Anti-inflammatory activity of Red and White Lotus seeds (*Nelumbo nucifera*) in Albino Rats

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Abstract

The present study was carried out to evaluate the anti-inflammatory activity of red and white lotus seeds in albino rats. The Carrageenin induced paw edema model was used for studying the anti-inflammatory activity. The cyclooxygenase-2 (COX-2) enzyme inhibition assay was carried out in spectrophotometer to identify the specific mode of action. Forty eight adult Sprague-Dawley rats were used in this experiment. They were divided into six groups of eight each and maintained under ideal laboratory conditions. Group I was taken as control and group II treated with the standard drug diclofenac potassium @ 3mg/kg/celecoxib @ 10mg/kg (in case of COX–2 assay) on 7th day of study. The methanolic extract of Nelumbo nucifera seeds of red and white varieties @ 400mg/kg and 600mg/kg were fed to group III, IV, V and VI respectively, for 7 days. All groups of lotus seed extracts were revealed anti-inflammatory activity in Carragenin induced inflammation as well as in COX-2 enzyme inhibition assay. While comparing all groups, the higher dose group of white lotus seed extracts, exhibited more pronounced inhibition than other groups.

Keywords: Anti-Inflammatory, Lotus Seeds, COX-2 Assay, Wistar Rat.

Introduction

A variety of drugs have been used to minimize the discomfort arising due to the inflammatory process. In the past, chiefly the natural and synthetic steroids were employed in the anti-inflammatory therapy. Because of the occurrence of undesirable side effects with steroids, the attention was directed towards the currently available non-steroidal anti-inflammatory drugs (NSAID) agents. The anti-inflammatory action of NSAID rest in their ability to inhibit the activity of cyclooxygenase (Vane, 1971). Most of the currently available drugs inhibit both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX 2) activities and thereby the production of prostaglandins and thromboxane. The inhibition of COX-1 results in unwanted side effects would lead to gastric ulcers, while COX-2 selective inhibition should specifically alleviate pain and inflammation. The COX-2 selective drugs lowers gastro toxicity than non-selective NSAID but the finding of elevated incidence of myocardial infarction raises the question whether all of the side effects that may be associated with these drugs are known. It is well known fact that the herbal component has been a better alternative to the allopathic agents. So it's ideal to look for a new herbal compound for eliciting the COX -2 specific actions with fewer side effects. Varieties of plants have been used for treating the ailments. Nelumbo nucifera (lotus) is one of the plants that have been used for its medicinal properties since ancient times. The rhizomes, flowers, stalk and leaves of lotus are used in the form of infusion in fever as refrigerant and diuretic (Mitra et al., 1973). Hence, the present study is aimed to prove scientifically the anti-inflammatory activity of lotus seeds (*Nelumbo nucifera*) in reference to COX-2 Enzyme inhibition as well as to compare the activity of red and white lotus seeds.

Materials and Methods

Experimental Animals:

The study was carried out on 48 adult Sprague-Dawley rats (150-200 g) of either sex, maintained under ideal feeding and management practices in the laboratory.

Experiment Protocol:

The Sprague-Dawley Rats were divided into six groups of eight each and maintained for anti-inflammatory study. Five per cent gum acacia were fed to Group I and II in which the Group II received the diclofenac potassium@3mg/kg/celecoxib@10mg/kg (In the case of COX-2 Assay) on the 7th day before carrageenin administration. The methanolic extract of N.nucifera seeds of red and white varieties @ 400mg/kg and 600mg/kg were fed to group III, IV, V and VI respectively, for 7 days. The paw oedema was induced after half an hour of extract/ drug administration by

Table-1. Percentage inhibition of oedema in carrageenin induced paw oedema model in rats.

Time Interval (min.)	Group II	Group III	Group IV	Group V	Group VI
60	60.75d	36.92b	41.12b	40.42b	50.47cd
120	62.67f	38.35b	41.24c	45.59d	47.76e
180	61.09c	44.22b	44.76b	45.44b	47.21b

injection of 0.05 ml of 2 per cent w/v carrageenin suspension in normal saline into plantar aponeurosis of the left hind paw of rat. The hind paw volume was measured by the method of Chattopadhyay et al. (1986) at 0 and 3 hours after carrageenin injection using a Plethysmometer.

Estimation of Cyclooxygenase 2 (COX-2) Enzyme Inhibition: The COX-2 protein extraction was done based on the method described by Anderson et al. (1996).Cyclooxygenase is a bifunctional enzyme exhibiting both the cyclooxygenase and peroxidase activities. The cyclooxygenase-2 inhibition assay measure the peroxidase activity of cyclooxygenase enzyme. It is assayed colorimetrically by monitoring the appearance of oxidized TMPD at 600 nm in spectrophotometer (Copeland et al., 1994).The tissue extract incubated in enzyme reaction buffer (100 mM Tris-Hcl buffer, pH 6.5, containing 0.1% Tween-20, gelatin at 1 mg/ml, 3 uM hematin, and 100 uM TMPD). One unit of enzyme activity is defined as the amount of enzyme required to cause a change in TMPD absorbance at 600 nm. After the indicated preincubation time (5 minutes), the enzymatic reaction was initiated with 100 uM arachidonic acid, and the initial velocity of the reaction was measured following the oxidation of TMPD at 600 nm. The velocities observed at different inhibitor treated tissue extracts were divided by the velocity observed for enzyme samples preincubated for the same time (without any treatment), and this ratio was multiplied by 100 to yield per cent control activity.

Statistical Analysis of Data: Results were analyzed by using one-way ANOVA test as described by Snedecor and Cochran (1985). Significance in the difference of the means was tested using Least Significant Difference (LSD). Results were expressed as mean ± standard error.

Results and Discussion

Effect on Carrageenin induced paw volume: Both red and white lotus seeds treated groups showed significant reduction of paw volume on the second and third hour after carrageenin injection (Table.1). The higher dose groups (Group IV and VI-600mg/kg) showed more activity than corresponding lower doses (Group III and V-400mg/kg) in both phases of inflammation. The carrageenin induced oedema is a biphasic response. The initial phase is attributable to the release of histamine, serotonin and kinin in the first hour after carrageenin injection; a more pronounced second phase is attributable to the release of prostaglandin like substance in second to third hour after carrageenin injection (Vinegar et al., 1969). A significant reduction in percentage of oedema from first to three hour after carrageenin injection in the present study revealed that both phases of inflammatory reactions were affected by methanolic extracts of red and white lotus seeds.

Effect on Cyclooxygenase-2 enzyme inhibition: In order to find out the specific mode of anti-inflammatory action of lotus seeds, another more advanced assay, based on cyclooxygenase-2 (COX-2) enzyme inhibition was performed in spectrophotometer. The results revealed the inhibition of COX-2 enzyme by methanolic extracts of lotus seeds in a significant manner (Table.2). The higher dose groups showed more inhibition of COX-2 enzyme than its corresponding lower dose group. The celecoxib, the specific COX-2 inhibitor showed more significant effect than others. Masferrer et al., (1994) reported that the COX-2 enzyme is prominently expressed in inflamed tissues, where it produces proinflammatory prostaglan-dins. Zhang et al. (1997) also reported that the carrageenin induced paw oedema was accompanied by the induction of COX-2 enzyme. From the above findings and present results it is revealed that the methanolic extracts of red and white lotus seeds exhibited its anti-inflammatory activity through inhibition of cyclooxy-genase-2 enzyme.

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Table-2. Percentage inhibition of cyclooxygenase-2 in spectrophotometric assay.

Particulars	Group II	Group III	Group IV	Group V	Group VI
% Inhibition	69.28d	37.68c	42.31c	42.02c	44.63b

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