

## EVALUATION OF THE ANTI-INFLAMMATORY AND ANTIPYRETIC ACTIVITIES OF THE PLANT *ALPINIA NIGRA* (FAMILY: ZINGIBERACEAE)

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### ABSTRACT

*Alpinia nigra* is an important medicinal plant having application in jaundice, fever and various other disorders. The aim of this study was to evaluate the anti-inflammatory and antipyretics activities of the whole plant *A. nigra*. The roots, stems, barks and the leaves of the plant *A. nigra* was sun dried and extracted using methanol. The anti-inflammatory activity was evaluated using the carageenan induced paw edema in rats. The crude methanolic extract at a dose of 600mg/kg showed very potent anti-inflammatory activity in carageenan induced rat paw edema model with 68.59% inhibition of paw edema after the fourth hour of study. The Antipyretic activity was evaluated by the yeast induced pyresis method. The crude methanolic extract at both 600mg/kg and 300mg/kg dose showed significant Antipyretic effect.

**KEYWORDS:** *Alpinia nigra*, carageenan, paw edema, yeast induced pyresis.

### INTRODUCTION

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses [1]. Although it is a defense mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases [2]. On

the other hand pyrexia is a common medical sign characterized by an elevation of temperature above the normal range of 36.5–37.5 °C (98–100 °F) due to an increase in the body temperature regulatory set-point. This increase in set-point triggers increased muscle tone and shivering. Drugs that are currently used for the management of inflammation and pyrexia are non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. All these drugs carry potential toxic effects. One study suggests that risk of gastrointestinal bleeding was significantly associated with acute use of non-steroidal anti-inflammatory drugs (NSAIDs) like regular-dose aspirin, diclofenac, ketorolac, naproxen or nimesulide. Piroxicam increased the risk of bleeding in both acute and chronic therapy. On the contrary many medicines of plant origin had been used since ages without any adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop more effective and cheaper drugs. *A. nigra* is an important medicinal plant having application in jaundice [3], fever [4] and constipation [5]. It is also known to be a blood purifier and is also reported to have anti-viral use [4]. The present study was designed to investigate the anti-inflammatory and Antipyretic potential of the crude methanolic extracts of the whole plant *Alpinia nigra*.

## MATERIALS AND METHOD

### Collection of the plant sample

Fresh plant of *Alpinia nigra* was collected from Sherpur, Mymensingh, Bangladesh in October, 2010. This plant was identified by the taxonomist of the Botany Department of the University of Dhaka. The reference sample for the plant was DUSH Accession Number 3616 and Call no 02.

### Preparation of plant extract

The stem-bark and leaves were sundried for 5 days. The plant materials were then oven dried for 24 hours at low temperature. 960 gm of powdered material (Roots, stem-bark and leaves) was macerated with 2.5 L of methanol in two 4 L round bottom flask. The containers were sealed with cotton plug and aluminum foil at room temperature for 15 days with occasional shaking. The mixture was filtered through cotton and then evaporated to dry (45°C) under reduced pressure by rotary evaporator. The dried extract was preserved in refrigerator.

### Drugs and Chemicals

Diclofenac was obtained from ACI pharmaceuticals. carrageenan was purchased from Sigma-Aldrich, Germany. Yeast was obtained from Gonoshastho Pharmaceuticals Ltd., Dhaka, Bangladesh.

### Experimental animal

Albino Wistar rats (150-200 g) were obtained from the Animal Research Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR, B). The animals were housed in polyvinyl cages and received feed, formulated by ICDDR, B and water *ad libitum*. To keep the hydration rate constant, food and water were stopped 12 hours before the experiments. The ethics for use of experimental animals were followed carefully.

### Anti-inflammatory study

In the present study, anti-inflammatory activity was determined in albino wistar rats of either sex according to the method of Winter [6]. All drugs were given orally to the respective groups as a suspension in gum acacia one hour before carrageenan injection. The procedure followed was, acute inflammation produced by injection of carrageenan (0.1 ml of 1% w/v suspension) [7], in the right hind paw of the rats under the plantar aponeurosis. It was injected +1h after the oral administration of the drug. The inflammation was quantified in terms of ml i.e. displacement of water by edema using a digital plethysmometer immediately before and after carageenan injection at +1, +2, +3, and +4 h. The percentage inhibition of edema was calculated for each group with respect to its vehicle-treated control group [8], [9].

$$\text{Percentage inhibition of paw edema} = (1 - V_t/V_c) \times 100$$

Where  $V_c$  represent average increase in paw volume (average inflammation) of the control group of rats at a given time; and  $V_t$  was the average inflammation of the drug treated (i.e. plant extracts or test drug aspirin) rats at the same time. The difference in the initial 0h and volume at +1h indicate paw edema at 1h following carageenan administration. Accordingly paw edema at +2, +3, and +4h was calculated [10]. Then percentage inhibition of paw edema was calculated.

### Antipyretic study

Antipyretic activity on albino rats was studied with fever induced by 15% brewer's yeast [11]. Healthy wistar strain albino rats weighing about 120-150 grams were taken. They were fasted overnight with water *ad libitum* before inducing pyrexia and just before induce pyrexia

animals were allowed to quiet in the cage for some time and after that their basal rectal temperature were measured by using a clinical digital thermometer by insertion of thermometer to a depth of one inch into the rectum. After taking the temperature Pyrexia was induced by injecting subcutaneously 15% w/v suspension of Brewer's yeast in distilled water at a dose of 10ml/kg body weight in the back below the nape of the neck. The site of injection was massaged in order to spread the suspension beneath the skin and returned to their cage and allowed to feed. After 18 hrs of Brewer's yeast injection the rise in rectal temperature was recorded. Only rats which were shown an increase in temperature of at least 0.6°C (or 1°F) was used for further experiment. The animals were divided into four groups, each group contain five animals. The control, standard and test extracts (300 mg/kg, 600mg/kg) were administered orally to the animals. After the drug was administered, the temperature of all the rats in each group was recorded after 1 hr, 2 hr, 3hr and 4hr. The difference in temperature between 0 hour and at the end of 4 hour was compared and analyzed.

#### **Statistical analysis**

Data was expressed as mean  $\pm$  S.D. The results were analyzed statistically by ANOVA followed by Dunnett's test.

### **RESULTS AND DISCUSSION**

#### **Anti-inflammatory study**

The mean percentage increase of paw volume for the different samples at different time intervals and the % increase in paw edema are given in table 1. The percentage inhibition of paw edema is also given in the table within parenthesis. The results indicated that the crude methanol extract showed significant anti-inflammatory action in a dose dependent manner at the 1<sup>st</sup>, 3<sup>rd</sup> and 4<sup>th</sup> hour of the study. The crude methanol extract at a dose of 600mg/kg showed a paw edema inhibition of 40.00%, 30.30%, 42.00% and 68.59% in 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> hour respectively. Similarly the crude methanol extract at a dose of 300mg/kg showed significant anti-inflammatory activity with a paw edema inhibition of 24.24%, 19.14%, 23.24% and 62.09% in 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> hour respectively.

**Table 1: Average increase in paw edema for the various samples at different time intervals and the percent inhibition of paw edema at different time intervals.**

Sample	Average % increase in paw volume $\pm$ SEM (Percent inhibition of paw edema)			
	1 <sup>st</sup> hour	2 <sup>nd</sup> hour	3 <sup>rd</sup> hour	4 <sup>th</sup> hour
1)Control (saline)	44.96 $\pm$ 5.2	50.74 $\pm$ 3.74	65.67 $\pm$ 6.93	75.27 $\pm$ 10.16
2)Crude Methanolic extract (600mg/kg)	35.90 $\pm$ 5.09* (40.60%)	32.212 $\pm$ 6.35 (30.30%)	31.42 $\pm$ 6.10* (42.00%)	24.95 $\pm$ 6.45* (68.59%)
3)Crude methanolic extract (300mg/kg)	30.91 $\pm$ 4.07* (24.24%)	38.49 $\pm$ 4.73 (19.14%)	36.4 $\pm$ 3.99* (23.24%)	23.39 $\pm$ 1.72* (62.09%)
4)Standard(dicloenac 100 mg/kg)	25.616 $\pm$ 2.87* (42.42%)	16.55 $\pm$ 3.82* (64.36%)	11.59 $\pm$ 1.62* (82.85%)	6.25 $\pm$ 2.42* (92.41%)

Probability values (calculated as compared to control using one way-ANOVA followed by Dunnett's test):\* indicates  $P < 0.05$ . All values are means of individual data obtained from five rats ( $n = 5$ ).

### Antipyretic study

The effect of methanolic extract of *Alpinia nigra* on normal body temperature in rats is presented in table 2. The results showed that the leaves extract at doses of 600 mg/kg and 300mg/kg caused significant lowering of the body temperature up to 4 hours. The normal mean temperature 100.58 °F at 0 hour was reduced to 98.62 °F after 4 hours. Lowering of body temperature was noticed at 300 mg/kg of the plant extract as the mean temperature of 100.24 °F was reduced to 98.88 °F within a 4 hour period. The average rectal temperature at different time intervals is listed in Table 2.

**Table 2: Average rectal temperature of the rats for the different samples at different time intervals**

Group	Initial rectal temperature Before yeast injection	Rectal temperature after 18 hours of yeast injection and after the administration of thee samples				
		0 hour	1 <sup>st</sup> hour	2 <sup>nd</sup> hour	3 <sup>rd</sup> hour	4 <sup>th</sup> hour
1)Control (saline)	99.0 $\pm$ 0.173	100.6 $\pm$ 0.25	100.7 $\pm$ 0.28	100.7 $\pm$ 0.2	100.68 $\pm$ 0.19	100.7 $\pm$ 0.19
2)Crude methanol extract 600mg/kg)	100.58 $\pm$ 0.23	100.58 $\pm$ 0.24	99.8 $\pm$ 0.19*	99.62 $\pm$ 0.21*	98.94 $\pm$ 0.26*	98.62 $\pm$ 0.19*
3)Crude methanol extract(300mg/kg)	100.24 $\pm$ 0.17	100.24 $\pm$ 0.28	100.1 $\pm$ 0.19*	99.8 $\pm$ 0.37*	99.16 $\pm$ 0.20*	98.88 $\pm$ 0.12*
4)Standard (Paracetamol 100mg/kg)	98.56 $\pm$ 0.10	100.56 $\pm$ 0.33	99.82 $\pm$ 0.12*	99.2 $\pm$ 0.12*	98.86 $\pm$ 0.051*	98.32 $\pm$ 0.10*

Probability values (calculated as compared to control using one way-ANOVA followed by Dunnett's test):\* indicates  $P < 0.05$ . All values are means of individual data obtained from five rats ( $n = 5$ ).

## CONCLUSION

The study proves that the crude methanolic extracts of *Alpinia nigra* exhibit both anti-inflammatory and Antipyretic properties. It is well known that inflammation is caused by substances like prostaglandin and bradykinin [12]. The anti-inflammatory action may be attributed to the presence of some endogenous compounds which is responsible for the inhibition of prostaglandin and bradykinin synthesis. This may be caused by the inhibition of the cyclo-oxygenase enzyme present in the cell membrane. It is well known that most of the anti-inflammatory, analgesic drugs possess antipyretic activity. *Alpinia nigra* revealed strong antipyretic effect at doses of 300 and 600 mg/kg in Brewer's yeast induced febrile rats. In general, non-steroidal anti-inflammatory drugs produce their antipyretic action through inhibition of prostaglandin synthesis within the hypothalamus [13]. Although, there is no direct evidence of *Alpinia nigra* to interfere with prostaglandin synthesis in hypothalamus but further study may be directed towards the mechanism of antipyretic effect of *A. nigra*.

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