Anti-Inflammatory And Anti-Arthritic Potential Of Essential Oil From Kaempferia Galanga Rhizome

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Abstract

This research aims to provide pharmacological investigation of Kaempferia galanga rhizome essential oil, specifically focusing on its therapeutic effects across various invitro and in vivo models of inflammation and arthritis. The powdered crude rhizome maintained all the standard parameters . The dried rhizome were subjected to steam distillation to isolate the essential oil and the percent yields of oils obtained in this study were 1.14%w/w. The phytochemical analysis of KGEO were performed using GC-MS and FT-IR. Acute toxicity study, conducted as per OECD 423 guidelines, demonstrated that the oil was safe up to 2000 mg/kg body weight. Evaluation of in vitro anti inflammatory activity by protein denaruration method using egg albumin were conducted, the standard reference drug diclofenac and the plant oil showed concentration dependent inhibition of protein denaturation. However the effect of diclofenac was found to be less when compared with Kaempferia galanga essential oil, which was confirmed with IC_{50} values ie. IC_{50} values 82 µg/ml and 62 µg/ml respectively. In in vivo carrageenan induced paw oedema model, oral administration of the essential oil at doses 200 and 400 mg/kg significantly suppressed the paw edema at 2, 3, 4 and 5 h after carrageenan injection in rats. Diclofenac at dose 10mg/kg,p.o significantly suppressed paw edema at 2,3,4 and 5 h after carrageenan administration ($p< 0.001$).In rats given Freund's adjuvant, oral administration of oil at 200 and 400 mg/kg and prednisolone at 10 mg/kg significantly decreased paw oedema during the second stage of inflammation. On day 13^{th}, 200 mg/kg of oil results in a little decrease in the amount of paw oedema ($p<0.05$), but 400 mg/kg has an effect that is comparable to that of the standard drug prednisolone at a dose of 10 mg/kg ($p<0.01$).

Key words: Galanga oil, paw edema,egg albumin, adjuvant arthritis

Introduction

Plants had been used for medicinal purposes long before recorded history. Ancient Chinese and Egyptian papyrus writings describe medicinal uses for plants as early as 3,000 BC. Indigenous cultures (such as African and Native American) used herbs in their healing rituals, while others developed traditional medical systems (such as Siddha, Ayurveda, Unani and traditional Chinese medicine (TCM) in which herbal therapies were used [1]. Recently there has been a shift in universal trend from synthetic to herbal medicine, which we can say 'Return to Nature'. Since ancient times, medicinal plants have been considered as a rich source of therapeutic substances for the prevention of diseases and ailments and they have been honoured worldwide. The rising burden of mental health and chronic inflammatory conditions has led to an intensified search for effective, natural, and multi-targeted therapeutic agents. Conventional pharmacotherapy for inflammatory disorders often comes with limitations such as side effects, tolerance development, and potential resistance, highlighting the need for alternative or complementary treatments. In this context, plant-based medicines have emerged as promising sources of bioactive compounds due to their broad-spectrum efficacy and favourable safety profiles.

Kaempferia galanga L., a member of the Zingiberaceae family, is a rhizomatous plant widely used in traditional medicine, particularly in Southeast Asia. Known as "galanga" or "sand ginger," it has long been valued for its diverse medicinal properties, including its diuretic, anti-inflammatory, and analgesic effects. The essential oil extracted from K. galanga rhizomes has been found to contain various bioactive compounds such as volatile oils, terpenoids, flavonoids, and phenolic constituents. These compounds are believed to contribute to the plant's multifaceted therapeutic potential [2].

The plant traditionally used for various conditions such as anxiety, insomnia, inflammation, arthritis, and as a diuretic. In some countries, particularly in Southeast Asia (Eg., Indonesia, Thailand, Malasia, India etc.) Kaempferia galanga has gained attention for its potential as a natural remedy for the above illness. However it was confirmed that most studies have concentrated on the plant extract. The anti-inflammatory and anti-arthritic works were reported using plant rhizome extract and some limited resources available with oil. More over so many works were seen regarding the antioxidant nature of the oil from K.Galanga rhizome.So this work concentrated on the in vitro ,in vivo anti-inflamatory activity and in vivo antiarthritic activity of the essential oil from the rhizome of Kaempferia galanga by steam distillation method [3,4,5,6].

Material and methods

Plant material

Freshly harvested and matured rhizomes of Kaempferia galanga were collected from Kuninji, Parappuzha village, Thodupuzha, Idukki district, Kerala, India. Dr. K.V George, Head of the Botany Department at C.M.S College, Kottayam, verified the authenticity of the entire plant with rhizome. A voucher specimen (DPS/HERB/2024/1/KG) has been kept in the Herberium of the Department of Pharmaceutical Sciences, Cheruvandoor for future reference. After proper identification, fresh matured rhizomes were separated, carefully washed with distilled water, sliced into little pieces, and allowed to air dry for seven days at room temperature. A mechanical grinder was used to grind the shade-dried rhizomes into a somewhat fine powder. After that, the powders were kept out of the light in an airtight container until further use.

Isolation of *Kaempferia galanga* rhizome oil by Steam distillation

Utilising a modified reported procedure, the essential oil from the Plant rhizomes was extracted through steam distillation. At first, 50 g of air-dried K. galanga rhizome powder was steam-distilled for four hours in a Clevenger

apparatus. After collecting the distillate, add 50 ml of n hexane to the seperatory funnel shake gently then allow the layers to separate. The organic layer (upper layer) will contain the essential oil. Repeat the extraction with n hexane two more times. After all three extractions combine the three hexane layers in to a beaker. The organic layer was then dried with anhydrous sodium sulphate, allowed to stand overnight, filtered, and evaporated using a rotary vacuum evaporator. The essential oil obtained was then weighed to determine the mass, and stored in light resistant vials at 4-6° C to preserve freshness until use [7]

Phytochemical analysis

Gas chromatography – Mass spectrometry analysis (GC-MS)

Using the GC-MS method, the rhizome oil of Kaempferia galanga L. was investigated for the presence of certain volatile substances. The GC model (Clarus 680) equipped with a fused silica column and packed with elite-5MS (5% phenyl & 95% dimethyl polysiloxane, 30 m × 0.25 mm ID × 250µm df) was used to perform the GC analysis. Helium was used as a carrier gas steady flow rate of 1 ml/min. 260° C was the injector temperature specified for the chromatographic run. The device was injected with 1µl of the sample oil. The oven was set to 60 °C for two minutes, then 300° C at a rate of 10° C per minute, and finally 300° C, which is maintained for six minutes. The parameters for the mass detector were as follows: 230° C for the transfer line, 230 °C for the ion source, 70 eV for the ionisation mode electron impact, 2 seconds for the scan period, and 0.1 seconds for the scanning interval. The run time was 31.14 minutes overall. More research was done using the peak areas that were discovered.The components spectrum found in the sample were compared to the published literature as well as the database of known component spectrum kept in the GCMS NIST (2008) library and other reference books[8,9]

Fourier transform infrared spectroscopy (FT-IR)

The essential oil was analysed using a FT-IR spectrometer (model –Perkin Elmer Spectrum Two) equipped with an ATR accessory. A background spectrum was first recorded. A drop of the essential oil was placed directly on the ATR crystal, ensuring good contact. The sample spectrum was collected in the mid-IR region (4000–400 cm^{-1}) and analysed for characteristic absorption bands. The spectrum was compared with standard reference spectra to confirm the presence of key compounds [10]

Acute toxicity studies as per OECD guidelines 423

Acute toxicity test was conducted in accordance with OECD guideline 423 for chemical testing. Based on biometric evaluation using fixed doses that are sufficiently separated, the acute toxic class technique allows a chemical to be graded for classification and hazard assessment. The method as adopted in 1996 and was thoroughly validated in vivo using LD50 data that were obtained from the literature on a national and international level.

Animals

20–25 g female albino mice, aged 7-8 weeks, were obtained from the animal house of Department of Pharmaceutical Sciences, C.P.A.S, Puthuppally, Kerala. The animals were housed in polypropylene cages with a 12-hour light and dark cycle, 25 ± 2°C temperature, and 50 ± 5% relative humidity. They were given an unlimited supply of water and a regular laboratory animal feed. Prior to the test, the animals were adjusted to the laboratory environment. The Committee for the Control and Supervision on Experiments on Animals (CCSEA) and the Institutional Animal Ethical Committee (IAEC NO: MGU/DPS/IAEC/2016/PhD/03) have approved the design and conduct of the experiments in accordance with ethical standards. Three hours before the dose, the animals were fasted (just food, not water, was

withheld). All test animals were divided into several groups (n=3) first group given a single oral dose of 5 mg/Kg. However if there was no mortality, the procedure was repeated with increased doses, such as 50, 300, and 2000 mg/kg of KGEO with 1% CMC using an oral feeding needle. After the administration of test substance, food for the mice was withheld for 2hr and for the first 30 minutes, each animal was observed alone. After that, they were watched for the next 24 hours, with special attention given to them during the first 4 hours. After that, they were watched every day for a total of 14 days to look for signs of toxicity/mortality, such as changes in the skin, fur, eyes, mucous membranes, respiratory, salivation, lacrimation, circulatory, scrotal licking, abdominal contraction, autonomic, and central nervous systems, as well as behavioural pattern [11]

Evaluation of anti-inflammatory activity of Kaempferia galanga rhizome oil

Protein denaturation by using egg albumin (in vitro)

5 ml of the reaction mixture contained 0.2 ml of fresh hen's egg albumin, 2.8 ml of phosphate buffered saline (PBS, pH 6.4), and 2 ml of the test drug KGEO in various concentrations, resulting in final concentrations of 31.25, 62.5, 125, 250,500 &1000 µg/ml. A control sample consisted of the same volume of double-distilled water. After that, the mixtures were heated for five minutes at 70º C after being incubated for fifteen minutes at 37±2º C in a BOD incubator. After cooling their absorbance was measured at 660 nm with a vehicle served as the blank. As a reference drug, diclofenac (final concentrations of 31.25, 62.5, 125, 250, 500, and 1000 µg/ml) was treated in a similar manner for the purpose of determining absorbance (SHIMADZU, UV 1800). Using the following formula, the percentage inhibition of protein denaturation was determined: % inhibition =100 x (Vt / Vc– 1) where, Vt is the test sample's absorbance &Vc is the control sample's absorbance. The oil/reference drug concentration for 50% inhibition (IC50) was determined by plotting percentage inhibition with respect to control against treatment concentration (standard calibration curve)[12,13]

Carrageenan induced paw edema in rats

Wistar albino rats weighing between 180 and 200 g were used. All the animals were procured from Agricultural University, Mannuthy, Thrissur, Kerala and they were maintained in the animal house of Department of pharmaceutical sciences, Puthuppally. The Institutional Animal Ethical Committee officially approved the experimental protocols, which were carried out in compliance with the CCSEA guidelines (IAEC NO: MGU/DPS/ IAEC/2016/ PhD/03).

All the animals were divided into five groups, each with six animals. Group-I : –ve control which received vehicle (1% CMC, 1ml/kg, p.o.) only, Group-II : +ve control which received 1% w/v solution of carrageenan (0.1ml, s.c) ,Group-111 and IV: Test groups which received KGEO (200 and 400mg/kg p.o. respectively) .Groups-V : Standard group which received diclofenac, 10 mg/kg, p.o. Anti-inflammatory activity was assessed by the method described earlier by Winter et al.,1968. The rats were divided into six-animal per group. Oral test drugs were administered to the various groups, along with standard and vehicle-based controls. The rats were given a subcutaneous injection of 0.1 ml of a 1% w/v carrageenan solution into the left paw's sub plantar area after 30 minutes. The paw was inked at the level of the lateral malleolus and then immersed in mercury. Up to the mark Using a volume transducer (model no. vt-2723) connected to a strain gauge coupler of the Student Physio-Graph (model no. PG-02, INCO, Ambala, India), the paw volume was measured at 0, 1, 2, 3, 4,5 and 24 hours following the carrageenan injection. The actual volume of paw edema was determined by the difference between the initial and subsequent readings [14]

Statistical analysis

Graph Pad Prism software (version 10.3.1(509), Graph Pad, San Diego, USA) was used for statistical analysis. The data were expressed as mean ± standard error of mean (S.E.M.) at 95 % confidence level. One way ANOVA followed by Dunnett's test was applied to test difference among the groups. $p < 0.05$ was considered statistically significant.

Evaluation of anti-arthritic activity of Kaempferia galanga rhizome oil by Freund's adjuvant induced arthritis in rats.

Healthy Wistar albino rats weighing 200–220 g and both sex were used. The Agricultural University, Mannuthy, Thrissur, Kerala, provided all of the animals. Before being tested, every animal was acclimatised in the laboratory animal room for one week. The chosen rats were kept in polypropylene cages with conventional temperature ranges of 20 to 25^{o} C, fed a standard rodent meal, and given unlimited access to water. The Institutional Animal Ethical Committee officially approved the experimental protocols, which were carried out in compliance with the CCSEA guidelines (IAEC NO: MGU/DPS/ IAEC/2016/ PhD/03).

The rats were divided into five groups, each with six animals. Group I was used as a negative control group and was given CMC 1% exclusively. Group II served as a positive control group, and its only treatment was the 0.1 ml of Complete Freund's adjuvant. Group III & IV (Test group) were given KGEO 200 and 400 mg/kg, p.o., respectively Groups V was given prednisolone 10 mg/kg, p.o., served as a standard group. Rat adjuvant arthritis and human rheumatoid arthritis are quite similar. When complete Freund's adjuvant is injected into the rat paw, the predominant lesion is inflammation, which maximum three to five days later. After delays of around, secondary lesions develop in rats weighing between 180 and 200 g at first. Day 1 involves injecting them subcutaneously (s.c.) with 0.1 ml of complete Freund's adjuvant (GENEI, Bangalore), which is suspended Mycobacterium tubercle (heat killed bacilli) in heavy paraffin oil at a concentration of 6 mg/ml. Dosing for both the standard or the test drugs begins the same day and continued for 12 days. On the day of injection, the volumes of both sides' paws and the total weight of the body are measured. The volume of the paws is measured using a volume transducer (model no. vt-2723) that is connected to the strain gauge coupler of the Student PhysioGraph (PG-02, INCO, Ambala, India). The volume of the injected paw is measured again on days 2, 3, 4, 5, 9, 13, 15, 19, and 21. This data reveals the primary lesion and the impact of treatment drugs during this period. Measurement of the noninjected paw (secondary lesions) using a volume transducer is used to determine the severity of the induced adjuvant disease. The animals are intentionally not dosed with the test drug or the standard between days 13 and 21. On the 21st day the rats were anaesthetised by administering combination of ketamine+Xylazine (70 mg/kg,i.p.+10 mg/kg,i.p.).Blood samples were taken from all animal groups (retroorbital puncture) on the 22nd day animals were weighed and sacrificed by ketamine and Xylazine over dosage. Rat knee joint samples were obtained for histopathological analysis. These samples were used to evaluate a number of microscopical parameters. Evaluation of joint inflammation was done by a third party who was not aware of the course of treatment [15,16]

Statistical analysis

Data were statistically analysed by the student's t-test and one way analysis of variance(ANOVA) followed by Dunnet multiple comparisonbased on the number of groups involved and are reported as the mean ±S.E.M. $p < 0.05$ are regarded as statistically significant.

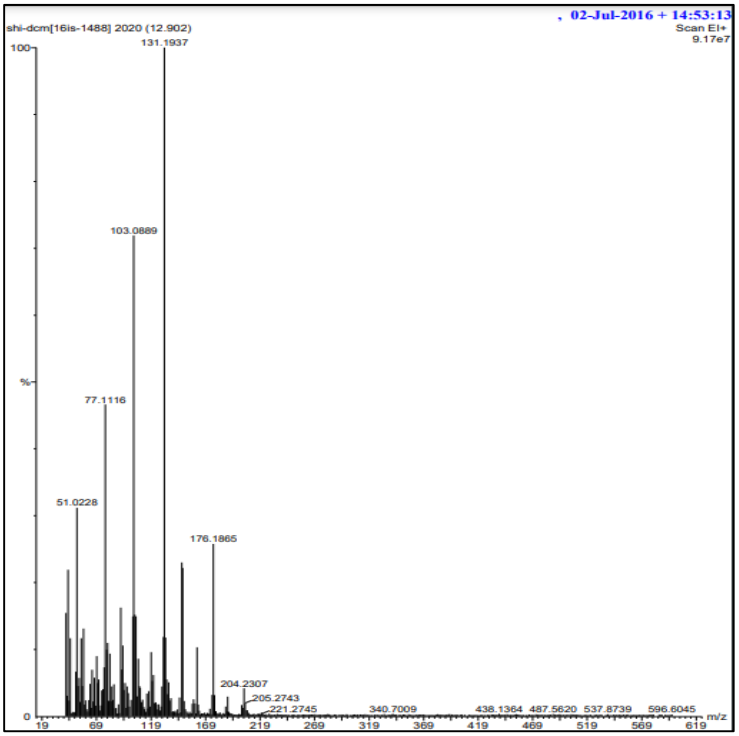
Results

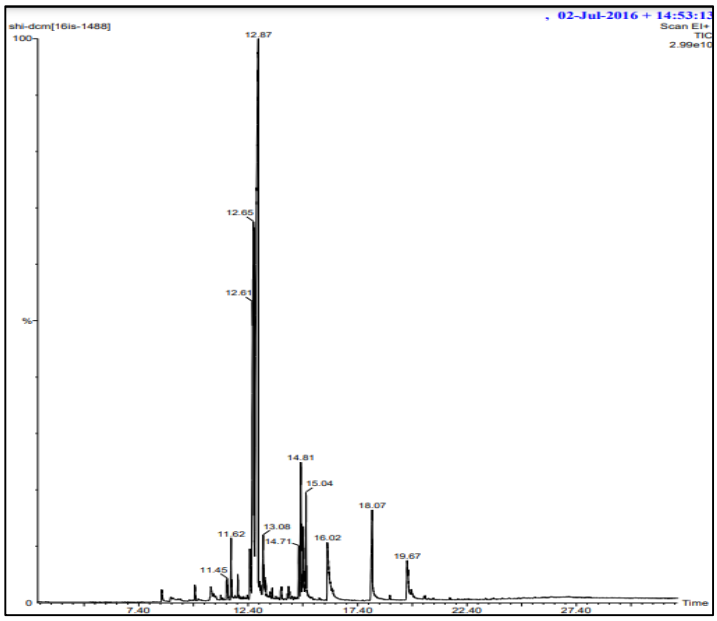
Table 1. Percentage yield of KGEO

Name of plant	**Plant part used**	**Method of Isolation**	**Solvent used for Isolation**	**Percentage yield (%w/w)**
Kaempferia galanga	Rhizome	Steam distillation	n Hexane was used to separate the oil from aqueous phase	1.14

Phytochemical analysis analysis of Kaempferia galanga essential oil

Gas Chromatography Mass Spectrometry (GC-MS)

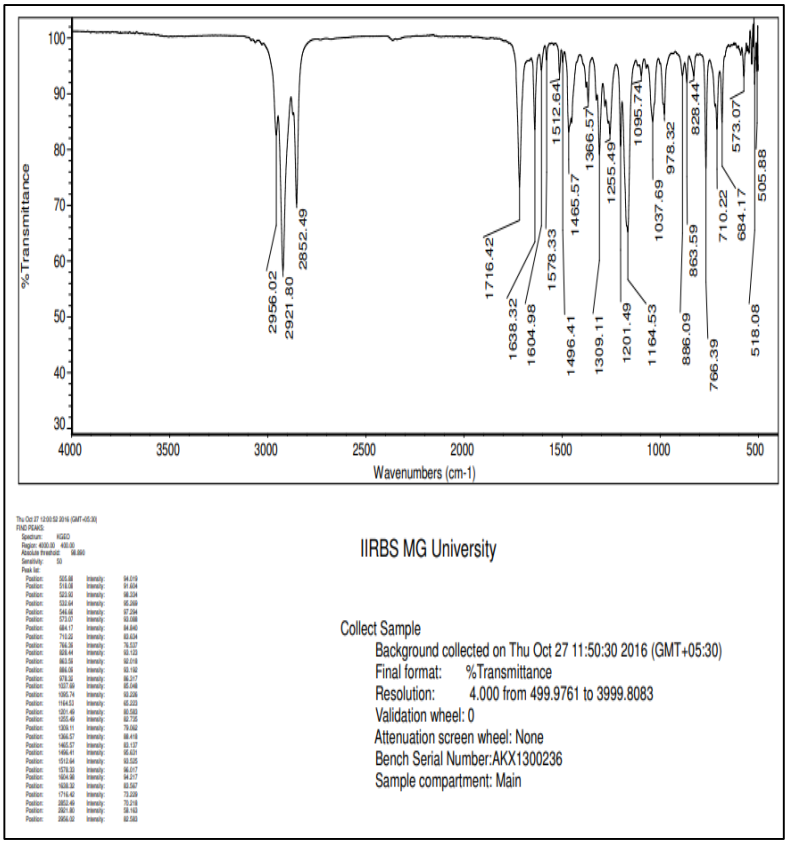




Chromatogram (1) GC-MS spectrum of KGEO

Fourier-transform infrared spectroscopy (FTIR)

IR spectrum of KGEO was recorded by FTIR spectrophotometer and compared with standard functional group frequencies.



Chromatogram (2) FTIR spectrum of KGEO

Table 2.

Functional groups	**Characteristic peaks(cm^{-1})**	**Observed peaks(cm^{-1})**
C=0 stretching of α,β unsaturated ester	1715-1730	1716
C-H Stretching	2848-3000	2921
C=C Stretching of alkene	1626-1662	1638
>C=C<S	1580 &1500	1578&1512
C-O-C	1310-1210	1309

Toxicological profile of Kaempferia galanga essential oil

Acute toxicity studies as per OECD guidelines 423

The KGEO were dissolved in CMC 1% and administered orally to mice. No acute mortality was observed even at 2000mg/kg. All the animals were found to be normal, healthy and there were no gross behavioural changes and other observational changes at the end of observation period (14 days). From these results, LD_{50} or the maximum tolerated dose was found to be 2000mg/kg. From this 1/5th and 1/10th of the maximum tolerated dose was selected for the screening of various in vivo pharmacological activities.

Table (3) Acute toxicity study data and dose selection of KGEO

Drug	**LD50 or MTD(mg/kg)**	**ED50 or 1/5th of LD50 /MTD(mg/kg)**	**ED50 or 1/10th of LD50 /MTD(mg/kg)**
KGEO	2000	400	200

Effect of anti-inflammatory activity Kaempferia galanga rhizome oil

Protein denaturation by using egg albumin (in vitro)

Table (4) Influence of KGEO against protein Denaturation

Concentration (µg/ml)	**% Inhibition**
31.25	39.30±0.006
62.5	48.68±0.012
125	70.51±0.006
250	78.40±0.015
500	85.7±0.006
1000	94.33±0.006

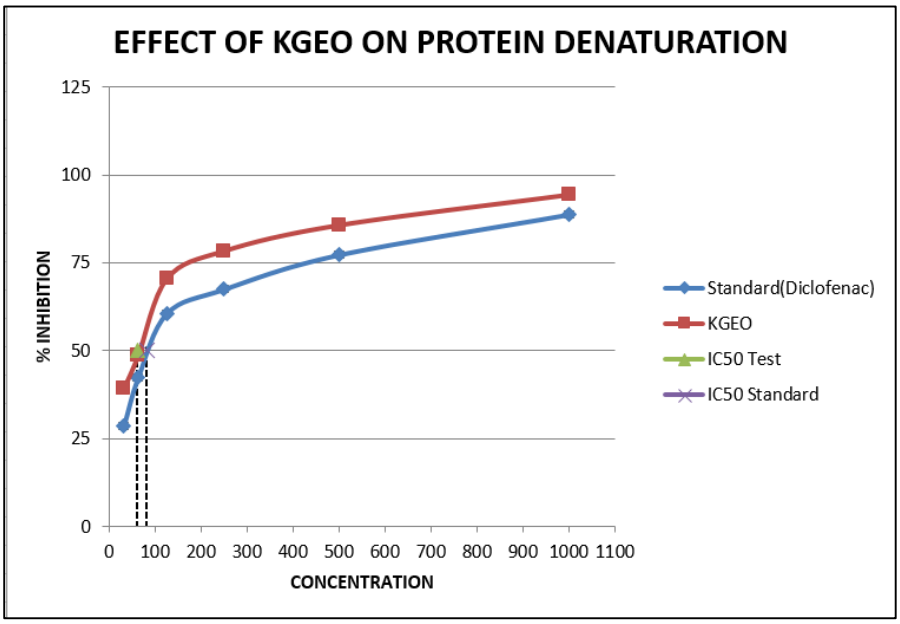
Values represent mean ± SD (n = 3)

Table (5) Influence of diclofenac against protein denaturation

Concentration (µg/ml)	**% Inhibition**
31.25	28.58±0.036
62.5	42.35±0.031
125	60.66±0.015
250	67.53±0.009
500	77.29±0.007
1000	88.77±0.007

Values represent mean ± SD (n = 3)

Graph (1) Effect of KGEO and diclofenac on protein denaturation



Values represent mean ± SD (n = 3)

Table (6) IC50 values of KGEO and Diclofenac against protein denaturation

Treatments	**IC50 values (µg/ml)**
KGEO	62
Diclofenac	82

Evaluation of anti-inflammatory activity of Kaempferia galanga rhizome oil by carrageenan- induced paw edema in rats

Table(7). Effect of KGEO on carrageenan- induced paw edema in rats

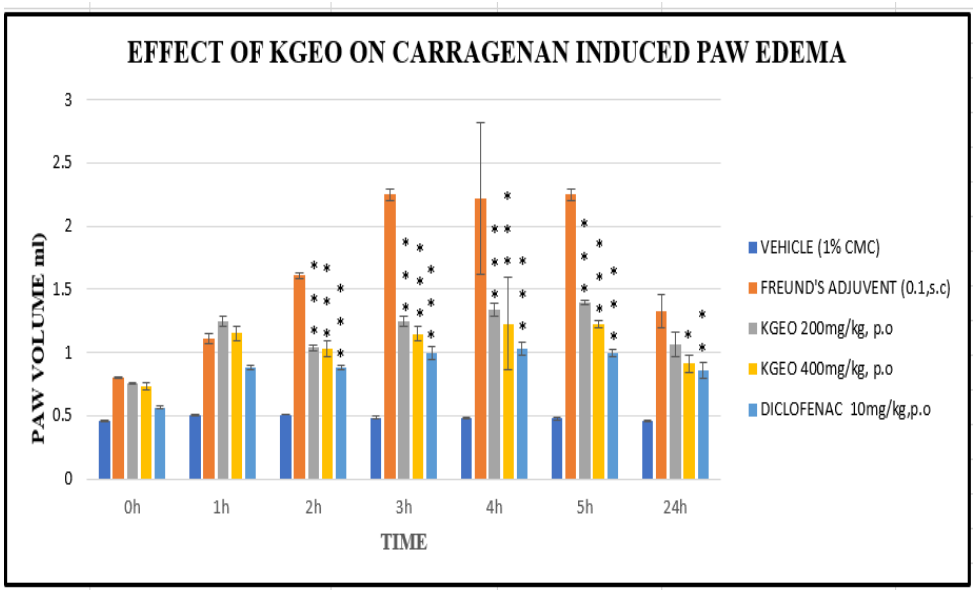
Treatment	**Dose (mg/kg,p.o.)**	**Paw edema volume at different time interval (in ml)** [a]					
		1 h	**2 h**	**3h**	**4h**	**5 h**	**24 h**
-ve control (CMC1%)	-	0.51 ± 0.003	0.51 ± 0.003	0.49 ± 0.009	0.48 ± 0.008	0.48 ± 0.010	0.48 ± 0.008
+ve control (Carrageenan1%)	0.1ml,s.c	1.11 ± 0.04 [b]	1.61 ± 0.022 [b]	2.25 ± 0.049 [b]	2.21 ± 0.601 [b]	2.25 ± 0.043 [b]	1.33 ± 0.133 [b]
KGEO + Carrageenan1%	200 + 0.1ml,s.c.	1.25 ± 0.039	1.04 ± 0,022***	1.25 ± 0.037***	1.34 ± 0.052***	1.40 ± 0.018***	1.07 ± 0.097

KGEO + Carrageenan1%	400 + 0.1ml,s.c	1.15 ± 0.056	1.03 ± 0.065***	1.15 ± 0.056***	1.23 ± 0.368***	1.22 ± 0.026***	0.91 ± 0.070*
Diclofenac+ Carrageenan1%	10	0.88 ± O.021	0.88 ± 0.022***	1.00 ± 0.053***	1.03 ± .053***	0.995 ± 0.028***	0.86 ± 0.64**

[a] The results given are mean ± SEM; no of animals used (n=6)

[b] The carrageenan induced inflammatory group was compared with that of vehicle treated group, showed significant difference using student's t test. [b]($p<0.001$). *,**,*** significant differences from carrageenan induced rats using one way analysis of variance(ANOVA) followed by Dunnet multiple comparison (compare all vs.control) n=6,at $p<0.05$, $p<0.05$and $p<0.001$respectively

Histogram (1)



The results given are mean ± SEM; no of animals used (n=6).

*,**,*** significant differences from carrageenan induced rats using one way analysis of variance (ANOVA) followed by Dunnet multiple comparison (compare all vs.control) n=6,at $p<0.05$, $p<0.05$and $p<0.001$respectively

Effect of Kaempferia galanga rhizome oil on adjuvant induced arthritis in rat

Table (8). Effect of KGEO on adjuvant- induced primary inflammation in the injected paw of rat

Groups	Dose (mg/kg, p.o.)	Injected left paw edema volume (ml) [a]			
		Day 1	Day 2	Day 3	Day 5
Vehicle (1% CMC)	---	0.43 ± 0.00	0.46± 0.01	0.46 ± 0.01	0.45± 0.01
Freund's adjuvant (CFA)	(0.1ml, s.c)	0.52 ± 0.03 [b]	0.57 ± 0.03 [b]	0.58 ± 0.03 [c]	0.62 ± 0.01 [d]
CFA + KGEO	(0.1ml, s.c) + 200	0.48 ± 0.03	0.53 ± 0.04	0.55± 0.03	0.57 ± 0.03
CFA + KGEO	(0.1ml, s.c) + 200	0.50 ± 0.03	0.55±0.02	0.57± 0.02	0.60± 0.02
CFA + prednisolone	(0.1ml, s.c) + 10	0.53± 0.02	0.58± .03	0.60± 0.02	0.63 ± 0.02

[a] The results given are mean ± SEM; no of animals used (n=6).The adjuvant induced arthritis group was compared with that of vehicle treated group, showed significant difference using student's t test. [b] ($p<0.05$). [c] ($p<0.01$), [d]($p<0.001$).

Table (9). Effect of KGEO on adjuvant- induced secondary inflammation in the injected paw of rat

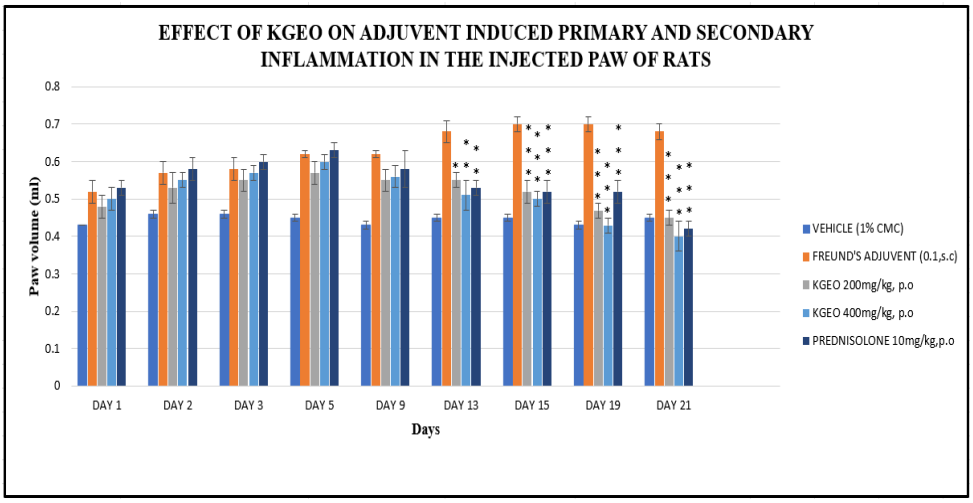
Groups	Dose (mg/kg, p.o.)	Injected left paw edema volume (in ml) [a]				
		Day 9	Day 13	Day 15	Day 19	Day 21
Vehicle (1% CMC)	-	0.43 ± 0.01	0.45 ± 0.01	0.45 ± 0.01	0.43 ± 0.01	0.45 ± 0.01
Freund's adjuvant (CFA)	(0.1ml, s.c)	0.62 ± 0.01 [b]	0.68 ± 0.03 [b]	0.70 ± 0.02 [b]	0.70 ± 0.02 [b]	0.68 ± 0.02 [b]
CFA	(0.1ml, s.c)	0.55	0.55	0.52	0.47	0.45

+ KGEO	+ 200	± 0.03	± 0.02*	± 0.03***	± 0.02***	± 0.02***
CFA + KGEO	(0.1ml, s.c) + 400	0.56 ± 0.03	0.51 ± 0.04**	0.50 ± 0.02***	0.43 ± 0.02***	0.40 ± 0.04***
CFA + prednisolone	(0.1ml, s.c) + 10	0.58 ± 0.05	0.53 ± 0.02**	0.52 ± 0.03***	0.45 ± 0.03***	0.42 ± 0.02***

[a] The results given are mean ± SEM; no of animals used (n=6)

The adjuvant induced arthritis group was compared with that of vehicle treated group, showed significant difference using student's t test($p<0.001$). *,**,*** significant differences from freund's adjuvant-induced arthritic in rats using one way analysis of variance(ANOVA) followed by Dunnet multiple comparison (compare all vs. Control) n=6,at $p<0.05$, $p<0.05$and $p<0.001$respectively

Histogram (2)



The results given are mean ± SEM; no of animals used (n=6).

*,**,*** significant differences from freund's adjuvant-induced arthritic in rats using one way analysis of variance(ANOVA) followed by Dunnet multiple comparison (compare all vs.control) n=6,at $p<0.05$, $p<0.05$and $p<0.001$respectively.

Table(10). Effect of KGEO on adjuvant induced primary inflammation in non-injected hind paw of the rats.

Group	Dose (mg/kg, p.o.)	Non injected Right paw edema volume(ml) [a]		
		Day 5	Day 9	Day 13
Vehicle (1% CMC)	-	0.44±0.010	0.45±0.004	0.45±0.01
Freund's adjuvant(CFA)	(0.1ml, s.c)	0.48±0.00[b]	0.56±0.016 [b]	0.65±0.019 [b]
CFA + KGEO	(0.1ml, s.c) + 200	0.49±0.004	0.51±0.006*	0.50±0.004***
CFA + KGEO	(0.1ml, s.c) + 400	0.55±0.034	0.55±0.017	0.52±0.016***
CFA + prednisolone	(0.1ml, s.c) + 10	0.57±0.042	0.50±0.005*	0.52±0.030***

[a]The results given are mean ± SEM; no of animals used (n=6).[b] The adjuvant induced arthritis group was compared with that of vehicle treated group, showed significant difference ($p<0.001$) using student's t test.*,*** significant differences from freund's adjuvant-induced arthritic in rats using one way analysis of variance(ANOVA) followed by Dunnet multiple comparison (compare all vs.control) n=6,at $p<0.05$ and $p<0.001$respectively.

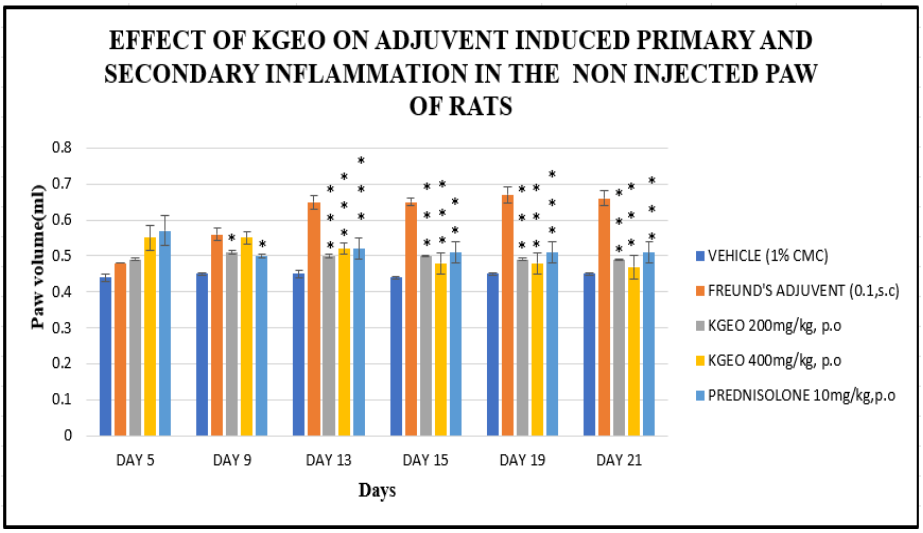
Table(11).Effect of KGEO on adjuvant induced secondary inflammation in non-injected hind paw of the rats

Group	Dose (mg/kg, p.o.)	Paw edema volume (in ml) [a]			
		Day 13	Day 15	Day 19	Day 21
Vehicle (1% CMC)	-	0.45 ± 0.01	0.44 ± 0.004	0.45 ± 0.004	0.45 ± 0.004
Freund's adjuvant .1 ml, s.c.)	-	0.65 ± 0.019 [b]	0.65 ± 0.01 [b]	0.67 ± 0.022[b]	0.66 ± 0.02 [b]
CFA + KGEO	200	0.50 ± 0.004***	0.50 ± 0.003***	0.49 ± 0.003***	0.49 ± 0.002***

CFA + KGEO	400	0.52 ± 0.016***	0.48 ± 0.030***	0.48 ± 0.030***	0.47 ± 0.033***
Prednisolone	10	0.52 ± 0.030***	0.51 ± 0.030**	0.51 ± 0.030***	0.51 ± 0.030**

[a] The results given are mean ± SEM; no of animals used (n=6).[b] The adjuvant induced arthritis group was compared with that of vehicle treated group, showed significant difference ($p<0.001$) using student's t test.**, *** significant differences from freund's adjuvant-induced arthritic in rats using one way analysis of variance (ANOVA) followed by Dunnet multiple comparison (compare all vs.control) at $p<0.01$ and $p<0.001$.

Histogram (3)



The results given are mean ± SEM; no of animals used (n=6)**, *** significant differences from freund's adjuvant-induced arthritic in rats using one way analysis of variance (ANOVA) followed by Dunnet multiple comparison (compare all vs. control) at $p<0.01$ and $p<0.001$.

CFA 0.1 ml, s.c. KGEO 200 mg/kg.p.o. KGEO 400 mg/kg.p.o. Prednisolone 10 mg/kg.i.p

Discussion

The dried rhizomes were subjected to steam distillation to isolate the essential oil and the percent yield of oil obtained in this study was 1.14%w/w. The phytochemical analysis of KGEO was performed using GC-MS and FT-IR. The m/z peaks of the mass spectra of oil showed the presence of ethyl p methoxy cinnamate and ethyl cinnamate with peaks 205.27 and 176.18 respectively and it can seen in Chromatogram 1. In FT-IR analysis the observed functional group were matched with the characteristic peak of the above two components. So spectral analysis using GC-MS and FT-IR showed the presence of ethyl p methoxy cinnamate and ethyl cinnamate as the major two components in the essential oil of *Kaempferia galanga* (Chromatogram 1&2).

Acute toxicity studies, conducted as per OECD 423 guidelines, demonstrated that the oil was safe up to 2000 mg/kg body weight, with no mortality, clinical signs of toxicity, or any observable changes, classifying it as non-toxic under GHS standards (The data shown in Table3).

One of the well-established causes of arthritic and inflammatory disorders is denaturation of tissue proteins. Denaturation of tissue proteins in vivo may be the cause of auto-antigen production in some arthritic conditions [17,18]. Therefore, it would be helpful to develop anti-inflammatory drugs that contain agents that can prevent protein denaturation. It has been reported that one of the features of several non-steroidal anti-inflammatory drugs is their ability to stabilize (prevent denaturation) heat treated albumin at the physiological pH (pH: 6.2-6.5)[19,20].Protein denaturation has also been prevented by a number of pharmaceutical drugs and herbal remedies[21].The current study provides an overview of the in vitro evaluation of anti-inflammatory properties of KGEO in relation to egg albumin denaturation. The results were summarised in table 4, 5 and IC_{50} values can be found in table 6 & graph 1). The standard reference drug and the plant oil showed percentage dependent inhibition of protein denaturation. However the effect of diclofenac was found to be less when compared with KGEO essential oil, which was also confirmed with IC_{50} values ie. IC_{50} values 82 µg/ml and 62 µg/ml respectively. The increments in absorbance's of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein (albumin) denaturation by KGEO and reference drug diclofenac[22].From the IC_{50} values it becomes evident that KGEO was more acEffects of KGEO and on carrageenan induced paw edema in rats are shown in Table 22&Histogram 6. Oral administration of the KGEO at doses 200 and 400 mg/kg significantly suppressed the paw edema at 2, 3, 4 and 5 h after carrageenan injection in rats. Diclofenac at dose 10mg/kg,p.o significantly suppressed paw edema at 2,3,4 and 5 h after carrageenan administration ($p< 0.001$) (Table 22). In the +ve control group, paw edema volume was maximum at the fifth hour after which the paw volume decreased gradually however readings up to the 24 h were recorded and compared.

The in vitro and in vivo anti-inflammatory activities of KGEO might be attributed to the presence of Ethyl cinnamate(EC) and ethyl p-methoxy cinnamate (EPMC) which are already reported to exhibit anti-inflammatory effects(in vitro) using the BSA method by komala et al.,2018[23]. Umar MI.,2014 proved the invitroantiinflammatory effect of ethyl p-methoxy cinnamate by cotton pellet granuloma assay[24].Therefore

the in vitro and in vivo anti-inflammatory activities of KGEO might be attributed to the presence of EC and EPMC and the present findings align with the traditional use of *Kaempferia galanga* for its anti-inflammatory properties and the reported activities of its extracts [25,26,27,28,29]. The current work extends previous in vitro studies on EC and EPMC by demonstrating their potential contribution to anti-inflammatory effects in vivo, thereby supporting their role as active components in the oil.

An established model for screening compounds for possible anti-inflammatory activity is the inhibition of carrageenan-induced inflammation in rats [30].When carrageenan is injected subplantarly into the rat paw, it causes plasma extravasation [31], inflammation marked by increased exudation of tissue water and plasma proteins, neutrophil extravasation, and arachidonic acid metabolism by the enzyme pathways of cyclooxygenase and lipoxygenase[32]. Histamine and serotonin, two cytoplasmic enzymes, are released from mast cells within an hour of carrageenan administration, causing the initial phase of carrageenan-induced paw oedema to develop. An increased release of prostaglandins in the inflamed area causes the second phase to start after one hour and last for three hours. Kinins provide continuity between the two phases [33].While some NSAIDs and hydrocortisone effectively block the second phase of carrageenan-induced oedema, others act against both phases [34]. According to table 7, KGEO at doses of 200, and 400 mg/kg p.o. appear to be efficacious only in the second phase. As a result, KGEO may inhibit the release of bradykinin and/or prostaglandins instead of serotonin and/or histamine. Diclofenac also has shown similar effect only at second phase, being effective in lower concentrations.

Table 8 & 9 summarises the effects of vehicle, +ve control, KGEO, and prednisolone on Freund's adjuvant-induced arthritis in rats. In rats given Freund's adjuvant, oral KGEO at 200 and 400 mg/kg and prednisolone at 10 mg/kg significantly decreased paw oedema during the second stage of inflammation. On day 13, 200 mg/kg of KGEO results in a little decrease in the amount of paw oedema ($p<0.05$), but 400 mg/kg has an effect that is comparable to that of the standard drug prednisolone at a dose of 10 mg/kg ($p<0.01$)(Table 9).

The paw volume difference in comparison to the control and adjuvant-induced rats is shown in Table 8, 9 &Histogram 2. After the administration of Freund's adjuvant, the paw volume increased significantly[35].In this anti-arthritic studies the long term administration of KGEO for the first 12 days at the dose of 200 & 400 mg/ kg, marked by inhibited the development of paw edema during the acute phases and the effect last long termly until the 21 st day (Table 8&9).0n days 15 ,19& 21 both doses of KGEO produce maximum effect that is equally to that of prednisolone 10 mg/kg($p<0.001$)

The adjuvant-treated group showed a significant inflammatory effect, as evidenced by the CFA-induced paw inflammation .The immune response caused by histamines, serotonin, prostaglandins, brandykinins, and hyaluronidase, whose release was induced by the adjuvant, was the cause of the joint swelling. It is clear from the above reaction that the inflammation has been induced by Mycobacterium tuberculosis. The paw oedema caused by Freund's adjuvant injection was found to be biphasic in animals with arthritis. The development of oedema in the paw after an adjuvant injection has been widely used to assess the severity of the illness. Inhibition of paw edema in adjuvant-induced arthritic rats is a hallmark for anti-inflammatory drug action [36].

According to earlier reports, the arthritic rats' inflammatory states were shown to be divided into two stages. On day five post inoculation, there was evidence of an acute phase. From day thirteen to day twenty-one, there were delayed sustained and chronic phases. On the fifth day following adjuvant injection, all animals showed acute inflammation; on the thirteenth day, they developed chronic polyarthritis. On the fifth day, arthritic rats showed significant oedema, which decreased on the ninth day. The injected paw's oedema volume increased much more on the thirteenth day, along with the non-injected paw also had noticeable oedema [37]. In this study non injucted paw of the CFA treated group rats shows edema when compared to vehicle treated group. Both test groups and standard group significantly reduced the paw edema volume (Table 10,11& Histogram 3)

The KGEO showed a similar pattern of amelioration throughout the chronic phase of arthritis, suppressing the oedema on the 19th and 21st day by $p>0.001$, (Tables 8 & 9).When compared to the control group, the right paw of the rat receiving adjuvant treatment showed a notable change during this stage, with an increase in paw volume. Comparing the KGEO-treated group of animals to the Freund's adjuvant-only treated group (Tables 8 &9), the KGEO-treated group showed a significant reduction of the right paw's paw volume ($p<0.001$), which is almost similar to the prednisolone-treated group's inhibition ($p<0.001$).

CONCLUSION

The present investigation was aimed at determining the spectrum of activity of anti-inflammatory (carrageenan induced paw edema) and anti-arthritic (freund’s adjuvant-induced arthritis) parameters in experimental animals. The essential oil was isolated from the rhizomes by steam distillation and was used as the test drug in this work.

The invitro study of KGEO against protein denaturation using egg album showed a dose dependent inhibitory effect which paved a torch light to carry out its in vivo activity. The KGEO at doses 200 and 400 mg/kg, p.o. showed promising effect in reducing the carrageenan induced paw edema volume (2h, 3h, 4h, 5h) in rats when compared positive control group.

The KGEO at a dose of 200 mg/kg, p.o. and 400mg/kg, p.o., showed significant and consistent reduction in rat paw edema in freund’s adjuvant-induced arthritis of both injected and non injected paw (days 13, 15, 19 &21).The primary and secondary inflammation was measured in injected and non injected paw of the rat during the experiment.

Hence finally, it can be concluded that the essential oil of *Kaempferia galanga* rhizome, at doses of 200 mg/kg and 400 mg/kg, demonstrated significant potential in managing inflammation, arthritis . The presence of ethyl cinnamate and ethyl p methoxy cinnamate might be the reason behind the present activities . However, further studies are warranted to elucidate the binding specificity of these active compounds to individual enzymes. Isolation and purification of these constituents from the plant, along with alternative in-vitro synthesis through synthetic mechanisms, could pave the way for their development into target-specific dosage forms, enhancing therapeutic precision,efficacy ,reduced toxicity and unwanted effects.

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