

Original Article

ASSESSMENT OF ANALGESIC ACTIVITY OF *NELUMBO NUCIFERA* FRUIT ETHANOL EXTRACT

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ABSTRACT

Objective: Utilization of herbal remedies rich in flavonoids and vitamins have increased significantly these days to treat various disorders, thus existing research work encircled to appraise the analgesic effect of *Nelumbo nucifera* fruit (NNF) for evaluating its traditional use pharmacologically in disorders which are associated with pain and inflammation.

Methods: Central analgesic activity in mice was assessed by tail flick test and the latency time i.e. the removal of tail from the stimulus was recorded. Similarly acetic acid induced writhing test was also conducted for the assessment of peripheral analgesic effect in mice and number of writhes was counted along with percent inhibition of writhes.

Results: In tail flick test the peak anti-nociceptive effect at all doses of fruit was observed at 90 min. However, the percentage of tail elongation time was highest at a dose of 200 mg/kg i.e. 82% at 90 min. Number of writhes was highly significantly reduced at all doses of NNF but maximum effects were observed at dose 200 mg/kg as compared to control, indicating 48.41 % inhibition of writhes.

Conclusion: NNF have exhibited strong analgesic effect in both animal models, which may be connected with the synergistic actions of flavonoids, saponins and tannins on arachidonic acid pathway inhibition. Hence NNF seems to have a great potential in disorders associated with pain but more experimental trials in this field are required to confirm these findings.

Keywords: *Nelumbo nucifera*, Tail flick test, Analgesic, Arachidonic acid

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INTRODUCTION

Lots of individuals who experience severe, inexorable and excruciating pain, for instance, that resulting from cancer or injury has to rely on morphine, in spite of its established adverse outcomes. Similarly unceasing anti-inflammatory conditions such as rheumatoid arthritis and osteoarthritis are mostly treated with non-steroidal anti-inflammatory drugs (NSAIDs). Although these synthetic agents are dominating the market but issue of toxicity with prolonged use of these agents cannot be ruled out, the most frequent being GIT bleeding and ulcers [1, 2]. Hence there is a need to develop new, safe, effective, economical and innocuous analgesics [3].

The uses of herbal drugs are becoming progressively more popular as they are supposed to be natural, advantageous and lack unwanted effects [4]. Mostly the plant-derived drugs are taken randomly by local population for the treatment of various diseases without having adequate information regarding its usefulness. Hence for proper guidance of the general population, especially users of natural products, there is a need to scientifically prove the effectiveness of these medicinal plants [5].

Nelumbo nucifera, a *Nymphaeaceae* family plant is commonly cultivated in the hot and humid climatic zones of Thailand, Pakistan, India and China [6]. Its fruit contains seeds plus pods (lotus bulbs). The green-colored pods offer an add-on to the seeds, which are black, firm and round egg-shaped. They are organized in spirals and edible portion of seeds have to be peeled separately before they are eaten [7, 8].

Its seeds are a wonderful source of protein, starch, fat, unsaturated fatty acids and asparagines. The key active principles in seeds are flavonoids, alkaloids, principally lines nine, lotusine, isoliensinine, dauricine, pronuciferine, nuciferine, roemerine, procyanidin, neferine plus armepavine. The seeds also have carbohydrates, Gallic acid and isoquininolinol and contain ample amount of various minerals as well such as potassium, magnesium, calcium, sodium, iron, chromium, manganese, copper and zinc [6, 9, 10].

Recently conducted study on NNF pods has shown the existence of numerous active bioactive principles in them for instance flavonoids, alkaloids, saponins, terpenoids and tannins [11]. Procyanidin (alkaloid) was also squeezed from NNF pods [6].

The fruits are commonly used up as a healthy component of Asian cuisine and also as a traditional cure of various ailments e. g. hypertension, palpitation, arrhythmia, fever, pain, inflammation, sleep disorders, chronic diarrhea, spermatorrhea, leucorrhoea, bad breath, leprosy and menorrhagia [12, 13]. Current research work revealed LD₅₀ value of NNF higher than 5g/kg whereas its Neuropharmacological role was also established as an anxiolytic, antidepressant and antiepileptic agent [11, 14]; however very inadequate literature is obtainable concerning its effects as analgesic agent, therefore existing study was centered towards the evaluation of analgesic effect of NNF in order to justify its traditional use pharmacologically in disorders which are connected with pain.

MATERIALS AND METHODS

Research design

Research work was executed utilizing the laboratory facilities of Pharmacology department and the Research Institute of Pharmaceutical Sciences, University of Karachi, following approval of synopsis and after getting permission for the use of laboratory animals from the Board of Advance Studies and Research (BASR/02149/Pharm, dated 30th December 2014). Albino mice (49) of either sex weighing 20-25 g were used for tail-flick test and were divided into seven groups. Likewise albino mice (49) of either sex weighing 20-25 g were used for acetic acid-induced writhing test and were equally divided into seven groups.

Animal care

Animals were housed in polycarbonate cages with cage enrichment and were allowed to acclimatize for three weeks before the start of the experiment. The temperature was kept at about 25 °C with a relative

humidity of 50 to 60% in alternating twelve-hour light and dark cycle. Each mouse was provided access to normal diet and water. The animals were carried to the laboratory about an hour prior to the initiation of experiments [14]. Animals were used in line with protocol from NAELAR (National Advisory Committee for Laboratory Animal Research) and NIH (National Institute of Health) [15, 16].

Preparation of extract/Chemicals

After obtaining fruits from the native fruit bazaar of Hyderabad, Pakistan in August 2015, they were initially presented to Pharmacognosy department, Karachi University for identification and authentication and afterward receipt no NNF-03 was deposited in the same department.

Crude extract was prepared through a cold extraction procedure [17, 18]. Six kg fruits were initially rinsed with tap water and the seeds were separated from the pods manually. The seeds have high contents of water that's why they need to be chopped first then left for 06 d for drying out in shade. The dried material obtained was thick so again needs to be ground into fine powder. In contrast pods were chopped once only and were allowed to dry in shade for just 03 d. The dried pod material takes a coarse powder form. So for better separation and collection of NNF constituents (secondary metabolites) they need to be chopped and dried separately before soaking up together in ethanol (95%) for ten days with occasional shaking.

Afterwards it was filtered using filter paper Whatman No. 1. Subsequently it was evaporated using rotary machine under condensed pressure at 40 °C to 45 °C. The condensed material was freeze dried in a freeze dryer at -30 °C. The material so gained was preserved at -20 °C until further use in doses of 50, 100 and 200 mg/kg orally. The ultimate amount of the extract acquired was 400 g of dry weight.

Tragacanth gum was acquired from Merck whereas aspirin was obtained from one of the well-known pharmacy shops at Karachi.

2% tragacanth gum in powder form was acquired from Merck which was consumed to make suspensions of 3 different doses of test drug i.e. NNF 50, 100 and 200 mg/kg. It was given to the control group as placebo in the dose of 10 ml/kg orally. 100 ml of warm distilled water was added in 2 g tragacanth gum to make 2% suspension. At each occasion new suspensions were prepared for dosing [19, 20].

Aspirin 300 mg tablets were trampled and suspended in gum tragacanth (2%) which was then administered per oral through orogastric tube in a dose of 50, 100 and 200 mg/kg in tail flick test and acetic acid-induced writhing test [21].

0.7% acetic acid in the dose of 10 ml/kg was administered IP [22].

Experiments

Tail flick test

Analgesia is the loss of ability to feel or react to painful stimulus such as chemical, thermal or mechanical [21]. In the current study central analgesic activity in mice was assessed by tail-flick test and tail-flick latency difference (TFLD) i.e. the time in seconds taken by mouse to remove its tail clearly out of water was noted as the reaction time [23].

The test was conducted on 49 white albino mice of either sex which were equally placed in seven groups ($n=7$). Group control was administered gum tragacanth as a vehicle; three groups served as test groups and were given NNF at a dose of 50, 100 and 200 mg/kg and three groups served as reference groups and were given aspirin 50, 100 and 200 mg/kg. All drugs were administered PO. NNF extract was initially tested in a dose of 20 mg/kg for 15 d but no significant effects were observed.

Each mouse was kept in a particular restrainer with only the tail extending out. Afterwards 1/3rd of its tail was submerged in a digital constant temperature water tank maintained at 51 °C. The 1st reading was taken straightaway prior to the administration of drugs and then after 30, 60, 90, 120, 150 and 180 min. Animals which did not remove their tail in 10 seconds were discarded from the experiment in order to avoid tissue damage [24]. Data acquired was

calculated utilizing the average values of every group and average increase in latency following dosing was measured. Lastly % time elongation of tail with respect to the control group was evaluated using the following formula as previously described [25].

Average tail-flick time for sample

$$\% \text{ Time elongation of tail} = \frac{\text{Average tail flick time for Control}}{\text{Average tail flick time for sample}} \times 100$$

Acetic acid-induced writhing test

Acetic acid-induced writhing test is a valuable test conducted for the assessment of peripheral analgesic effects in mice by the already described method [26]. A population of 49 white albino mice of both sexes was equally placed in seven groups ($n=7$). Control animals were given gum tragacanth as vehicle, whereas three groups served as test groups and were given NNF extract at a dose of 50, 100 and 200 mg/kg. Another three groups served as reference groups and were given aspirin in a dose of 50, 100 and 200 mg/kg. All drugs were given orally. NNF extract was initially tested in a dose of 20 mg/kg for 15 d but no significant effects were observed.

30 min after the administration of drugs, 0.7% acetic acid 10 ml/kg was given IP. Mice were placed immediately in a plastic transparent box (13 cm height×12 cm width×23 cm length) and number of writhes which includes abdominal muscle contraction, periodic arching of body, stretching and drawing up of hind limbs were counted for twenty minutes and lastly percent inhibition of writhes was assessed using the following formula [21].

$$\% \text{ Inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

Where:

V_c = Average Number of writhes for control animals

V_t = Average Number of writhes for treated animals

Statistical analysis

The tables obtained through experiments were used to take an average and standard error to the average utilizing two sample student T-test and values of P less than 0.05 were reflected as significant and P less than 0.005 as extremely significant. All numerical methods were accomplished with SPSS software version 20 [27].

RESULTS

Tail flick test

Table-1 has depicted the analgesic activity of NNF and aspirin using the tail-flick test. The table has summarized the results of NNF at doses 50, 100 and 200 mg/kg against control and also with similar doses of aspirin. It was revealed that NNF at dose 50 mg/kg exhibited extremely noteworthy analgesic effects at 30, 60 and 90 min and noteworthy effects at 120 and 150 min in comparison to control. Whereas NNF at doses 100 and 200 mg/kg exhibited highly noteworthy analgesic effects from 30 to 180 min as compared to control. Aspirin, in contrast, revealed extremely noteworthy analgesic effects at 50, 100 and 200 mg/kg from 30 to 180 min as compared to control. Table 2 has demonstrated % tail elongation time at 90 min after administration of NNF and aspirin with respect to control. NNF showed highest % tail elongation time at 200 mg/kg dose i.e. 82% followed by 76% and 42% for extract doses 100 and 50 mg/kg. Aspirin, on the other hand, exhibited % tail elongation time of 81, 84 and 95 % at doses 50, 100 and 200 mg/kg.

Acetic acid-induced writhing test

Table-3 revealed the analgesic effects of NNF and aspirin using acetic acid-induced writhing test. Number of writhes was highly significantly reduced at 50, 100 and 200 mg/kg doses of NNF extract but maximum effects were observed at extract dose of 200 mg/kg i.e. 11.42±0.57 as compared to control 22.14±0.46, indicating 48.41 % inhibition of writhes. On the other hand aspirin also decreased highly significantly number of writhes at doses 50, 100 and 200 mg/kg, representing 65.8, 72.89 and 79.35 % inhibition of writhes as compared to number of writhes in control animals.

Table 1: Analgesic effect of NNF and aspirin in tail-flick test demonstrating tail-flick latency difference in mice

Groups/ doses	Analgesia TFLD or average increase in latency after drug administration±SEM						
	Pre-drug	30 min	60 min	90 min	120 min	150 min	180 min
Control	0.9±	0.9±	0.9±	0.9±	0.9±	0.9±	0.9±
10 ml/kg	0.01	0.05	0.01	0.02	0.03	0.02	0.02
NNF 50 mg/kg	1.0±	1.6±	1.6±	1.6±	1.3±	1.3±	1.2±
	0.01	0.10**	0.12**	0.12**	0.14*	0.11*	0.14
NNF 100 mg/kg	1.0±	2.7±	3.5±	3.9±	3.2±	2.4±	2.3±
	0.02	0.33**	0.24**	0.40**	0.21**	0.15**	0.17**
NNF 200 mg/kg	0.9±	2.8±	3.9±	5.1±	4.5±	3.3±	3.1±
	0.04	0.26**	0.15**	0.26**	0.46**	0.30**	0.12**
Aspirin	1.0±	3.2±	4.3±	4.8±	4.2±	3.2±	2.8±
50 mg/kg	0.03	0.13**	0.60**	0.62**	0.42**	0.23**	0.64**
Aspirin 100 mg/kg	1.0±	3.7±	5.7±	5.7±	4.8±	3.6±	3.0±
	0.02	0.06**	0.51**	0.37**	0.15**	0.29**	0.19**
Aspirin	1.104±	2.695±	4.75±	6.411±	4.249±	4.199±	4.064±
200 mg/kg	0.14	0.21**	0.45**	0.49**	0.23**	0.31**	0.24**

n=7, The expressions were calculated by taking mean±standard error to the mean, **p*-value less than 0.05 was counted as significant in comparison to control, ***p* value less than 0.005 was counted as extremely significant in comparison to control

Table 2: Analgesic effect of NNF and aspirin in tail-flick test demonstrating % tail elongation time in mice

Groups/Doses	% tail elongation at 90 min
Control 10 ml/kg	-
NNF 50 mg/kg	42
NNF 100 mg/kg	76
NNF 200 mg/kg	82
Aspirin 50 mg/kg	81
Aspirin 100 mg/kg	84
Aspirin 200 mg/kg	95

Table 3: Analgesic effect of NNF and aspirin in acetic acid-induced, writhing test in mice

Drugs	Dose	Number of writhes	% Inhibition
Control	10 ml/kg	22.1±0.46	-
NNF	50 mg/kg	16.3±0.29**	26.46
NNF	100 mg/kg	12.5±0.20**	43.22
NNF	200 mg/kg	11.4±0.57**	48.41
Aspirin	50 mg/kg	7.5±0.20**	65.8
Aspirin	100 mg/kg	6.0±0.31**	72.89
Aspirin	200 mg/kg	4.5±0.20**	79.35

n=7, The expressions were calculated by taking mean±standard error to the mean, **p* value less than 0.05 was counted as significant in comparison to control, ***p* value less than 0.005 was counted as extremely significant as compared to control

DISCUSSION

In current study analgesic effects of NNF was evaluated utilizing couple of animal models against aspirin as it is a remarkably well-recognized analgesic agent. Aspirin produces analgesia via non-selective and irreversible inhibition of cyclooxygenase enzyme (COX) which in turn decreases the synthesis of prostaglandins and reduces prostaglandins induced pain, inflammation and fever [28]. Despite the fact that aspirin does have a central component of action but it primarily produces analgesia via its peripheral action [29].

Tail flick test is efficient in evaluating the potency and efficacy of centrally acting analgesics [30]. In tail-flick test NNF exhibited highly noteworthy analgesic action at all doses; nevertheless the effects were especially intense at doses 100 and 200 mg/kg. The peak antinociceptive effect at all doses of fruit extract was observed at 90 min after which gradual decline in analgesic activity was recorded which was highly similar in pattern with aspirin. The percentage of tail elongation time was highest at a dose of 200 mg/kg i.e. 82% at 90 min. The higher the % elongation time of the group the greater is the group's central analgesic effect [25]. Hence it can be stated that NNF at dose of 200 mg/kg revealed strong central analgesic activity at 90 min after its administration.

Acetic acid-induced writhing test is a widely used method for the evaluation of visceral pain model in rodents [31]. It is also known as

the abdominal constrictor response and is very perceptive in detecting anti-nociceptive activities of agents at dose levels that may appear inactive in other procedures [32, 33].

The present study depicted and confirmed analgesic effects of NNF using acetic acid-induced writhing test. The number of writhes was highly significantly reduced at doses of 50, 100 and 200 mg/kg of NNF but maximum effects were observed at extract dose of 200 mg/kg in comparison to control, indicating 48.41 % inhibition of writhes.

Local presence of peritoneal receptors is assumed to be partially involved in abdominal constrictor response [34]. This method is linked with enhanced levels of PGE₂ and PGF_{2α} along with lipoxigenase products in abdominal fluid [35, 36]. Acetic acid intraperitoneal administration also causes the release of inflammatory mediators i.e. bradykinin and histamine which excites nerve fibers responsible for transmitting signals to the advanced centers of the brain and spinal cord which amalgamate and modulate nociception [37].

Since flavonoids, saponins and tannins are important secondary metabolites of NNF and have exhibited inhibitory effects on arachidonic acid metabolism and prostaglandin synthesis [38-40]. Therefore the results of the tail flick test and acetic acid induced writhing test strongly recommend that the mechanism of the analgesic effect of NNF is connected with the blockade of

arachidonic acid metabolism and inhibition of prostaglandin synthesis.

CONCLUSION

NNF has demonstrated remarkable analgesic activity in both experiments i.e. tail-flick test and acetic acid-induced writhing test, which was comparable with aspirin and that is perhaps both segments of the NNF i.e. seeds and pods are rich in flavonoids, saponins and tannins. Hence NNF seems to have a great potential for therapeutic applications especially in the treatment of disorders associated with pain and strongly supports more experimental trials in this field.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally in this piece of work

CONFLICT OF INTERESTS

The authors declared no conflict of interest

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