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Anti-inflammatory and acute toxicity studies of the extracts from the rhizomes of *Alpinia galanga* Willd.

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ABSTRACT

Petroleum ether, Chloroform, Methanolic and Aqueous methanolic (1:1) extracts of Alpinia galanga Willd. were investigated for anti-inflammatory activity in carrageenan induced paw edema in Wistar rats, and compared to a positive control drug, Ibuprofen. These extracts were given orally at a concentration of 500 mg/kg b.w. 1 hr before carrageenan injection. Methanolic extract of Alpinia galanga showed maximum inhibition of 79.51 % on carrageenan induced rat paw edema. The effect was significant ($p< 0.001$) and near to the maximum inhibition of standard Ibuprofen (83.25 %). Petroleum extract, Chloroform extract and Aqueous methanolic extracts produced 28.19, 30.13, 23.34 % of maximum inhibition, which was not significant when compared to the control. The inhibition exhibited by methanolic extract was similar with respect to onset, duration of action as compared to the standard drug Ibuprofen. The study strengthens the claim of anti-inflammatory activity for the plant, the effect of methanolic extract may be due to the inhibition of prostaglandin synthesis. The acute toxicity study of the methanolic extract on Wistar male rats exhibited an LD_{50} value of more than 5000 mg/kg with no behavioural changes.

Key Words: Carrageenan, LD_{50}, Methanolic extract, Ibuprofen, Phytochemical screening.

INTRODUCTION

It is unambiguous that the survival and enhanced life span of human beings is made possible by the medicinal plants. The dependence of early man on medicinal plants is as old as the civilization itself. The therapeutic property of the medicinal plants is the outcome of the active constituents, these pharmacologically active constituents were synthesized and stored in different plant parts. Researchers are trying to explore this treasure of bio active molecules to convert the natural chemicals in a form useful for modern systems of medicine. The chemical constituents of herbal drugs were believed to have better compatibility with the human body and hence less side effects associated with them. Hence there is a growing trend in screening new herbs with subsequent isolation of the bioactive molecules from them. The traditional medicines have been

derived from rich traditions of ancient civilizations and heritage. Indigenous systems of medicine across the world have enriched the present knowledge about the secondary metabolites and hence much of the scientific investigations are associated and relay on traditional systems of medicine. Indian system of medicine is considered as one of the richest ethnobotanical source and the work presented herein is a study carried out on a plant frequently used in Ayurvedic system of medicine.

Alpinia galanga Willd. (Fam: Zingiberaceae) is a herb used in Ayurvedic system of medicine in the name of Rasna, Sugandhamula and Greater galangal. It is also a part of Unani, Chinese and Thai folk medicine in the preparations of food and cosmetics. The pungent, spicy and zinger like odour of the drug is due to rich essential oils like cineole, methyl cinnamate, myrecene and methyl eugenol, which are the constituents of the family Zinziberaceae in general. Zingiberaceae family constitutes a vital group of rhizomatous medicinal and aromatic plants characterised by the presence of volatile oils and oleoresins. The important genera coming under Zingiberaceae are *Curcuma, Kaempferia, Hedychium, Amomum, Zingiber, Alpinia, Elettaria* and *Costus.* The genus *Alpinia* is a wide genus with 230 species distributed in Sri lanka, India, China, Japan, Australia and south east asian countries [1-4]. The rhizomes and fruits are aromatic, tonic, stimulant, nutritive, were found to be useful in conditions like rheumatoid arthritis, inflammations, cough, asthma, dyspepsia, obesity, diabetes and fevers [5].

A. *galanga* is reported to have essential oils, flavones such as galangin, alpinin, kampferide and 3-dioxy-4-methoxy flavones [6,7]. It is also reported to possess antimicrobial, antioxidant, antifungal, anti-cancer and gastroprotective activities [8-10].
B.
In view of the popularity of *A. galanga* in various Ayurvedic preparations the present work was planned to investigate anti-inflammatory activity of the extracts of its rhizomes in carageenan induced paw edema of Wistar rats and acute toxicity of methanolic extract to know LD_{50}.

MATERIALS AND METHODS

Plant material

The fresh rhizomes of *A. galanga* were collected from M/s Natural Remedies, Bangalore, India during October 2009 and were authenticated by Dr P.R. Shanta at Regional Research Institute, Bangalore, a voucher specimen is deposited in the college herbarium in the Department of Pharmacognosy, MMU College of Pharmacy, Ramanagaram (MMU/AG-04/2009) for future reference. The rhizomes were air dried for two weeks under shade and pulverized using mechanical blender until coarse, the coarse powder was kept in airtight container until used.

Preparation of Extracts

Air dried powdered plant material of rhizomes (500 g) was extracted successively using petroleum ether(60-80^{o} C), chloroform, methanol and aqueous-methanol (1:1) in soxhlet apparatus for 6 hrs which produced corresponding residues of 7.14 g, 14.38 g, 25.46 g and 19.43 g respectively after filtration, distillation, evaporation and drying of the extracts in vaccum using rotary evaporator. The dried brownish residues were stored in dessicator and were used further in the study for the pharmacological and phytochemical screening.

Phytochemical screening

A small quantity of stored extracts of *A. galanga* was diluted using corresponding solvents and was subjected to standard phytochemical screening for various constituents. The extracts were tested for the presence of phytoconstituents like Alkaloids, Glycosides, Flavonoids, Fixed oils,

Carbohydrates, Tannins, Saponins and Volatile oils. The phytochemical screening of the extracts revealed the presence of Carbohydrates, Glycosides, Flavonoids, Saponins, Phenolics and Tannins. The tests were done as per the procedures given by C.K. *Kokate* [11 and the results were given in **Table 1**.

Table 1: Phytochemical screening of the extracts of *Alpinia galanga* Willd

Constituent tested	Petroleum ether extract	Chloroform extract	Methanolic extract	Aqueous methanolic ext.
Alkaloids	-	-	+	+
Glycosides & Carbohydrates	-	-	+	+
Phytosterols	+	-	-	-
Fixed oils and fats	+	-	-	-
Tannins	-	-	+	+
Flavonoids	-	-	+	+
Saponins	-	-	+	+
Mucilage	-	-	-	+
Volatile oils	+	-	-	-
Terpenoids	-	+	-	-

+ = Positive, - = Negative

Acute Toxicity Studies

Acute oral toxicity study was carried out on methanolic extract residue as per OECD guidelines [12]. The selection of methanolic extract was done based on the quantity of the extract and the presence of phytoconstituents in it. Male Wister rats weighing 150-200 g were selected by random sampling technique for the study and were divided into 5 groups of 5 animals each. The animals were fasted overnight prior to administration of test sample, provided only water, a single oral dose of the extract was administered at the dose level of 5 mg/kg body weight and the group was observed for any toxic symptoms, behavioural changes, locomotion, convulsions and mortality for 72 hrs. The methanolic extract in the study had no signs of toxicity in the first group tested and hence higher doses of 50, 300, 2000 and 5000 mg/kg were administered to the groups by single oral administration and the animals were observed initially for any behavioural changes during 72 hrs and subsequently for toxic symptoms and mortality for a period of 14 days. The dose of 5000 mg/kg was studied in the present study to know the LD_{50} between 2000-5000 mg/kg as the drug is popular and extensively used, the information will help in safeguarding the human health. As there is no mortality even at the highest dose tested the LD_{50} was estimated to be more than 5000 mg/kg and accordingly a dose of 500 mg/kg was chosen for the anti-inflammatory study.

Anti-inflammatory activity

Albino rats of Wistar strain of either sex weighing 150-200 g were procured from the Department of Pharmacology, Al-Ameen College of Pharmacy, Bangalore, India. The animals were kept for acclimatization under laboratory conditions in polypropylene cages for one week before the start of study and allowed food and water *ad libitum*. The standard environmental conditions of temperature 27 ± 2^{o} C with humidity of 50-60% RH and light/dark cycle of 12hr/day, were used throughout the period of anti-inflammatory activity study. The experimental protocol was approved by Institutional Animal Ethics Committee, MMU College of Pharmacy, Ramanagaram, India.

The extracts of *A. galanga* were evaluated for anti-inflammatory activity by carrageenan induced rat paw edema method [13]. The rats were randomly distributed into six groups of six animals each. The first group served as control and received 2 ml of 0.5% w/v gum acacia, second group

served as positive control and standard (Ibuprofen 50 mg/kg, p.o), while third, fourth, fifth and sixth groups received petroleum ether, chloroform, methanolic and aqueous methanolic extracts at 500 mg/kg body weight respectively. 500 mg/kg extracts were suspended in 2 ml of 0.5% w/v gum acacia and was administered orally to rats 1 hr before subcutaneous injection of carrageenan. After 1 hr 0.1ml of 1% w/v suspension of carrageenan was injected into sub-plantar region of the left hind paw to all the groups. The paw volume was measured at 1, 2, 3, 4, and 5 hr using Plethysmometer (Model 7150 UGO Basile, Italy). Edema was expressed as the mean increase in paw volume relative to control animals. The percentage inhibition of edema was calculated by the following equation:

$$\% \text{ Inhibition is } = 100\ (1 - Vt/Vc)$$

Where Vc= Edema volume of control
Vt= Edema volume of test

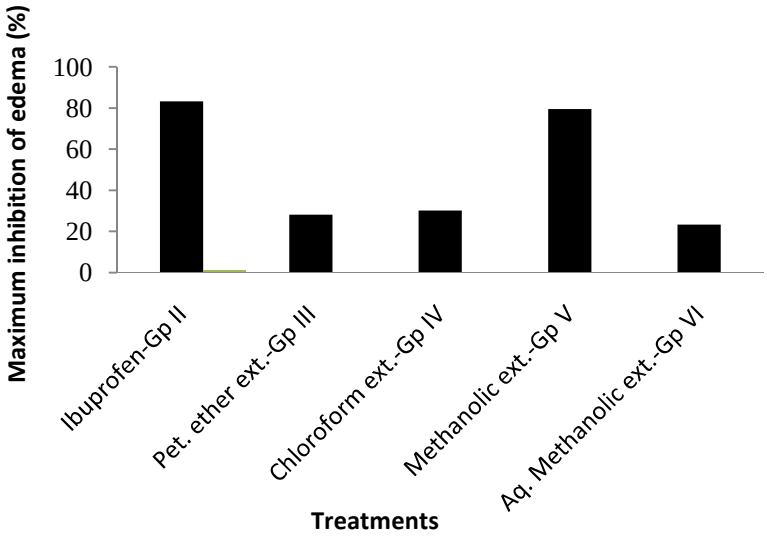
The results were given in **Table 2**

Table 2: % Inhibition of edema by the extracts of *Alpinia galanga* Willd. at different time intervals

Group	Dose	% Inhibition of edema at different time intervals				
		1 hr	2 hr	3 hr	4 hr	5 hr
Group II (Standard)	50 mg/kg	42.93**	68.65***	80.16***	81.60***	83.25***
Group III (Petroleum ether extract)	500 mg/kg	18.47	20.72	24.51	27.69	28.19
Group IV (Chloroform extract)	500 mg/kg	15.42	22.34	24.14	28.14	30.13
Group V (Methanolic extract)	500 mg/kg	40.76**	62.39**	76.52***	78.43***	79.51***
Group VI (Aqueous Methanolic ext.)	500 mg/kg	16.84	18.39	20.17	21.14	23.34

*Statistically significant using Students's t-test, compared with control, **p< 0.01,***p<0.001*

Fig 1: Comparison of the maximum inhibitions of Ibuprofen and the extracts



Statistical Analysis
The experimental results were statistically analysed by one-way ANOVA, p values < 0.01 were considered significant.

RESULTS AND DISCUSSION

Alpinia galanga is a plant frequently cultivated for their rhizomes in tropical countries. The rich essential oils present in the rhizomes enables them to be used as flavouring, antispasmodic, carminative, stimulant, and anti inflammatory agents. In Ayurveda the use of the plant in preparations like arishtas, asavas, kasayas, churnas and tailas to cure vata and kapha is highly recommended. Being a member of Zingiberaceae family the plant is rich in volatile oils and other phytoconstituents. The extracts of the rhizomes were evaluated for the presence of phytoconstituents before the anti-inflammatory study and the results were as given in the **Table 1**. Methanolic and aqueous methanolic extracts gave positive reactions for alkaloids, glycosides, tannins, flavonoids and saponins, whereas the chloroform extract for terpenoids and petroleum extract for phytosterols, volatile oils and fixed oils.

To assess the acute toxicity of the plant, methanolic extract was selected as it gave positive tests for most of the constituents during phytochemical screening. The extract was also chosen because of its high extractive value. The animals tested in acute toxicity had no symptoms of toxicity and signs of behavioural changes for 72 hrs and later for 14 days. According to OECD if mortality was observed in 2 or 3 animals in a group of 5 animals then the dose administered should be considered as a toxic dose, if mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses. The OECD suggests and discourages the dose of 5000 mg/kg keeping the welfare of the animals, but recommends only under special conditions. In view of the popularity and extensive use of the herb in Ayurvedic preparations it was felt appropriate to test higher dose to know the toxicity. Common side effects like diarrhoea, depression and loss of weight were not observed. In the methanolic extract study LD_{50} of the extract was found to be more than 5000 mg/kg.

Carrageenan induced paw edema is the most frequently used technique to screen the anti-inflammatory activity**.** Edema developed after the injection of carrageenan serves as an index of acute inflammatory changes and can be determined from differences in the paw volume measured immediately after carrageenan injection and then every hour upto 5 hrs. Edema induced by carrageenan occurs in two phases in the first phase serotonin and histamine release occurs and will last for 1 hr, the second phase involves the release of prostaglandins, cyclooxygenase products [14,15]. The anti-inflammatory effect of *A. galanga* rhizome extracts against carrageenan induced paw edema is shown in **Table 2**. To evaluate the anti-inflammatory property of the extracts on carrageenan induced paw edema, Ibuprofen at 50 mg/kg body weight was suspended in 0.5 % w/v gum acacia and 2 ml of the suspension was given as single oral administration 1 hr before the carrageenan injection. The control groups received only the gum acacia suspension. Ibuprofen significantly ($p<0.01$) reduced the rat paw edema by 42.93 % in the 1 hr, 68.65 % and 80.16 % ($p<0.001$) respectively during the 2 and 3 rd hrs. The highly significant anti-edematous property of Ibuprofen after 2 hr clearly justifies the role of the drug in decreasing the inflammatory mediators of the second phase, prostaglandins. Thus Ibuprofen inhibits the cyclooxygenase and their by reducing the inflammation associated with prostaglandins. Different extracts of *A. galanga* as single oral dose at 500 mg/kg body weight using gum acacia as a suspending agent were given to group III, group IV, group V and group VI. The petroleum ether extract was given to group III, chloroform extract to group IV,

methanolic extract to group V and aqueous methanolic extract (1:1) to group VI. Petroleum ether extract showed an insignificant inhibition of 18.47 at the 1 hr, 20.72 at 2 hr, 24.51 at 3 hr, 27.69 at 4 hr and a maximum inhibition of 28.19 % at the 5 hr. Similarly the chloroform extract exhibited 15.42, 22.34, 24.14, 28.14 and 30.13 % during 1, 2, 3, 4, and 5 hr respectively which was not significant. Methanolic extract significantly decreased rat paw volume by 40.76 % at the 1 hr and 62.39 % ($p<0.01$) at 2 hr. The decrease was highly significant in comparison with control at 3, 4 and 5 hr with 76.52, 78.43, 79.51 % inhibition ($p<0.001$) respectively. The anti-inflammatory effect of methanolic extract was higher at 2 and 3 hr similar to the Standard Ibuprofen which has cyclooxygenase inhibitory property. This indicated that the methanolic extract followed similar approach in inhibiting rat paw volume, probably by inhibiting prostaglandins. However, in contradiction the aqueous methanolic extract was not significant in reducing the inflammation throughout the period. The effect may be due to high concentration of anti-inflammatory constituents in methanolic extract than in aqueous methanolic extract. The maximum inhibition (**Fig 1**) of rat paw edema was least in aqueous methanolic extract (23.34 %), followed by petroleum ether extract (28.19 %), chloroform extract (30.13 %) and methanolic extract (79.51 %).

CONCLUSION

Thus the present study supports the anti-inflammatory effect of *A. galanga* in carrageenan induced rat paw edema model. The magnitude of inhibition, onset and the period of action suggest that the anti-inflammatory mechanism of the methanolic extract may be through inhibition of prostaglandin synthesis by reduced action of cyclooxygenase. The non toxicity of the extract also brings us to the conclusion that the plant is a valuable candidate to investigate and isolate active constituents responsible for the anti-inflammatory effect to counter various disorders of inflammation. Further studies are in progress to isolate probable active constituents contributing to anti-inflammatory activity.

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REFERENCES

[1]. K.R. Kirtikar, B.D. Basu; Indian Medicinal Plants. (Internat. Book Distributors, Dehra Dun, **1987**) 2444-2449.
[2]. J.S. Gamble; Flora of the presidency of Madras. Vol.III. (Bishen Singh Mahendra Pal Singh, Dehra Dun, India, **1987**) 1478-1493.
[3]. R.N. Chopra, S.L. Nayar, I.C. Chopra; Glossary of Indian Medicinal Plants. (CSIR, New Delhi, **1956**) 330.
[4]. W. John Kress, Ai-Zhong Liu, M. Newman, Qing-Jun Li, *Am. J. Bot*., **2005**, 92(1), 167-178.
[5]. P.K.Warrier, V.P.K. Nambiar, C. Ramankutty, Indian Medicinal Plants. Vol.1-5. Orient Longman Ltd., Madras. **1995**.
[6]. The Wealth of India, National Institute of Science Communication and Information Resources Council of Scientific and Industrial Research, New Delhi, **2005**.
[7]. Z. Cui, Determination of chemical constituents of the essential oil from *Alpinia galanga* (L.) by GCMS., *Lixueban*, **2003**, 38, 104-107.
[8]. A.M. Janssen, J.C. Scheffer, *Planta Medica*, **1985**, 507-511.
[9]. O. Jirawan, S.Tomoko, LWT-Food and Science Technology, **2006**, 39, 1214-1220.

[10]. H. Matsuda, T. Morikawa, *Euro. J. Pharmacol.*, **2005**, 471, 59-67.
[11]. C.K. Kokate, Practical Pharmacognosy, 3rd edn: Vallabh Prakashan, New Delhi, **1994**, 107-109.
[12]. OECD: OECD guideline for the testing of Chemicals, Acute Oral Toxicity-Fixed dose procedure 420 adopted 17th December **2001**.
[13]. C.A.Winter, E.A. Risley, G.W. Nuss, *Proc. Soc. Exp. Biol. Med.*, **1962**,111, 544-547.
[14]. J.B. Perianayagam, S.K. Sharma, K.K Pillai, *J. Ethnopharmacol.*, **2006**, 104(3), 410-414.
[15]. R. Vinegar, W. Scheirber, R. Hugo, *J. Pharmacol. Exp. Ther.*, **1969**, 166(1), 96-103.