Original Research Article

An Evaluation of Potential Therapeutic Activity of *Nelumbo nucifera* as Anti Inflammatory and Analgesic Agentin Rat Model

Abstract

Although inflammation is the body's defensive action against cell or tissue damage in order to prevent and treat diseases like infections, chronic or excessive inflammation can be dangerous and can develop several health issues. Nowadays, conventional drugs for inflammation are growing more expensive with increased levels of risks and can only provide specific treatments. On the contrary, medicinal plants are highly beneficial natural sources for anti-inflammatory and analgesic treatments which lessen the cost and risks in medications. Thus, studies were conducted and carried out in vivo and in silico to assess the analgesic and anti-inflammatory effects of *Nelumbo nucifera*, commonly known as "Sacred Lotus" in rats.

Nelumbo nucifera plants were collected and then extracted with 70% ethanol for the research on its anti-inflammatory and analgesic activity. Moreover, carrageenan, acetic acid, standard drugs (Ibuprofen and Aspirin) and blood serum measuring kits were made available for the study. Additionally, Wister albino rats were separated into 5 groups and subjected to the in vivo testingand ensuring strict compliance with the Institutional Animal Ethics Committee (IEAC) guidelines. In this study, carrageenan induced tests were observed to evaluate the anti-inflammatory activity and for the evaluation of analgesic effect writhing tests upon acetic acid induction and tail flick tests were performed. Correspondingly, the results of the test *N. nucifera* plant showed similar potential with improved safety measures in anti-inflammatory and analgesic activity with the traditional medicines.

In the test of anti-inflammatory efficacy, we observed a highly significant decrease ($p< 0.05$) in paw volume in the rats treated with a high dose of *Nelumbo nucifera*. When *Nelumbo nucifera* was administered in both the writhing test and the tail flick test, where rats were given acetic acid, we also noticed a significant result in a dose-dependent manner. Furthermore, with high doses of *N. nucifera* demonstrated highly statistical significance. *Nelumbo nucifera* substances demonstrated the maximum binding affinity whereas control medicines displayed a lower binding affinity according to in silico analysis. Thus, it may be concluded that *N. nucifera*extract had enhanced anti-inflammatory and analgesic effects in rats compared to standard medications. In conclusion, frequent and progressive studies should be conducted in order to increase the

prevalence for integration of medicinal plants in the treatment of inflammation and pain caused in various health conditions.

Keywords: *Nelumbo nucifera,* traditional mednicine, carrageenan, ftail lick test, extract

Introduction

The immune system activates in response to a wide variety of stimuli, such as injury or infection that leads to inflammation. **[1]** Inflammatory disorders include autoimmune conditions like rheumatoid arthritis, cardiovascular conditions, such as heart disease and high blood pressure, gastrointestinal conditions such as ulcerative colitis, Crohn's disease, and irritable bowel syndrome, asthma and other lung conditions like chronic obstructive pulmonary disease (COPD), Mental disorders like depression, Type 2 diabetes metabolic condition, Parkinson's disease a neurodegenerative disease and cancer which further inflicts great pain. **[2]** Recent studies have found that inflammation plays a role in almost all chronic diseases, which impact 133 million Americans, or more than 40% of the country's population. **[3]**

In addition, Nonsteroidal anti-inflammatory medications (NSAIDs), narcotic drugs, corticosteroids, and immune selective anti-inflammatory derivatives (mSAIDs) are the standard drugs that has potential risks and are associated with side effects in the field of medicine for the management of pain and inflammation. Diclofenac, Naproxen ketorolac and ibuprofen, two widely used NSAIDs, exhibit anti-inflammatory properties via binding to both COX1 and COX2. As a result, it transforms arachidonic acid into a prostaglandin that is anti-inflammatory. **[4]**

However, The usage of medicinal plants is rising in prevalence as they are thought to be beneficial, natural, and free of side effects. Herbal medicines use the entire plant, in contrast to modern conventional medicine, which aims to use only the plant's active component. The herbalists contend that the combination of compounds found in the entire plant produces a better result (referred to as synergy) than a single active ingredient. The ability of plants to digest genetic material and build complex proteins that can be exploited to create more potent medicines is superior. **[5]** Many medicinal plants, including *Acokantheraoppositifolia, Vangueriainfausta, Nigella sativa, Agathosmabetulina, Berchemia zeyheri, Commiphorawightii, Turbina oblongata, Brocchia cinerea, Euphorbia pulcherrima* etc. are used in traditional medicine to alleviate inflammation and pain. **[6]**

Nevertheless, *Nelumbo nucifera* under Nelumbonaceae family has powerful cooling and astringent effects in addition to being opulent and attractive, making it an excellent source of herbal medicine. In South East Asia, where the seeds and leaves are often consumed, lotus is also highly revered and so known as a sacred lotus. **[7]** The primary edible components of *N. nucifera* are the rhizome and seeds. Traditional medicine has employed various portions of *N. nucifera* to

treat a variety of conditions, including fever, inflammation, sleeplessness, neurological disorders, epilepsy, hypertension, cardiovascular illnesses, obesity, and hyperlipidemia. Numerous bioactive substances, such as alkaloids, flavonoids, polyphenols, terpenoids, steroids, and glycosides, are thought to be in charge of the substance's wide range of biological and pharmacological actions, including antioxidant, anti-inflammatory, immune-modulatory, antiviral, hepatoprotective, cardioprotective, antiarrhythmic, anticancer and hypoglycemic effects. **[8]**

Moreover, several bioactive substances found in N. nucifera provide it a variety of beneficial properties, including flavonoids and polyphenols (Arbutin, syringetin, p-hydroxybenzoic acid, (E)-Ferulic acid, dauricine, Isoliensinine, Myricetin 3-O-galactoside, isorhamnetin, Quercetin 3-O-β-D−glucopyranoside, Betulinic acid, Kaempferol, Dihydrophaseic acid etc.), alkaloids (N−nornuciferine, nuciferine, roemerin, 2−hydroxy−1−methoxyaporphine, (S)−armepavine, (+)−1(R)−coclaurine, (−)−1(*S*)-norcoclaurine, lotusine, isoliensinine, liensinine, neferine, liriodenine, asimilobine, pronuciferine, oleracein E, demethyl-coclaurine, dauricine, *cis-N*-feruloyltyramine and *trans-N*-coumaroyltyramineetc), *β*-palmitic acid-linoleic acid, *β*-sitosterol, methionine , valine, terpenoids, steroids, and glycosides and so on. **[9]**

Furthermore, Nelumbo nucifera have been reported to have a variety of pharmacological effects by researchers. Accumulation of ascorbic acid and cholesterol from the extract of the plant can give antifertility activity by suppression of hypothalamic gonadotropin releasing hormone production, reduction in *δ*-5-3-*β*-hydroxysteroid dehydrogenase and glucose-6-phosphate dehydrogenase activity in the ovary and testis. **[8]**

The chemical constituent extracted from N. nucifera, Flavonoids have an anti-inflammatory impact via reducing neutrophil breakdown and preventing the production of arachidonic acid from neutrophils and other immune cells. Arachidonic acid is converted by lipoxygenase-containing neutrophils into chemotactic substances that promote the release of cytokines. Certain flavonoids have the ability to lower complement activation, which in turn lowers the adhesion of inflammatory cells to the endothelium and, ultimately, lowers the inflammatory response. The same tannins and saponins found in N. nucifera have been reported to have inhibitory effects on the metabolism of arachidonic acid. Recent research has demonstrated the analgesic effectiveness of N. nucifera in multiple animal models and hypothesized that this activity may be related to the synergistic effects of flavonoids, saponins, and tannins on the suppression of the arachidonic acid pathway. **[9]**

To conclude, Commercially available medications and N. nucifera ingredients, such as its polyphenols and flavonoids, exhibit comparable pharmacological actions against inflammation. As a result, it suggests that N. nucifera exhibits similar behaviors to those of synthetic medicines. This study's goal is to perform an in silico experiment. A carrageenan-induced paw edema will

be used in an in vivo investigation, however, to gauge how well N. nucifera works to reduce inflammation. To measure the analgesic activity, the team will additionally perform the tail freak test and acetic acid writhing test. Overall, the team's research will undoubtedly help other researchers take the appropriate actions to modify the behavior of the N. nucifera.

Materials and Methods

Drugs, Chemicals and Instruments:

Ibuprofen and aspirin were provided from Healthcare Pharmaceutical Limited as a gift sample. However, carrageenan, acetic acid, tween 80 and ethanol had been originally purchased from Sigma-Aldrich in Germany. Plethysmometer and analgesia meters were employed, respectively, to measure the anti-inflammatory and analgesic activity.

Plant Collection and Extract Preparation:

The N. nucifera plants were gathered at the Botanical Garden in Dhaka. Following taxonomic confirmation and identification, the specimen was certified by the Department of Botany at the University of Dhaka.

The plants were initially carefully cleaned before being allowed to dry in the sun for 7–10 days. They were then kept in an oven for a further seven days at a temperature of 40°C. After the dried herbs were roughly ground, fine powder was produced. After being ground into powder, the plants were immersed in 70% ethanol for an additional 21 days while being shaken occasionally. Once the extract had finished soaking, it was then filtered in every three days, and the filtered liquid was eventually collected. A rotary evaporator was then used to concentrate the extracted solution. The dried extract was then carefully collected for later use. This procedure was repeated numerous times to improve the extracted sample's accuracy. Lastly, 5g of the extracted preparation were separated to conduct further pharmacological experiments.

Experimental Animal Handling:

Healthy, male wistar adult rats, weighing between 125–200 g were obtained from the pharmacy at Jahangirnagar University in Dhaka, Bangladesh, and kept at the University of Dhaka's Institute of Nutrition and Food Science under a 12-hour light/dark cycle at a constant temperature of 25 °C and 55% relative humidity. A standard pellet meal and clean water were provided on a regular basis. The rats were put there for two weeks prior to the start of the investigation to acclimate. Guidelines of the Institutional Animal Ethics Committee (IEAC) were adhered to in all rat trials. Animals were handled and treated humanely in accordance with the standards established by the Swiss Academy of Medical Sciences (SAMS) and the Swiss Academy of Sciences (SCNAT).

Experimental Guidelines:All experiments were carried out in accordance with the 2013 Declaration of Helsinki's ethical standards.

Experimental Design: Following individual weighting of rats, the rodents were divided into five-rat groups with an even distribution of rodents based on their body weight.

Evaluation of Anti-Inflammatory Activity:

Carrageenan was used to induce inflammation in rodents in order to investigate the anti-inflammatory activity of the reference medication and the extract of Nelumbo nucifera (N. nucifera).

Table 1: Analysis of anti-inflammatory activity in carrageenan-induced rats

Group Number	Group Specification	Treatment species	Dose Treatment species (mg/kg)	Abbreviation of Groups
1	Carrageenan Control	N/A	N/A	Car
2	Carrageenan + Ibuprofen	Ibuprofen	10	$Car+Ib_{10}$
3	Carrageenan + *N. nucifera*	*N. nucifera*	500	$Car+Nn_{500}$
4	Carrageenan + *N. nucifera*	*N. nucifera*	750	$Car+Nn_{750}$
5	Carrageenan + *N. nucifera*	*N. nucifera*	1000	$Car+Nn_{1000}$

Carrageenan-Induced Acute Inflammatory Model:

Researchers became interested in rodent paw edema because carrageenan induction is a method frequently used to evaluate the effectiveness of anti-inflammatory medicines. Plethysmometer were used to conduct the anti-inflammatory test, making them a special kind of equipment. The volume of the paws of each rodent was initially determined. Then, to generate edema, sub planar tissue of the rat's left rear paw was injected with 0.1 mL of the freshly prepared carrageenan solution per 100 g of body weight. It was then kept for an hour. Rats were then given a range of dosages of the test drug and extracts. The paw volume was estimated 0–6 hours after the carrageenan infusion using a plethysmometer. The rate of edema inhibition was then determined by the following formula 1.

$$\text{Percentage Inhibition} = \frac{V_{Pc} - V_t}{V_{pc}} \times 100 \quad \text{Equation 1}$$

Here,

V_{PC} = volume of animals' paw in Positive Control rat

V_0= volume of animals' paw in Treatment Group

Evaluation of Analgesics Activity:

Through the acetic acid-induced writhing test (Table 2) and the tail-flick test (Table 3), the rat is given pain sensation.

Table 2: Analysis of analgesic activity in rats using acetic acid-induced writhing test

Group Number	Group Specification	Treatment species	Dose Treatment species (mg/kg)	Abbreviation of Groups
1	Acetic Acid Control	Physiological Saline	10 ml/kg	Ace
2	Aspirin + Acetic Acid	Aspirin	100	As_{100}+Ace
3	*N. nucifera* + Acetic Acid	*N. nucifera*	500	Nn_{500}+Ace
4	*N. nucifera* + Acetic Acid	*N. nucifera*	750	Nn_{750}+Ace
5	*N. nucifera* + Acetic Acid	*N. nucifera*	1000	Nn_{1000}+Ace

Acetic acid-induced Writhing Test:

Peripheral analgesic activity was examined using the acetic acid-induced writhing technique. Various test samples were administered 30 minutes prior to the intraperitoneal infusion of acidic acid. Along with painful stimuli, the rats received an intraperitoneal injection of 0.9% acidic acid (10 ml/kg). Following the administration of acetic acid, the number of writhes (muscular contraction ions) was counted for 20 minutes before counting again started. Writhing movements such as abdominal muscle contraction, pulling up of hind limbs toward abdominal walls, stretching of hind limbs, and periodic body arching were counted for twenty minutes prior to calculating the percentage of inhibition of writhes. Formula 2 was used to determine the percentage of writhes, one of the measures to show analgesic activity.

$$\left\{\frac{A.\ Control\ mean - Treatment\ mean}{A\ Control\ mean}\right\} \times 100 \quad \text{Equation 2}$$

Where, $T\ Control$ = the mean number of the writhing of each test group

$A\ Control$ = The mean number of the writhing of acetic acid control group

The "Tail Flick Method" is used to assess the extract's analgesic potency on the same experimental rat model after a seven-day interval. The injection of acetic acid had stopped working by this point.

Table 3: Analysis of analgesic activity in rats using Tail Flick Method

Group Number	Group Specification	Treatment species	Dose Treatment species (mg/kg)	Abbreviation of Groups
1	Tail Flick Stress (control)	Physiological Saline	10 ml/kg	TFS
2	Aspirin + Tail Flick Stress	Aspirin	100	As_{100}+TFS
3	*N. nucifera* + Tail Flick Stress	*N. nucifera*	500	Nn_{500}+TFS
4	*N. nucifera* + Tail Flick Stress	*N. nucifera*	750	Nn_{750}+TFS
5	*N. nucifera* + Tail Flick Stress	*N. nucifera*	1000	Nn_{1000}+TFS

Tail Flick Method:

The tail-flick experiment, a nociceptive assay developed by Love and Smith in 1941, is used to assess how animals react behaviorally to noxious stimuli. The length of time it took for the avoidance reaction to heat stimuli to be delayed was measured using a UGO BASILE® (Germany) radiant heat regulated tail-flick analgesia meter. A 4 Amp steady current was then delivered through the exposed nichrome after a heat controller helped the device's nichrome wire reach the right temperature. This method involves heating the center of the rats' tails with radiant heat to cause pain. The time needed for the tail-flick response to appear in control rats that were provided the test treatment has been calculated. The test was performed 0, 15, 30, 45, and 60 minutes after the test medications were administered to the rats.

Statistical analysis:

In accordance with several study criteria, all of our findings (raw data) were separated into multiple groups and documented on a spreadsheet using the MS Excel application. The mean SD was used to display the findings of descriptive statistics applied to the data. The "One Way Anova Test" function of the SPSS 1600 program was used to determine the statistical significance of the inter-group heterogeneity in terms of a number of biological variables. The

occurrences were considered statistically significant when the "p" value was less than 0.05 ($p<0.05$).

Result and Discussion

1. **Anti inflammatory activity of *Nelumbo nucifera:***

Table 4: Anti inflammatory activity of Nelumbo nucifera (*presents the level of significance of result)

Groups specification	Time µL				
	0 Minute(Just before carrageenan injection)	**1 hour (just before treatment**	**2 Hours**	**3 Hours**	**4 Hours**
Car	104.32±4.56	114.56±4.56	127.39±5.83	134.23±5.46	138.56±6.39
Car+Ib_{10}	106.23±3.29	118.29±4.56	121.39±4.43 (04.71%)	116.25±5.39 (13.39%)	110.23±6.29 (21.05%)
Car+Nn_{500}	109.29±3.46	122.23±5.66	125.25±4.53 (01.67%)	130.24±6.39 (02.97%)	134.26±4.78 (03.10%)
Car+Nn_{750}	105.56±5.36	117.23±4.47	122.2339±4.40 (04.04%)	125.25±5.56* (06.69%)	121.23±6.12* (12.50%)
Car+Nn_{1000}	108.26±5.83	120.23±4.49	122.36±4.55 (03.94%)	118.32±4.26* (11.85%)	113.46±4.16* (18.11%)

The above Table 4 illustrates the rise in paw volume following the administration of carrageenan (Car) and the subsequent decrease in paw volume following the treatment of paw edema with Ibuprofen (Ib) and Nelumbo nucifera (Nn).

Before carrageenan induction, the paw volume in each group ranged from 104 to 113 ml. And following the administration of carrageenan for an hour, the amount rose to between 114 and 125

ml. After 4 hours, volume in the untreated group (Car) increased by as much as 138 ml. However, in the Ibuprofen-treated group (Car+Ib_{10}), the volume non-significantly reduced near the initial range after 2 hours. But after 3 hours and 4 hours of Ib therapy, it decreased significantly giving anti inflammatory effect compared to untreated carrageenan induction group.

In the N. nucifera treated groups, at low dose (**Car+Nn_{500}**) the volume decreased non-significantly to some extent in comparison to the untreated group. However, a moderate (750mg) dose of Nn resulted in significant decreases of 6.69% after 3 hours and 12.50% after 4 hours. In the case of high dose (**Car+Nn_{1000}**) the decrease in volume of paws was highly significant resulting in 11.85% after 3 hours and 18.11% after 4 hours. Hence, N. nucifera treated group upon carrageenan induction statistically resulting in significant decrease (maximum 18.11% on 1000mg dose) in paw volume giving anti inflammatory activity was not as much as Ibuprofen treated group.

2. **Analgesic Activity of *Nelumbo nucifera:***

Writhing Test:

The result of the acetic acid writhing test is shown above in table 5. The table illustrates that the number of writhing is highest (88.24) in the Nn-treated (Nn_{500}+Acetic Acid) group . In the Aspirin-treated group, the number reduced by 38.34% whereas in Nn-treated groups, the number reduced by 13.96-29.25% in a dose-dependent manner. Thus, all the Nn-treated groups showed statistical significance in low (Nn_{500}+Acetic Acid), moderate (Nn_{750}+Acetic Acid), and high (Nn_{1000}+Acetic Acid) doses in reducing the number of writhing and high dose of 1000mg showed the highest reduction. Therefore, all the Nn-treated groups had better analgesic effect than the Aspirin-treated group in the reduction of number of writhing in acetic acid induced rats.

Table 5: Writhing test (*presents the level of significance of result)

Group specification	Dose	Number of writhing	% Inhibition
Ace	N/A	102.56±6.23	N/A
As_{100}+Acetic Acid	100	63.23±4.25	38.34%
Nn_{500}+Acetic Acid	500	88.24±6.99*****	13.96%
Nn_{750}+Acetic Acid	750	81.26±3.79*****	20.76%
Nn_{1000}+Acetic Acid	1000	72.56±5.29*****	29.25%

Tail Flick Test (TFT) :

The result of the Tail Flick test is shown in table 6. Here we can see that all the groups treated with Nn significantly increased the pain threshold of rats in a dose-dependent manner and the effect was also more than the standard drug, Aspirin. However, the high dose (1000mg) achieved the maximum statistical significance reaching the highest pain threshold thus giving analgesic effect. Hence, N. nucifera maximizing the pain threshold provides better analgesic effect compared to Aspirin.

Table 6: Tail Flick Test (*presents the level of significance of result)

Group No	**Group Specification**	**Basal Reaction**	**Reaction time in second**			
			After 30 minutes	**After 1 Hour**	**After 2 Hour**	**After 4 Hour**
1	TFT	2.80±0.77	2.40±0.95	3.36±0.82	3.39±0.65	4.50±0.38
2	As_{100}+TFT	1.79±0.65	3.89±0.92 -62.08%	4.38±0.87 -30.35%	5.08±1.09* -50.29%	5.59±1.90 -24.22%
3	Nn_{500}+TFT	2.23±0.90	4.20±0.81* -75%	4.50±0.0.63* -33.92%	5.39±0.76* -58.99%	6.04±0.0.39* -34.55%
4	Nn_{750}+TFT	2.29±0.82	3.86±0.52* -60.83%	4.58±00.73* -36.30%	5.32±0.69* -56.93%	6.22±0.77* –38.22%
5	Nn_{1000}+TFT	2.60±0.87	4.08±0.50* -70%	4.89±0.93* -45.53%	5.83±1.29* -71.97%	6.77±1.23* –50.44%

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