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Original Article

Evaluation of phytochemical and pharmacological properties of seeds of *Nephelium lappaceum* L.

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Abstract: This study aimed to evaluate the antioxidant, analgesic, neuropharmacological, antihyperglycemic, anti-inflammatory, and antidiarrheal properties of the methanolic extract of *Nephelium lappaceum* L. seeds (MSNL). *In vitro* antioxidant was determined using the DPPH free radical scavenging test. Analgesic, antihyperglycemic, antidiarrheal, and anti-inflammatory activities were evaluated using the acetic acid-induced writhing test, oral glucose tolerance test, castor oil-induced diarrhea, and carrageenan-induced paw edema, respectively, using rodents. Neuropharmacological activity was investigated in mice using both open-field and hole-cross methods. MSNL demonstrated strong DPPH scavenging capacity (IC_{50} = 53.92 µg/mL) compared to standard ascorbic acid (IC_{50} = 41.10 µg/mL). In the acetic acid-induced writhing test, the highest dose (600 mg/kg) showed 59.89% inhibition of abdominal constrictions compared to indomethacin (81.97%). MSNL showed a significant ($P<0.05$, $P<0.01$) diminution in the locomotion of rodents in both open field, and hole cross methods at the highest dose compared to the control group. MSNL significantly reduced blood glucose levels in mice ($P<0.01$, $P<0.05$) in a dose-dependent manner. The highest dose 600 mg/kg of MSNL showed a significant 53.46% reduction in diarrhea. MSNL 600 mg/kg displayed significant inhibition of inflammation at the 3rd hour (65.22%). The findings demonstrated that the extract has potential bioactivities and can be considered as the benchmark for the isolation of pure bioactive compounds from this plant to discover new drugs.

Keywords: *Nephelium lappaceum L.;* Antioxidant; Analgesic; Neuropharmacological activity; Anti-inflammatory; Antihyperglycemic properly.

1. Introduction

Researchers are always looking for novel medications with enhanced or superior therapeutic effects. Medicinal plants are a great source of lead compounds for the development of novel, noble medications with comparatively few side effects or unfavorable effects [1, 2, 3]. Since ancient times, traditional medicines have been widely used for their reliability in treating various illnesses and sufferings in humans. Medicinal plants yield a variety of bioactive natural chemicals that are used as starting points for the development of novel drugs [4, 5, 6]. Different kinds of medicinal plants are used to synthesize phytochemicals, which are used in the treatment of various disorders. These phytochemicals include alkaloids, saponins, carbohydrates, glycosides, flavonoids, gums, steroids, tannins, phenolic compounds, volatile oils, etc. The potential health advantages of natural compounds with antioxidants, as well as their antibacterial, analgesic, neuropharmacological, anti-inflammatory, anticancer, and anti-diabetic properties, have received much attention in the past few years. As a result, there has

been a massive surge in studies on many medicinal plants to identify the key chemicals causing this pharmacological activity [3, 4, 5, 6].

The fruit known as rambutan, or *Nephelium lappaceum* L., is indigenous to tropical areas including Indonesia, China, India, Australia, Malaysia, Mexico, and Thailand. It belongs to the *Sapindaceae* family [7]. The fruit known as rambutan is also called hairy lychee since its name comes from the Malay-Indonesian word "rambut", which means hairy [8]. Its fruit is an ovoid berry with colors ranging from brilliant crimson, maroon, yellow, and orange-red [9]. Its leathery skin, about 3 mm thick, is completely covered in splinters ranging from 0.5 to 2.0 cm. The flesh has a sweet to slightly sour flavor and is juicy and translucent white in color. The almond line seed in the fruit core is rectangular, measuring 2.5–3.4 cm in length and 1.0-1.5 cm in breadth [10, 11]. There are several bioactive ingredients in the rambutan peel, seed, and pulp. Phenolic chemicals were the most significant phytochemical component examined. Among other phytoconstituents, the phytochemical investigations identified reducing sugar, monosaccharides, carbohydrates, phenols, proteins, tannins, alkaloids, flavonoids, steroids, saponins, and glycosides. Antioxidant, antibacterial, anticancer, antidiabetic, antiviral, anti-inflammatory, and antiproliferative actions are just a few of the medicinal and nutritional qualities of phytochemicals [12, 13, 14, 15].

Traditionally, not much thought has been given to how the seeds and rind, which are frequently the wasted portion of the fruits, may be recycled or utilized instead of being thrown away. Interestingly, some fruits' seeds and rinds contain more vitamins, fibers, minerals, and other vital components than the pulp parts [15, 16]. Because rambutan seeds don't contain any toxins and provide protein, fat, and carbohydrates that the body requires, they are safe to eat [16]. Rambutan seeds have a high polyphenol content. The chemicals known as polyphenols, which include anthocyanins, complex polyphenols, leucoanthocyanidin (3%), and catechin (3%), are highly astringent [16]. The primary components of polyphenols are flavonoids and tannins, which have been identified as ellagic acid, geraniin, and corilagin [14, 15, 16]. The various polyphenols, such as antioxidant, anti-inflammatory, anticarcinogenic, and other bioactivities, demonstrated suggest that they may have beneficial effects on human health and provide protection against such chronic diseases as cardiovascular diseases, neurodegenerative disorders, and cancers [16].

The literature review revealed that, despite the abundance of bioactive components, there had been relatively little research on *N. lappaceum* seeds. This motivated us to investigate the antioxidative, analgesic, neuropharmacological, antihyperglycemic, anti-inflammatory, and antidiarrheal properties of rambutan seeds and their methanolic extractive in a comprehensive manner, keeping in mind the need for natural mineral supplements and bioactive compounds from seeds on a global scale.

2. Materials and Methods

2.1 Collection, authentication, and extraction of plant sample

The Fresh ripe *N. lappaceum* fruits were collected from Dhaka district, Bangladesh, in April 2024, and were identified by a taxonomist from the Bangladesh National Herbarium in Mirpur, Dhaka (Accession no. DCAB 36575). After precise washing, seeds were separated from fruits. Following a week of drying in the shade, they were ground into a fine powder and kept in sealed containers in a dark, cold, and dry room until they were processed. Extraction was carried out based on the maceration method [17]. Five hundred grams of powdered sample was macerated in two liters (95%) of methanol for 14 days with random shaking and stirring. After two weeks, the entire mixtures were separately filtered using a clean cotton bed and Whatman filter paper number 1. The filtrate was concentrated to dryness in a rotary evaporator at 40°C under reduced pressure. The dried methanolic extract of *N. lappaceum* fruit (MSNL) was preserved in the laboratory to conduct *in vivo* and *in vitro* pharmacological experiments.

2.2 Chemicals

Diazepam, Indomethacin, Metformin, and Loperamide were generous gifts from Bangladesh's Square Pharmaceuticals Ltd. We bought DPPH, ascorbic acid, acetic acid, and methanol (95%) from Merc (Germany). Analytical grade materials were used for all other reagents in the investigations.

2.3 Experimental Animals

All *in vivo* pharmacological studies were conducted on young, healthy Swiss-albino mice aged 4-5 weeks (weight, 25-30 g) and rats aged 3-4 months (weight, 120–130 g) of both sexes. They were purchased from the Animal Resources Division of the International Center for Diarrheal Disease Research, Bangladesh (ICDDR, B). The rodents had free access to ICCDR, B-formulated rodent feed, and water while being housed in a conventional laboratory environment (room temperature of (25±1)°C, relative humidity of 56%–60%, and a 12-hr light/12-hr dark cycle). Prior to pharmacological investigations, all rodents were housed under the mentioned conditions for approximately a week to acclimate to the laboratory environment.The animal ethical committee of the Southeast University (Dhaka, Bangladesh) authorized each animal experiment protocol (SEU/Pharm/CERC/111/2023).

2.4 Phytochemical screening

Conventional techniques were employed to investigate whether the investigated extract included any distinct bioactive components [18, 19]. Color or foaming was visually examined to determine if a particular phytochemical group was present or missing [20].

2.5 Evaluation of antioxidant activity

The antioxidant activity of *N. lappaceum* methanol seed extract was assessed using a slightly modified version of the previously published quantitative DPPH-scavenging approach [21]. In summary, 3.0 mL of a methanolic solution (20 g/mL) containing DPPH was combined with plant materials at different concentrations (500.0 to 0.977 g/mL). After vortexing the reaction mixture, it was kept in the dark for half an hour. The absorbance of each mixture was then measured at 517 nm using a UV-Vis spectrophotometer. Using the following formula, the free radical quenching capacity was determined:

$$\textbf{\% of inhibition} = \frac{\mathbf{A_{blank} - A_{sample}}}{\mathbf{A_{blank}}} \times \mathbf{100\%}$$

In this equation, A is the absorbance for each group. The IC_{50} value (50 percent inhibition) for each plant sample was then determined using a graph of the percent inhibition of DPPH scavenging vs. concentration of the test materials.

2.6 Evaluation of analgesic activity

The acetic acid-induced writhing test was used to measure the peripheral analgesic activity in accordance with the Haque et al., 2020 approach [5]. The experimental mice were given 200, 400, and 600 mg/kg body weight of the plant sample in addition to standard indomethacin orally during this test. Each mouse was given an intraperitoneal injection of 1% acetic acid at a dose of 10 mL/kg body weight, forty minutes after starting the treatments in order to induce writhing (abdominal constrictions). Each animal's writhes were counted over the course of the following thirty minutes. The following equation was used to compute the proportion of the treated group's writhing inhibition:

$$\textbf{\% of inhibition of writhing} = \frac{\mathbf{N_{Control} - N_{Test}}}{\mathbf{N_{Control}}} \times \mathbf{100\%}$$

Where N represents the average number of writings for each group.

2.7. Evaluation of neuropharmacological activity

The neuropharmacological activity of the plant extract was evaluated using the open-field and hole-cross methods.

2.7.1 Open field method

The open field behavioral test is widely used to assess the emotional state and locomotor activity of rats [22]. With a few minor modifications, the test was carried out utilizing the methodology outlined in Moniruzzaman et al. [23]. A half-square-meter hardwood field with rows of squares painted in black and white alternately used as the open field device. It was kept in a dimly lit room with a wall 50 centimeters high. The mice were free to move about the center of the box throughout the duration of the three-minute pretreatment reading, and the number of squares they visited was recorded. After the reading was taken, the rats were given a vehicle, extracts (200, 400, and 600 mg/kg), or diazepam (1 mg/kg) therapy. After that, they were viewed on a regular basis at 30, 60, 90, and 120 minutes later.

2.7.2 Hole cross method

The most persistent behavioral shift occurs from an intense emotional response to a novel setting. The hole-cross test was carried out using the Shahed-Al-Mahmud and Lina [22] protocol. A cage measuring 30 cm × 20 cm × 14 cm, with a partition in the middle. The apparatus is constructed from hardwood planks. A 3 cm-diameter hole was drilled in the center of the cage at a height of 7.5 cm. After placing a mouse in the middle of the cage and administering oral treatments (vehicle, extracts, and standard), the number of mice that passed through the aperture connecting one chamber to the next was counted for three minutes at 0, 30, 60, 90, and 120 minutes.

2.8 Evaluation of Anti-inflammatory Activity

By slightly altering the technique of Mondal et al. [24], inflammation caused by carrageenan in rat paws was used to test the anti-inflammatory properties of MSNL. Five groups of five rats each were randomly assigned, while group I was kept as the control group and was given only distilled water. The conventional medicine, indomethacin (5 mg/kg), was administered to Group II. The test sample was administered to groups III, IV, and V at doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg body weight, respectively. Each rat received an injection of 1% carrageenan in saline into its left hind paw thirty minutes after the test materials were given orally. After carrageenan was administered, the amount of paw edema was measured using a water plethysmometer at 1, 2, and 3 hours. For comparison, the right hind paw was used as the non-inflamed reference. We computed the average percent increase in paw volume over time and compared the results to the control group. Percent inhibition was calculated using the formula:

$$\textbf{\% Inhibition of paw edema} = [(Vc - Vt) / Vc] \times 100.$$

Where Vc and Vt represent the average paw volume of the control and treated animals, respectively.

2.9 Hypoglycemic Test (Oral Glucose Tolerance Test-OGTT)

The Tesfaye et al. technique [25] was followed by an overnight fast (18 hours) before healthy mice were given the oral glucose tolerance test (OGTT). Five groups of mice, one for each sex, were randomly assigned. 1% tween 80 in normal saline, metformin, and extracts/fractions were given to the test, positive, and negative control groups, respectively. Mice in all test groups were given a 10% glucose solution orally, 30 minutes after the plant extract/drug was administered orally. A glucometer was then used to measure each animal's blood glucose levels at zero, 30, 90, and 150 minutes after the glucose load. The equation below can be used to calculate the percent drop in blood sugar level caused by *N. lappaceum* extract:

$$\textbf{\%reduction in blood glucose} = \frac{\mathbf{BGL_{control} - BGL_{test}}}{\mathbf{BGL_{control}}} \times 100$$

Here, BG is the average blood glucose level for each group.

2.10 Evaluation of Antidiarrheal activity

The method of Haque et al. [5] was followed for conducting the castor oil-induced diarrheal test. To put it briefly, 30 minutes after the proper doses and treatments were administered, each mouse was given 1 mL of castor oil orally to induce diarrhea. The mice were then kept in cages covered in absorbent materials for four hours in order to check for diarrhea, which is defined as sloppy, unformed feces. The control group's results were considered to be 100%. The percentage inhibition (%) of diarrhea was used to assess each group's performance. The percentage of defecation inhibition was estimated as follows:

$$\% \text{ Inhibition of defecation} = [(A\text{-}B)/A] \times 100$$

Where A represented the average number of feces induced by castor oil, and B represented the average number of feces caused by the drug or extract.

2.11 Statistical analysis

The findings were shown as mean ± SEM. The statistical analysis was carried out using the SPSS 26 program, and one-way analysis of variance (ANOVA) and Dunnett's post hoc test were utilized. Differences between groups were deemed significant at a level of $p < 0.01$ and $p < 0.05$.

3. Results

3.1 Preliminary phytochemical screening

Table 1 summarizes the findings of the phytochemical screening of MSNL extract. The crude methanolic extract showed the presence of numerous beneficial secondary metabolites. All of the examined phytochemicals had been identified in the experimented sample. Carbohydrate, glycoside, resin, steroid, terpenoids were the least abundant secondary metabolites among all the bio constituents while fixed oil, protein, phenol were the most prevalent the studied sample.

Table 1. Phytochemical screening test of methanolic extract of *N. lappaceum* seed

Phytocompounds	MSNL
Carbohydrate	+
Glycoside	+
Tannin	++
Protein	+++
Alkaloid	++
Saponin	-
Resin	+
Phenol	+++
Flavonoid	++
Steroid	+
Terpenoids	+
Fixed oil	+++

Here, "+" specifies the existence, and "-" shows the absence of any phytochemical group. Bioavailability key: (+++) ve = strong intensity, (++) ve = Moderate intensity, (+) ve weak intensity, (−) ve = Absence

3.2 DPPH radical scavenging assay

In the antioxidant experiment, the studied extract demonstrated concentration-dependent quenching characteristics against the DPPH radical. The ability of plant extracts to quench DPPH radicals is shown by the IC_{50} values (50 percent inhibition), which are displayed in **Table 2**. At every concentration point, ascorbic acid, a well-known antioxidant, had a higher level of free radical-scavenging activity than the plant extract. The normal ascorbic acid had an IC_{50} value of 41.10 µg/mL, while the seed methanol extract had an IC_{50} value of 53.92 µg/mL.

Table 2. DPPH scavenging capacity of MSNL extract

Sample	IC_{50} (µg/mL)
Standard (Ascorbic acid)	41.10
MSNL	53.92

3.3 Acetic acid induced writhing test

The tested extract considerably ($p<0.01$; $p<0.05$) reduced the number of writhes in mice in the acetic acid-induced writhing technique when compared to the negative control (**Table 3**) at all doses. Additionally, the reference medication, indomethacin, demonstrated a significant ($p<0.01$) antinociceptive activity compared to the negative control group. The MSNL extract showed a maximum percent of inhibition of 69.59% at the higher dose of 600 mg/kg.

Table 3. Effect of MSNL Extract on acetic Acid-induced writhing in mice

Group	Treatment	Number of writhing	% of inhibition
Negative control (I)	Tween 80 solution	30.83 ± 0.98	----------
Positive control (Standard) (II)	Indomethacin 10 mg/kg	6.33 ± 0.83**	84.11
III	MSNL 200 mg/kg	30.00 ± 0.33*	24.68
IV	MSNL 400 mg/kg	21.17 ± 1.33*	46.85
V	MSNL 600 mg/kg	14.50 ± 1.05**	69.59

The values are revealed as mean±SD (n=5); One-Way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.05, **P<0.01 significant compared to the negative control.

3.4 Neuropharmacological activity

3.4.1 Open field method

The outcomes of the open field test are given in **Table 4**. At dosages of 200, 400, and 600 mg/kg, MSNL significantly reduced the locomotor activity in mice ($p < 0.01$; $p< 0.05$), and this effect was noticed from the initial observation (0 min) period and persisted through the fifth observation period (120 min). From the second to the fifth observation, mice were given diazepam (1 mg/kg, i.p.) showed a noticeably reduced ability to move around.

Table 4. Neuropharmacological effect of *N. lappaceum* L. seed extract on mice in the Open Field method

Groups	Treatment	Number of movements				
		0 min	**30 min**	**60 min**	**90 min**	**120 min**
Negative control (I)	Tween 80 solution	152.4 ± 0.83	150.0 ± 0.63	152.2 ± 0.74	149.4 ± 0.66	150.6 ± 0.33
Positive control (Standard) (II)	Diazepam 1 mg/kg, i.p.	142.80 ± 0.84*	100.2 ± 0.53**	69.2 ± 1.3**	62.2 ± 0.83**	10.2 ± 0.66**
III	MSNL 200 mg/kg	136.33 ± 0.52	99.8 ± 0.54*	83.0 ± 1.64*	76.0 ± 0.89**	50.60 ± 1.58*
IV	MSNL 400 mg/kg	130.00 ± 1.52*	87.2 ± 1.48**	72.4 ± 1.14**	66.2 ± 1.648*	49.0 ± 1.87**
V	MSNL 600 mg/kg	129.6 ± 0.89*	76.8 ± 0.83	70.2 ± 1.30	62.2 ± 1.58**	40.0 ± 0.71*

The values are revealed as mean±SD (n=5); One-Way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.05, **P<0.01 significant compared to the negative control.

3.4.2 Hole cross method

The findings demonstrated that practically MSNL extract considerably (p 0.01; p 0.05) reduced the number of holes the mice crossed over time from their initial value. From the second to the fifth observation hour (30 to 120 min), at a dose of 600 g/kg, the locomotor activity of the experimental mice was noticeably decreased in the examined sample. With time, the CNS-depressant effects were significantly (p<0.01; p<0.05) diminished. The MSNL extract effectively reduced locomotor activity, and the results were significant compared to those obtained using diazepam as the reference medication (**Table 5**).

Table 5. Neuropharmacological effect of *N. lappaceum* L. seed extract on mice in hole cross test

Groups	Treatment	Number of movements				
		0 min	**30 min**	**60 min**	**90 min**	**120 min**
Negative control (I)	Tween 80 solution	28.0 ± 0.64	26.6 ± 0.80	29.2 ± 0.75	26.20 ± 0.49	26.8 ± 0.52
Positive control (Standard) (II)	Diazepam 1 mg/kg, i.p.	29.8 ± 0.75*	20.0 ± 1.40*	10.8 ± 0.75**	3.2 ± 0.40**	1.6 ± 0.80**
III	MSNL 200 mg/kg	30.4 ± 0.49*	25.2 ± 0.74*	20.2 ± 0.89*	15.60 ± 0.89	12.60 ± 0.89*
IV	MSNL 400 mg/kg	27.41 ± 1.02*	17 ± 1.66*	14.0 ± 0.97*	11.40 ± 0.49	9.40 ± 0.80**
V	MSNL 600 mg/kg	26.0 ± 1.02*	14.63 ± 0.48*	10.80 ± 0.49*	9.60 ± 0.80**	4.0 ± 0.63*

The values are revealed as mean±SD (n=5); One-Way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.05, **P<0.01 significant compared to the negative control

3.5 Anti-inflammatory activity evaluation

Table 6 displays the anti-inflammatory effect of the investigated plant extract in the rat paw edema technique caused by carrageenan. According to the current investigation, after three hours, MSNL (600 mg/kg dose) significantly reduced the amount of edema (56.52%). At three hours, the standard anti-inflammatory medication (Indomethacin 5 mg/kg dose) showed effective inhibition (71.01%).

Table 6. Anti-inflammatory effect of *N. lappaceum L*. seed extract in the rat paw edema technique

Groups	**Treatment**	**Paw volume (mm)**	
		Change in paw edema mean (mm)	**% Edema inhibition relative to control at 3rd hr**
Negative Control (I)	Normal saline 0.9% 0.3 ml	1.38 ± 0.05	---
Positive control (Standard) (II)	Indomethacin 5 mg/kg	0.4 ± 0.02**	71.01
III	MSNL 200 mg/kg	0.9 ± 0.05*	34.78
IV	MSNL 400 mg/kg	0.7 ± 0.02*	49.27
V	MSNL 600 mg/kg	0.6 ± 0.02*	56.52

The values are revealed as mean±SD (n=5); One-Way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.05, **P<0.01 significant compared to the negative control

3.6 Hypoglycemic test

From 30 minutes on, MSNL extract in the OGTT significantly reduced the plasma glucose levels (**Table 7**). In comparison to Metformin, the tested sample showed a substantial (p<0.01, p<0.05) and concentration-dependent glucose-lowering impact that continued for up to 150 minutes after the loading dosage.

Table 7. Effect of *N. lappaceum* L. seed extract on OGTT in healthy rodents

Groups	**Treatment**	**Blood glucose level in different time**			
		0 min	**30 min**	**90 min**	**150 min**
Negative control (I)	Tween 80 solution	8.32 ± 0.49	26.78 ± 0.48	20.5 ± 0.71	18.33 ± 0.62
Positive control (Standard) (II)	Metformin 10 mg/kg, i.p.	8.78 ± 0.63**	15.53 ± 0.67**	11.78 ± 0.77**	5.22 ± 0.89**
III	MSNL 200 mg/kg	9.58 ± 0.58	22.67 ± 0.69*	17.28 ± 0.38*	14.55 ± 0.25*
IV	MSNL 400 mg/kg	9.67 ± 0.66	19.78 ± 0.69**	12.27 ± 0.29**	10.95 ± 1.16**
V	MSNL 600 mg/kg	9.3 ± 0.36	16.27 ± 0.53	10.10 ± 0.35	9.2 ± 1.38*

The values are revealed as mean±SD (n=5); One-Way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.05, **P<0.01 significant compared to the negative control

3.7. Antidiarrheal activity evaluation

At all tested doses, MSNL was found to be efficacious in a dose-dependent manner in treating experimental mice's diarrhea caused by castor oil. The extract significantly reduced the amount of diarrhea in albino mice, measured by the defecation rate and feces' consistency, at all doses (200, 400, and 600 mg/kg bodyweight). Compared to the standard drug Loperamide, which showed an 80.32% suppression of diarrhea, the highest dose of the extract (600 mg/kg) demonstrated a 59.03% inhibition of diarrhea (**Table 8**).

Table 8. Effect of MSNL extract at various dose levels on castor oil-induced diarrhea in mice

Groups	Treatment	Total no. of feces in 4 hours	% of inhibition
Negative control (I)	1% Tween 80 solution	20.33 ± 1.21	----------
Positive control (Standard) (II)	Loperamide 2 mg/kg	4.0 ± 0.63**	80.32
III	MSNL 200 mg/kg	15.56 ± 0.75*	21.84
IV	MSNL 400 mg/kg	12.16 ± 1.05*	40.19
V	MSNL 600 mg/kg	8.33 ± 0.84*	59.03

The values are revealed as mean±SD (n=5); One-Way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.05, **P<0.01 significant compared to the negative control

4. Discussion

The use of medicinal plants has emerged as a fascinating avenue for the development of traditional and modern medications, and research has demonstrated the actual medical benefits of herbal medicines [26, 27, 28]. Our current study's objective was to look into the general *in vitro* and *in vivo* bioactivities of *N. lappaceum* L. seeds. The biological effectiveness of medicinal plants is mainly dependent on their phytochemical content. A key factor in the discovery of novel, uncommon, and active chemicals is phytochemical analysis. The existence of secondary metabolites in plants is associated with their biological significance [27]. The crude seed extract of the experimental plant exhibited the presence of numerous valuable secondary metabolites such as alkaloids, glycosides, tannins, reducing sugars, steroids, fixed oil, terpenoids, flavonoids, and phenols (**Table 1**). *N. lappaceum* L. plant parts are already reported as an ailment for various diseases in the traditional system [12, 13, 14, 15]. The seed of the plant is reported to have a diverse nature of compounds, including anthocyanins, complex polyphenols, leucoanthocyanidin, catechin, ellagic acid, geraniin, corilagin, etc. [14, 15, 16].

The most used technique for assessing the antioxidant capacity of plant materials is the DPPH scavenging assay. Based on the ability of 1, 1-diphenyl-2-picrylhydrazyl (DPPH), a persistent free radical that decolorizes in the presence of antioxidants, the DPPH antioxidant experiment was carried out [24]. The results of the DPPH scavenging test suggest that the plant may contain active ingredients that, because of their redox characteristics, exhibit antioxidant activity and are essential for absorbing and neutralizing free radicals. Using DPPH, we found, as previously reported by Mohan et al. [29], a direct correlation between dose and radical quenching potential in MSNL extract. Our study was able to show that the extract has a considerable scavenging effect in a dose-dependent manner, even if it is less active than ascorbic acid (IC_{50} 41.10µg/mL) (IC_{50} 59.92 µg/mL; **Table 2**). Free radicals are recognized to have a significant impact on a wide range of clinical symptoms. This becomes well-known when an excess of it occurs in a living organism and causes oxidative damage. It also weakens the body's defenses against disease, leading to the manifestation of a range of ailments such as ageing, cancer, Alzheimer's, atherosclerosis, angina pectoris, metabolic disorders, Parkinson's, complications from diabetes, rheumatoid arthritis, etc. Free radical-squelching antioxidants are essential for treating this pathological condition. Because of this, there is a growing interest in creating natural antioxidants derived from plants that can shield the body from oxidative damage brought on by free radicals [5, 30]. Previous studies have demonstrated the importance of bioactive phytochemical components, especially phenolic compounds (flavonoids, phenolic acids, and tannins), for plants' ability to scavenge free radicals and act as antioxidants (these compounds were also found in our studied extracts; **Table 1**) [2, 30, 31].

Acetic acid is the primary inducer of pain in an animal model, and the acetic acid-induced writhing response technique is a commonly used approach to assess the peripheral analgesic activity of any plant portion [32]. The acetic acid-induced writhing test is a useful paradigm for evaluating the peripheral analgesic potentials of test compounds because of its sensitivity and capacity to identify antinociceptive effects of natural products and test compounds at dose levels that are inert for other techniques [33]. Acetic acid injected intraperitoneally stimulates and irritates the peritoneal cavity, which in turn causes the production and release of a number of endogenous inflammatory mediators, including histamine, serotonin, bradykinin, substance P, and PGs [34, 35]. These different endogenous inflammatory mediators produced chemically induced visceral discomfort, which is characterized by the body lengthening and the forelimbs extending together with the abdominal muscles contracting. The acetic acid-induced writhing test is regarded as a model of visceral pain because of this [35], and furthermore, linked to elevated PGE and PGF2α levels in this model. Raising PG levels in the peritoneal cavity increases the intensity of inflammatory pain by activating primary afferent nociceptors and widening capillary permeability [33, 35]. When compared to the negative control, MSNL extract at all three dosages (200, 400, and 600 mg/kg) significantly ($p < 0.05$ and $p < 0.01$) demonstrated peripheral analgesic effects by lowering the number of writhing (**Table 3**) with respective values of 24.68%, 46.85%, and 69.59%. These results demonstrated that the extract's dosage-dependent peripheral analgesic effectiveness rose from the lower dose (200 mg/kg) to the higher dose (600 mg/kg). A rise in concentration of phytoconstituents that exhibit analgesic activity at the highest dose may be the cause of the extract's increasing analgesic effect with higher doses. The extract may have inhibited the synthesis and release of different endogenous inflammatory mediators as well as the sensitivity of peripheral nociceptors in the peritoneal free nerve endings to chemically induced pain, which could be the mechanism by which it produced peripheral analgesia in this model. These suggested pathways are consistent with the guiding principles, which claim that any substance that reduces the amount of writhing will exhibit analgesia by preventing the production and release of PGs and by preventing the transmission of pain to the peripheral areas [32, 33, 34, 35].

Mice's naturalistic locomotor behavior was used to study the CNS depressed effect of MSNL extract using two neuropharmacological models: open field and hole cross. These paradigms represent established conventional approaches to investigating neuropharmacological activity [35, 36]. The investigated extracts significantly induced substantial locomotor inhibition at the tested doses, and in both tests, this effect persisted for 30 to 120 minutes during the research period (**Tables 4 and 5**). Reduced locomotor activity is thought to be an indicator of awareness and is a symptom of CNS-depressing activity [37]. The CNS-depressing impact of the plant extract may be responsible for this decrease in spontaneous motor activity [38]. In the brain and central nervous system, GABA is an essential inhibitory neurotransmitter that plays a role in physiological processes related to neurological and psychiatric disorders [39]. Many medications can modify the GABA system at the molecular level by increasing GABA-mediated postsynaptic inhibition through an allosteric modification of GABA receptors. It can either improve chloride conductivity or increase GABA-induced chloride conduction when the voltage-triggered Ca2+ channel is blocked. Therefore, the extracts are probably going to cause a decrease in the firing rate of important brain neurons by either directly activating GABA receptors or amplifying GABAergic suppression in the CNS through membrane hyperpolarization [36, 38]. Prior studies on phytochemicals indicate that flavonoids, neuroactive steroids, triterpenoids, and saponins have agonistic effects at the GABA-A receptor complex. The depressive activity of the extracts is caused by the secondary metabolites (flavonoids, terpenoids, saponins, etc.) of the plant, which may have synergistic effects at one or more target sites related to a physiological function [36, 37, 38, 39]. These phytoconstituents may play a role in the CNS depressive effects observed in mice. Further investigation is required to pinpoint the specific phytoconstituents responsible for the neuropharmacological activities and the corresponding mechanisms of action.

An *in vivo* experimental model of acute inflammation known as carrageenan-induced paw edema has been widely used to assess the anti-inflammatory properties of novel investigational medicines. The urge to concentrate on herbal drugs with fewer side effects arises from the rise in the use of synthetic drugs and their associated adverse effects. According to the study's findings, methanolic seed extract of *N. lappaceum* L. has a strong anti-inflammatory effect on rats' paw edema caused by carrageenan. It is thought that rats' paw edema caused by carrageenan is biphasic. Bradykinin, protease, prostaglandin, and lysosome release are the causes of the second

phase, whereas histamine or serotonin release is responsible for the first. Other chemical mediators, such as hydroxyl radicals (OH^-) and oxygen-derived free radicals like superoxide anion (O^{2-}), are also generated during the late phase of inflammation and are crucial to the onset and development of acute inflammation [32, 33]. Beginning one hour after carrageenan induction, the extract at all test dosages used (200, 400, and 600 mg/kg) significantly ($p < 0.05$ and $p < 0.01$) reduced the formation of edema, and the effects lasted until the 150 minutes of observation. The extract's effects persisted from the first phase of inflammation, which lasted for one hour, until the second phase, which lasted three hours. This observation implied that the extract's bioactive components might inhibit the release and/or activation of chemical mediators, hence suppressing both stages of acute inflammation. At the third time of observation (150 minutes), the most significant percentage of edema inhibition by all extract doses was noted, with the corresponding values being 34.78%, 49.27%, and 56.52% (**Table 6**). These results confirmed that the extract has a dose-dependent anti-inflammatory effect. At the 3rd period of observation, the edema inhibition potential exhibited by the larger dose of the extract (600 mg/kg) exhibited good inhibition in comparison to that of the standard drug (Indomethacin 5 mg/kg), with respective values of 56.52% and 71.01%. Since endogenous inflammatory mediators like serotonin and histamine are involved in the early phase of inflammation, the extract and standard medication, indomethacin 5 mg/kg, both demonstrated significant anti-inflammatory effects. Additionally, the extract's effects on edema inhibition peaked at the third time point, suggesting that both the extract and the standard medication have potent anti-inflammatory effects against a variety of endogenous inflammatory mediators that involve in the late phase of inflammation such as, COX, different PG analogues, BK and/or leukotriene or they could have, free radical scavenging activities [33, 34, 35]. It follows that the MSNL extract may have inhibited carrageenan-induced inflammation by inhibiting the enzyme cyclooxygenase, which in turn may have inhibited the manufacture of prostaglandins.

A drug that effectively cures diabetes will be able to control the rise in blood sugar through several different pathways, and a glucose-loaded hyperglycemic mode may be used to evaluate an extract's potential to prevent hyperglycemia. The OGTT measures the speed at which the body can remove exogenous glucose from the blood after it has been eaten and is a commonly used test to diagnose diabetes mellitus [40, 41, 42]. This method is called physiological induction of diabetes mellitus [41] because it momentarily raises the animal's blood glucose level without harming the pancreas. Tracking changes in blood glucose levels in response to an oral glucose challenge is a common use for it [40]. During the glucose tolerance test, the crude MSNL extract showed a considerable hypoglycemic action ($p < 0.05$, $p < 0.01$; **Table 7**) in mice compared to the reference metformin, which lasted up to three hours. It is believed that secondary metabolites (tannin, flavonoids, phenol, sterol, etc.) enhance regulatory systems by an action akin to that of insulin, most likely by raising peripheral glucose consumption or cell glucose sensitivity [5, 40, 41]. We also validated the presence of hypoglycemic terpenoids, flavonoids, and tannins in our qualitative phytochemical screening of MSNL extract (**Table 1**). These compounds may work alone or in combination to lower blood glucose levels.

One of the most widely used methods for *in vivo* studies of the antidiarrheal properties of medicinal herbs is castor oil, which is used to cause diarrhea in animals. By promoting intestinal peristalsis and obstructing the absorption of fluids and electrolytes, castor oil causes diarrhea [5, 43]. Thus, the most crucial aspect of managing diarrhea is preventing castor oil-induced diarrhea. The crude extract of *N. lappaceum* L. seeds was administered, and the number of wet feces decreased, and the beginning of diarrhea was significantly ($p < 0.05$, $p < 0.01$) delayed. These results suggest that the extract had an antidiarrheal effect at the test dosages used. The ability of MSNL extract to enhance the gastrointestinal tract's absorption of fluids and electrolytes is one possible explanation for its anti-diarrheal properties. The antidiarrheal properties of crude extract (ME) and solvent fractions may be attributed to phytochemicals such as alkaloids, tannins, saponins, phenols, terpenoids, and flavonoids, per results from many investigations [5, 43, 44]. The reduction of total feces, including the wet and watery components, implies that an antisecretory mechanism may be involved in the antidiarrheal effect of the MSNL extract. Moreover, the examined extract's antidiarrheal properties may be explained by its inhibition of nitric oxide and platelet-activating factor synthesis [45, 46]. In all of the models used in this investigation, the MSNL demonstrated antidiarrheal action by lowering castor oil-induced diarrhea. The quantity and weight of wet and watery fecal matter were significantly reduced ($p < 0.05$, $p < 0.01$), and the onset of diarrhea was delayed. The most plausible explanation could be the presence of different phytochemicals in MSNL extract [47, 48]. For

instance, the antidiarrheal effect of flavonoids and phenolic compounds is probably due to their antioxidant characteristics [5, 43, 44, 45, 46, 47, 48, 49]. These phytochemicals may reduce the amount of fluid produced by castor oil by blocking enzymes or slowing the metabolism of arachidonic acid [48, 49, 50]. There have been prior reports of the anti-diarrheal effects of tannins and saponins. Furthermore, the results of this investigation are in line with previous studies on a variety of crude plant extracts that demonstrated dose-dependent antidiarrheal properties [5, 43, 44, 45, 46, 47, 48, 49, 50]. These might be explained by the fact that the test plants included a wide variety of phytochemicals.

5. Conclusions

Throughout human history, customary herbal remedies have been utilized to both prevent and cure a diverse array of ailments. Researchers are reportedly considering the development of plant-based medications as a major and demanding area of attention by considering the therapeutic benefits of herbs. The secondary bioactive metabolites found in large quantities in *N. lappaceum* L. seeds, including glycosides, alkaloids, tannin, flavonoids, terpenoids, resin, and others, have been shown to have a range of health benefits, including analgesic, anti-inflammatory, CNS depressant, hypoglycemic, and antidiarrheal properties. There is an instantaneous, widespread, and statistically significant effect at every experimental dosage that is investigated. Our research leads us to conclude that pharmaceutical companies may succeed in developing new, safer, more effective, and less toxic candidate drugs from the seeds of *N. lappaceum* L., hence reducing the cost of treating sickness. We will conduct an additional study to identify the bioactive compound(s) and comprehend the exact molecular mechanisms to develop a safe and effective dosage and confirm the likelihood of its usage in the prevention and treatment of various diseases.

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