Turk J Pharm Sci 2021;18(1):56-60
DOI: 10.4274/tjps.galenos.2019.47108

ORIGINAL ARTICLE

Evaluation of Antiinflammatory Activity of Ethanol Extract of *Nelumbo nucifera* Fruit

Nelumbo nucifera Meyvesinin Etanol Ekstresinin Antiinflamatuvar Aktivitesinin Değerlendirilmesi

Muhammad Ali RAJPUT[1]*, Tabassum ZEHRA[2], Fizzah ALI[2], Gunesh KUMAR[3]

[1]Multan Medical and Dental College, Department of Pharmacology, Multan, Pakistan
[2]Liaquat National Medical College, Department of Pharmacology , Karachi, Pakistan
[3]Liaquat University of Medical and Health Sciences, Department of Pharmacology, Sindh, Pakistan

ABSTRACT

Objectives: In recent times, the use of natural remedies, which are rich in varieties of vitamins and flavonoids, for treatment of inflammation has increased substantially. These natural remedies are expected to be safe and economical when compared with other conventional allopathic drugs. Thus, existing research investigated the anti-inflammatory effect of *Nelumbo nucifera* fruit (NNF), in view of estimating its traditional and pharmacologic use against disorders associated with pain and inflammation.

Materials and Methods: To estimate the antiinflammatory effect of NNF, carrageenan-induced paw edema method was employed with equally distributed (n=7) Wistar male rats (N=35). The paw edema was measured by volume displacement method with plethysmometer.

Results: The NNF extract significantly reduced the inflammation of the paw and decreased the edema volume in rats administered carrageenan at all doses from the 3rd to 5th hour when compared to control, whose maximum percent reduction of edema was estimated as 100 mg/kg dose (that is, 73.92% at the 5th hour after administration of carrageenan).

Conclusion: NNF exhibited a strong antiinflammatory effect, due to its phytochemical constituents, including flavonoids, saponins, and tannins, all of which synergistically exert inhibitory effects on arachidonic acid metabolism, neutrophil degranulation, and enzyme systems that promote cell proliferation and regulation of complement system. However, more preclinical and clinical evaluations are mandatory to validate these findings.

Key words: *Nelumbo nucifera*, flavonoids, carrageenan, arachidonic acid

ÖZ

Amaç: Son zamanlarda enflamasyon tedavisinde vitaminler ve flavonoidler açısından zengin çeşitli doğal ilaçların kullanımı önemli oranda artmıştır. Bu doğal ilaçların diğer geleneksel allopatik ilaçlarla karşılaştırıldığında güvenli ve ekonomik olması beklenmektedir. Bu nedenle, bu çalışmada, geleneksel ve farmakolojik olarak ağrı ve enflamasyon ile ilişkili bozukluklara karşı kullanılan *Nelumbo nucifera* meyvesinin (NNF) antiinflamatuvar etkisi araştırıldı.

Gereç ve Yöntemler: NNF'nin antiinflamatuvar etkisini belirlemek için, carrageenan kaynaklı pençe ödemi yöntemi, eşit olarak dağıtılmış (n=7) Wistar erkek sıçanlarda (N=35) kullanıldı. Pençe ödemi, pletismometre ile hacim değiştirme yöntemi ile ölçüldü.

Bulgular: NNF ekstresi, ödemdeki maksimum azalma yüzdesi 100 mg/kg doz olarak tahmin edilen kontrole kıyasla 3. ile 5. saatte tüm dozlarda carrageenan uygulanan sıçanlarda pençenin enflamasyonunu önemli ölçüde azalttı ve ödem hacmini azalttı (carrageenan uygulamasından sonraki 5. saatte %73,92).

Sonuç: NNF, flavonoidler, saponinler ve taninler de dahil olmak üzere fitokimyasal bileşenleri nedeniyle güçlü antiinflamatuvar etki gösterdi; bunların tümü, hücre proliferasyonunu ve kompleman sistem regülasyonunu tetikleyen araşidonik asit metabolizması, nötrofil degranülasyonu ve enzim sistemleri üzerinde sinerjistik inhibitor etki gösterdi. Bununla birlikte, bu bulguların pre klinik ve klinik deneyler ile doğrulanması gerekmektedir.

Anahtar kelimeler: *Nelumbo nucifera*, flavonoidler, carrageenan, araşidonik asit

***Correspondence:** drmuhammadali2016@gmail.com, **Phone:** +0923463881721 **ORCID-ID:** orcid.org/0000-0003-3688-8518
Received: 10.10.2019, **Accepted:** 12.12.2019

INTRODUCTION

A lot of individuals who experience severe, inexorable, and excruciating pain resulting from any deleterious painful condition, such as myocardial infarction or injury secondary to accidents, rely primarily on opioids, in spite of their established adverse effects. Similarly, unceasing anti-nflammatory conditions, such as rheumatoid arthritis and osteoarthritis, are generally cured with non-steroidal antiinflammatory drugs (NSAIDs). Although NSAIDs are dominating in the market, the toxicity associated with their prolonged use cannot be overlooked. The most frequent toxicity associated with NSAIDs includes gastro-intestinal tract bleeding and ulcers.[1,2] Therefore, this instigates the need to develop new, safe, effective, economical, and innocuous analgesics.[3]

The use of herbal drugs is progressively becoming more popular, since they are natural and have no adverse effects.[4] Basically, plant-derived drugs are taken randomly with no adequate knowledge of their local application for the treatment of various diseases. It is therefore important to appropriately guide the general population on the use of natural products. In addition, it is necessary to scientifically prove the effectiveness of these medicinal plants.[5]

Nelumbo nucifera (NNF) (Lotus), a member of the *Nymphaeaceae* family of plants, is commonly cultivated in hot and humid climate zones of Thailand, Pakistan, India, and China.[6] Its fruit contains seeds and pods. The green colored pods offer add-on to the seeds, which are usually black in color, tough, and roundish in shape (diameter: 1.5x1.0 cm). They are organized in whorls.[7] The seeds are the edible part of the fruit, which have to be skinned separately before consumption.[8]

The seeds of NNF are good sources of protein, fat, asparagines, unsaturated fatty acids, and starch. The key active components in the seeds are flavonoids, alkaloids, carbohydrates, gallic acid, and ample amount of various minerals, in addition to zinc, iron, potassium, sodium, and calcium.[9,10]

A recently conducted study on NNF pods revealed the existence of numerous active bioactive principles, such as flavonoids, alkaloids, saponins, terpenoids, and tannins.[11] Procyanidin (flavonoid) was also extracted from NNF pods.[6]

Customarily, the fruits are used as a healthy component of Asian cuisine. They are also used as a traditional cure for various ailments, such as hypertension, palpitation, arrhythmia, fever, pain, inflammation, sleep disorders, chronic diarrhea, spermatorrhea, leucorrhoea, bad breath, leprosy, and menorrhagia.[12,13] Recent research revealed that the LD_{50} value of NNF was higher than 5g/kg, whereas its neuropharmacological role was also established to be anxiolytic, antidepressant, and antiepileptic.[11,14] However, there insufficient information in literature regarding its ameliorative effect on inflammation. In this regard, this study aimed to evaluate the antiinflammatory activity of NNF, in view of justifying its traditional and pharmacologic use against disorders associated with pain and inflammation.

MATERIALS AND METHODS

Study design and methodology

This study was performed with the assistance of the laboratory services of the Department of Pharmacology, UoK, following approval from the Board of Advance Studies and Research, UoK.

Animals care

The research board, which comprise members of Faculty of Pharmacy, permitted the use of animals for the experiments in accordance with the protocols described by NIH and NACLAR.[15,16]

Animals were kept in plastic cages and maintained at a temperature near 25°C and humidity of 50% to 60% in an interchanging twelve hour cycle. Each mouse was given free access to normal diet and water. Rats were carried to the workroom about an hour prior to the trials initiation. Prior to administration of the dose, complete health of the rats was assessed based on the absence of movements, edema, diarrhea, and ulceration during the acclimatization process using the laboratory settings for 7 days.

Preparation of extract

After obtaining fruits from the domestic fruit bazaar of Hyderabad, Pakistan in July 2015, they were initially presented to Pharmacognosy Department, UoK for identification and authentication and, afterward, the receipt no NNF-03 was assigned and deposited in the same department.

Crude extract was prepared through cold extraction procedure.[17,18] In brief, fruits (6 kg) were initially rinsed with clean water and seeds were manually separated from the fruit. Since the seeds have high contents of water, they need to be chopped first and left for 6 days to dry out in shade. The dried material obtained was thick and, as a result, the seeds need to be ground into fine powder. In contrast, pods were chopped once and allowed to dry in shade for 3 days. The dried pod material was in a coarse powder form. For better separation and collection of NNF constituents (secondary metabolites), they need to be chopped and dried separately before soaking them in ethanol (98%) for 30 days with occasional shaking.

Afterward, it was filtered with whatman no. 1 filter paper. Then, it was evaporated with a rotary evaporator under condensed pressure at a temperature of 40°C-45°C. The condensed material was freeze-dried with a freeze dryer (-30°C) and stored in a refrigerator. The final yield of the extract was 0.4 kg (dry weight).

Grounding of drugs

Carrageenan and gum tragacanth were obtained from Merck. Aspirin was purchased from a well-known pharmacy in Karachi.

2% tragacanth gum (powder form) was procured from Merck, which was used to prepare suspensions of 3 different dosages for the test group (NNF 50, 100, and 200 mg/kg). Control group was given tragacanth gum (10 mL/kg PO) as placebo. On each occasion, new suspensions were prepared for the dosage.[19,20]

Carrageenan suspension (0.1 mL of 1% w/v) was prepared in normal saline and inoculated via the planter aponeurosis of right hind paw to induce hind paw edema in the experimental rats.[21]

Aspirin (300 mg tablet) was crushed and suspended in tragacanth gum (2%), which was then administered to the rats at the dosage of 150 mg/kg PO (as reference agent) using an orogastric tube.[22]

Hind paw edema method

Hind paw edema method is an established technique for assessing the antiinflammatory effect of NNF extract, following the induction of edema in the hind paw of rat by carrageenan. Generally, carrageenan is used as a phlogistic agent, a substance that induces inflammation or edema.[23]

The test was performed on 35 Wistar rats that were equally distributed into 5 groups (n=7). The control group was administered 2% tragacanth gum; the reference group was administered 150 mg/kg aspirin; and the 3 test groups were administered 50, 100, and 200 mg/kg NNF. All drugs were administered orally (PO) at an hour prior to the delivery of carrageenan injection. Carrageenan suspension (0.1 mL of 1% w/v) prepared in normal saline was introduced under the planter aponeurosis of right hind paw of rats to induce hind paw edema in the experimental rats.[21]

The paw edema was estimated by volume displacement technique using a plethysmometer (UGO Basile 7140, Italy), which, perhaps, is the most efficient method for estimating the antiinflammatory effect of a drug. Plethysmometer is a volume meter that is composed of water-filled Perspex cell, in which the hind paw of rat was submerged with the transducer, which records small changes in water level caused by volume displacement, as well as with a digital meter, which displays the exact volume of water being displaced by the edema in the hind paw of rats. The swelling was estimated as mL of edema at different time interval, that is, the paw volume before carrageenan administration (time=0 baseline) and paw volume after carrageenan administration from the 1st to 5th hour was noted. Changes in the paw volume estimated earlier, as well as the succeeding administration of phlogistic agent points toward the significance of edema. Lastly, percent reduction of paw swelling was estimated in terms of % inhibition as follows:[24]

% inhibition: A-B/A x100

Where;

A: The average paw volume for test groups

B: The average paw volume for control group

Statistical analysis

The statistical analysis was done by applying Student's independent samples t-test to the mean and standard error of mean. P values less than 0.05 were considered significant and p values less than 0.005 were considered highly significant. Analysis of data was performed by using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, N.Y., USA).

RESULTS

Hind paw edema method

Table 1 and 2 showed that the carrageenan injection produced a localized edema at the right hind paw of rats, which attained its peak at the 3rd hour after the carrageenan administration, but gradually reduced after this period. Administration of 50, 100, and 200 mg/kg NNF significantly reduced the paw edema volume from the 3rd to 5th hour when compared to control, whose maximum percent reduction of edema was estimated as 100 mg/kg (that is, 73.92% at the 5th hour after administration of carrageenan). The initial phase of the edema (1st and 2nd hour) was not affected by the fruit extract. On the other hand, 150 mg/kg aspirin substantially decreased paw edema volume from the 1st to 5th hour when compared to control. Maximum percent inhibition of edema (88.17%) was estimated at the 5th hour after the carrageenan administration.

DISCUSSION

Several individuals who experience severe, inexorable, and excruciating pain resulting from cancer, injury, or from various autoimmune disorders and other degenerative diseases rely on opioids, such as morphine, in spite of their established adverse effects. Although NSAIDs are dominating in the market, toxicity associated with their prolonged use cannot be overlooked.[1,2] This instigates the need to develop novel, safe, and effective substances for ameliorating the effect of inflammation.

Acute inflammation induced by carrageenan is the most appropriate technique for screening antiinflammatory agents.

Table 1. Antiinflammatory activity of NNF and aspirin estimated by hind paw edema method in rats

Groups	Average paw size (mL) ± SEM						Average rise in paw volume (mL) ± SEM				
	Pre drug	1 h	2 h	3 h	4 h	5 h	1 h	2 h	3 h	4 h	5 h
Control	2.0±0.02	2.8±0.10	3.5±0.17	4.3±0.18	4.2±0.16	3.8±0.16	0.8±0.07	1.5±0.14	2.3±0.15	2.1±0.13	1.8±0.13
NNF 50 mg/kg	2.1±0.03	3.3±0.21	3.9±0.18	3.2±0.18**	2.8±0.20**	2.9±0.21**	1.2±0.18	1.8±0.15	1.1±0.15**	0.8±0.16**	0.8±0.18**
NNF 100 mg/kg	2.0±0.01	2.8±0.10	3.1±0.11	3.0±0.18**	2.6±0.07**	2.5±0.18**	0.7±0.08	1.1±0.09	1.0±0.16**	0.65±0.05**	0.48±0.16**
NNF 200 mg/kg	1.9±0.02	2.8±0.18	3.4±0.14	3.0±0.11**	2.8±0.14**	2.6±0.16**	0.84±0.15	1.43±0.11	1.11±0.08**	0.9±0.11**	0.66±0.13**
Aspirin 150 mg/kg	1.8±0.04	1.9±0.05**	2.1±0.18**	2.3±0.06**	2.1±0.06**	2.0±0.05**	0.19±0.02	0.35±0.14	0.5±0.03**	0.34±0.02**	0.22±0.01**

n=7, data are expressed as mean ± SEM, **: P value less than 0.005 was considered highly significant. SEM: Standard error of mean, NNF: *Nelumbo nucifera* fruit

Table 2. Percent inhibition of edema in rats treated with NNF and aspirin

Groups	% inhibition of edema		
	3 h	4 h	5 h
Control	-	-	-
NNF 50 mg/kg	51.29	63.30	55.91
NNF 100 mg/kg	55.12	70.18	73.92
NNF 200 mg/kg	52.15	58.71	64.51
Aspirin 150 mg/kg	78.44	84.40	88.17

n=7, NNF: *Nelumbo nucifera* fruit

The time required for edema to appear in carrageenan-induced hind paw edema model in rats is often estimated by a biphasic curve. The initial phase of inflammation arises within an hour of carrageenan administration, due to trauma at the injection site, serotonin, and histamine element.[25] The 2nd phase of edema starts from the 3rd hour and is sensitive to cyclooxygenase (COX) inhibitors, such as NSAIDs.[3]

This study revealed the antiinflammatory effect of NNF, which incredibly decreased the paw edema volume at all doses from the 3rd to 5th hour as compared to control, with maximum percent reduction of edema estimated at 100 mg/kg (that is, 73.92% at the 5th hour after administration of carrageenan). The first phase of the edema (that is, 1st and 2nd hour) was not affected by the fruit extract. Hence, in the present study, there was no inhibition of histamine and serotonin at the first phase of the test; however, COX pathway was effectively inhibited at the second phase of the test.

Flavonoids, which are also one of the significant constituents of NNF, exert its antiinflammatory effects via several mechanisms. One of these mechanisms pertains to their proposed capability of diminishing neutrophil degranulation. This represents the shortest possible approach to inhibit the liberation of arachidonic acid by neutrophils and other immune cells. Neutrophils carrying lipoxygenase produce chemotactic factors from arachidonic acid, which also stimulate the release of cytokines.[26,27]

Certain flavonoids are capable of reducing complement system activation, thus diminishing the attachment of inflammatory cells to the endothelium, thus reducing the inflammatory response.[28]

Saponins and tannins, which are also present in NNF, have also been reported to show inhibitory effects on arachidonic acid breakdown.[29] A recently conducted study has revealed the analgesic activity of NNF in various animal models, postulating that it may be connected with the synergistic actions of flavonoids, saponins, and tannins on arachidonic acid inhibition.[30] Therefore, it can be stated that the antiinflammatory effects of NNF are largely due to their flavonoids, saponins, and tannins content, which is believed to synergistically exert inhibitory effects on arachidonic acid metabolism, neutrophil degranulation, and enzyme systems that promote cell growth and regulates complement system.

CONCLUSION

The antiinflammatory activity of NNF demonstrated in this study was due to the existence of phytochemical constituents, which make it a useful agent for the treatment of patients with chronic inflammatory disorders. However, further preclinical and clinical evaluation is required to validate these findings.

ACKNOWLEDGEMENTS

The authors are thankful to the Chairman of Pharmacognosy Department, UoK for identifying and authenticating the NNF. We also appreciate the support of the Director, International Center for Chemical and Biological Sciences, for permitting us to use their facilities to complete this piece of work.

Conflicts of interest: No conflict of interest was declared by the authors. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1. Yesilada E, Ustun O, Sezik E, Takaishi Y, Ono Y, Honda G. Inhibitory effect of Turkish folk remedies on inflammatory cytokines: Interleukins-1-alpha, interleukins-1-beta and tumor necrosis factor-alpha. J Ethnopharmacol. 1997;58:59-73.
2. Corley DA, Kerlikowske K, Verma R, Buffler P. Protective association of aspirin/NSAIDS and esophageal cancer: A systemic review and meta-analysis. Gastroenterology. 2003;124:47-56.
3. Singh M, Kumar V, Singh I, Gauttam V, Kalia AN. Anti-inflammatory activity of aqueous extract of *Mirabilis jalapa* Linn. leaves. Pharmacognosy Res. 2010;2:364-367.
4. da Costa Lopes L, Albano F, Augusto Travassos Laranja G, Marques Alves L, Fernando Martins e Silva L, Poubel de Souza G, de Magalhães Araujo I, Firmino Nogueira-Neto J, Felzenszwalb I, Kovary K. Toxicological evaluation by *in vitro* and *in vivo* assays of an aqueous extract prepared from Echinodorus macrophyllus leaves. Toxicol Lett. 2000;116:189-198.
5. Agbaje EO, Adeneye AA, Daramola AO. Biochemical and toxicological studies of aqueous extract of Syzigium aromaticum (L.) Merr. & Perry (Myrtaceae) in rodents. Afr J Tradit Complement Altern Med. 2009;6:241-254.
6. Mukherjee PK, Mukherjee D, Maji AK, Rai S, Heinrich M. The sacred lotus (*Nelumbo nucifera*) - phytochemical and therapeutic profile. J Pharm Pharmacol. 2009;61:407-422.
7. Sridhar KR, Bhat R. Lotus: a potential nutraceutical source. J Agri Technol. 2007;3:143-155.
8. Carlo FM, Masami Y, Yoshiaki K, Ganesh KA, Randeep Rakwal. Lotus - A Source of Food and Medicine: Current Status and Future Perspectives in Context of the Seed Proteomics. IJLS 2013;7:1-5.
9. Indrayan AK, Sharma S, Durgapal D, Kumar N, Kumar M. Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. Curr Sci. 2005;89:1252-1255.
10. Pal I, Dey P. A review on lotus (*Nelumbo nucifera*) seed. IJSR. 2015;4:1659-1666.
11. Rajput MA, Khan RA. Phytochemical screening, acute toxicity, anxiolytic and antidepressant activities of Nelumbo nucifera fruit. Metab Brain Dis. 2017;32:743-749.

12. Chopra RN, Nayar SL, Chopra IC, Asolkar LV, Kakkar KK, Chakre OJ, Varma BS. Glossary of Indian Medicinal Plants. New Delhi: Council of Scientific and Industrial Research, 1996;3:174.

13. Varshney CK, Rzoska J. Aquatic weeds in South East Asia. 1st ed. New Delhi: Springer; 1976; p. 39.

14. Rajput MA, Khan RA, Assad T. Anti-epileptic activity of *Nelumbo nucifera* fruit. Metab Brain Dis. 2017;32:1883-1887.

15. National Advisory Committee for Laboratory Animal Research. Guidelines on the care and use of animals for scientific purposes. 2004; p. 24.

16. National Institute of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research. Guide for the care and use of laboratory animals. Prepublication draft. 8thed. The National Academies Press Washington DC 2010; 6, 47.

17. Hossain MS, Ahmed M, Islam A. Hypolipidemic and hepatoprotective effects of different fractions of ethanolic extract of immature leaves of *Mangifera indica* Linn in alloxan induced diabetic rats. IJPSR. 2010;1:132-138.

18. Assad T, Khan RA, Rajput MA. Effect of *Trigonella foenum-graecum* Linn seeds methanol extract on learning and memory. Metab Brain Dis. 2018;33:1275-1280.

19. Madhu A, Keerthi PHV, Singh J, Shivalinge GKP. To evaluate the anti-epileptic activity of aqueous root extract of *Hemidesmus indicus* in rats. Arch Pharm Sci Res. 2009;1:43-47.

20. Khan RA, Rajput MA, Assad T. Effect of Nelumbo nucifera fruit on scopolamine induced memory deficits and on motor coordination. Metab Brain Dis. 2018;34:87-92.

21. Ocete MA, Risco S, Zarzuelo A, Jimenez J. Pharmacological activity of the essential oil of Bupleurum gibraltaricum: anti-inflammatory activity and effects on isolated rat uteri. J Ethnopharmacol. 1989;25:305-313.

22. Ahmed S, Naved A, Khan RA, Siddiqui S. Analgesic Activities of Methanol Extract of *Terminalia chebula* Fruit. Pharmacology Pharmacy 2015;6:547-553.

23. Winter CA, Risely EA, Nuss GW. Carrageenan induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Proc Soc Exp Biol Med. 1962;111:544-547.

24. Palanichamy S, Nagarajan S. Anti-inflammatory activity of Cassia alata leaf extract and kaempferol 3-O-sophoroside. Fitoterapia. 1990;61:44-47.

25. Crunkhorn P, Meacock SC. Mediators of the inflammation induced in the rat paw by Carrageenan. Br J Pharmacol. 1971;42:392-402.

26. Hoult JR, Moroney MA, Paya M. Actions of flavonoids and coumarins on lipoxygenase and cyclooxygenase. Methods Enzymol. 1994;234:443-454.

27. Tordera M, Ferrandiz ML, Alcaraz MJ. Influence of anti-inflammatory flavonoids on degranulation and arachidonic acid release in rat neutrophils. Z Naturforsch C J Biosci. 1994;49:235-240.

28. Friesenecker B, Tsai AG, Intaglietta M. Cellular basis of inflammation, edema and the activity of Daflon 500 mg. Int J Microcirc Clin Exp. 1995;15(Suppl):17-21.

29. Ahmadiani A, Hosseiny J, Semnanian S, Javan M, Saeedi F, Kamalinejad M, Saremi S. Anti-nociceptive and anti-inflammatory effects of *Eleagnusangusti folia* Fruit Extract. J Ethnopharmacol. 2000;72:287-292.

30. Rajput MA, Tabassum, Z, Fizzah A, Gunesh K. Assessment of analgesic activity of *Nelumbo nucifera* fruit ethanol extract. Int J Pharm Sci. 2019;11:1-5.