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Assessment of analgesic activity of red and white lotus seeds *(Nelumbo nucifera)* in albino rats

P. Vikrama Chakravarthi, N. Gopakumar *, A. M. C. Nair, A. D. Joy

Dept.of Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala.

Abstract

Lotus (*Nelumbo nucifera)* is one of the plants that have been used for its medicinal properties since ancient times. Almost all parts of the plants are used for the treatment of conditions associated with health abnormalities. In order to prove the analgesic effect of lotus seeds scientifically, a study was conducted in albino rats using the chronic pain model (experimental neuropathy). In this experiment, forty eight adult Sprague-Dawley rats were used. All the rats were subjected to surgery under anasthesia as per the standard neuropathy model protocols. After the surgery the rats were divided into six groups of eight each and group I was taken as control, group II was treated with the standard drug diclofenac potassium @ 3 mg/kg on the last day of experiment. The methanolic extract of lotus seeds of red and white varieties @ 400 mg/kg and 600 mg/kg were fed to group III, IV, V and VI respectively, for 7 days. After the dosage periods, the number of foot withdrawal reflex of each group was calculated using acetone induced cold stimulus and the responses analyzed statistically to findout the analgesic activity. It was revealed that the white lotus seed higher dose group (600 mg/kg.b.wt) exhibited significant analgesic activity than other lotus seed treated groups.

Keywords: Lotus Seeds, Analgesic, Neuropathy model.

1. Introduction

Pain can be considered in two categories viz. nociceptive and neuropathic pain. Nociceptive pain alerts the body to potential or actual tissue damage. In contrast, neuropathic pain, which results from injury or damage to the peripheral or central nervous system which persists long even after all signs of the original injury have disappeared. Neuropathic pain is due to aberrant processing of information in the injured nervous system and it can be described in terms of characteristics of the pain, the site of injury, or the presumed site of aberrant neural activity. Peripheral neuropathic pain is caused by injury to a peripheral nerve. After tissue damage the healing process accompanied by a period of hyperalgesia (increased sensivity to pain) and allodynia (nociceptive reaction to normally non noxious stimulus). This hyperalgesia is due to the proliferation of regenerating nerve fibres. The allodynia observed since the second day after surgery in mononeuropathy model, and the sensitivity was significantly higher during the 14 days of the study. The chronic administration of topiramate, at the dose of 50 mg/kg/day,

* Corresponding author
Email: ngk38@yahoo.com

significantly diminished the mechanical sensitivity and shortened the period of allodynia in rats [1].

In nociceptive and neuropathic pain, the receptors get sensitized because of the presence of autacoids. These receptors are more prone to mechanical and other stimulus. The NSAIDs arrest the sensitization of nociceptors to such stimulus by preventing the production of arachidonic acid cascade products and useful for treating the conditions associated with mild to moderate pain. Diclofenac has been found to be an effective analgesic agent and the potassium salt of diclofenac providing rapid onset of action after oral administration and also gives rapid pain relief within 60 to 90 minutes [2]. Due to the drawbacks associated with long term use of NSAIDs such as gastrointestinal tract ulceration, disturbances in platelet function, changes in renal function etc. has led to the search for better alternative especially herbal drugs.

The herbal medicine consists of natural plant substances which are used for prevention and treatment of ailments. This practice has existed since prehistoric times and flourishes today as the primary form of medicine. The use of herbal drugs remains a good alternative to allopathic agents, with fewer side effects.

Varieties of plants have been used for the purpose of analgesic activity in herbal medicine. *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 [3]. Hence, the present study is aimed to prove the analgesic property of lotus seeds scientifically as well as to compare the activity of red and white lotus seeds.

2. Materials and methods

2.1 Extracts Preparation and Phytochemical Screening

The lotus seeds powder was extracted in methanol using Soxhlet Extraction apparatus. The prepared methanolic extracts of red and white lotus seeds were tested for the presence of various active principles namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins as per the procedure [4].

2.2 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. They were housed in plastic cages with proper bedding material. After approval of institutional ethics committee the experiment was conducted in rats.

2.3 Chronic Pain Model - Experimental neuropathy

Procedure: The rats were subjected to surgery as per the standard protocol [5]. Anaesthesia was induced in Sprague-Dawley rats by injection of thiopentone sodium (40 mg/kg, i.p.) and maintained with subsequent injection if needed. After a local incision, the biceps femoralis of each hind leg was bluntly dissected at midthigh to expose the sciatic nerve. Each nerve was mobilized with care to avoid undue stretching. Four 4-0 chromic gut sutures were tied loosely with a square knot around the sciatic nerve. Both incisions are closed layer to layer with silk sutures and the rats allowed to recover. During the next days, the animals show a mild eversion of the affected paw, mild-to-moderate degree of foot drop and postural abnormalities. The rats were allowed a period of atleast 7 days to recover from surgery before behavioral testing began.

2.4 Experimental Design

The forty eight adult rats were divided into 6 groups of eight each and treated as follows. The dosing was started on 8th day of study, after the stable reductions in the foot withdrawal reflex.

Group I - Vehicle alone (5 per cent gum acacia @ 1 ml /kg body weight) administered orally for 7 days.

Table. Effect of treatments on number of foot withdrawal reflex in albino rats.

Groups	Control (Group I)	Diclofenac Treated @ 3mg/Kg (Group II)	Red Lotus seed @ 400mg/Kg (Group III)	Red Lotus seed @ 600mg/Kg (Group IV)	White Lotus seed @ 400mg/Kg (Group V)	White Lotus seed @ 600mg/Kg (Group VI)
Mean ± SE	4.88 ± 0.61^{a}	2.13 ± 0.36^{c}	3.75 ± 0.46^{ab}	3.63 ± 0.50^{ab}	3.75 ± 0.52^{ab}	3.50 ± 0.33^{b}

Means bearing same superscript (ab) do not differ significantly at P<0.05. Means bearing different superscript (a/b/c) differ significantly at P<0.05.

Group II - Vehicle alone for 7 days + diclofenac potassium (3 mg/kg body weight) administered orally on 7th day.

Group III - Methanolic extract of red lotus seeds @ 400 mg/kg body weight administered orally for 7 days.

Group IV - Methanolic extract of red lotus seeds @ 600 mg/kg body weight administered orally for 7 days.

Group V - Methanolic extract of white lotus seeds @ 400 mg/kg body weight administered orally for 7 days.

Group VI - Methanolic extract of white lotus seeds @ 600 mg/kg body weight administered orally for 7 days.

On the last day after dosing the sensitivity of the ipsilateral and contralateral hindpaw to cooling was assessed by the application of a drop of acetone on plantar region of the foot [6]. Each trial consisted of five applications of acetone, separated by a period of 5 min. The number of foot withdrawal reflex to cold stimulus (acetone) was noted and represented as datas.

2.5 Statistical Analysis of Data

Results were analyzed by using one-way ANOVA test for comparison between control groups and treatment groups II, III, IV, V and VI as per the procedure [7]. Significance in the difference of the means was tested using Least Significant Difference (LSD). Results were expressed as mean ± standard error.

3. Results

3.1 Phytochemical Screening

The phytochemical analysis of lotus seeds revealed the presence of alkaloids, flavonoids, glycosides, steroids, phenolic compounds, diterpenes and triterpenes in them. As per the findings [8], it was proved that the active principles of *N.nucifera* seeds contain alkaloids, saponins, phenolics and carbohydrates.

3.2 Effect of treatments on foot withdrawal reflex

In the present study the cold stimulus induced by application of acetone on the plantar region of operated paw was taken as pain reflex measurement. The foot withdrawal reflex exhibited by the animal after cold stimulus is considered as an index of nociception. The data for experimental neuropathy was given in the table as follows.

4. Discussion

The physiology of nociception involves a complex interaction of peripheral and central nervous system (CNS) structures. The pathophysiology of chronic pain shows alterations of normal physiological pathways, giving rise to hyperalgesia or allodynia. Peripheral mononeuropathy model was demonstrated in adult rats by placing constrictive ligatures around the common sciatic nerve [3]. The postoperative behaviour of these rats indicated that hyperalgesia; allodynia and possibly spontaneous pain (or dysthesia). The

hyperalgesic responses and allodynia were evident on 2nd postoperative day and lasted for over 2 months. In the present study of neuropathy model the hyperalgesia & allodynia behaviors observed in rats to indicate pain response. These behavioral parameters can be exploited to study the pharmacology and modulation of neuropathic pain. In this experiment a non-significant reduction in foot withdrawal reflex was noticed in all the lotus seed treated groups except group VI, which showed a significant reduction, indicating the analgesic activity of white lotus seeds at a dose above 600 mg/kg even though it was not comparable to diclofenac (Group II).

In inflammatory and neuropathic pain models the involvement of centrally mediated nociception and upregulation of COX-2 enzyme were noted. The administration of ketorolac a mixed COX-1 and COX-2 inhibitor reduces the allodynia induced in neuropathic pain models [9]. Also, the prostaglandins play a significant role in different phases of inflammatory pain reactions. Prostaglandins elicit pain by direct stimulation of sensory nerves and also sensitize sensory nerves to other pain provoking stimuli [10]. The flavonoids were reported to have analgesic activity [11] by reduced availability of prostaglandins. Hence, the presence of flavonoids in the methanolic extract of red and white lotus seeds authenticates that the analgesic activity of the lotus seed extracts may be due to prevention of prostaglandin production through cyclooxygenase enzyme inhibition. From the results of present study it can be inferred that methanolic extract of red and white lotus seeds may be used as analgesic agents. While comparing the lotus seed extracts, the white lotus seed @ 600 mg/kg body weight revealed higher analgesic activity than others.

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