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Research Article

EVALUATION OF EFFECT OF AQUEOUS EXTRACT OF *ZINGIBER OFFICINALE ROSCOE* (GINGER) ON ACUTE AND CHRONIC INFLAMMATION IN ADULT ALBINO RATS

NAGARAJ B MALIPATIL[1]*, MANJUNATH S[2], SHRUTHI DP[3]

[1]Department of Pharmacology, Mahadevappa Rampure Medical College, Gulbarga, Karnataka, India, [2] Department of Pharmacology, S Nijalingappa Medical College, Bagalkot, Karnataka, India, [3] Department of Orthodontics and Dentofacial Orthopaedics, Dayananda Sagar College of Dental Sciences, Bengaluru, Karnataka, India. Email: nagaraj.malipatil@gmail.com

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ABSTRACT

Objective: *Zingiber officinale* (ZO) Roscoe (Ginger) is known to have many medicinal properties. The present study was carried out to evaluate the anti-inflammatory activity of aqueous extract of ginger in adult albino rats, both in acute and chronic inflammatory settings and to compare the same with standard anti-inflammatory agent diclofenac sodium.

Methods: Anti-inflammatory activity of ginger at the dose of 500 mg/kg body weight administered orally was evaluated in adult albino rats divided into different groups as control, test and standard. Effect of ginger on acute inflammation was evaluated by carrageenan in induced rat paw edema method and chronic inflammation was evaluated by rexin pellet granuloma method. Histopathological analysis was also done to evaluate effect of ginger on leukocyte migration and lymphocyte accumulation at the site of acute and chronic inflammation respectively.

Results: Aqueous extract of ginger decreased the signs of both acute and chronic inflammation. The percent inhibition of edema (for acute inflammation) with ZO extract was 28.80%, whereas with diclofenac sodium 63.46%. Percentage inhibition of granulation tissue (for chronic inflammation) for ginger was 31.04% and 63.42% for diclofenac sodium.

Conclusion: Aqueous extract of ginger decreased the signs of both acute and chronic inflammation and was comparable to standard anti-inflammatory drug diclofenac sodium. As currently available anti-inflammatory drugs are associated with number of side-effects, ginger can be potentially explored as an anti-inflammatory agent with minimal or no side-effects.

Keywords: *Zingiber officinale* Roscoe, Ginger, Acute inflammation, Chronic inflammation, Rat paw oedema, Rexin pellet granuloma, Diclofenac sodium.

INTRODUCTION

India, the cradle of ancient civilization had acquired a high degree of knowledge, on the nutritional and medicinal properties of large number of plant products. *Zingiber officinale* (ZO) Roscoe (Ginger) is widely used spice and functional food. Ginger being aromatic and pleasantly pungent is commonly used in the preparation of condiments, curries and ginger bread. Ginger is cultivated in many part of India; on a large scale in the warm, moist regions, chiefly in Madras, Cochin and Travanancore, and to somewhat less extent in Bengal and the Punjab [1]. Ginger (ZO Roscoe, Zingiberacae) is a medicinal plant that has been widely used in Chinese, Ayurvedic and Tibb-Unani herbal medicines all over the world, since antiquity, for a wide array of unrelated ailments.

Toxonomical position [2]

Kingdom	Plantae
Sub kingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Zingiberidae
Order	Zingiberales
Family	Zingiberaceae
Genus	Zingiber mill
Species	Z. officinale Roscoe

Ginger is extremely valuable in dyspepsia, flatulence, colic, spasms and other painful affection of stomach [1]. The pharmacological effects of ZO have been reported to have hypolipidemic [3], anti-inflammatory [4,5], antioxidant [6], hypoglycemic [7], analgesic [7], anti-platelet [8], antiemetic [9], antithrombotic [10], anti-tumorigenic [11], radio protective [12], antimicrobial [13], antifungal [13] actions. Considering the vast variety of actions of ginger, this study has been done to evaluate the effect of aqueous extract ZO on inflammation in adult albino rats. Signs of inflammation such as redness (rubor), swelling (tumor), heat (calor), pain (dolor), leukocytic migration were reduced to a significant level by ginger when compared to standard anti-inflammatory drug diclofenac sodium.

Chemistry

The constituents of ginger are numerous and vary depending on the place of origin and whether the rhizomes are fresh or dry. The odor of ginger depends mainly on its volatile oil, the yield of which varies from 1% to 3%. Over 50 components of the oil have been characterized and these are mainly monoterpenoids (β-phellandrene, (+) – camphene, cineole, geraniol, curcumene, citral, terpineol, borneol) and sesquiterpenoids (α-zingberene (30-70%), β-sesquiphellandrene (15-20%), β-bisabolene (10-15%), (E-E)-α-farnesene, arcurcumene, zingiberol) [14]. Important constituents include gingerols including (6) - gingerol (usually <1% of the roots weight) [15], (6)-Shagol (a dehydroxylated analog of (6) gingerol), (6)- and (10)-dehydrogingerdione, (6)- and (10)-gingerdione, (6)-paradol, vallinoids galanals A and B, and zingerone. Other compounds present include carbohydrates, fats, minerals, oleoresins, vitamins, waxes and zingibain (A proteolytic enzyme). The rhizome of ginger contains pungent vanillyl ketones, including (6) - gingerol and (6) paradol, and has been reported to possess strong anti-inflammatory activity as well as anti-tumor promoting properties [16]. Inducible nitric oxide synthase (iNOS), a proinflammatory enzyme responsible for the generation of nitric oxide, has been implicated in the pathogenesis of inflammatory disease, and gingerols are known to produce anti-inflammatory effects *in vitro* by inhibiting the generation of iNOS [17].

METHODS

Chemicals

ZO Roscoe (Ginger), pure powder form was obtained from Vidya Herbs, Bangalore. Diclofenac sodium obtained from Biocon Pharmaceuticals, Bangalore. Gum acacia (4%), carrageenan (1%) and normal saline (0.9%) were procured locally.

Preparation of extract

Aqueous extract of the ginger was used in present study.

Animals

Study was carried out in healthy albino rats of Wistar strain (*Rattus norvegicus*) of either sex weighing 150-200 g each procured from Central Animal House, Mahadevappa Rampure Medical College, Gulbarga, Karnataka. A total of 30 rats were used, 15 for study of acute inflammation i.e. rat paw edema method, 15 for study of chronic inflammation i.e. rexin pellet granuloma method. Rats were housed in polypropylene cages in room where the congenial temperature 27±1°C and 12 hrs light and dark cycles were maintained. The animals were allowed to acclimatize to the environment for 7 days and supplied with a standard pellet diet manufactured by Hindustan Lever Ltd., Bangalore.

Dose and route of administration

ZO Roscoe (ginger) 500 mg/kg [3] in rats per oral, diclofenac sodium 4.5 mg/kg in rats [18] per oral, gum acacia (4%) 2 ml/kg of rat, per oral and carrageenan 1%, 0.1 ml subcutaneously in the paw of rat.

Analysis

Effect of aqueous extract of ginger on acute inflammation was evaluated by rat paw oedema method as described by Wilhelmi and Domenjoz, which was further modified by Sisodia and Rao [19]. Its effect on chronic inflammation was evaluated by rexin pellet granulation method described by Meir, Schuler and Desaules and modified by Finney and Somers [20]. Histopathological analysis was also done to evaluate effect of ginger on leukocyte migration and lymphocyte accumulation at the site of acute and chronic inflammation respectively.

RESULTS

Acute and chronic models inflammation were used and histopathological study was done to compare the anti-inflammatory activity of the test drug with that of the standard drug (diclofenac sodium).

Rat paw edema method for acute inflammation [21]

The results obtained from the standard and test drugs are shown in Table 1. The percent inhibition of edema in rats treated with diclofenac sodium, ZO is calculated with reference to the control group by applying "unpaired *t*-test." The percent inhibition of edema at the end of 3 hrs with diclofenac sodium was 63.46%, whereas with ZO extract 28.80%.

On comparison of anti-inflammatory effects of standard and test drug against control, the anti-inflammatory activity was highly significant with diclofenac sodium ($p<0.001$) and significant with ZO extract ($p<0.01$). When compared anti-inflammatory effect of test drug with that of standard, the anti-inflammatory activity was significant with ZO extract ($p<0.01$).

Rexin pellet granuloma method for chronic inflammation

The result obtained from the standard and test drugs are shown in Table 2. The percent inhibition of granuloma formation was determined by weighing the rexin pellets after 7 days of their implantation in the subcutaneous tissue.

Histopathological study

The sections obtained from the subcutaneous tissue rexin pellet containing granulation tissue from control (Fig. 1), standard (Fig. 2) and test (Fig. 3) groups were subjected to haematoxylin and eosin staining and the following histopathological observations were noted. Section from control sample showed immediately surrounding the pellet, many acute inflammatory cells, many neutrophils, few monocytes, lymphocytes and occasional eosinophils. There was a good amount of granulation tissue in the periphery consisting of many capillaries, fibroblasts and variable collagen.

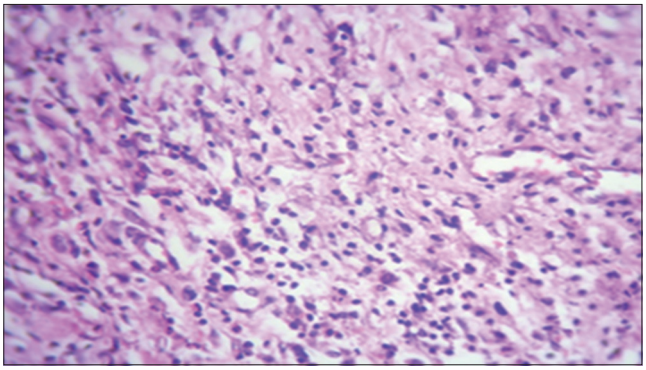

Fig. 1: Microphotograph of granulation tissue (H and E stain, ×100), control showing exuberant granulation tissue, inflammatory cells and fibroblasts

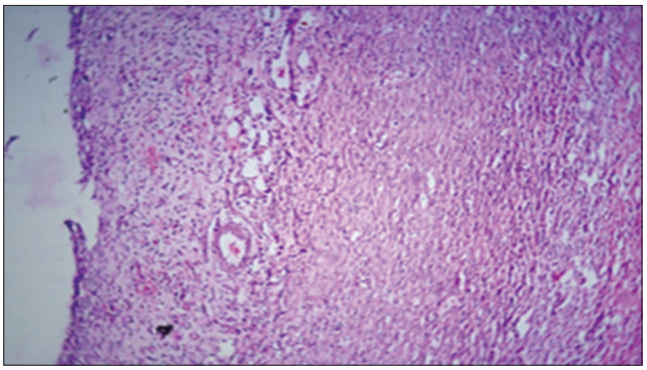

Fig. 2: Microphotograph of granulation tissue (H and E stain, ×100), standard (diclofenac sodium), marked decrease in granulation tissue, inflammatory cells and fibroblasts as compared to control group

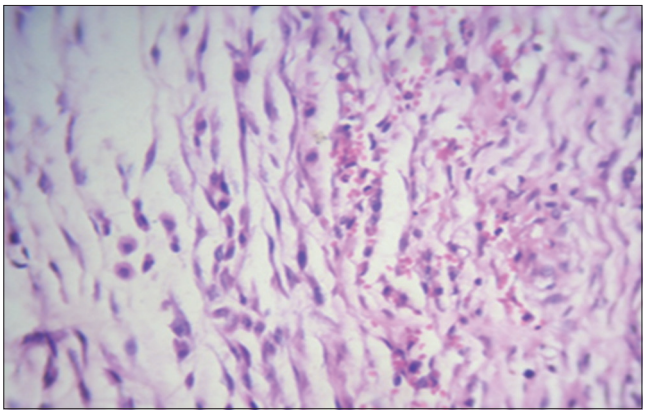

Fig. 3: Microphotograph of granulation tissue (H and E stain, ×100) test (ginger), moderately decreased granulation tissue, inflammatory cells and fibroblasts as compared to control group

DISCUSSION

In carrageenan induced rat paw edema method, rats were divided into three groups of five animals each. These rats received orally 4%

Table 1: Effects of diclofenac sodium and ZO extract on carrageenan induced rat paw edema

Groups	Control group			Standard group			Test group		
Drugs	4% gum acacia			Diclofenac sodium			ZO extract		
	Edema volume (ml)			Edema volume (ml)			Edema volume (ml)		
Rat	Baseline	At the end of 3 hrs	Difference in volume (ml)	Baseline	At the end of 3hrs	Difference in volume (ml)	Baseline	At the end of 3 hrs	Difference in volume (ml)
1	1.05	1.45	0.4	1.09	1.33	0.24	1.18	1.56	0.38
2	1.02	1.55	0.53	1.04	1.22	0.18	1.27	1.65	0.38
3	1.14	1.65	0.51	1.07	1.34	0.27	1.20	1.5	0.30
4	1.12	1.81	0.69	1.02	1.10	0.08	1.09	1.51	0.42
5	1.08	1.57	0.49	1.08	1.29	0.21	1.08	1.47	0.39
Mean			0.52			0.19			0.37
Percentage inhibition of edema				63.46			28.80		
Standard deviation	0.105			0.073			0.045		
Standard error	0.047			0.0326			0.02		
*t-value	-			5.78			2.86		
*p-value	-			<0.001			<0.02		
**t-value	5.78			-			4.76		
**p-value	<0.001			-			<0.01		

*Comparison of anti-inflammatory effects of standard and test drug against control (unpaired *t*-test), ** Comparison of ant-inflammatory effects of test drug with that of standard (unpaired *t*-test), ZO: *Zingiber officinale*

Table 2: Effect of diclofenac sodium and ZO extract on rexin pellet granuloma method

Group (n=5)	Control group		Standard group		Test group	
Drugs	4% Gum acacia		Diclofenac sodium		ZO	
Rats (4 pellets/rat)	Gain in weight of individual rexin pellet (mg)	Mean weight gain (mg)	Gain in weight of individual rexin pellet (mg)	Mean weight gain (mg)	Gain in weight of individual rexin pellet (mg)	Mean weight gain (mg)
1	40, 29, 17, 18	25.75	11, 15, 06, 10	10.5	15, 26, 21, 24	21.5
2	21, 36, 30, 22	27.25	9, 12, 10, 15	11.5	20, 17, 21, 25	20.75
3	26, 36, 25, 28	28.75	10, 11, 10, 8	9.75	26, 19, 17.21	20.75
4	35, 39, 30, 31	33.75	12, 13, 9, 12	11.5	15, 19, 16, 19	17.25
5	34, 31, 32, 37	33.5	9, 10, 12, 14	11.25	21, 18, 28, 23	22.5
Total mean		29.8		10.9		20.55
Percentage inhibition of granulation tissue			63.42		31.04	
Standard deviation	3.58		0.77		1.98	
Standard error	1.6		0.344		0.89	
*t-value	-		11.55		5.08	
*p-value	-		<0.001		<0.001	
**t-value	11.55		-		10.16	
**p-value	<0.001		-		<0.001	

*Comparison of anti-inflammatory effects of standard and test drug against control (unpaired *t*-test), **Comparison of ant-inflammatory effects of test drug with that of standard (unpaired t-test), ZO: *Zingiber officinale*

gum acacia, diclofenac sodium and *Z. officinale* extract respectively, 1 hr before carrageenan injection into rat paw. The paw volume was measured with plethysmograph after 3 hrs and percent inhibition of edema in various groups calculated. In carrageenan induced rat paw edema method, the percentage inhibition of edema at the end of 3 hrs with diclofenac sodium was 63.46%, whereas the ZO extract was 28.80%. This study showed that ZO extract has significant anti-inflammatory activity as compared to diclofenac sodium (standard). In rexin pellet granuloma method, four rexin pellets were implanted into dorsum skin of each rat of three groups (n=5), which include control, diclofenac and ZO extract respectively. 4% gum acacia and drugs were given orally to these rats, once a day for 7 days and on 8th day rexin pellets were removed after sacrificing rats and dried in incubator at 60°C for overnight. Pellets were then weighed and percentage inhibition of granulation tissue was calculated. Granulation tissue was observed for the histopathological changes. The percentage inhibition of granuloma formation in rexin pellet granuloam method was 63.42% with diclofenac sodium, whereas with ZO extract 31.04%. ZO extract showed highly significant anti-inflammatory activity in chronic model as well.

The histopathological study of acute and chronic models of inflammation in rats, using standard drug (diclofenac sodium) exhibited marked decrease in inflammation. In comparison ZO extract also exhibited significant anti-inflammatory activity but lesser than standard drug (diclofenac sodium).

The results obtained from this study revealed that, ZO extract exhibited significant anti-inflammatory activity in both acute as well as chronic models of inflammation, on comparison with standard drug diclofenac sodium. According to Penna *et al*. [8], they evaluated the anti-inflammatory activity of ZO hydralcoholic extract on albino rats. Results suggested that hydralcoholic extract of ZO at a dose of 186 mg/kg/day by intra-peritoneal injection has significant anti-inflammatory activity

in serotonin induced hind paw edema in rats. Until now no study has been conducted for studying effect of ZO extract on inflammation. Previous studies have used methanolic and ethanolic extract of ZO and in the present study the effect of ginger on chronic inflammation is also studied, which was not done before. Histopathological study of acute and chronic inflammation is also done in the present study, which was not done previously.

CONCLUSION

ZO extract has got prominent anti-inflammatory activity at a dose of 500 mg/kg body weight in adult albino rats in both acute and chronic models evidenced by percentage inhibition of acute rat paw edema and granulation tissue mass. Histopathology study also revealed, strong anti-inflammatory activity of ZO extract in both acute and chronic models. Not many studies have been undertaken, to fully evaluate the molecular and biochemical basis of anti-inflammatory action of ginger. Thus, ginger ZO being a commonly used natural product, deserves further evaluation from the stand point of its anti-inflammatory effect in therapy. Hence, emphasis should be laid upon, discovery of different active principles in ginger for the control of inflammation as well as their role various diseases.

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