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SAFETY EVALUATION OF GALANGAL (*ALPINIA GALANGA*) EXTRACT FOR THERAPEUTIC USE AS AN ANTIMICROBIAL AGENT

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ABSTRACT: Hexane extract of Galangal (*Alpinia galanga*) rhizome was evaluated for acute dermal, oral and intraperitoneal toxicities using OECD guidelines. The undiluted crude galangal extract showed negligible irritation on non-abraded skin of New Zealand white rabbits with 0.25 primary irritation index whereas the abraded skin of the rabbits showed irritation for all tested dilutions of galangal extracts; 0.75 g/ml, 0.5 g/ml, 0.25 g/ml and 0.125 g/ml. The single oral dose of the Galangal extract at 2000 mg/kg did not produce mortality or significant changes in the general behavior, body weights, feed intake and biochemical analysis (ALT, AST, BUN and creatinine levels) of Wistar rats compared to the control. However, 2000 mg/kg and 50 mg/kg body weight of galangal extract were highly toxic to Wistar rats when administered intraperitoneally. Galangal extract with concentrations <20 mg/ml were non-irritant on Hen's egg chorioallantoic membrane which is an alternative to the Draize eye irritation test.

INTRODUCTION: Some functional values of plants rich in phytochemicals and other secondary metabolites have been found *in-vitro* and developed into products to prevent or cure diseases. Further, medicinal plant extracts have provided enomorous opportunities towards developing various new drugs with different purposes in the modern pharmacological industry [1]. However, many medicinal plants are known to possess toxicological effect in both humans and animals [2].

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Hence, overall safety of plant derived constituents intended for human consumption needs to be established with respect to toxicity studies using animal models. It is necessary to investigate the toxic effect that may be caused by a plant extract which is intended to be used in natural products such as preservatives, disinfectants, herbal cream, antiseptics or ointments and to establish the safe limits for recommended usage.

Determining the irritant levels of a plant extract or its chemical constituents on the skin and eye of experimental animals provide scientists with useful information on production of safe natural products. Further, the assurance of pharmacological safety is important if a plant extract is expected to be used as an anticancer, antiviral, antibacterial or any other therapeutic drug in the pharmaceutical industry or

as an ingredient in the food industry for human consumption. Therefore, safety evaluation of a natural substance is important to increase the confidence in its safe use for humans, and to provide protection against possible hazards to human beings. Galangal (*Alpinia galanga*); a native plant in Southern China and Southeast Asia belongs to family Zingiberaceae [3]. The rhizome of galangal is widely used as a spice for food flavoring due to its characteristic fragrance and pungency.

Galangal rhizome is also being used in traditional medicine such as Ayurveda, Unani, Chinese and Thai folk medicine. Galangal has been studied extensively for its various functional constituents possessing different pharmacological activities such as antimicrobial activity, anti-inflammatory and analgesic action, anticancer and antimelanogenic potentials, antioxidant activity and apoptosis activity [4, 5, 6]. There are not many studies on safety evaluation of galangal except toxicity studies on mice and human cell lines. An acute toxicity study of Galangal ethanol extract on rodents has suggested that the galangal extract falls under the hazard category at 2000 mg/kg body weight [7]. However, further research is needed to confirm the safety levels of galangal in terms of overall safe usage.

Toxicity levels of a certain substance can be evaluated using a variety of modern methods including non-animal alternative methods. In toxicology experiments these modern methods can be used as full or partial replacements of animals. Using primary culture, human cell lines and tissues for experiments are known as *in-vitro* methods. The use of isolated animal tissues and organs are known as *ex-vivo* methods. *In-silico* methods include computer simulations and mathematical models [8]. However, the test guidelines program of the Organization for Economic Cooperation and Development (OECD) has major influence on the current experimentation techniques. In terms of the applicability, a wide range of chemicals, substances and mixtures can be tested using OECD guidelines. Further, these guidelines are particularly important for the reduction in the number of test animals used for testing and particularly concern about animal welfare. Hen's Egg Test - Chorioallantoic Membrane (HET-CAM) method is a non animal alternative method to Draize eye irritation test which can be used to evaluate the potential ocular irritancy of a certain test substance. This is an *in-vitro* test method proposed by Luepke and Kemper [9, 10] to identify eye injury hazard potential of chemicals and products if the test substance is suspected to be corrosive or severe irritant. The irritancy level can be classified in accordance with the ability of the test substance to induce toxicity in the chorioallantoic membrane (CAM) of a chicken [11]. The objective of this study is to investigate potential safe levels of galangal hexane extract with respect to skin, eye and oral toxicities and to confirm its safe use in herbal products or to be used as a preservative, pharmaceutical or a disinfectant. The methods used in this study are such guidelines that point out the critical experimental points.

MATERIALS AND METHODOLOGY:

Animals: The ethical clearance to conduct toxicity studies was obtained from the ethics review committee of Medical Research Institute, Sri Lanka (43/2011). The acute dermal irritation tests were carried out using New Zealand White rabbits while the acute oral toxicity test was carried out using Wistar rats. The number of animals and sex used for each test was determined considering both OECD guidelines and the opinion of the ethics review committee of Medical Research Institute, Sri Lanka based on the 3R concept. All animals were maintained under standard laboratory conditions including 20 ºC to 24 ºC temperature, 50% to 70% relative humidity and a light sequence of 12 h light-dark throughout the experimental period. Standard size, polycarbonate, transparent cages were used for the housing of animals. Three Wistar rats from the same sex were kept in one cage while New Zealand White rabbits were housed individually. Sterilized wood shavings were used as the bedding material. All animals were fed with MRI rabbit and rat formula prepared according to WHO guideline given by Saboudry [12]. The formulas were prepared at the medical research institute using locally available ingredients.

Identification, Collection and Extraction of Plant Material: Different plant parts including flowers, pods, leaves and infloresence were collected from the herbarium of Nature's Secret (Pvt.,) Ltd., Horana. The morphological characters of the collected plant materials were used to

identify and authenticate the plant according to a key described by Dassanayake and Forceburg [13]. Fresh galangal rhizomes were washed under running water and the outer skin was removed. The rhizomes were then sliced and dried (INC/75/55/DG, Genlab) at 40 ºC overnight and subjected to grinding (MX-T110PN, National, Thaiwan) for 10 min to make a fine powder. Hexane extracts were prepared by mixing galangal and hexane at a ratio of 1: 10 and agitating for 24 h at 28 ºC in a rotary shaker (Stuart orbital shaker SSLI, UK). The mixture was then centrifuged at 10000 g for 10 min and the supernatant was filtered using Whattman no. 1 filter papers. Hexane extract was evaporated to dryness under vacuum at 40 ºC using a rotary evaporator (Bouchi Labortecnik RV 5, Switzerland), followed by nitrogen fluxing to obtain a hexane free galangal extract. The pure extract was stored at 4 ºC until use.

Phytochemical Analysis of the Extract: The chemical composition of the galangal hexane extract was determined using gas chromatography - mass spectrometry analysis to identify the major chemical compound. The mass selective detector agilent 6890N series was used for the analysis. The capillary column of Rtx-wax cross bond carbowax polyethylene glycol (0.25 mm × 0.25 µm, 30 m) was used and the temperature was first held at 50ºC for 2 min and then raised to 250 ºC at a rate of 10ºC/min and held at 250 ºC for 8 min. The carrier gas was helium at a flow rate of 0.9 ml/min. The injection volume was 1 µl. The components of the extract were recognized by the retention time of the chromatogram peaks and by their mass spectra. The percentage of each compound was calculated as the ratio of the peak area to the total chromatographic area. Wiley W9N08 database was used as the reference library.

Acute Dermal Irritation Study for Non-Abraded Skin: Acute dermal irritation study for crude galangal extract was carried out according to the method described in OECD guideline no 404 [14]. Three healthy young New Zealand White rabbits from each sex within the weight range of 2.0 kg to 3.5 kg were selected for the experiment at the start of dosing. The test was performed using one animal initially. After confirming non corrosiveness of the extract, two additional animals were used for the confirmatory test.

Skin reactions prior to the application of the crude galangal extract was observed after removing hair on the dorsal side of animals, 24 h before the commencement of the experiment. The exposed skin was gently wiped with a clean moistened paper towel and 0.5 ml of undiluted crude galangal extract and commercial saline solution were applied on approximately 6 cm^2 area of the left and right dorsal sides of the skin as the test substance and the control respectively. Then the applied area was covered with a sterile gauze pad and the entire trunk of the rabbit was wrapped with non-irritant adhesive plaster. After 4 h of exposure period the wrapping was removed and the skin was wiped with clean moistened paper towel to remove any residual test substance. Skin reaction at the site of exposure was observed immediately for erythema and edema in accordance with the method described in OECD guideline 404.

Further, the responses were observed and scored at 60 min, 24 h, 48 h and 72 h after exposure period. To determine the reversibility, the animals were observed upto 14 days after removal of the patches. In addition to the skin reactions other toxicity determinants such as body weight, feed and water intake were recorded throughout the observation period. Dermal reactions were graded according to the grades given in OECD guideline 404 [14]. The Score of Primary Irritation (SPI) was calculated for each rabbit using following formula for both treatment and the control site.

$$SPI = \sum \frac{\text{Erythema and oedema grades at 24,48 and 72 h}}{\text{Number of observations}}$$

The Primary Irritation Index (PII) was calculated to determine the response category of irritation in rabbits according to the following formula and the irritation degree was categorized based on the PII.

$$PII = \frac{\sum SPI\ (test) - \sum SPI\ (control)}{\text{No of animals}}$$

Acute Dermal Irritation Study for Abraded Skin: The same method described in non-abraded skin was repeated for the abraded skin irritation study using New Zealand White rabbits [14]. Five female New Zealand white rabbits in the weight range of 2.0 kg to 3.5 kg were treated with five concentrations of galangal extracts ranging; 0.75 g/ml, 0.5 g/ml, 0.25 g/ml and 0.125 g/ml including

undiluted galangal extract. Galangal dilution series were made using glycerol as the carrier. The hair of the dorsal side of each animal was removed and a slight scarification was made on the skin, 24 h prior to the commencement of the test.

Hen's Egg Test - Chorioallantoic Membrane: The HET-CAM test was carried out according to Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recommended test method protocol [11] with some modifications. Nine day old fertile, fresh, clean White Leghorn chicken eggs weighing 50-60 g, were obtained from a commercial source (Nidarshanee Breeder Farm, Radawana). Eggs were transported under conditions (at 38 ºC temperature and 58% of relative humidity) that did not affect embryo viability or development. The eggs were candled prior to the experiment on the same day to eliminate the use of nonviable, defective eggs including misshapen eggs or eggs with cracked or thin shells and the air cell of the eggs were marked on viable eggs after candling. The marked section was cut carefully with a rotating dentist saw blade and the outer egg shell was carefully removed to expose the inner membrane which was moistened with 0.9% NaCl using a disposable pipette.

The inner membrane of the egg was then removed carefully with a forcep, after allowing the membranes to be moistened for a few minutes and decanting the 0.9% NaCl. Then 0.3 ml of crude galangal extract from each concentration (0.625 mg/ml, 1.25 mg/ml, 2.5 mg/ml and 5 mg/ml) was applied directly onto the CAM surface which was prepared on the same day using olive oil as the carrier. Olive oil served as the media control while 0.1N NaOH and 0.9% NaCl served as the positive and negative control respectively. Three eggs were used as replicates for each dilution of galangal extract including positive, negative and media control. Then the reaction endpoints for irritancy such as hemorrhage (bleeding from the vessels), vascular lysis (blood vessel disintegration) and coagulation (intra and extravascular protein denaturation) on the CAM were observed over a period of 300 s.

The time for the appearance of each endpoint were monitored and recorded in seconds. Time was recorded as the start time in seconds for each of the above reactions on CAM. The evaluation of test results were carried out using irritation score [section B] analysis method, which is the ICCVAM-recommended HET-CAM protocol for prospective studies [15].

Acute Oral Toxicity Study: Acute oral toxicity study of crude galangal extract was carried out according to the method described in OECD guideline no. 423 [16]. Three-month old female Wistar rats, weight ranging from 150 - 200 g, were randomly divided into two groups as the control (n=4) and the treatment (n=6). They were maintained in an air conditioned and light controlled room with access to water and MRI rat feed *ad libitum* throughout the experimental period. The day prior to starting the experiment, animals were kept fasting overnight with free access to water. Animals were individually weighed and a dosage of 2000 mg/kg body weight crude galangal extract was administered in a single dose using gastric lavage method. Olive oil was used for the control group. Animals were observed individually for mortality and pharmacotoxic signs frequently during the first 30 min, periodically during first 24 h with special attention given during first 4 h and thereafter, daily for 14 days. Animals were observed for any signs of toxicity including changes in the skin, fur, eyes and mucous membrane, tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. In addition, the body weights and feed intake were recorded throughout the observation period. At the end of the observation period blood samples were drawn from rats and the serum was separated for determination of biomedical parameters. The serum creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and blood urea nitrogen (BUN) were measured with commercially available estimation kits (Pointe Scientific) using a semi automated biomedical analyser (Stat Fax 3300).

Statistical Analysis: All quatitative data for acute oral toxicity study were expressed as mean ± standard deviation (SD) of triplicates. Statistical analysis was carried out using one way ANOVA and significant difference between means were compared using student's t-test at $P < 0.05$.

RESULTS:

Phytochemical Analysis of the Extract: According to the GC-MS analysis results 1' acetochavicol acetate (1' ACA) was recorded as the major chemical compound which was found in highest concentration (82.88%) in hexane extract of galangal. Trans form of beta farnesene (2.68%) and 9-octadecenoic acid (2.63%) were found to be the second and the third highest chemical compounds present in the galangal hexane extract. The rest of the chemical compounds; 1, 8- cineole, beta. -bisabolene, beta- sesquiphellandrene, 2-methoxy-4-methyl benzaldehyde and hexadecanoic acid / palmitic acid were present in < 2% in galangal hexane extract.

Acute Dermal Irritation Study for Non-Abraded Skin: The observation results for irritation study on non abraded skin of rabbits are shown in **Table 1**. According to the test results the SPI for treatment and the control were 1.5 and 0 respectively. The PII was calculated as 0.25 which falls under the category of negligible according to the response categories of irritation in rabbits.

TABLE 1: SCORE OF IRRITATION FOR ERYTHEMA AND OEDEMA, AFTER APPLICATION OF CRUDE GALANGAL EXTRACT ON NON-ABRADED SKIN

		Score for skin reaction							
Rabbit no.	Reaction	Undiluted crude galangal extract				Control			
		60 min	24 h	48 h	72 h	60 min	24 h	48 h	72 h
1	Erythema	1	0	0	0	0	0	0	0
	Oedema	0	0	0	0	0	0	0	0
2	Erythema	1	0	0	0	0	0	0	0
	Oedema	0	0	0	0	0	0	0	0
3	Erythema	1	0	0	0	0	0	0	0
	Oedema	0	0	0	0	0	0	0	0
4	Erythema	1	0	0	0	0	0	0	0
	Oedema	0	0	0	0	0	0	0	0
5	Erythema	1	0	0	0	0	0	0	0
	Oedema	0	0	0	0	0	0	0	0
6	Erythema	1	0	0	0	0	0	0	0
	Oedema	0	0	0	0	0	0	0	0

Acute Dermal Irritation Study for Abraded Skin: The abraded skin test results showed that the undiluted galangal extract and all tested dilutions were irritant to the rabbits, with discoloration of the test area after patch removal **Fig. 1**. Scab formation was observed on abraded skin of all the rabbits where crude galangal was applied. The reversibility of the irritation effect on abraded skin (scab formation and skin discoloration) was not observed even after 14 d for undiluted crude galangal extract and all dilutions.

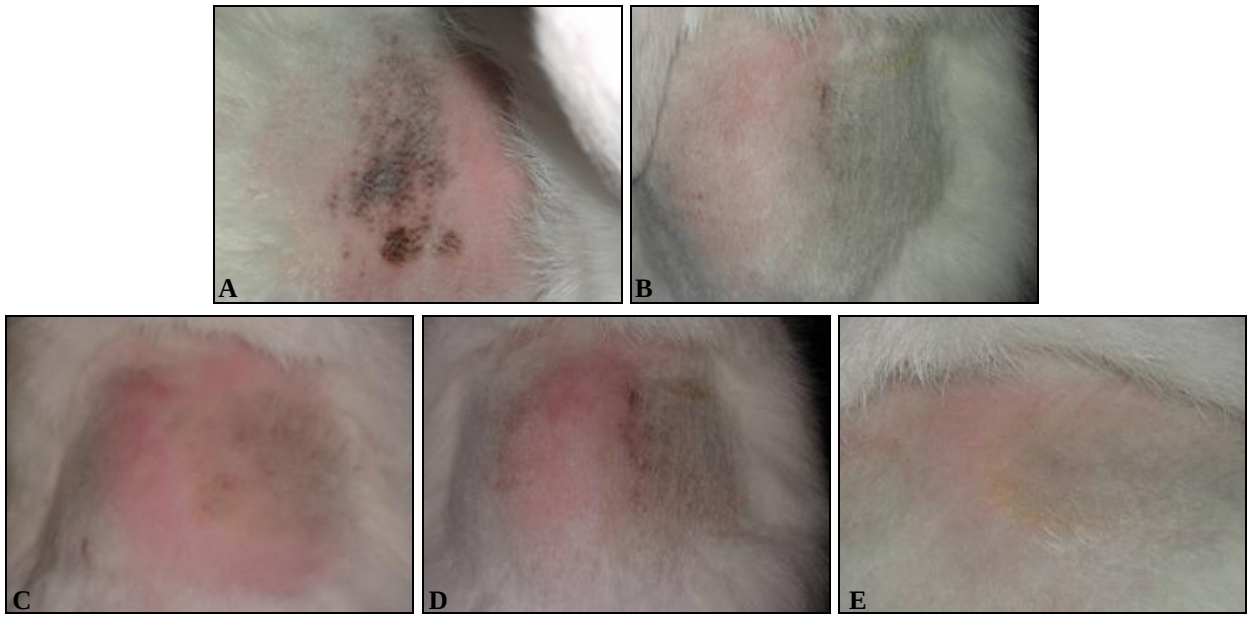

FIG. 1: SKIN IRRITATION REACTION ON ABRADED SKIN OF NEW ZEALAND WHITE RABBITS AT 24 h AFTER APPLICATION OF CRUDE GALANGAL EXTRACT. A) UNDILUTED B) 75% C) 50% D) 25% AND E) 12.5% OF CRUDE GALANGAL EXTRACT

Hen's Egg Test - Chorioallantoic Membrane: The observation results on chorioallantoic membrane surface of Hen's egg test are shown in **Fig. 2**. As illustrated in the **Fig. 2C** 40 mg/ml of galangal extract showed signs of hemorrhage on CAM within first 5 min. Both 5 mg/ml and 20 mg/ml of galangal extract did not show any signs of irritancy on CAM within first 5 min **Fig. 2A** and **B**. Positive control as illustrated in **Fig. 2D** showed all three irritation reactions; hemorrhage, vascular lysis and coagulation.

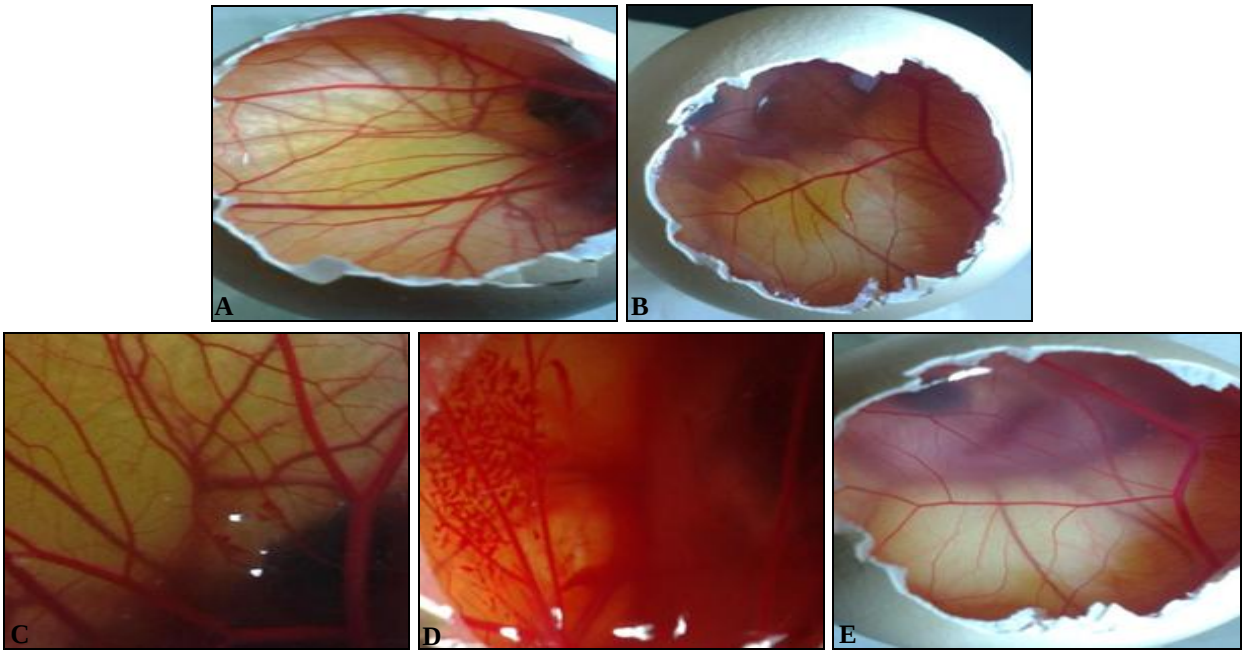

FIG. 2: EFFECT OF DIFFERENT DOSAGES OF GALANGAL EXTRACT ON CAM SURFACE A) 5 mg/ml B) 20 mg/ml C) 40 mg/ml D) POSITIVE CONTROL E) NEGATIVE CONTROL

According to the irritation category of the HET-CAM the irritation scores obtained for 5 mg/ml and 20 mg/ml concentrations of crude galangal extract were found to be non irritant on CAM surface. The negative and the media control also showed no irritancy on CAM surface. Further, the test results for 40 mg/ml of galangal extract gave a mean irritation score of 2.18 ± 0.76 which falls under the category of weak or slight irritation. The positive control gave a mean irritation score of 19.68 ± 0.46 which falls under the category of strong or severe irritation.

Acute Oral Toxicity Study: The oral administration of 2000 mg/kg body weight of crude galangal extract showed no mortality or clinical signs of toxicity in Wistar rats throughout the observation period which indicate that the LD_{50} value of galangal extract is greater than 2000 mg/kg body weight. The observation results for acute oral toxicity study are shown in **Table 2**. There was no significant difference (P>0.05) in body weights of Wistar rats during the observation period between control and treated group.

TABLE 2: EFFECT OF ORAL ADMINISTRATION OF CRUDE GALANGAL EXTRACT ON BODY WEIGHT AND FEED INTAKE OF WISTAR RATS

Group		Day 1	Day 2	Day 3	Day 7	Day 14
Treatment (n=6)	Body weight	160 ± 8.10^{a}	158.67 ± 4.32^{a}	162.67 ± 7.86^{a}	165.67 ± 5.54^{a}	177 ± 8.65^{a}
	Feed intake	0 ± 0^{x}	6.67 ± 1.03^{x}	9 ± 3.35^{x}	8.33 ± 1.63^{x}	8.17 ± 1.33^{x}
Control (n=4)	Body weight	177 ± 17.08^{a}	178.75 ± 16.68^{a}	180 ± 16.30^{a}	182.5 ± 15.86^{a}	189 ± 18^{a}
	Feed intake	24.5 ± 3.11^{y}	25 ± 2.71^{y}	23.75 ± 1.5^{y}	25 ± 1.82^{y}	24 ± 1.41^{y}

Values are expressed as mean ±standard deviation. Within the column, mean values followed by the same lowercase letter are not significantly different at P >0.05 level.

However, the feed intake of the treated group showed a significant reduction compared to the control group throughout the observation period. As shown in **Table 3**, the results for biochemical parameters tested after 14 d of oral administration of crude galangal extract showed no significant difference between control group and treatment group.

TABLE 3: EFFECT OF ORAL ADMINISTRATION OF CRUDE GALANGAL EXTRACT ON BIOCHEMICAL PARAMETERS OF WISTAR RATS

Group	ALT (U/L)	AST (U/L)	Creatinine (mg/dl)	BUN (mg/dl)
Treatment (n=6)	36.13 ± 7.69[a]	158.9 ± 16.34[a]	0.75 ± 0.10[a]	38.85 ± 4.92[a]
Control (n=4)	37.30 ± 2.67[a]	175.28 ± 7.67[a]	0.75 ± 0.05[a]	37.00 ± 0.89[a]

Values are expressed as mean ± standard deviation. Within the column, mean values followed by the same lowercase letter are not significantly different at P >0.05 level.

DISCUSSION: Dermal irritation test results showed that undiluted galangal extract was not irritant on non abraded skin of New Zealand white rabbits. However, the same extract and its dilutions; 0.75 g/ml, 0.5 g/ml, 0.25 g/ml and 0.125 g/ml were irritant on the abraded skin of rabbits. According to the single harmonised corrosion category of OECD guideline 404, Dermal corrosion is defined as "the production of irreversible damage of the skin; namely, visible necrosis through the epidermis and into the epidermis, following the application of a test substance for up to 4 h. Corrosive reactions are typified by ulcers, bleeding, bloody scabs, and, by the end of observation at 14 d, by discoloration due to blanching of the skin, complete areas of alopecia, and scars" [14]. Therefore, the test results of the current study indicated that high concentrations of crude galangal extract could be corrosive (category 1) on abraded skin of the rabbits. Further, these levels fall under subcategory 1C where the animals were exposed to the extract between 1 h and 4 h and observations up to 14 d. However, this study revealed that the dermal application of undiluted galangal extract is not associated with any toxic effect on non abraded skin of rabbits and these findings could provide satisfactory evidence to launch a clinical trial of the plant extracts on human volunteers. It is also important to carryout further studies to find the non-irritable concentrations of galangal on abraded skin of rabbits.

In-vivo local toxicity study such as Draize rabbit eye test results and HET-CAM test results showed a good correlation and therefore, proposed the HET-CAM test to be used as a pre-screen method of eye irritation tests [17, 18]. The test is also proposed as a model for living membrane such as the conjunctiva since it comprises a functional vasculature. Further, the test is proposed to provide information on the effects that may occur in the conjunctiva following exposure to a test substance [19]. It is suggested that anti-irritant properties of a natural material could be screened using physiological CAM assays and the HET-CAM assay had been used in previous studies as an *in-vitro* safety assessment method [20, 21]. Thus, HET-CAM was used as an alternative method to eye irritation test in our study and the test results revealed that 5 mg/ml and 20 mg/ml of galangal extract could be considered within the safe limits with respect to eye irritation. However, these concentrations produced slight engorgement of blood vessels on CAM and an increased rate of pulsation in the embryo. The same reactions were observed within less time after application of 40 mg/ml of galangal extract.

The test procedure for acute oral toxicity study using OECD guideline 423, allows the exposure ranges for expected lethality of the test substance to be determined. In particular, the test does not provide the precise calculation of LD_{50} value and considers the death of a proportion of the animals tested as the major end point. According to a previous study's results highest the starting dose of 2000 mg/kg body weight galangal extract was unlikely to cause mortality in Wistar rats where the test was performed as a limit test [7]. According to OECD guideline 423, a limit test may be carried out using six animals of a single sex (normally females) at 2000 mg/kg body weight dose level.

Though a limit test does not provide a precise LD_{50} value it could provide evidence on the dose level of the extract at which animals are expected to survive [22]. Since, there were no moribund condition or dead animals found at LD_{50}> 2000 mg/kg body weight, galangal extract could be classified under the category 5 in the globally harmonised system which was considered the lowest toxicity class for acute toxicity. However, the animals showed signs of distress such as excessive grooming, restlessness and nervousness during first 30 min. During the next 3 h the animals were drinking water excessively, eating wood shavings and showing lethargy and sleepiness. However, all treated rats showed normal behaviour within 24 h.

The body weight changes are considered to be markers of adverse effects of drugs and chemicals. If the weight loss is more than 10% of the initial body weight it will be considered as statistically significant [23]. In our study none of the animals showed such drastic reduction in body weight during the observation period. This revealed that there was no effect of oral administration of galangal extract on body weights of rats in terms of toxicity. Further, the feed intake of the treated group showed a significant change compared to the control which indicate that the oral administration of 2000 mg/kg body weight of galangal extract promoted any appetite suppression, slight gastric irritation or any unfavourable effect on Wistar rats during observation period. Investigating the effect of a test substance on liver and kidney functions are important in toxicity evaluation as they are considered to be vital organs of an organism [24]. Therefore, clinical biochemistry analysis was carried out to investigate the possible changes in hepatic and renal functions of the tested animals. Interestingly, the results revealed that the oral administration of galangal extract did not produce any significant changes in ALT, AST levels indicating that there is no adverse effect of galangal extract on hepatocyte functions of tested animals. The normal levels of BUN and creatinine levels also reflected that there was reduced likelihood of renal problems.

According to chemical compositional data, 1' ACA was recorded as the major chemical compound of galangal extract which is also known to possess pharmacological activities including antimicrobial activity [25, 26, 27, 28]. However, there are not much data on toxicological effect or any irritancy levels of 1'ACA recorded so far. Therefore, further studies need to be conducted to find the non-irritant levels of purified 1' ACA which could be used in pharmaceutical or food industry. The dermal application of galangal extract does not show any toxicologically adverse effect on rabbits without wounds and scratches. Therefore, the undiluted extract may have the potentials to be used in topical dermal applications after testing clinically with human volunteers. In terms of eye irritation, the galangal extract with lower concentrations (< 20 mg/ml) are found to be non irritant on CAM which could be similar to conjunctiva.

Thus, these data can be taken into considerations on standardized formulations of galangal extract which could be used in disinfectants with effective dose to kill pathogenic microorganisms such as *Staphylococcus aureus* or *Listeria monocytogenes*. Further, the oral toxicity results suggest that the administration of galangal extract at a single dose of 2000 mg/kg body weight was not toxic in Wistar rats. Hence, the lower concentrations (<2000 mg/kg bodyweight) of galangal extract can be used in pharmaceutical formulations.

CONCLUSION: All tested concentrations of galangal extract in terms of acute skin irritation; acute eye irritation and acute oral toxicity are well above the therapeutic levels for antimicrobial activity of galangal extract against food-borne pathogens including *Staphylococcus aureus* and *Listeria monocytogenes* [29]. Therefore, the current toxicity study revealed the importance of determining the safe effective dosage of galangal extracts before using them for the development of herbal products with antimicrobial activity for dermal applications, pharmaceuticals or food additives. Detailed experimental analysis of chronic toxicity with long term use of galangal extract is suggested for further confirmation of these data.

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CONFLICT OF INTEREST: The authors declared that there is no competing interest.

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