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EVALUATION OF ANTI-ARTHRITIC POTENTIAL OF *ZINGIBER OFFICINALE* IN EXPETIMENTAL RATS

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ABSTRACT

Rheumatoid arthritis (RA) is an autoimmune disease particularly affecting elderly people which leads to massive bone destruction with consequent inflammation, joint pain, and debility. Allopathic medicine can provide only symptomatic relief. However, *Zingiber officinale* is a rhizome belonging to the Zingiberaceae family, which has traditionally been used for treatment of RA in alternative medicines of many countries. Phytochemical screening revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoid and phlobotannins in both the extracts. LD_{50} studies for both the extracts upto the maximum of 2000 mg/kg dose level no mortality was observed in the animals that indicate practically nontoxic nature of rhizome. The results of the current investigation concluded, Alcoholic and Aqueous extracts of *Zingiber officinale* possess a significant anti-arthritic activity against formaldehyde induced arthritis model and justifying its therapeutic role in arthritic condition. The observed antiarthritic activity may be due to the presence of phytoconstituents such as alkaloid and flavonoids.

KEYWORDS: *Zingiber officinale*, anti-arthritic activity, Rheumatoid arthritis (RA), indomethacin.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease that causes chronic inflammation in synovial tissue and joints, which leads to impaired joint function, severe pain and reduced life expectancy. This disease affects about 1% of the human population, women three times more often than men. The etiology and pathogenesis of this disease are not yet fully understood but it seems likely that an autoimmune-mediated attack on joints plays a crucial role lead to inflammation and bone destruction. Therefore, therapeutic agents developed for anti-inflammatory and immunosuppressant activity will be useful and indispensable for RA therapy. Currently, non-steroidal anti-inflammatory drugs (NSAIDs) such as Indomethacin are commonly used to treat RA disease. However, these drugs produce unwanted effects including gastric ulcer and dysfunctioning or the risk of cardiovascular disease.[1]

Presently many non steroidal, steroidal and immunosuppressive drugs are used to control inflammatory symptoms and pain**;** they are associated with certain undesirable side effects. With these difficulties, the field of arthritis research has progressed exponentially towards herbal therapies that have been considered safe and effective in all elevating chronic pain associated with arthritis.[2]

Ginger (*Zingiber officinale*) is a flowering plant, in the family Zingiberaceae whose rhizome, ginger root or simply ginger, is widely used as a spice or a folk medicine.[3] Phytochemical screening of extracts showed presence of alkaloid, phlobotannins, flavanoids, glycosides, saponins, tannin and terpenoids and absence of steroids.

The anti-inflammatory properties of ginger have been known and valued for centuries. The original discovery of ginger's inhibitory effects on prostaglandin biosynthesis in the early 1970s has been repeatedly confirmed. This discovery identified ginger as an herbal medicinal product that shares pharmacological properties with non-steroidal anti-inflammatory drugs. Ginger is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals. It is considered a safe herbal medicine with only few and insignificant adverse/side effects.[4] From the literature it was found that *Zingiber officinale* has been traditionally indicated for treatment of arthritis. Hence extracts of this plant was select for the study of anti-arthritic activity in experimental rats.

MATERIALS AND METHODS

Plant material

Rhizomes of *Zingiber officinale* collected in the month of March and dried in shade at room temperature then subjected to size reduction to a fine powder.

Chemicals
Indomethacin and formaldehyde were purchased from Sun Pharma Ltd., Mumbai and Sigma-Aldrich, Mumbai respectively.

Animals
Albino rats (Wistar strain) of either sex weighing between 150-200 g was used for the study. The animals were acclimatized for 7 days under standard animal husbandry condition. i.e.

Room temperature	-	26 ± 2^0 C
Relative humidity	-	45-55%
Light/ dark cycle	-	12:12 h

The animals were fed with a standard diet from Amrut Laboratories & Pranav Agro Industries Ltd. Sangli. Water was allowed *ad libitum* under strict hygienic conditions. All animal studies were performed in accordance to guidelines No. 425 of CPCSEA and Institutional Animal Ethical Committee (IAEC) and all the procedures were followed as per rules and regulations.

Preparation of extracts
Preparation of alcoholic extract
The rhizome powder was packed in a soxhlet apparatus and extracted with 95% alcohol for 18 h. Appearance of colourless solvent in the siphon tube was taken as the completion of extraction. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 40-45^{o}C to get alcoholic extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated [5].

Preparation of aqueous extract
About 100 g of powder was taken in a round bottom flask (2000 ml) and macerated with 500 ml of distilled water with 10 ml of chloroform (preservative) for 7 days with occasional shaking for every hour in a closed vessel. Then the marc was removed by filtering the extract and then it was concentrated on a water bath maintained at 40-45^{o}C.[5]

These two extracts were stored in an airtight containers in a dessicator below 10^{o}C. The two extracts were examined for their colour and consistency. Their percentage yield was calculated with reference to air-dried powder sample used for the extraction.

Toxicity studies
The acute toxicity of *Zingiber officinale* was determined by using albino rats of either sex (150-200 g), maintained under standard animal husbandry conditions. The animals were fasted for 3 h prior to the experiment and were administered with single dose of individual extracts of *Zingiber officinale* and observed for the mortality upto 48 h study period (Short term toxicity). Based on the short-term toxicity profile, the next dose of the individual extracts was determined as per OECD guidelines No. 425. From the LD_{50} doses 1/20,1/10 and 1/5 doses were selected and considered as low, medium and high dose respectively.[6]

Determination of Anti-arthritic Activity
Formaldehyde induced arthritis[7]
Albino rats weighing between (150-200 g) each group containing six animals were divided into 8 groups.

Group A	Toxicant Control (Formaldehyde 2% v/v)
Group B	Standard (Indomethacin 10mg / kg p.o)
Group C	AERZO (low dose, {100 mg/kg} p.o)
Group D	AERZO (medium dose, {200 mg/kg} p.o)
Group E	AERZO (high dose, {400 mg/kg} p.o)
Group F	AQERZO (low dose, {100 mg/kg} p.o)
Group G	AQERZO (medium dose, {200 mg/kg} p.o)
Group H	AQERZO (high dose, {400 mg/kg} p.o)

Experimental Procedure
Albino rats weighing between (150-200 g) were divided into 8 groups of six rats in each. Group A served as toxicant control, given with 0.1 ml of formaldehyde (2% v/v) into the hind paw and Group B served as standard, was given with indomethacin. Animals in Groups C, D, and E were treated with three different doses (low, medium and high) of AERZO and animals in groups F, G and H were treated with three different doses (low, medium and high) of AQERZO. Groups B, C, D, E, F, G, and H were intoxicated with 0.1 ml of Formaldehyde (2% v/v). Daily the paw volume was measured for the next 10 days.

Stastical analysis
All the recorded results are expressed as mean ± SEM from 6 animals. Statistical difference in mean was analyzed by using one-way ANOVA (analysis of variance) followed by Post hoc test (Dunnett's 't' test). P value >0.05 was considered as non-significant (ns), P<0.05 as significant (*), P<0.01 as more significant (**) and P< 0.001 as highly significant (***).

RESULTS
The preliminary phytochemical analysis of the AERZO and AQERZO revealed the presence of carbohydrates, sterols, flavonoids, glycosides, fixed oils, fats, saponins and alkaloids. The result of oral administration of alcoholic and aqueous extracts at 100, 200 & 400 mg/kg b.w on % change in paw oedema volume in formaldehyde induced arthritic rats represented Table-1 and Figure-1.

In toxicant control (formaldehyde treated group) 83.5% increase in oedema volume was recorded on 1st day and was gradually increased to a maximum of 89.02% on 10th day.

Standard drug indomethacin has significantly reduced oedema volume on 1st day it self (16.03%) and time

dependent reduction in oedema volume was recorded up to 10th day and the maximum percentage reduction in oedema volume was recorded as 58.63%.

AERZO with different doses exhibited time dependent significant reduction in oedema volume recorded as minimum and maximum of (1.04%,10.98%), (6.90%,26.18%) and (14.99%,58.11%) respectively on 1st and 10th day of the experimental study. similarly AQERZO also exhibited dose dependent and time dependent significant reduction in oedema volume on different time intervals of the experimental study and reduction of oedema volume with three different doses of AQERZO on 1st and 10th days of the experimental study are recorded as (2.11%,13.08%), (2.11%,27.21%) and (14.36%,57.59%) respectively.

Table.1. Percentage change in oedema volume at different time intervals (days).

Groups	Treatment	Oedema Volume (mean±SD)			
		1 day	4 day	7 day	10 day
1	Toxicant control (Formaldehyde 2% v/v)	83.5±5.68	87.40±5.21	89.02±2.31	89.02±2.31
2	Standard (Indomethacin 10 mg/kg)	16.03±3.39**	17.12±7.78**	42.3±2.19**	58.63±2.35**
3	AERZO(100mg/kg)	1.04±2.43ns	5.22±1.59*	9.94±3.64*	10.98±5.12*
4	AERZO(200mg/kg)	6.90±4.31**	7.32±4.72**	24.16±2.84**	26.18±2.71**
5	AERZO(400mg/kg)	14.99±2.05**	19.35±2.95**	41.34±2.0**	58.11±2.5**
6	AQERZO(100mg/kg)	2.11±2.45 *	4.16±2.55*	9.91±3.61*	13.08±1.25*
7	AQERZO(200mg/kg)	2.11±2.45**	7.33±4.73**	24.57±2.84**	27.21±2.43**
8	AQERZO(400mg/kg)	14.36±3.78**	19.43±2.44**	38.21±6.67**	57.59±3.19**

AERZO- Alcoholic extract of rhizome of *Zingiber officinale.*
AQERZO -Aqueous extract of rhizome of *Zingiber officinale.*
n=6, Significant at *P*<0.05*, *P* <0.01** and ns-not significant vs. control group.

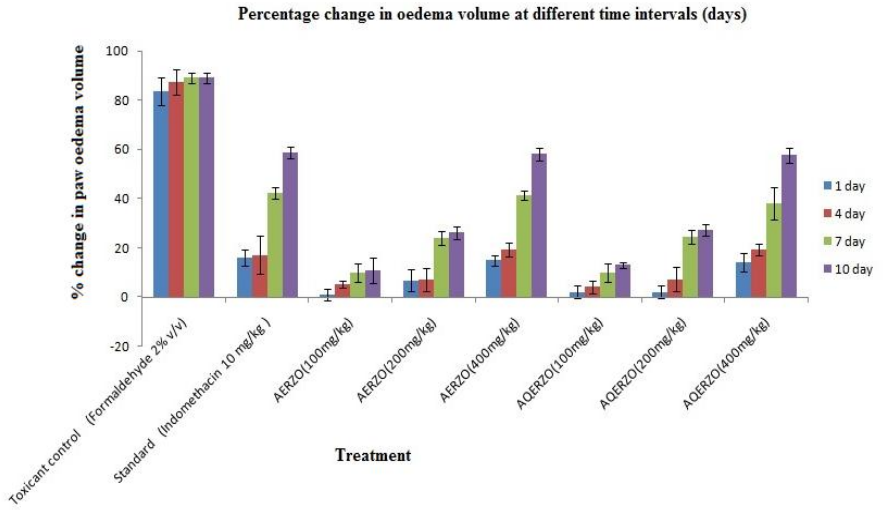

Figure. 1.

DISCUSSION

RA is a chronic inflammatory disease affecting about 1% of the population in developed countries. The acute stage of arthritis is characterized by signs of hyperalgesia, lack of mobility and a pause in body weight gain; during the acute period, the hind and fore paw joint diameters increase. In chronic stages of the disease rats with arthritis are often relatively immobile due to the severity of paw swelling.[8,9] Eventhough various categories like immunosuppressants, NSAIDs, steroidal anti inflammatory drugs are being used till now, but the potential side effects give a limitation for their use. Traditional medicines derived mainly from plants play major role in the management of arthritis as they are effective, non-toxic, with less or no side effects and are considered to be excellent candidates for arthritic therapy.[10] Hence, there was a need to explore for more naturally available alternatives, so that their therapeutic values can be assessed and expanded.

It is well known that inhibition of formaldehyde induced paw edema in rats is one of the most suitable test

procedures to screen anti-inflammatory and anti-arthritic agents. Antiarthritic activity was reported to be mediated either by inhibition of phospholipase A2 (PL-A2) activity or cyclooxygenase pathway as the cyclooxygenase enzyme participates in syntheis of prostaglandins from arachidonic acid and also by blocking the release of vasoactive substances such as histamine, serotonin and kinins.

Formaldehyde induced arthritis is one of most commonly used acute model for assessing anti-arthritic potential of plant extract. The development of oedema in the paw of the rat after injection of formaldehyde (0.1ml, 2%v/v) is due to the release of histamine, serotonin and the prostaglandin like substances at the site of injection. Both histamine and prostaglandin are the key mediators in inflammatory hyperalgesia that is mediated through the activation of local pain receptors and nerve terminals producing hypersensitivity in the area of injury.[11,12] Inhibition of paw edema observed in formaldehyde induced arthritic model may be due to the ability of the AERZO and AQERZO to inhibit histamine, serotonin and the prostaglandin which are responsible for inflammation. In present study Indomethacin is used as standard drug. The Indomethacin is a NSAIDs acts by inhibition of prostaglandins (PGs) synthesis by blocking COX enzymes responsible for inflammation.

CONCLUSION

AERZO and AQERZO medium and high dose (200,400 mg/kg) but not low dose (100 mg/kg) have shown significant anti arthritic activity against Formaldehyde induced arthritis in rats. The present study concluded that AERZO and AQERZO in 400mg/kg dose exhibited almost similar effect on %change in oedema vlume on 10^{th} day 58.11% & 57.59% respectively as compared to Indomethacin (10mg/kg) 58.63%.

Our photochemical investigation revealed that the presence of carbohydrates, sterols, flavonoids, glycosides, fixed oils, fats, saponins and alkaloids in AERZO and AQERZO. Presence of wide range of constituents indicates the good efficacy of this plant in various pathological disorders. Beside these flavonoids has been reported to inhibit the cyclooxygenase enzyme thereby inhibiting prostaglandin synthesis which are responsible for development of arthritis. Pharmacological studies indicate that flavonoids and saponin have anti-inflammatory and antiarthritic activity.[13,14]

Thus, in the light of above facts, it can be demonstrated that the AERZO and AQERZO may serve as an effective anti-arthritic drug and the effect might be speculated due to phytochemicals such as saponins and flavonoids. This study warrants the investigation to isolate and identify the active principles and to investigate the exact mechanism of action of *Zingiber officinale* against arthritis.

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CONFLICT OF INTEREST

There is no conflict of interest.

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