

ANTI-INFLAMMATORY EFFECT OF *ZINGIBER OFFICINALE* ON SPRAGUE DAWLEY RATS

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## ABSTRACT

**Objective:** To investigate the anti-inflammatory effect of aqueous extract of *Zingiber officinale* on carrageenan-induced inflammation on Sprague Dawley (SD) rats.

**Methods:** SD rats were divided into six of five groups and allowed to acclimatize for 1 week. Inflammation was induced on the animal by injecting the right hand paw with carrageenan (0.1 ml of 1%). Group 1 was given normal saline and served as a control. Group 2 was fed with food and water and served as the carrageenan control. Group 3 was given 200 mg/kg aqueous extract of ginger, Group 4 with 400 mg/kg aqueous extract of ginger, and Group 5 with 150 mg/kg diclofenac sodium (reference drug for inflammation).

**Results:** The paw edema in carrageenan-induced SD rats was considerably reduced by treating with 400 mg/kg aqueous ginger extracts when compared to the untreated SD rats ( $p < 0.001$ ).

**Conclusion:** This study indicates that aqueous extract of *Z. officinale* possesses anti-inflammatory properties.

**Keywords:** Anti-inflammatory, Sprague Dawley rats, *Zingiber officinale*, Carrageenan, Edema

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## INTRODUCTION

Inflammation is a body resistance reaction to eradicate or limit the spread of injurious agent and it is a local response of living mammalian tissues to injury [1-3]. There are various mechanisms to an inflammatory reaction, which can contribute to the associated symptoms and tissue injury. Edema, granuloma formation, and leukocyte infiltration signify such components of inflammation. However, it is a defense mechanism. The complex events and mediators involved in the inflammatory reaction can induce or aggravate many reactions [3-5]. According to the WHO report, about 70-80% of the world's population depend on nonconventional medicine predominantly from herbal sources in their primary health care [6,7]. Especially, in developing nations where the cost of consulting a physician and fee of medicine are beyond the edge of most people, thereby the demand is increasing day by day. These drugs are anti-inflammatory and used to ease pain in various conditions including arthritis, muscle, and ligament pains [8,9].

## METHODS

## Plant material

Ginger (*Zingiber officinale*) rhizomes were collected during March-April 2016 from Sungai petani vegetable market Kedah Malaysia. The plant material was taxonomically identified by a botanist from faculty of Biotechnology at AIMST University Malaysia. (Halia in Malay. Scientifically as *Z. officinale*). The voucher specimen was maintained in AIMST University, Faculty of Medicine research laboratory for future reference. *Z. officinale* rhizome was collected and aqueous extract for use in the study.

Preliminary phytochemical screening test for *Z. officinale*

Phytochemical screening of the plant extract was carried out to investigate the present of secondary metabolites such as flavonoid, terpenoids, saponins, tannins, and phenol using standard procedure by Evans method [10].

## Test for terpenoids (Salkowski test)

About 2 ml of chloroform was added to about 1 ml of plant extract. To this mixture, 3 ml of concentrated sulfuric ( $H_2SO_4$ ) acid is added

carefully without jerking. Development of a reddish brown coloration at the interface indicates the presence of terpenoids.

## Test for flavonoids

To 1 ml of the extract, 5 ml of dilute ammonia is added. In addition, of 1 ml concentrated  $H_2SO_4$  a yellow color solution is temporarily produced which indicate the present of flavonoid. As a confirm test for flavonoid, a few drops of 1% aluminum solution is added to the plant extract and formation of a permanent yellow color solution indicates the presence of flavonoid.

## Test for saponins

To 1 ml of extract, 1 ml of distilled water was added and shaken vigorously; a stable permanent form will develop indicating its presence. The result was positive for the test.

## Test for tannins

About 1 ml of the extract was mixed with 9 ml of distilled water to make up the volume of the solution to 10 ml; it was then subjected to boiling in a boiling bath for 5 minutes. After boiling, the mixture was cooled and filtered. To the filtrate, 1.0 ml of 0.1% ferric chloride is added. This will result in the formation of brownish green or blue-black coloration if tannin was presence.

## Test for phenols

A portion of the extract of the samples was diluted with distilled water in a ratio 1:4. Few drops of 10% ferric chloride solution were added. The appearance of green solution indicates the presence of phenol, which was present.

## Experimental animals

Female SD rats of weighing 160-200 g were procured from registered breeders (Universiti Sains Malaysia) and were housed in a clean polypropylene cages with not more than four animals per cage and maintained under standard laboratory conditions (temperature  $25 \pm 2^\circ C$  with dark/light cycle 12/12 hrs) at AIMST University animal house. They were fed with standard pellet diet and water *ad libitum*. The

animals were acclimatized to laboratory conditions for 10 days before experiment. All experimental procedures described were reviewed and approved by the Institutional Animal Ethics Committee.

#### Preparation of extract *Z. officinale*

Aqueous ginger extract was prepared from locally available Malaysian ginger roots. Ginger roots (20 g) were peeled and was cut into small pieces and homogenized in 75 ml cold, sterile 0.9% NaCl solution, and 25 ml ice-cold water to make the volume 100 ml. The homogenization was carried out in a blender for 12 minutes. The homogenized mixture was filtered three times through cheesecloth. The filtrate was centrifuged at 2000 rpm for 10 minutes, the clear supernatant fraction was separated, and volume made up to 100 ml with normal saline. The concentration of this ginger preparation was calculated to have 200 mg/ml based on the weight of the starting material [11]. The aqueous extract was stored in sample tubes at  $-20^{\circ}\text{C}$  until fed to rats.

#### Acute toxicity

The acute oral toxicity of aqueous extract of *Z. officinale*, in female Sprague Dawley (SD) rats was studied as per reported method [12]. These extracts were given to three groups (n=6) of animals at concentrations 1000, 1500 and 2000 mg/kg body weight. The treated animals were kept under observation for 2 days, for mortality and general behavior. No toxic effects were observed until the end of the study.

#### Evaluation of anti-inflammatory activity

The rats were divided into five groups (n=6). Group I served as normal non-inflammation control and all other groups were comprised carrageenan-induced inflammation rats. Group II served as carrageenan control. Groups III and IV received 200 mg/kg and 400 mg/kg b.w of *Z. officinale* orally. Group V received the reference drug diclofenac sodium (150 mg/kg b.w.,) once for 24 hrs.

#### RESULTS

Preliminarily, the presence of the phytochemicals was assessed to identify the potential constituents present in the extract. The following results show the phytochemical analysis of ethanolic (Table 1) and aqueous (Table 2) extracts of *Z. officinale*.

The results in Table 1 show the presence of flavonoids, saponins, tannins, terpenoids, and phenol in the ethanolic extract of *Z. officinale*. The aqueous extract of *Z. officinale* was also tested for the presence of phytochemicals by the methods described in the materials and methods section. Table 2 shows the presence of phytochemical constituents such as flavonoids, saponins, and terpenoids in the aqueous extract of *Z. officinale*.

#### DISCUSSION

The use of herbal medicines is becoming popular due to toxicity and side effects of allopathic medicines. Medicinal plants play an important role in the development of potent therapeutic agents. There are over 1.5 million practitioners of the traditional medicinal system using medicinal plants in preventive, promotional, and curative applications [13,14]. In this study, the results of the preliminary

phytochemical screening of both aqueous and ethanolic extracts of Malaysian ginger (*Z. officinale*) have shown to contain flavonoids, saponins, terpenoids and phenols. As shown in Tables 1 and 2, several studies also have shown that *Z. officinale* extracts have substantial amounts of flavonoid, terpenoids, saponins, and phenols [15-17].

To assess the safety of the plant material being used, the toxicity assay with the *Z. officinale* aqueous extracts was carried out. The acute oral toxicity of aqueous extract of *Z. officinale*, in female SD rats was studied with concentrations 1000, 1500 and 2000 mg/kg body weight the treated animals were kept under observation for 2 days, for mortality and general behavior. No toxic effect was observed until the end of the study.

Plants as natural anti-inflammatory agents unlike modern allopathic drugs, which are single active components that target one specific pathway, herbal medicines work in a way that depends on an orchestral approach. A plant contains a multitude of different molecules that act synergistically act on targeted elements of the complex cellular pathway [16]. Medicinal plants have been a source of wide variety of biologically active compounds for many centuries and used extensively as crude material or as pure compounds for treating various disease conditions [18,19].

Carrageenan-induced rat paw edema model is a suitable test for evaluating anti-inflammatory drugs, which has frequently been used to assess the anti-edematous effect of the drug [20]. Carrageenan is a strong chemical used for the release of inflammatory and pro-inflammatory mediators (prostaglandins, leukotrienes, histamine, bradykinin, TNF- $\alpha$ , etc.) [21]. Table 3 shows injection of carrageenan into the hind paw induced a progressive edema reaching its maximum at 3 hrs. In case of Group 1 animals paw thickness found at prestudy  $t=0$  was  $1.56\pm 0.02$  cm, and this remains constant at the end of 24 hrs.

**Table 1: Phytochemical analysis of ethanolic extract of *Z. officinale***

Phytochemicals	Observation
Flavonoids	+
Saponins	+
Tannins	+
Terpenoids	+
Phenol	+

*Z. officinale*: *Zingiber officinale*

**Table 2: Phytochemical analysis of aqueous extract of *Z. officinale***

Phytochemicals	Observation
Flavonoids	+
Saponins	+
Tannins	-
Terpenoids	+
Phenol	-

*Z. officinale*: *Zingiber officinale*

**Table 3 Anti-inflammatory effect of *Z. officinale* on carrageenan induced edema on rats**

Treatment Groups	Anti-inflammatory (cm)					
	Prestudy	1 h post dose	2 hrs post dose	3 hrs post dose	4 hrs post dose	24 hrs post dose
Group 1	1.56 $\pm$ 0.02	1.54 $\pm$ 0.02	1.56 $\pm$ 0.02	1.54 $\pm$ 0.02	1.56 $\pm$ 0.02	1.56 $\pm$ 0.02
Group 2	1.56 $\pm$ 0.04	2.18 $\pm$ 0.06 <sup>a</sup>	3.16 $\pm$ 0.05 <sup>a</sup>	3.06 $\pm$ 0.05 <sup>a</sup>	2.66 $\pm$ 0.05 <sup>a</sup>	2.1 $\pm$ 0.09 <sup>a</sup>
Group 3	1.56 $\pm$ 0.04	2.28 $\pm$ 0.10 <sup>a</sup>	2.1 $\pm$ 0.10 <sup>a</sup>	1.82 $\pm$ 0.04 <sup>b</sup>	1.64 $\pm$ 0.06	1.54 $\pm$ 0.04
Group 4	1.60 $\pm$ 0.07	2.44 $\pm$ 0.10 <sup>a</sup>	1.8 $\pm$ 0.05	1.80 $\pm$ 0.08 <sup>b</sup>	1.62 $\pm$ 0.06	1.52 $\pm$ 0.02
Group 5	1.58 $\pm$ 0.04	2.22 $\pm$ 0.09 <sup>a</sup>	1.56 $\pm$ 0.04	1.52 $\pm$ 0.02	1.50 $\pm$ 0.03	1.52 $\pm$ 0.02

All the values are mean $\pm$ SEM (n=5). <sup>b</sup>p<0.01 and <sup>a</sup>p<0.001 compared to Group I. One-way ANOVA followed by Tukey's *post-hoc* test was applied. Group 1: Normal control rats, Group 2: Diabetic control rats, Group 3: 200 mg/kg *Z. officinale* aqueous extract was administered intraperitoneally, Group 4: 400 mg/kg *Z. officinale* aqueous extract was administered intraperitoneally, Group 5: Diclofenac sodium 150 mg/kg was administered intraperitoneally, *Z. officinale*: *Zingiber officinale*

Group 2 animals had showed an increase in paw thickness at each hour, which was significant ( $p < 0.001$ ). At 24 hrs, the thickness was found to be  $2.1 \pm 0.09$  cm. The paw thickness of Group 3 animals was  $1.56 \pm 0.04$  cm at  $t=0$  hr, which showed a mild increase at the end of the first hour, that is,  $2.28 \pm 0.010$  cm. At the end of 24 hrs, it decreased to  $1.54 \pm 0.04$  cm at ( $p < 0.001$ ), respectively. Group 4 animals showed an increase up to the 1<sup>st</sup> hr.  $2.44 \pm 0.10$  cm but showed a decrease in the paw thickness at the end of 24 hrs. From these findings, Groups 3 and 4 indicate a significant decrease in paw thickness as compare to the control Group 1 ( $p < 0.001$ ). Group 5 which received 150 mg/kg diclofenac sodium shows a significant decrease in paw thickness at 2 hrs, 3 hrs, 4 hrs and also 24 hrs as compared to the control Group 1 ( $p < 0.001$ ).

## CONCLUSION

The above results indicate that aqueous extracts of *Z. officinale* have significantly decreased the carrageenan-Induced inflammation and related paw edema in our study on SD rats.

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