

A study of the anti-inflammatory effect of *Zingiber officinale* Roscoe (Gyin-sein) on albino rats

Khine Khine Lwin*, *Mya Mya Than*, **Khin Chit*
**Mu Mu Sein Myint*, **Aye Than* & **Aye Aye Thein*

*Pharmacology Research Division
Department of Medical Research (Lower Myanmar)
**Institute of Pharmacy, Mandalay

This study was aimed to investigate the anti-inflammatory activity of *Zingiber officinale* Roscoe (Ginger) on carrageenan-induced paw inflammation in albino rats. Both aqueous extract and ethanolic extract of the dried rhizomes of Ginger were used. Plethysmographic apparatus was used to measure the volume changes of carrageenan-induced rat's paws oedema. Anti-inflammatory actions of both extracts were investigated by using 3 dose levels i.e. 3g/kg, 1.5 g/kg, 0.75 g/kg body weight, orally and compared with that of aspirin 300mg/kg. Significant anti-inflammatory actions were found with both extracts of Ginger. It was found that the anti-inflammatory actions of both extracts were found to have dose-response relationship in nature. The results also indicated that anti-inflammatory action of ethanolic extract of Ginger was found to be superior to that of aqueous extract. But anti-inflammatory effects of both extracts of Ginger were inferior to that of standard reference drug, aspirin. The actions of ethanolic extract and aqueous extract were (0.49-0.66) times and (0.52-0.55) times that of aspirin respectively. Acute toxicity studies and general pharmacological screening tests of both extracts on albino mice showed no lethality and no abnormal changes. The actions of both extracts of Ginger may be due to one or more compounds obtained in these extracts i.e. saponins, alkaloids, resins, steroids and flavonoids according to the phytochemistry. Therefore, it can be concluded that both extracts of Ginger may be beneficial for use in joint pain and inflammation.

INTRODUCTION

Joint pain and inflammation are common problems among the people in the world. The non-steroidal (NSAIDs) and steroidal anti-inflammatory drugs are used in symptomatic treatment of joint pain and inflammation but have a number of undesirable side effects and toxicity [1]. Since there is yet no cure for arthritis in modern medicine, a large numbers of herbal drugs are being used for the treatment of arthritis in eastern countries like Myanmar, India and China. One of the medicinal plants, *Zingiber officinale* Roscoe (Zingiberaceae) (Gyin-sein) commonly known as "Ginger" has been reputedly used

in Myanmar and Ayurvedic medicine for the treatment of inflammatory disorders [2, 3]. Mascolo-N *et. al.* [4] reported that an ethanolic extract of the rhizomes of ginger reduced carrageenan-induced paw inflammation in albino rats.

In Myanmar, in spite of its long historical use, scientific information regarding effective dose, efficacy and toxicity of Ginger have not been fully documented yet. Thus, the objective of this study was to determine the effect of Ginger on the carrageenan-induced inflammation of rat's paw so as to determine its potential therapeutic efficacy in the treatment of arthritis.

MATERIALS AND METHODS

Extraction of Zingiber officinale Roscoe (Ginger)

Powder of dried rhizomes of Ginger were extracted separately using 50% ethanol and distilled water to get the required ethanolic and aqueous extracts of Ginger. The dried extracts were dissolved in distilled water before administration to animals.

The acute toxicity study of Ginger extracts

The lethal activity and the determination of LD₅₀ of the extracts of Ginger were done according to the standard method of Litchfield and Wilcoxon [5].

Fifty albino mice of both sexes, weighing (25-30g) were used in this study. They were divided into 5 groups and each group contained 10 mice. After overnight fast, five doses of ethanolic extract of Ginger were given orally to each group (i.e. 0.25 g/kg, 0.5 g/kg, 1 g/kg, 2 g/kg and 3 g/kg body weight). They were observed closely for 7 days after drug administration. Similarly, acute toxicity test of aqueous extract was also done as above.

General pharmacological screening tests of both extracts of Ginger

The standard method of Sandberg G. F [6] was used in this study. Both extracts of Ginger were tested for behavior, neurologic, and autonomic activity on albino rats following oral administration of different doses of extracts (i.e. 3 g/kg, 2 g/kg, 1 g/kg, and 0.5 g/kg).

In vivo experiment on albino rats

1. Induction of inflamed paws in albino rats

Freshly prepared suspension of Lambda carrageenan 0.1 ml (1.0% in 0.9% NaCl) was injected under the plantar aponeurosis of right hind paws of albino rats to produce

acute inflammation. Oedema formation was regarded as an indicator of inflammation. The volume of the paw was measured by plethysmometer (i.e., differential volume measuring instrument). The measurement of the paw volume was done immediately before and every one hour interval up to six hours after carrageenan injection [7].

Paw volume was measured by immersion of the foot in wetting fluid i.e. normal saline to an ink mark at the level of the lateral malleolous. The volume of paw was expressed in terms of milliliter (ml).

2. Carrageenan-induced inflammation on right hind paws of albino rats (control group)

In this study, female albino rats (Wistar strain) weighing 130-230g maintained on the same standard laboratory diets were used. A total of 10 rats were used and they were fasted overnight about 16 hours before the experiment.

3. The effects of both extracts of Ginger and standard reference drug, acetylsalicylic acid (aspirin) on carrageenan-induced paws inflammation

The above procedure was repeated with both extracts of Ginger and standard reference drug, aspirin. Three dose levels of ethanolic and aqueous extract (i.e., 3 g/kg, 1.5 g/kg, and 0.75 g/kg) were used to each group of rats.

One positive control group in which standard drug, aspirin, 300 mg/kg was given. All drug vehicles and extracts were given orally, one hour before sub planter injection of Lambda carrageenan.

4. Determination of ED₅₀ of anti-inflammatory effects of both extracts of Ginger

The ED₅₀ of both extracts were determined by using the standard method [5]. ED₅₀ was calculated from the volume reduction effect

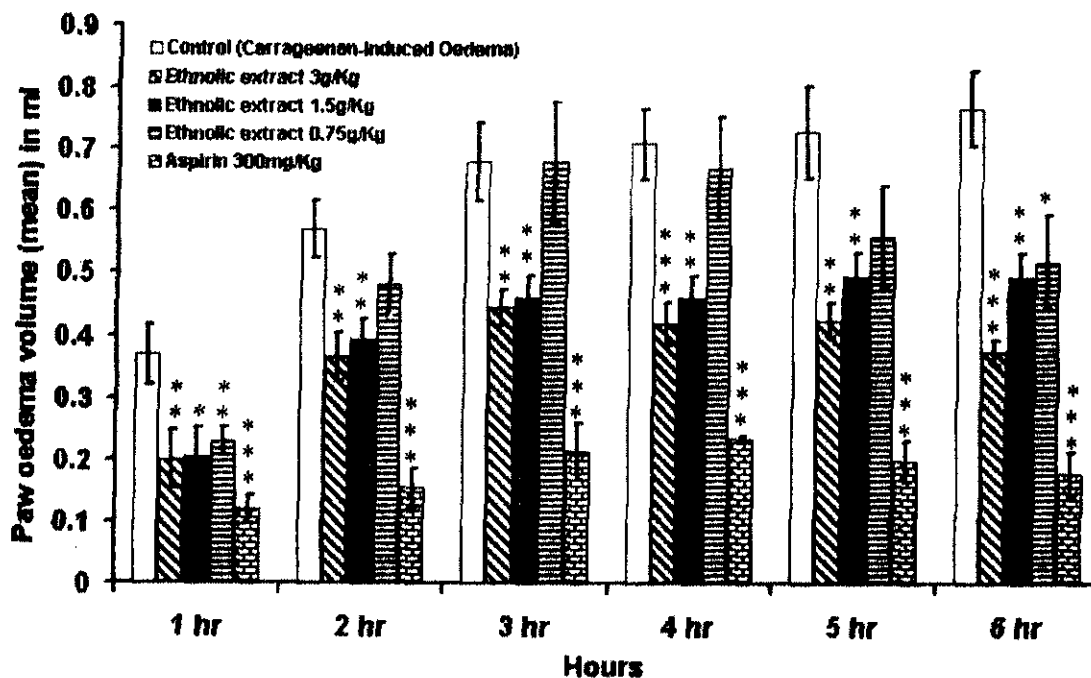


Fig. 1. Effects of ethnolic extracts of *Zingiber officinale* Roscoe and standard drug (Aspirin) on carrageenan-induced paw oedema volume (mean) in ml (mean \pm S.E) (n=10) (statistical comparison between control and tested groups - * P<0.05, ** P<0.01, ***P<0.001)

of both extracts of Ginger on right hind paw of albino rats at three dose levels (i.e. 0.75 g/kg, 1.5 g/kg, 3 g/kg).

Phytochemical analysis of both ethanolic and aqueous extracts of Ginger

Both ethanolic and aqueous extracts of Ginger were tested qualitatively for the presence of saponins, alkaloids, resins, tannins, cyanogenic glycosides, starch and flavonoids by using the standard method [8].

Data management and analysis

Unpaired student 't' test was used to observe the significance of difference between the means of control and experimental groups.

RESULTS

Extraction of rhizomes of *Zingiber officinale* Roscoe (Ginger)

Both the aqueous and ethanolic extraction of dried rhizomes powder of Ginger (100 g) yielded 15 g/100 g or 15%.

Acute toxicity study of Ginger extracts

The acute toxicity tests showed no lethality in mice observed up to 7 days, even with the maximum doses (3 g/kg) of the both aqueous and ethanolic extracts. Thus, both extracts of Ginger were considered non-toxic up to the dose level of 3 g/kg and the median lethal doses (LD₅₀) of both extracts were greater than 3 g/kg.

General pharmacological screening tests of both extracts of Ginger

In present study, no abnormal motor activities, screen grips and gaits were observed and neither fit nor tremor was detected. No other abnormalities were seen.

Effect of carrageenan on right hind paws of the albino rats

The effects of carrageenan on right hind paws of the albino rats were shown in Fig 1. Sub plantar injection of 0.1 ml of 1% carrageenan produced statistically significant rise in paw oedema volume, starting from 1 hour up to 6 hours after carrageenan injection (P<0.001).

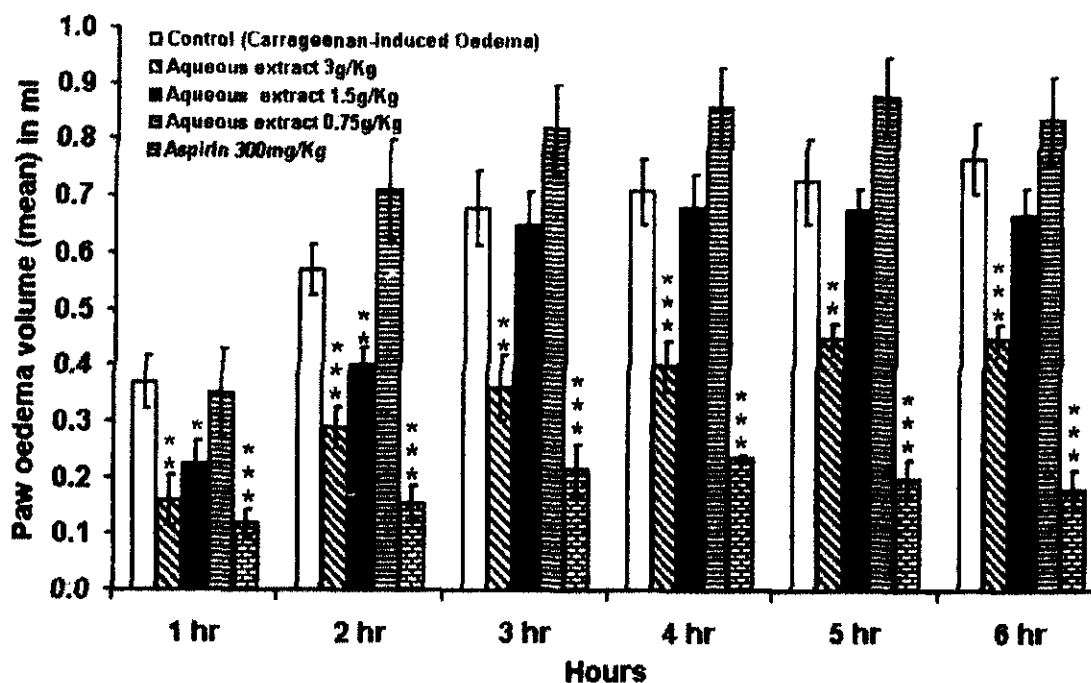


Fig. 2. Effects of aqueous extracts of *Zingiber officinale* Roscoe and standard drug (Aspirin) on carrageenan-induced paw oedema volume (mean) in ml (mean \pm S.E) (n=10) (statistical comparison between control and tested groups - * P<0.05, ** P<0.01, ***P<0.001)

The effects of both extracts of Ginger and standard drug, aspirin on carrageenan - induced paws oedema

The results of ethanolic extract of Ginger were shown in Fig. 1. Ethanolic extract of Ginger, at the dose level of 3 g/kg and 1.5 g/kg, showed statistically significant decrease in rats' paws oedema volumes compared with the control. This was found to start from 1 hour to 6 hours (P<0.05-P<0.001) after carrageenan injection. With 0.75 g/kg of ethanolic extract, statistically significant decrease in paw oedema volume was found at 1 hour (P<0.01) and at 6 hours (P<0.05) only.

In Fig. 2, aqueous extract of Ginger, at the dose level of 3 g/kg showed that statistically significant decrease in rat's paws oedema volumes compared with control were found to start from 1 hour to 6 hours (P<0.01-P<0.001) after carrageenan injection. With 1.5 g/kg of aqueous extract, statistically significant decrease in paw oedema volume was found at 1 hour (P<0.05) and 2 hours (P<0.01) only. With 0.75 g/kg of aqueous

extract, there was no statistically significant decrease in paw oedema volume compared with control group.

Aspirin (acetylsalicylic acid 300 mg/kg) showed that statistically significant decrease in paw oedema volume was found at 1 hour after carrageenan injection up to 6 hours (P<0.001).

ED₅₀ of anti-inflammatory effects of both extracts of Ginger

The result of ED₅₀ of ethanolic extract of Ginger was 0.98 g/kg and its confidence limit was (0.81 g/kg-1.186 g/kg). ED₅₀ value of aqueous extract of Ginger was 1.2g/kg and its confidence limit was (1.026 g/kg-1.404 g/kg).

Phytochemical analysis of both extracts of Ginger

Phytochemical study of Ginger had shown that both extracts of ethanolic and aqueous extracts of Ginger contained saponins, alkaloids, tannins, steroids and flavonoids.

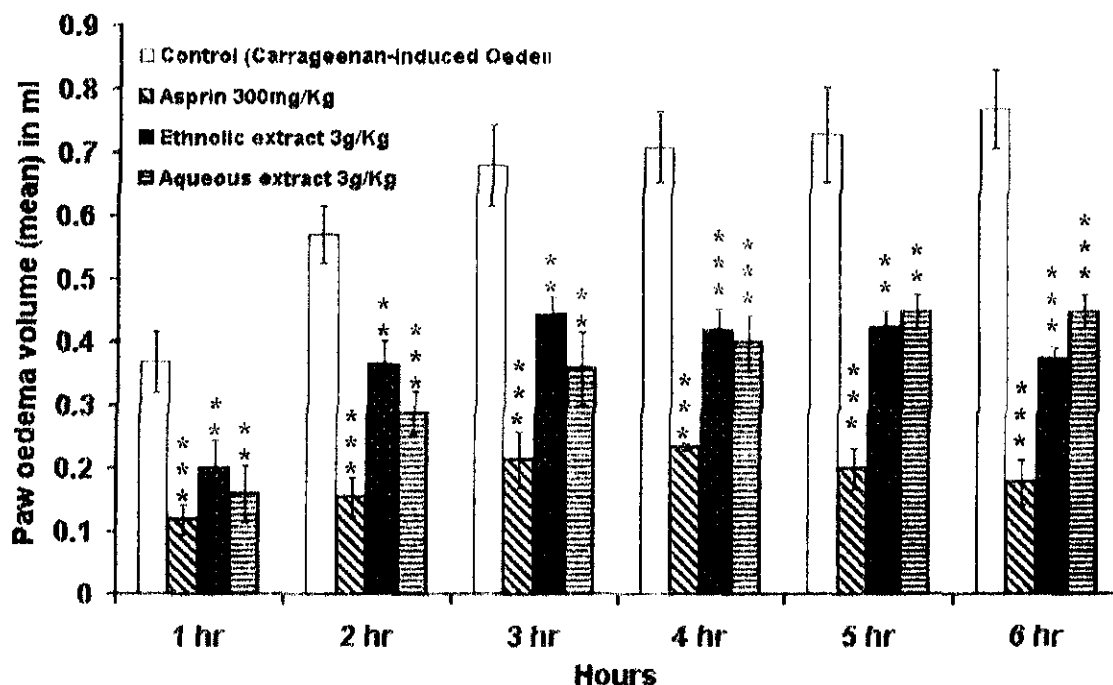


Fig. 3. Effects of ethnolic and aqueous extracts of *Zingiber officinale* Roscoe and standard reference drug (aspirin) on carrageenan-induced paw oedema volume (mean \pm S.E) (n=10) (statistical comparison between control and tested groups - * P<0.05, ** P<0.01, ***P<0.001)

The 50% ethnolic extract also contained resins (Table 1).

Table 1. Phytochemical analysis of both extracts of Ginger

No.	Compounds	50% Ethnolic extract	Aqueous extract
1.	Saponins	+	+
2.	Alkaloids	-	+
3.	Tannins	+	+
4.	Flavonoids	+	+
5.	Steroids	+	+
6.	Resins	+	-
7.	Cyanogenic glycosides	-	-

+ Presence - Absence

DISCUSSION

To study the anti-inflammatory effect, carrageenan-induced inflammation on hind paw of albino rat was used as an acute inflammation model. Both ethnolic and aqueous extracts of Ginger were used in this study. Aspirin was used as standard

reference drug for comparison of anti-inflammatory actions of Ginger extracts.

According to the results, both extracts of Ginger on albino mice showed no acute toxic effects up to the dose level of 3g/kg. Therefore, both extracts can be said to be free from acute toxic effect.

The results showed that both extracts of Ginger had significant anti-inflammatory actions and also had dose-response relationship in actions ($P<0.05$ - $P<0.001$). It was found that ED_{50} value of ethnolic extract was lower than that of aqueous extract. The result also showed that the anti-inflammatory actions of ethnolic extract was greater than that of aqueous extract ($P<0.01$ - $P<0.05$) (Fig. 3).

Even though both extracts of Ginger showed significant anti-inflammatory actions, the actions were found to be inferior to that of aspirin (i.e. ethnolic extract and aqueous extract were found to be 0.49-0.66 times and 0.52-0.55 times that of aspirin respectively).

Steroids, alkaloids, phenolic compounds, saponins, resin and flavonoids were reported that they could give anti-inflammatory action [9]. So, the actions of both extracts of Ginger may be due to one or more compound obtained in those extracts (i.e. saponins, alkaloids, resins, steroids and flavonoids constituents of Ginger as shown in the phytochemistry study).

Gingerol, the only known active principle of Ginger has been reported to be a resin with oily liquid [10]. The presence of resin in the ethanolic extract may be the reason why its action was more potent than that of aqueous extract.

Both extracts of Ginger at the dose level of 3 g/kg, showed significant anti-inflammatory effects started from 1 hour up to 6 hours after sub plantar injection of carrageenan on the hind paws of albino rats. The patterns of inhibition of both extracts were similar to that of standard drug, aspirin. Therefore, the mechanisms of anti-inflammatory actions of both extracts were suggested to be similar to that of aspirin.

In conclusion, the results of the above experiment have provided useful preliminary information on use of Ginger in the treatment of joint pain and inflammation.

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