100	11.5±2.10	13.50±1.94 **(34.59%)**	16.17±2.33 **(39.21%)**	21±2.82** **(55.57%)**
	200	7.83±0.79	13.67±1.89 **(35.41%)**	21±2.35* **(53.19%)**	23.67±1.47** **(60.58%)**
PEF	100	8±1.15	11±2.25 **(19.73%)**	19±2.46* **(48.26%)**	22.17±2.02* **(57.92%)**
	200	8.17±1.30	14.67±2.51 **(39.81%)**	23.5±2.64** **(58.17%)**	30.5±3.54** **(69.41%)**
CHF	100	9.83±1.14	13.17±1.42 **(32.95%)**	19±1.75* **(48.26%)**	21.5±1.92** **(56.60%)**
	200	8.83±0.91	13.5±2.44 **(34.59%)**	20.83±2.38* **(52.81%)**	25±2.76** **(62.68%)**
MEF	100	8±0.93	10.5±1.17 **(15.90%)**	17±2.01 **(42.17%)**	18.33±1.72* **(49.10%)**
	200	8.5±1.42	17.5±2.51* **(49.54%)**	21.5±3.64* **(54.28%)**	21.67±3.21** **(56.94%)**
ACL	100	10.17±1.10	12.5±0.43 **(29.36%)**	22±1.69 **(55.32%)**	21.5±1.99* **(56.60%)**
	200	9.33±1.35	14.5±1.05 **(39.10%)**	25.83±3.27** **(61.94%)**	25.33±4.12** **(63.17%)**

Control group received water 5ml/kg body weight (p.o.), standard groups received Morphine 5 mg/kg body weight (i.p.), test groups ACR, PEF, CHF, MEF and ACL were treated with 100 and 200 mg/kg body weight of the extracts (p.o.) respectively. Values are mean ±SEM, (n=6); $^{*}p < 0.05$, $^{**}p < 0.001$, *Dunnett t-test* as compared to control. **ACR**=Acetone extract of rhizome, **PEF**= Petroether fraction of rhizome, **CHF**=Chloroform fraction of rhizome, **MEF**=Methanol fraction of rhizome and **ACL**=Acetone extract of leaves.

3.2. Antinociceptive Activity

3.2.1. Acetic Acid Induced Writhing Test

The results of the acetic acid induced writhing test showed that the different extracts of *K. galanga* at all doses produced significant ($p<0.001$) inhibition of writhing reaction in a dose dependent manner. In this test, ACR, PEF, CHF, MEF and ACL (200 mg/kg) inhibited maximum 70.12%, 65.24%, 81.22%, 70.12%, and 60.30% writhing respectively, whereas the writhing inhibition of the standard drug Diclofenac-Na (25 mg/kg) and Aspirin (100 mg/kg) were 80.72% and 61.94% respectively (Table 1).

3.2.2. Hot Plate Test

Results of hot plate test are presented in Table 2. All doses of the extracts produced a dose dependent increase in latency time when compared with the control. The results were found to be statistically significant ($p < 0.05$-0.001). In the hot plate test, ACR, PEF, CHF, MEF and ACL (200mg/kg) showed maximum 60.58%, 69.41%, 62.68%, 56.94%, and 63.17% nociception inhibition of thermal stimulus respectively, whereas the standard drug Morphine (5 mg/kg) displayed maximum 53.91% nociception inhibition.

3.2.3. Tail Immersion Test

The tail withdrawal reflex time following administration of the extracts of *K. galanga* was found to increase with increasing dose of the sample. In this test maximum effect was observed after 60 and 90 min of drug administration. The results were statistically significant ($p < 0.05$-0.001). In tail immersion test, maximum 80.67%, 80.69%, 80.90%, 74.53% and 81.69% nociception inhibition of thermal stimulus were exhibited with the ACR, PEF, CHF, MEF and ACL (200 mg/kg) respectively. In this study morphine (69.89% inhibition) was used as standard (Table 3).

Table 3. Effect of different extracts of *K. galanga* on tail immersion test

Group	Dose (mg/kg)	Mean Reaction Time (s)			
		0 min	30 min	60 min	90 min
Control	5ml/kg	1.77±0.11	1.66±0.15	1.83±0.24	1.77±0.11
Morphine	5	2.44±0.11	4.94±0.63** **(66.39%)**	5.11±0.40** **(64.18%)**	5.88±0.25** **(69.89%)**
ACR	100	1.99±0.15	4.55±0.92* **(63.52%)**	5.22±0.44** **(64.94%)**	5.94±0.30** **(70.20%)**
	200	2.28±0.13	4.61±0.20* **(63.99%)**	6.61±0.31** **(72.32%)**	9.16± 0.47** **(80.67%)**
PEF	100	2.22±0.38	2.99±0.34 **(44.48%)**	4.55±0.57** **(59.78%)**	5.44±0.68** **(67.46%)**
	200	2.05±0.20	4.88±0.37** **(65.98%)**	7.22±0.32** **(74.65%)**	9.17±0.36** **(80.69%)**
CHF	100	1.66±0.17	3.72±0.34* **(55.37%)**	4.11±0.33* **(55.47%)**	5.77± 0.72** **(69.32%)**
	200	2.27±0.18	4.94±0.42** **(66.39%)**	6.67±0.56** **(72.56%)**	9.27±0.53** **(80.90%)**
MEF	100	2.33±0.17	2.94±0.10 **(43.54%)**	3.99±0.35* **(54.14%)**	3.99±0.27** **(55.64%)**
	200	2.05±0.16	4.38±0.51** **(62.10%)**	5.77±0.76** **(68.28%)**	6.95±0.40** **(74.53%)**
ACL	100	2.27±0.55	3.77±0.16** **(55.97%)**	5.05±0.44** **(63.76%)**	.05±0.76** **(74.89%)**
	200	2.17±0.18	4.72±0.29** **(64.83%)**	7.11±0.25** **(74.26%)**	9.67±0.38** **(81.69%)**

Control group received water 5ml/kg body weight (p.o.), standard groups received Morphine 5 mg/kg body weight (i.p.), test groups ACR, PEF, CHF, MEF and ACL were treated with 100 and 200 mg/kg body weight of the extracts (p.o.) respectively. Values are mean ±SEM, (n=6); * $p < 0.05$, ** $p < 0.001$, *Dunnett t-test* as compared to control. **ACR**=Acetone extract of rhizome, **PEF**= Petroether fraction of rhizome, **CHF**=Chloroform fraction of rhizome, **MEF**=Methanol fraction of rhizome and **ACL**=Acetone extract of leaves.

4. Discussion

The present study demonstrates that different extracts of *K. galanga* possess potent antinociceptive activity in chemical and heat induced models. No acute toxicity was observed after oral administration of *K. galanga* even at the dose of 2000 mg/kg in mice.

Acetic acid-induced writhing test is a visceral pain model. The pain is induced at the peritoneal receptors by the increasing amount of endogenous mediators of pain, such as prostaglandin E2, prostaglandin E2α, kinins, and the like [13,14]. The stimulation of the nociceptive neurons, which is sensitive to NSAIDs and narcotics, by the endogenous mediators also contribute in the pain induction [15]. Acetic acid-induced abdominal writhing is widely used to screen peripherally acting analgesics [16]. The findings of the present study indicate that different extracts of *K. galanga* possess a significant peripheral antinociceptive activity.

The hot plate test is used to evaluate the centrally acting analgesics [17]. The paw-licking or jumping responses in hot plate are complex supraspinally organized behavior of mice [18]. So, a decrease in licking or increase in latency indicates the centrally acting analgesic properties of the treatment. The results of the hot plate test showed that *K. galanga* extracts produced antinociceptive effect against heat induced pain. The effect was evident from the elongation of the latency time till the 4th observation (90 min).

Tail immersion model is an acute pain model. The tail-withdrawal response is predominantly considered to be selective for centrally acting analgesics, whereas the peripherally acting drugs are known to be inactive on

heat-induced pain [19]. The significant increase ($p < 0.05$-0.001) in tail-withdrawal time by the extracts suggests centrally acting analgesic activity of *K. galanga*. Both tail immersion and hot plate test measure the latency time of mice to thermal stimuli. Therefore, the results of the present study indicate that the central antinociceptive effect of *K. galanga* may be prominent. Influx of calcium ions at the terminal of the axon of the afferent nerve by different compounds in the extracts may also decrease the activity of adenylyl cyclase. This may lead to decreased cAMP level, potassium ion efflux and subsequent hyperpolarization of the nerves and give the antinociceptive effect [20]. The antinociceptive effect of the extracts in these three models implies that the extracts contain pharmacologically active phytoconstituents that may act both centrally and peripherally.

5. Conclusion

Finally it can be concluded that, the different extracts of *K. galanga* possesses a significant antinociceptive activities and the results tend to corroborate the traditional use of this plant in the treatment of pain. However, further investigations are required to identify the active constituents and to verify the therapeutic merits of the active constituents.

Acknowledgments

The authors are acknowledge to the director of Animal Research Division of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) for supplying mice and the National Herbarium of Bangladesh for identifying the plant sample.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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