



## Anti-inflammatory activity of *Adenanthera pavonina* L., Fabaceae, in experimental animals

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**RESUMO: “Atividade anti-inflamatória de *Adenanthera pavonina* L., Fabaceae, em animais experimentais”.** *Adenanthera pavonina* L., Fabaceae, conhecida na região de Bengala por “rakta kombol”, é uma planta medicinal endêmica do sul da China e da Índia e amplamente difundida na Malásia, África Ocidental e Oriental, bem como na maioria das ilhas de ambas as regiões do Pacífico e Caribe. Esta planta tem sido utilizada na medicina tradicional para o tratamento da asma, febre, diarreia, gota, inflamações, reumatismo tumor, úlceras, e como tônico. Cascas secas e moídas de *A. pavonina* foi submetida a extração sucessiva com éter de petróleo (PE), diclorometano (DCM), acetato de etila (AcOEt) e metanol (MeOH), e os extratos foram avaliados *in vivo* para atividade anti-inflamatória induzida por carragenina tendo como ensaio modelo de inflamação edema de pata em ratos.. Os extratos foram administrados por via oral em doses de 200 e 400 mg/kg de peso corporal, e foram observadas efeitos anti-inflamatórios de maneira dose-dependente, estatisticamente significativos ( $p < 0,001$ ). O extrato metanólico (400 mg/kg) apresentou 37,10% ( $p < 0,01$ ) de inibição da inflamação na primeira hora do estudo e, o extrato DCM (400 mg/kg) apresentou 33,11% ( $p < 0,001$ ) na inibição da inflamação na terceira hora do estudo, que foi comparável ao resultado da substância de referência diclofenato de sódio. Os resultados deste estudo confirmaram alguns dos usos tradicionais desta planta medicinal.

**Unitermos:** *Adenanthera pavonina*, Fabaceae *alt.* Leguminosae, atividade anti-inflamatória, edema em ratos induzido por carragenanas.

**ABSTRACT:** *Adenanthera pavonina* L., Fabaceae *alt.* Leguminosae, Bengali name ‘rakta kombol’, is an Indian medicinal plant. It is endemic to Southern China and India, and widely naturalized in Malaysia, Western and Eastern Africa as well as in most islands of both the Pacific and Caribbean regions. This plant has been used in traditional medicine for the treatment of asthma, boil, diarrhoea, gout, inflammations, rheumatism, tumour and ulcers, and as a tonic. The dried and ground bark of *A. pavonina* L. was extracted, successively, with petroleum ether (PE), dichloromethane (DCM), ethyl acetate (EtOAc) and methanol (MeOH), and the resulting extracts were assessed *in vivo* for anti-inflammatory activity on carrageenan-induced rat hind paw oedema as a model of inflammation. The extracts were administered orally at the doses of 200 and 400 mg/kg body weight, and statistically significant ( $p < 0.001$ ) anti-inflammatory effects were observed in a dose dependant manner. The MeOH extract (400 mg/kg) showed 37.10% ( $p < 0.01$ ) inhibition of inflammation at the first hour of the study and, the DCM extract (400 mg/kg) exhibited 33.11% ( $p < 0.001$ ) inhibition of inflammation at the third hour of the study which was comparable with that of reference standard drug diclofenac sodium. The results of this study supported some of the traditional medicinal uses of this plant.

**Keywords:** *Adenanthera pavonina*, Fabaceae *alt.* Leguminosae, anti-inflammatory activity; Carrageenan-induced rat oedema.

## INTRODUCTION

*Adenanthera pavonina* L., Fabaceae *alt.* Leguminosae, commonly known in Bangladesh as 'rakta kombol', is an important medicinal plant from the 'Indian subcontinent'. This species is endemic to Southern China and India, and widely naturalized in Malaysia, Western and Eastern Africa as well as in most islands of both the Pacific and Caribbean regions (Balogun & Fetuga, 1985; GRIN Databases, 2009). While the wood of this plant has been used for decorative wood products, the fruits are consumed by local people (Benthall, 1946; Clark & Thaman, 1993). Various parts of this plant have also been used in traditional medicine for the treatment of asthma, boil, diarrhoea, gout, inflammations, rheumatism, tumour and ulcers, and as a tonic (Watt & Breyer-Brandwijk, 1962; Kirtikar & Basu, 1981; Burkil, 1994; Dr Duke's, 2009). The seeds of this plant have been found to be effective in treating cardiovascular diseases during pregnancy. Previous phytochemical studies on this plant revealed the presence of various secondary metabolites including mainly flavonoids, steroids, saponins and triterpenoids (Chandra et al., 1982; Yeoh et al., 1984). The antibacterial, antifungal, antioxidant, cytotoxic and blood pressure reducing activities of the extracts of *A. pavonina* have recently been reported (Jayasinghe et al., 2006; Rodrigo et al., 2007; Adedapo et al., 2009). We now report on the *in vivo* anti-inflammatory activity of various extracts of the bark of *A. pavonina* in carrageenan-induced rat oedema model.

## MATERIAL AND METHODS

### General

The solvents petroleum ether (PE), dichloromethane (DCM), ethyl acetate (EtOAc) and methanol (MeOH) were of the BDH laboratory (BDH) grade, dichlofenac sodium was supplied by Square Pharmaceutical Ltd, Bangladesh, and carrageenan and Tween-80 were of pharmaceutical grade.

### Plant material

The barks of *Adenanthera pavonina* L., Fabaceae, were collected from a mature plant from the Jahangirnagar University campus, Savar, Dhaka, Bangladesh, in March 2008. A voucher specimen representing this collection (accession N<sup>o</sup>-DACB34196) was deposited in the National Herbarium of Bangladesh.

### Extraction

The barks of *A. pavonina* were shade-dried, pulverized by a mechanical grinder and passed through a

60 mesh sieve to obtain fine powder, a portion (1.0 kg) of which was extracted, successively, with PE, DCM, EtOAc and MeOH (2 L each). All extracts were evaporated to dryness under reduced pressure. The yields of the PE, DCM, EtOAc and MeOH extracts were 2.1 g, 3.7 g, 2.5 g and 30.0 g, respectively.

### Animals

Long Evan rats of either sex (weighing 130-180 g) were collected from the International Centre for Diarrhoeal Diseases and Research, Bangladesh (ICDDR, B). The animals were kept in polyvinyl cages under controlled room temperature (25±2 °C) in the laboratory environment under 12 h dark and 12 h light cycle for seven days. The ICDDR, B formulated food pellets were supplied to the animals and water *ad libitum*. Animals were fasted overnight and weighed before the experiment. The study involving rats was approved by the Ethical Review Committee (approval number: SCB090221) Biological Science, University of Dhaka, Bangladesh, and the experiments were carried out strictly in accordance with the guidelines provided by the World Health Organization.

### Assessment of anti-inflammatory activity: the carrageenan-induced oedema model

The anti-inflammatory activity of the extracts of *A. pavonina* was measured by the carrageenan-induced rat paw oedema model (Winter et al., 1962). Experimental animals were randomly selected, irrespective of sexes, and divided into ten groups consisting of 5 rats in each group. The test compounds were administered orally to each group at 200 and 400 mg/kg body weight. After 1 h of treatment, acute inflammation was produced by sub-planter injection of 0.1 mL of 1% suspension of carrageenan in normal saline in the right hind paw of the rats. Another animal group was treated with dichlofenac sodium at a dose of 15 mg/kg per orally (*p.o.*) as a reference standard. The saline treated group was considered as control. The paw volume was measured plethysmometrically (Ugo Basile, Italy) at 1, 2, 3 and 4 h after the carrageenan injection. Results were expressed as percentage of inhibition of oedema calculated by the formula-  $(1 - V_t/V_c) \times 100$ , where  $V_t$  and  $V_c$  are the mean paw volume in the treated and controlled groups, respectively.

### Statistical analysis

Statistical analysis was performed using SPSS-11.5 statistical Software for Windows. Experimental values were expressed as mean±SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a *p* value<0.05 in all cases.

**Table 1.** Anti-inflammatory effects of the extracts of the barks of *Adenanthera pavonina* L. on the carrageenan-induced rat paw oedema.

Treatment	Dose mg kg <sup>-1</sup>	Carrageenan-induced rat paw oedema Mean±SEM*			
		+1h	+2h	+3h	+4h
Control	-	99.20±6.902	106.80±3.597	119.60±4.812	102.4±4.445
Dichlofenac sodium	15	73.80±3.056 (25.60) <sup>b</sup>	75.20±2.746 (29.59) <sup>c</sup>	76.00±3.821 (36.45) <sup>c</sup>	70.20±3.734 (31.45) <sup>c</sup>
	200	73.80±1.158 (25.60) <sup>b</sup>	83.60±2.943 (21.72) <sup>b</sup>	98.20±2.223 (17.89) <sup>b</sup>	80.40±1.806 (21.48) <sup>b</sup>
PE extract	400	71.60±3.763 (27.82) <sup>b</sup>	82.60±3.750 (22.66) <sup>b</sup>	97.60±2.786 (18.39) <sup>b</sup>	78.40±2.315 (23.44) <sup>b</sup>
	200	73.00±2.025 (26.41) <sup>b</sup>	76.60±2.600 (28.28) <sup>c</sup>	81.80±3.470 (31.61) <sup>c</sup>	77.20±3.382 (24.61) <sup>c</sup>
DCM extract	400	69.80±2.498 (29.64) <sup>b</sup>	72.80±2.332 (31.84) <sup>c</sup>	80.00±1.378 (33.11) <sup>c</sup>	73.00±3.647 (28.71) <sup>c</sup>
	200	78.20±2.615 (21.17) <sup>a</sup>	91.80±3.292 (14.04) <sup>a</sup>	104.40±5.921 (12.71) <sup>NS</sup>	86.60±4.632 (11.55) <sup>a</sup>
EtOAc extract	400	70.80±3.967 (28.63) <sup>b</sup>	89.40±4.118 (16.29) <sup>a</sup>	104.20±5.774 (12.88) <sup>NS</sup>	85.80±6.461 (16.21) <sup>NS</sup>
	200	68.60±2.768 (30.85) <sup>b</sup>	80.00±2.302 (25.09) <sup>c</sup>	95.00±2.000 (20.57) <sup>b</sup>	81.00±2.168 (20.89) <sup>b</sup>
MeOH extract	400	62.40±5.862 (37.10) <sup>b</sup>	76.80±4.964 (28.09) <sup>b</sup>	89.00±4.494 (25.59) <sup>b</sup>	80.40±4.915 (21.48) <sup>a</sup>

\*The number of animal was 5 in each group. Figure in parenthesis indicate percent inhibition in paw volume. The probability values were calculated using student's 't'-test: a<0.05, b<0.01, c<0.001, NS=Not significant.

## RESULTS AND DISCUSSION

The extracts of the barks of *A. pavonina* were evaluated for anti-inflammatory activity using the carrageenan-induced rat paw oedema model at dose levels of 200 and 400 mg/kg body weight, where dichlofenac sodium was used as a positive reference standard (Table 1). The results showed that the PE fraction exhibited 25.6% and 27.8% inhibition of the paw oedema at the doses of 200 and 400 mg/kg, respectively at the first hour of the study. The DCM fraction exhibited maximum inhibition at the third hour of the study with 31.61% and 33.11% inhibition at the doses of 200 and 400 mg/kg, respectively. The DCM extract exhibited significant ( $p<0.001$ ) inhibition of paw oedema at different time intervals. The EtOAc extraction also demonstrated the maximum inhibition (28.63%) after the first hour of the study at the dose of 400 mg/kg body weight only. On the other hand the MeOH extract displayed maximum anti-inflammatory effect at the first hour with 30.85% and 37.1% inhibition at the doses of 200 mg/kg and 400 mg/kg body weight, respectively, but at the second and third hour, the effects were observed in decreasing pattern. Among the extracts, the DCM extract showed the maximum effect at the third hour of the experiment, whereas the MeOH extracts exhibited the maximum effect at the first hour of the study at 400 mg/kg. Both these extracts exhibited the anti-inflammatory effect in a

dose dependent manner.

The carrageenan induced paw oedema model is known to be sensitive to cyclooxygenase inhibition which has been used to evaluate the effect of non-steroidal anti-inflammatory agents (Seibert et al., 1994; Appleton et al., 1995). The time response of oedema in rat is believed to be biphasic (Vinegar et al., 1969). The first phase which occurs between 0.5 to 2 h after injection of the fluogestic agent has been attributed to the release of histamine or serotonin. The second phase of the inflammatory reaction is caused by the release of bradykinin, protease, prostaglandin and lysosome (Crunkhorn & Meacock, 1971; Di Rosa & Willoughby, 1971). Therefore it can be assumed that the inhibitory effect of the MeOH extract on carrageenan induced inflammation could be due to the inhibition of histamine or serotonin. On the other hand, the maximum inhibitory activity of the DCM extract at the third hour of the study was due to the inhibition of the enzyme cyclooxygenase leading to the inhibition of prostaglandin synthesis.

The significant *in vivo* anti-inflammatory activities exhibited by the extracts of *A. pavonina* in the carrageenan-induced rat paw oedema model provide some scientific evidence in support of the traditional uses of this plant for treatment of various inflammatory conditions, e.g. gout, rheumatism and tumours.

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