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Study of anti-inflammatory activity of aqueous and methanolic extracts of fresh rhizome of *Zingiber Officinale* in Wistar rats

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Abstract---To evaluate the anti-inflammatory activity of aqueous and methanolic extracts of fresh rhizome of plant *Zingiber officinale*: 1. To evaluate anti-inflammatory activity of all extracts in two doses and the standard drug in wistar rats by using Carrageenan induced paw edema method. 2. To compare anti-inflammatory activity of both extracts with that of standard drug. The study was conducted after animal ethics committee approval. Animals were divided into six groups. Control group received 0.2ml NS IP while test groups received aspirin (150mg/kg) , Aqueous extract of fresh rhizome of *Z. officinale* (100 and 200mg/kg) , Methanolic extract of fresh rhizome of *Z. officinale* (100 and 200 mg/kg). Acute model of inflammation consisting of carrageenan induced rat paw edema method was used to investigate anti-inflammatory activity of Ginger. The present study clearly showed significant anti-inflammatory activity of aqueous and methanolic extracts of fresh rhizome ($p < 0.05$) in acute model of inflammation when compared to control and its activity was comparable to aspirin at all time interval. Aqueous and methanolic extracts of *gingiber officinale* have significant anti-inflammatory property.

Keywords---ginger, aspirin, anti-inflammatory, carrageenan.

Introduction

Latin word 'inflammacio from which the word inflammation is derived which means 'to set a fire' .¹ Cardinal signs of inflammation rubor, tumor, calor and dolor which were first listed by Celsus who was Roman writer from 1st century AD. Rubor means redness tumor suggests swelling, calor means heat and dolor suggests pain. Virchow added fifth clinical sign 'loss of function'.² It is body's one of the defence mechanisms by which there will be either removal or limitation of injurious agents like bacteria, viruses, parasites, fungi, antigen antibody complexes etc³. Drugs like Non-Steroidal Anti Inflammatory drugs have shown to be anti inflammatory drugs. They are also non specific analgesics and can also be used for acute or chronic pain.⁴ But they are having adverse effects like peptic ulcer, nephrotoxicity, hepatic damage. Whereas Ginger is digestive, anti-emetic and hepato protective.

Ginger, Botanical name *Zingiber officinale* (Family: Zingiberaceae) is a plant distributed worldwide⁵. It has been used as a spice, flavouring agent in food, it is also used by traditional Indian and Chinese medicine for more than 25 centuries. It is grown widely in India, Jamaica, Mexico, Hawaii. It is an underground root or rhizome, which is used in traditional medicine for antiemetic effect, improving blood circulation, stimulating digestion etc⁶. Various animal studies, pilot studies in human, clinical trials suggest the analgesic^{7,8}, anti inflammatory^{9,10}, hepatoprotective¹¹, hypouricaemic¹², antidiabetic¹³, anticancer¹¹ effects of either crude extract or pure gingerol. Use of Ginger extract for acute and chronic anti-inflammatory purpose is not established in modern medicine and not a documented in textbooks only in traditional medicine of different countries many uses of crude Ginger extract are mentioned. They are not yet proven. Different experimental studies have shown its anti-inflammatory effects in animals but few studies report no activity. If anti-inflammatory effect of Ginger extract is proved it will be step forward towards formation of new safe drug which will be useful for patients suffering from pain and inflammation.

Materials and Methods

Animals

Wistar rats of either sex weighing 150-200G were procured from the Central Animal House, KIMS, Karad. Total 6 groups of animals each having 6 animals were used for experiments. Total 36 wistar rats were used.

Drugs

Fresh rhizome of *Zingiber officinale* was obtained from local market. The rhizome was used after authentication by botanist. The rhizome was washed with tap water and shade dried. Aqueous and methanolic extracts of both powders were prepared by using Soxhlet Apparatus. All dried extracts were dissolved in 0.9 % normal saline for injection intraperitoneally. Following extracts and standard drug were used in two doses as given in following table.

GROUP	Name of Drug /Extract	Short form used	Dose
Group I	0.9% Normal Saline	NS	0.2ml i.p
Group II	Aspirin (Standard Control)	ASP	150mg/kg i.p
Group III	Aqueous extract of fresh rhizome of <i>Z. officinale</i>	AFZ	100mg/kg i.p
Group IV	Aqueous extract of fresh rhizome of <i>Z. officinale</i>	AFZ	200mg/kg i.p
Group V	Methanolic extract of fresh rhizome of <i>Z. officinale</i>	MFZ	100mg/kg
Group VI	Methanolic extract of fresh rhizome of <i>Z. officinale</i>	MFZ	200mg/kg

Doses were selected as per previous research for anti inflammatory activity of ginger extract.¹⁴ Acute toxicity study was done. No mortality was seen upto 2000mg/kg methanolic extract of zingiber officinale. All experiment were conducted after approval of institutional animal ethics committee. (Certificate No:IAEC/KIMS/2019/09). Experiment were conducted as per CPCSEA guidelines.

Evaluation of anti inflammatory activity

It was done by carrageenan induced paw edema method.¹⁵

Carrageenan-induced Oedema in rat hind paw

This method is based on the plethysmometric measurement of oedema produced by sub plantar injection of carrageenan into the hind paw of albino rats. Acute inflammation is produced by sub plantar injection of 0.1 ml of freshly prepared 1% suspension of carrageenan in normal saline in the right hind paw of the rats and paw volume is measured plethysmometrically hourly till the fourth hour after carrageenan injection. Percentage inhibition (protection) against oedema formation is taken as an index of acute anti-inflammatory activity. It is calculated as follows

$$\text{Percentage Inhibition} = (V_c - V_t / V_c) \times 100$$

Where:

V_c = Volume of paw oedema in control animals

V_t = Volume of paw oedema in treated animals.

Statistical analysis

Results were expressed as mean \pm standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's t-test for post-hoc analysis.

P < 0.05 considered statistically significant

All the statistical methods were carried out using the SPSS software.

Results

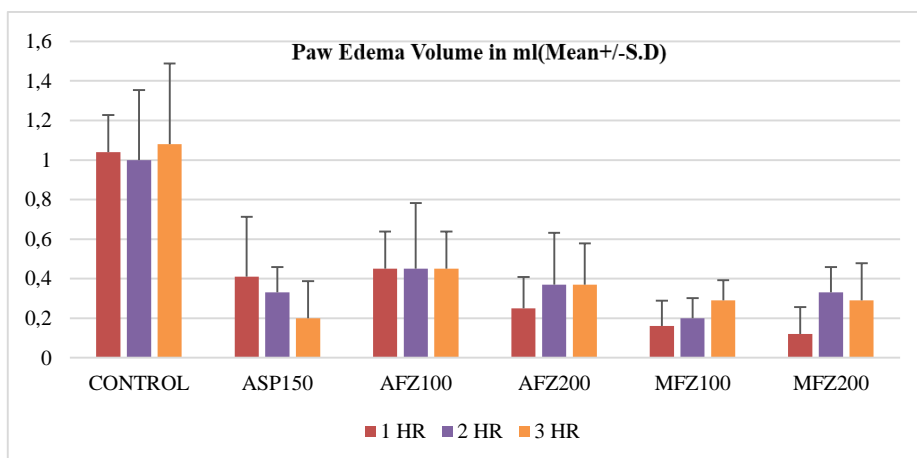
Table 1
Effect of ASP150, AFZ100, AFZ200, MFZ100, MFZ200 treatments on carrageenan induced paw edema compared with control

Time after carrageenan injection	Paw edema volume in ml (Mean ± SD)						ANOVA	
	Control	ASP 150	AFZ 100	AFZ 200	MFZ 100	MFZ 200	F value	P Value
1 hr	1.04 ± 0.188	0.41 ± 0.302*	0.45 ± 0.188*	0.25 ± 0.158*	0.16 ± 0.129*	0.12 ± 0.136*	18.327	<0.0001
2 hr	1 ± 0.353	0.33 ± 0.129*	0.45 ± 0.332*	0.37 ± 0.262*	0.20 ± 0.102*	0.33 ± 0.129*	8.150	<0.0001
3 hr	1.08 ± 0.408	0.20 ± 0.188*	0.45 ± 0.188*	0.37 ± 0.209*	0.29 ± 0.102*	0.29 ± 0.188*	11.344	<0.0001

Post hoc analysis by Dunnett's Test: * $p < 0.05$

ANOVA revealed statistically significant difference in study groups; control, ASP150, AFZ100, AFZ200, MFZ100, MFZ 200.

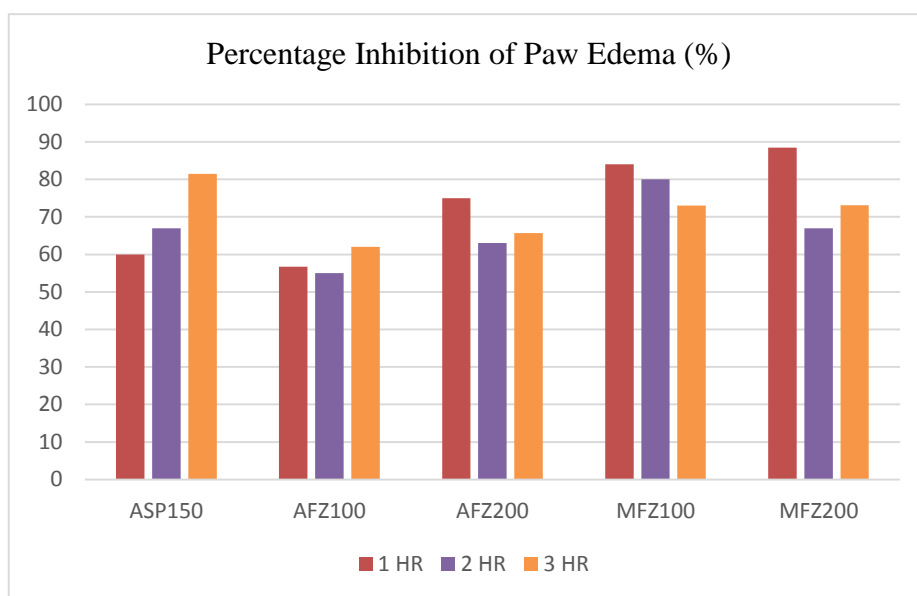
Post hoc analysis by Dunnett's Test revealed statistically Significant difference between all treatment groups when compared with Control at all time interval (P < 0.05). Bonferroni Multiple Comparison test revealed, no statistically significant difference in anti-inflammatory activity of all extracts compared with Aspirin at 1hr, 2hr, 3hr time interval (P > 0.05).



Graph 1. Effect of ASP150, AFZ100, AFZ200, MFZ100, MFZ200 treatments on carrageenan induced paw edema compared with control

Table 2
Percentage inhibition of paw edema in AFZ100, AFZ200, MFZ100, MFZ200 treatments treated group compared with Aspirin group at different time intervals

Groups	1hr	2hr	3hr
ASP	60 %	67 %	81.48 %
AFZ 100mg/kg	56.73 %	55 %	62.03 %
AFZ 200mg/kg	75 %	63 %	65.7 %
MFZ 100mg/kg	84 %	80 %	73 %
MFZ 200mg/kg	88.46 %	67 %	73.14 %



Graph 2. Percentage inhibition of paw edema in AFZ100, AFZ200, MFZ100, MFZ200 treatments treated group compared with Aspirin group at different time intervals

Discussion

In the present study, anti-inflammatory effects of different doses of methanolic and aqueous extracts of fresh rhizome of plant *Zingiber Officinale* were tested in experimental models of inflammation. For anti-inflammatory activity we selected Carrageenan induced paw edema method. There are minimum 115 constituents identified from fresh and dried Ginger. Among them Gingerols are the major ones. They are abundant in fresh Ginger and less in dry Ginger whereas Shogaols are abundant in dry than in fresh Ginger. They are referred to major Gingerol dehydration products.¹⁶ There are different possible mechanisms for anti-inflammatory effect of Ginger. Ginger has capacity to inhibit prostaglandin and leukotriene biosynthesis¹⁷. It is suggested that inhibition of Arachidonate 5 – lipoxygenase also help in anti-inflammatory activity of Ginger.¹⁸ Inhibition of cyclooxygenase 2 (COX 2) and¹⁹ proinflammatory cytokines by Gingerol²⁰ also claimed to be responsible for anti-inflammatory activity of Ginger.

Aqueous extract of fresh rhizome (AFZ 100 and AFZ 200)

In the present study, we found that aqueous extract of fresh rhizome of plant *Zingiber Officinale* at dose of 100mg/kg and 200mg/kg (AFZ 100 and AFZ 200) showed statistically significant inhibition of paw edema volume ($p < 0.05$) when compared to control at 1hr, 2hr and 3hr. There was no statistically significant difference in anti-inflammatory activity of aspirin and AFZ 100; aspirin and AFZ 200 at all-time interval ($p > 0.05$). It indicates anti-inflammatory activity of AFZ 100 and AFZ 200 is comparable to aspirin. AFZ 100 has shown decrease in inflammation by 56.73%, 55% and 62.03% at 1hr, 2hr and 3hr interval respectively whereas AFZ 200 has shown decrease in inflammation by 75%, 63%, 65.7% at 1hr, 2hr and 3hr respectively. Though anti-inflammatory activity of AFZ 100 is comparable to aspirin, it is less than aspirin at all time intervals whereas effect of AFZ 200 is more than aspirin only at 1 hr. In general, it was observed that anti-inflammatory activity increased dose dependently from 100mg/kg to 200mg/kg for aqueous extract of fresh rhizome of plant *Zingiber Officinale*.

Methanolic extract of fresh rhizome (MFZ 100)

We found that methanolic extract of fresh rhizome of plant *Zingiber Officinale* at a dose of 100mg/kg (MFZ 100) showed statistically significant inhibition of paw edema volume ($p < 0.05$) when compared to control at 1hr, 2hr and 3hr. There was no statistically significant difference in anti-inflammatory activity of aspirin and MFZ 100 at all-time interval ($p > 0.05$). It indicates anti-inflammatory activity of MFZ 100 is comparable to aspirin. MFZ 100 has shown decrease in inflammation by 84%, 80% and 73% at 1hr, 2hr and 3hr interval respectively. Though anti-inflammatory activity of MFZ 100 is comparable to aspirin, it is more than aspirin at 1hr and 2hr interval.

Methanolic extract of fresh rhizome (MFZ 200)

We found that methanolic extract of fresh rhizome of plant *Zingiber Officinale* at a dose of 200mg/kg (MFZ 200) showed statistically significant inhibition of paw edema volume ($p < 0.05$) when compared to control at 1hr, 2hr and 3hr. There was no statistically significant difference in anti-inflammatory activity of aspirin and MFZ 200 at all-time interval ($p > 0.05$). It indicates anti-inflammatory activity of MFZ 200 is comparable to aspirin at all time interval. MFZ 200 has shown decrease in inflammation by 88.46%, 67% and 73.14% at 1hr, 2hr and 3hr interval respectively. The percentage inhibition of paw edema by MFZ 200 is maximum amongst all the treatment groups at all time interval. When we compared percentage inhibition of paw edema at all time intervals, it was evident that action of aqueous as well as methanolic extract started early but reduced gradually till 3 hours whereas aspirin effect reached peak at 3 hours.

Thus, in conclusion, our results show extracts of *Zingiber officinale* have anti-inflammatory property. Anti-inflammatory properties are comparable to aspirin. Considering problems of gastric ulceration and hepatotoxicity associated with higher doses of aspirin and other NSAIDs, it will be justifiable to combine ginger extract with aspirin (NSAIDs) for chronic treatments of senile arthritis, low back ache, rheumatoid arthritis etc. Ginger due to its anti-inflammatory activities

might potentiate aspirin action, therefore we may be able to reduce dose of aspirin. Ginger due to its digestant and hepatoprotective properties might reduce chances of aspirin induced adverse effects. Thus, combination may prove to have quick onset, long duration, decrease gastric intolerance, side effects of aspirin and may also have additive anti-inflammatory action. Therefore, we propose that further clinical studies should be done to prove benefit of coadministration of ginger extract and NSAIDs in chronic arthritis conditions.

Summary and Conclusions

From the findings of present experimental study, we conclude that Extracts of *Zingiber officinale* have anti-inflammatory properties. 100mg/kg and 200mg/kg doses of aqueous and methanolic extracts of fresh rhizome have significant anti-inflammatory activity as compared to control in animal model of acute inflammation which is comparable to anti-inflammatory activity of aspirin. 200mg/kg of methanolic extract of fresh rhizome of plant *Zingiber officinale* has shown maximum anti-inflammatory activity among all the extracts and standard control. AFZ, MFZ have shown dose dependent anti-inflammatory activity. Present study predicts that use of ginger may have beneficial anti-inflammatory effect in various inflammatory conditions. As this study proves anti-inflammatory effect of ginger extracts, it will be a step forward towards formation of new safe drug which will be useful for patients suffering from pain and inflammation considering the adverse effects of conventional anti-inflammatory drugs such as NSAIDs and Corticosteroids. Considering digestant and hepatoprotective activities of ginger extracts, it may be a good adjuvant to aspirin or other NSAIDs therapies for chronic inflammatory conditions like arthrites. Aqueous and Methanolic extracts of ginger can be tried as anti-inflammatory agents in treating chronic inflammatory conditions such as Osteoarthritis, Rheumatoid arthritis. Although further studies need to be done in various other acute and chronic inflammatory models along with human studies to strengthen the results and prove their efficacy on long term administration as potential anti-inflammatory agent in routine clinical practice.

The limitations of the study

- Only single species i.e. wistar rats were used
- In our setup, we were unable to quantify active principle present in the extracts.
- The efficacy of the extracts as an anti-inflammatory agent can be further evaluated using other related models in different species and also in human studies.

Conflict of interest: None

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