

COMPARISON OF ANTI-INFLAMMATORY ACTIVITY OF ALPINIA GALANGAL IN THREE EXPERIMENTAL ANIMAL MODELS

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ABSTRACT: Inflammation is not a disease but a non-specific response of the body defence¹. Anti-inflammatory drugs have become popular because of their ability in controlling the inflammatory reaction and mitigating the suffering in such clinical situations. Edema represents the early phase of inflammation.² In the present study acute and subacute experimental methods were compared with standard drug Indomethacin. Rat hind paw edema, formalin induced peritonitis in mice were used as acute methods, paper disc induced granuloma in rats was used as subacute method. Institutional animal ethics committee permission was taken for all the methods as per CPCSEA guide lines. Inflammation was induced by the following 3 methods: i) Hind paw edema was produced by sub plantar injection of 0.1ml of 1% carrageenin³ and paw volume was measured by digital plethysmometer at 0, 3 hours. ii) Intraperitoneal injection of 1.5% formalin³ was given to albino mice in peritonitis method and ascitic fluid was measured after 6 hours by sacrificing the animals. iii) Under ether anesthesia, sterilized & weighed paper discs were implanted subcutaneously in each axilla and groin of male wistar rats sutured under sterilized conditions in granuloma⁴ method. The discs were cleared of extraneous tissue, dried and weighed on the 5th day by sacrificing the animals. In all the methods 6 animals in each group (test, standard and control) were taken. Results were tabulated in each method separately and statistical analysis was done by student t test. P value < 0.05 considered significant. Percentage inhibition in each method was calculated.

KEYWORDS: Alpinialgalangal(A.G), Indomethacin(I), carrageenin, formalin, phlogistic agent Plethysmography, ascites, granuloma pouch.

INTRODUCTION: The process of inflammation is one of the most fundamental responses of the vascularised living tissue to local injury. The word inflammation derived from Latin "inflammatic" and Greek "phlegmasia" is one might say as old as medicine itself. The term anti-inflammatory is said clinically to designate an agent which will lessen one or more of the various components of inflammatory reaction. Salicylates are the earliest drugs known for their antirheumatic activity. Of the currently available NSAIDS, Indomethacin, Diclofenac have been reported to inhibit the synthesis of both prostaglandins and leukotrienes.⁵ The most common purpose in setting up the experimental models is the search for newer compounds and their comparison for efficacy.

To produce inflammation, phlogistic agents like carrageenin, formalin, Freund's adjuvant were commonly employed in experimental studies. The chief source of carrageenin is *Chondrus crispus* and *Gigartinamamillosa*. The name carrageenin is originally derived from the Irish town Carrageen. The structure consists of alternating co polymers of β 1, 3 d galactose and

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1-4-3, 6 an hydrod or l galactose. There are many types of carrageenin iota and kappa being common ones used. Iota carrageenin is the highly sulfated and completely soluble in hot water. The inflammation produced will be peak at 3 hours. An irritant substance commonly used for inducing pleurisy or peritonitis are turpentine, mineral oil, Evans blue, formalin, dextran, kaolin etc., formalin induced peritonitis peaks at 6-8hrs. The formation of granuloma for a foreign body is used in granuloma method. Some of the agents used for inducing granuloma are cotton wool pellet, Rexene pellet, plastic ring, filter paper disc. Another related method, the granuloma pouch technique, where pouch is made by air, croton seed oil, and collection of exudate and measurement of volume and thickness of the pouch wall.

Some acute methods commonly used are:

- Carrageenin induced Rat paw edema (Seyle 1944, Buch 1959).
- Ultraviolet erythema in guinea pigs (Schiken 1932, Willhelmii 1949&1960).
- Formalin induced peritonitis in mice (Teotino et al 1963).

Some subacute/ chronic methods used are:

- Experimental Arthritis in rats (Gardener 1960, Murray 1964).
- Experimental granuloma⁴ in rats (Seyle 1953, Ganley et al 1958, Meir et al 1950).

The common laboratory animals employed for designing the experimental models are Albino mice, Wistar rats, Guinea pigs, Rabbits. Use of Pigeons, Monkeys and Swines is also reported.

Alpiniagalanga family zinziberaceae^{6,7} called Peddadumparastramu in Telugu, Kulanjan, Barakulanjan in Hindi, Greater galangal in English, Sugandhamula in Sanskrit, Doddarasagadde in Kannada and Arattai, Perarattai in Malayalam. It's a perennial plant growing upto 2 meters height. Rhizomes are aromatic^{6,7} and fruits are orange red in size of small cherries. The dried rhizome of this plant has reputation in indigenous system of medicine and is fairly used in southern India. The dried rhizomes are useful in rheumatism and catarrhal infections. Administration of this drug to young children relieved the distressing symptoms of bronchitis. Pilocarpine induced asthma in animals can be relieved by small doses of tincture of Alpiniagalanga. 50% ethyl alcohol extract of rhizome has anti-inflammatory and antifungal actions.

MATERIALS AND METHODS: Two acute and one subacute methods were compared in the present study. Acute toxicity testing was done for the potential toxicity of the test drug. The hexane extract of the test drug was yellow colored, obtained from Indian Institute of Chemical Technology (IICT), Hyderabad, using Soxhlet apparatus at 20⁰ C, concentrated under vacuum. The study was done after the approval from the Institutional Animal Ethics Committee, and animals were obtained from the central animal house. The dried rhizomes were obtained from a local herbal store.

2 grams of gumacacia powder was taken in a mortar constantly triturated with 100ml of distilled water into a uniform suspension of 2%. 1 gram carrageenin weighed and dissolved in

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100ml of 0.9% NaCl (normal saline) to a 1% solution. 20 mgs/ kg Indomethacin triturated in 5 ml of 2% gumacacia.

Rat paw edema^{4,8}: Male Wister rats 150 – 200 grams were obtained from central animal house of the institute, numbered and divided into 3 groups of 6 each, labelled Test(T), Standard(S) and Control(C), kept in separate cages. They were maintained 12 hour light and dark cycles, fed with food and water ad libitum. On the day of experiment a line was marked at the level of lateral malleolus of right hind paw of all three groups. Hexane extract suspended in 2% gum acacia of Alpinagalanga (250mg/kg), Indomethacin (20mg/kg) suspended in 2% gum acacia, 2% gum acacia suspension were given to test, standard and control groups orally with feeding tube. After 1 hour, 0.1ml of 1% carrageenin was injected subcutaneously into the plantar surface of the right hind paw of all the rats. The hind paw volume was measured immediately (0hr) and after 3 hours using digital plethysmometer.

The difference between 0 hr and 3 hrs indicated the actual volume inhibited in the 3 groups. The percentage inhibition of edema was measured by using the formula,

$$\frac{V_c - V_t / V_s}{V_c} \times 100.$$

V_c: Mean paw volume in control group.

V_t: Mean paw volume in test group.

V_s: Mean paw volume in standard group.

Student "t" test was used for statistical significance.

Formalin induced peritonitis⁸ in mice.

Male albino mice weighing 35 – 40grams were obtained from central animal house, made into 3 groups, marked as T, S, C kept in separate cages of 6 animals in each. The animals were given balanced diet for a week before the experiment and maintained weight under supervision. The test was performed according to the method of Teotinoetal, 1963. Hexane extract of Alpinagalanga suspended in 2% gum acacia 250 mg/kg, Indomethacin⁹ (20mg/kg) and 2% gum acacia suspension were given orally to the T, S, C groups. An intraperitoneal injection of 1.5% formalin of 0.33 to 0.4ml depending on the weight of the animal was given after 1 hr. Animals were sacrificed after 6 hours under ether anesthesia and the ascitic fluid was collected and volume was measured, after opening the abdominal cavity in each mouse. The fluid was collected in a funnel clamped over a graduated centrifuge tube, by placing the animal on the funnel. Here, inhibition of ascitic fluid is the parameter. Diminution of the fluid volume in the S, T groups in comparison with the C group was expressed as percentage inhibition. Student 't' test was performed for statistical significance.

Paper disc induced granuloma⁹ method: Male Wistar rats of 180 – 200 gm were obtained from central animal house, numbered and grouped as T, S, C for test, standard and control groups, kept in separate cages, fed with balanced diet and water ad libitum a week prior to the experiment and maintained the weight under supervision. In this method, filter paper discs were used to induce granuloma. Small paper discs weighed and implanted under aseptic conditions

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subcutaneously in each axilla and groin of each rat. Each paper disc was prepared by punching device, into discs of equal size and weight. Ten such discs were compressed in order to get a single paper disc/ pellet weighing 40 mg. These discs were sterilized with 70% ethyl alcohol. 0.5ml of test drug suspended in 2% gum acacia (250 mg/ kg), 0.75 ml of Indomethacin suspended in 2% gum acacia, 0.5 ml of 2% gum acacia suspension were given orally 1 hr prior to the procedure to the T, S and C groups respectively. Small linear incisions of 1 cm were made in each axilla and groin of each animal under ether anesthesia and aseptic conditions. Sterilized paper discs of 40 mg each were implanted, in each axilla and groin of all animals with the help of technician and the surgical wound were sutured with black silk thread. The animals were kept in clean cages and fed with food and water daily throughout the experiment. Alpinialgalanga extract 0.5 ml in 2% gum acacia, 0.75ml Indomethacin in 2% gum acacia and 2% gumacacia was given daily to the test, standard and the control groups respectively throughout the experiment. The animals were sacrificed on the 5th day under anesthesia and the paper pellets were taken out, cleared of extraneous tissue, dried in the oven and weighed and compared in all the groups. Percentage inhibition was calculated by decrease in weight of the paper pellets in each group. Student "t" test was used for statistical significance.

Results were tabulated:

Groups	Mean paw volume ± S.E After 3 hours(ml)	Percentage Inhibition
Control	0.8±0.1673	0%
Indomethacin*** (20mg/kg)	0.25±0.0341	68.75%
AlpiniaGalanga*** (250mg/kg)	0.38±0.09	52.5%

Table 1: Carrageenin induced hind paw edema model in Wister rats

No. of animals used in each group – 6. Student 't' test p < 0.001***, P < 0.01**, P < 0.05*.

Groups	Mean peritoneal fluid volume±S.E.M after 6 hours(ml)	Percentage Inhibition
Control	0.93±0.242	0%
Indomethacin*** (20 mg/kg)	0.26±0.01	72%
AlpiniaGalanga*** (250 mg/kg)	0.37±0.37	60.21%

Table 2: Inhibition of Ascitic fluid in Albino Mice

No. of animals in each group – 6. Student 't' test p value < 0.001***, P value < 0.01**, P value < 0.05*.

Groups	Mean granuloma weight± S.D on 5 th day(mg)	Percentage Inhibition
Control	20.5	0
Indomethacin (20mg/kg)	12.87±2.23	37.22
AlpiniaGalanga (250mg/kg)	13.25±2.24	35.4

Table 3: Paper pellet induced granuloma in male wister rats.

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No. of animals in each group – 6. Student 't' test p value <0.001***, Pvalue < 0.01**, P value < 0.05*.

DISCUSSION: A number of experimental procedures and animal models are available to evaluate the anti-inflammatory activity of a new compound. Every method has its own limitations and a single experimental method is not enough for determining the anti-inflammatory activity, consequently a battery of tests are essential. Evaluation of Anti-inflammatory compounds includes acute and chronic categories. In the present study 2 acute methods¹⁰ i.e, rat paw edema, Inhibition of ascitic fluid and one subacute method cotton pellet induced granuloma method were compared with Indomethacin. The dose of Alpiniagalanga was determined by doing a pilot study, as 250mg/kg body weight. The literature showed Indomethacin at a dose of 20mg/kg body weight. Acute toxicity testing was done and LD50 was found to be 1.25gm/kg body weight.

When a part of the body was swollen by accumulation of excess fluid in the intercellular tissue, in response to inflammatory stimulus, it is said to be edematous, which is immediate response. This was observed in rat paw edema and ascitic fluid models where carrageenin and formalin were used as Phlogistic agents. Inhibition of edema volume and fluid volume were the parameters used in these methods. In subacute model formation of granulation tissue which is late response, as in rheumatoid arthritis, the measurement of granulation tissue is the parameter.

In rat paw edema, 250mg/kg body weight Alpiniagalanga, and 20mg/kg body weight Indomethacin were comparable. The mean paw volume inhibited by A.G 0.38±0.09, and Indomethacin 0.25±0.0341. P value in both is < 0.001 i.e highly significant. Percentage inhibition for Alpiniagalanga was 52.5%, with Indomethacin 68.75%.

In inhibition of Ascitic fluid method, 250mg/kg A.G, 20mg/kg were compared. The mean peritoneal fluid inhibited by A.G. 0.37± 0.368 and Indomethacin 0.26±0.01. p value in both is < 0.001 i.e highly significant. Percentage inhibition for Alpinia galangal was 60.21% with Indomethacin 72%.

In paper pellet granuloma method, 250mg/kg A.G, 20mg/kg Indomethacin were compared. The mean granuloma weight by A.G 13.25±2.24, and Indomethacin 12.87±2.23.p value in both tests was < 0.001 i.e highly significant. Percentage inhibition with Alpinia galangal was 35.4%, with Indomethacin 35.4%.

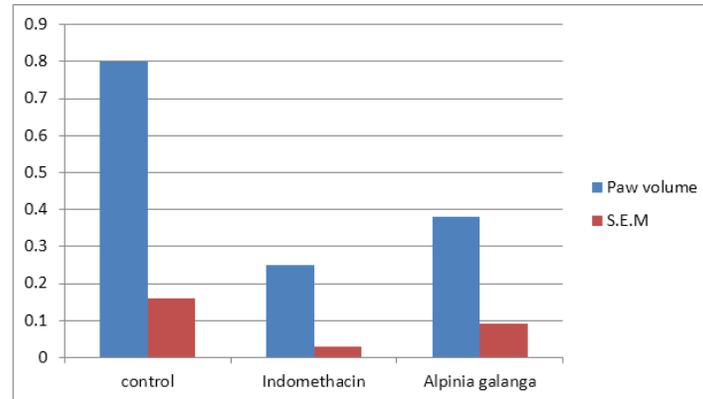
CONCLUSION: The dried rhizome of Alpiniagalanga plant has reputation in indigenous system of medicine. The dried rhizomes are useful in rheumatism and catarrhal infections. This is compared with Indomethacin in three models described in this study. In rat paw edema, 250mg/kg body weight Alpiniagalanga, and 20mg/kg body weight Indomethacin were comparable. The mean paw volume inhibited by A.G 0.38±0.09, and Indomethacin 0.25±0.0341. P value in both is < 0.001 i.e highly significant. Percentage inhibition for Alpiniagalanga was 52.5%, with Indomethacin 68.75%.

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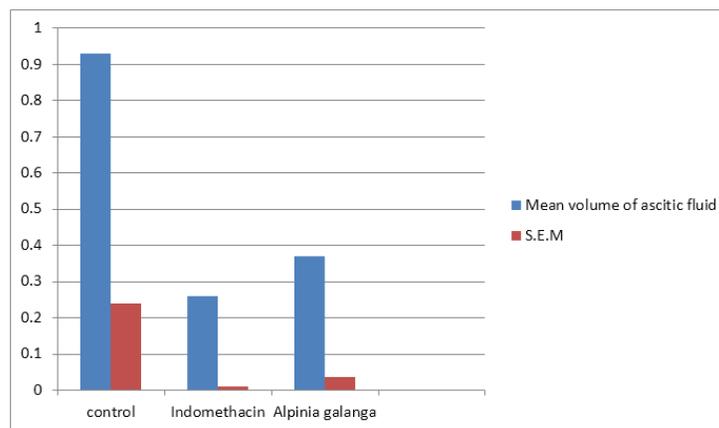
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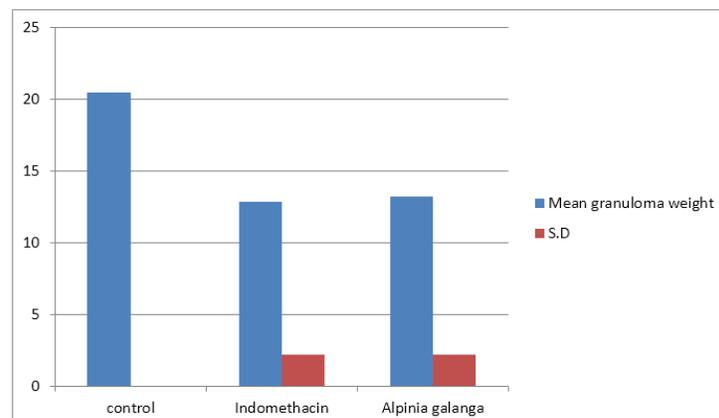
value in both tests was < 0.001 i.e highly significant. Percentage inhibition with *Alpinia galangal* was 35.4%, with Indomethacin 35.4%.



Graph 1: Showing inhibition of paw edema by control, Indomethacin, Alpinialangal after 3 hours



Graph 2: inhibition of Ascitic fluid in Albino mice by control, Indomethacin, Alpinialangala



Graph 3: Showing mean granuloma weight in Granuloma model in rats

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