

ORIGINAL RESEARCH ARTICLE

Evaluation of antimicrobial, analgesic, and hypoglycemic activities of *Commelina diffusa* (Commelinaceae)

 Tasnia Islam Anika¹, Md. Monirul Islam², and Mohammad Shawkat Ali^{1*}
¹Department of Clinical Pharmacy & Pharmacology, University of Dhaka, Dhaka, Bangladesh

²Department of Pharmacy, Noakhali Science and Technology University, Noakhali, Bangladesh

Abstract

Commelina diffusa, also known as the climbing dayflower or spreading dayflower, is an herbaceous plant from the Commelinaceae family, found throughout tropical regions, including Bangladesh. The crude methanol extract and different fractions of *C. diffusa* were evaluated for their antimicrobial, analgesic, and hypoglycemic activities. The whole plant was extracted with methanol by the cold extraction method. The concentrated extract was then partitioned into petroleum ether- and chloroform-soluble fractions. The antimicrobial test was performed using the disc diffusion method. The analgesic effects were evaluated through both writhing and tail-flick tests at doses of 100 and 200 mg/kg body weight. The oral glucose tolerance test (OGTT) was performed to observe the hypoglycemic effect. The chloroform and methanol soluble fractions showed potent antimicrobial activity against Gram-positive bacteria and fungi. Statistical evaluation of the tail-flick test confirmed that the chloroform soluble fraction (100 mg/kg body weight) of *C. diffusa* had a significant amount of central analgesic activity ($p < 0.001$). The petroleum ether soluble fraction showed significant central analgesic activity only at higher doses ($p < 0.01$; 200 mg/kg body weight). The acetic acid-induced writhing test also confirmed the peripheral analgesic activity of the samples. The maximum inhibition was noted for the chloroform soluble fraction (64.56%), followed by crude methanolic extract (56.96%) and petroleum ether soluble fraction (53.16%). However, all the extracts showed no significant hypoglycemic activity in the OGTT. This observation, derived from an acute model of non-diabetic animals, does not preclude the possibility of antidiabetic effects in disease-related conditions. Further investigation is warranted to explore the specific metabolites and their pharmacological activities in relevant disease models.

*Corresponding author:

 Mohammad Shawkat Ali
 (drshawkat@du.ac.bd)

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1. Introduction

Commelina diffusa (Family: Commelinaceae) is a herb traditionally used as a diuretic, blood coagulant, antidote for different poisons, heart tonic, anti-diarrheal, anti-inflammatory, and antipyretic across the world.¹ The Commelinaceae, or the spiderwort family, is one of the five families within the order Commelinales and comprises about

40 genera and 640 species.² *C. diffusa* is typically an annual herb with a wide range of medicinal applications.³

The increasing prevalence of antimicrobial resistance poses a significant global health threat, rendering many conventional antibiotics ineffective against resistant bacterial strains. Overuse and misuse of antibiotics in medicine and agriculture have accelerated the emergence of multidrug-resistant pathogens, leading to higher treatment failures, prolonged illnesses, and increased mortality rates.^{4,5} The slow pace of new antibiotic development further exacerbates this crisis, necessitating the exploration of alternative therapeutic agents. Natural products, particularly medicinal plants, offer a promising source of novel antimicrobial compounds due to their diverse bioactive constituents, such as alkaloids, flavonoids, and terpenoids; these compounds often act through multiple mechanisms, reducing the likelihood of resistance.⁶ In addition, plant-derived antimicrobials often exhibit synergistic effects, enhancing therapeutic efficacy while reducing side effects compared to synthetic drugs.^{7,8}

Pain is an unpleasant sensory and emotional experience that can originate from various parts of the body and different causes. Usually, opioids and non-opioid drugs are used for pain relief. In most instances, these analgesic drugs can only relieve half of the pain in only 30% of patients. Furthermore, many of these drugs may cause serious side effects.⁹ Medicinal plants may serve as a natural source of potent analgesic drugs with fewer side effects.¹⁰ Diabetes mellitus is an endocrine disorder characterized by aberrant blood glucose levels. The complexity of its pathophysiology necessitates numerous approaches for effective management.¹¹ Traditional antidiabetic medications work well, but they also have inevitable adverse effects. Medicinal plants might serve as an alternative source of antidiabetic substances.¹²

Several species of the Commelinaceae family have demonstrated significant bioactive potential, including antimicrobial, anti-inflammatory, and antidiabetic properties.¹³ Despite the extensive traditional use of *C. diffusa*, scientific validation of its pharmacological effects remains limited. Previous studies have reported the presence of flavonoids, alkaloids, tannins, and phenolic compounds in *C. diffusa*, all of which are associated with diverse biological activities.¹⁴ This study aims to evaluate the antimicrobial, analgesic, and hypoglycemic activities of *C. diffusa* to provide scientific evidence supporting its ethnomedicinal uses. The findings could contribute to the development of alternative treatments for infections, pain management, and diabetes, addressing the need for safer and more accessible therapeutic options.

2. Materials and methods

2.1. Chemicals

All solvents used in this study, including methanol (Merck, Germany), petroleum ether (Merck, Germany), chloroform (Merck, Germany), and Tween-80 (Merck, Germany), were of analytical grade. Morphine (Morphine G) was purchased from Gonoghahsto Pharmaceuticals (Bangladesh), while diclofenac sodium and glibenclamide were gifts from a renowned pharmaceutical industry.

2.2. Preparation of plant extract and partitioning

The whole plant of *C. diffusa* was thoroughly cleaned, chopped into tiny pieces, and allowed to air dry for a few days. Following a 24-h oven drying, the fragments were ground into a coarse powder using a high-capacity grinding machine. Approximately 800 g of the powdered material was cold-extracted using methanol, yielding 65 g of crude extract. Solvent-solvent partitioning was subsequently carried out utilizing the Kupchan method as modified by Van Wagenen.¹⁵ Five grams of the crude methanolic extract were dissolved in 10% aqueous methanol and successively extracted using petroleum ether and chloroform.

2.3. Antimicrobial activity

The antimicrobial activity was assessed by evaluating the ability of the test agents to stop the microbial growth surrounding the discs, resulting in distinct zones of inhibition.¹⁶ Kanamycin (30 µg/disc; Bioprom, Greece) was used as the reference antibiotic.¹⁷ After incubation, the diameter of the zones of inhibition was measured in mm using a scale and compared to the zones generated by the reference discs.

2.4. Experimental animal

Swiss albino mice of either sex, aged 4–5 weeks, were acquired from Jahangirnagar University. They were fed with water and rodent food formulated by ICDDR, B, and maintained in standard environmental conditions. The study was reviewed and approved by the Institutional Ethical Review Board (Reference number: DoP/RC/EC/2022/01/01).

2.5. Central analgesic activity

Using morphine as a positive control, the tail flicking method was used to assess the central analgesic activity.^{18,19} In this method, thermal stimulus is applied to the mouse tail, and the response time until tail withdrawal is recorded as a measure of analgesic effect.²⁰ Forty mice were randomly assigned into eight groups ($n = 5$ per group). The groups received the following treatments: control (normal

saline, 10 mL/kg body weight, orally), standard (morphine, 2 mg/kg body weight, intraperitoneally), crude methanolic extract of *C. diffusa* (100 and 200 mg/kg), chloroform-soluble fraction (100 and 200 mg/kg), and petroleum ether-soluble fraction (100 and 200 mg/kg). At time zero, the test samples and control treatments were administered orally using a feeding needle. Tail-flick latency was recorded at 30 and 60 min post-administration for each mouse, and the average latency for each group was calculated.

2.6. Peripheral analgesic activity

The acetic acid-induced writhing method was used to assess the peripheral analgesic efficacy.²¹ Pain was induced in the experimental animals via intraperitoneal injection of acetic acid, leading to writhing behavior, defined as contraction of the abdomen and elongation of the body. The analgesic effect of the test samples and the control was assessed by comparing their writhing inhibition relative to the standard drug. A total of 40 mice were randomly divided into treatment and control groups as described in Section 2.5. However, the standard group received diclofenac sodium 10 mg/kg BW. At time zero, the mice received the respective treatments orally through a feeding needle. After 40 min, all animals were administered 1% acetic acid intraperitoneally. Five minutes post-injection, the number of writhes was recorded for each mouse over a 10-min period. The inhibition of writhing was calculated as a percentage using Equation I.

$$\% \text{Inhibition} = \frac{\text{Average writhing of control} - \text{Average writhing of sample}}{\text{Average writhing of control}} \times 100 \quad (I)$$

2.7. Oral glucose tolerance test (OGTT)

OGTT is one of the best ways to assess hypoglycemic activity. The test measures the rate at which glucose is removed from the circulation following administration.²² A total of 25 experimental animals were chosen at random and split into five groups (*n* = 5 per group): Control, standard, methanol extract, petroleum ether-soluble fraction, and chloroform-soluble fraction. Each mouse was accurately weighed before treatment to ensure accurate dosage adjustment. The standard group received glibenclamide (5 mg/kg body weight),²³ the control group received 1% Tween-80 in normal saline, and the other three groups were given 200 mg/kg body weight of crude methanol extract, petroleum-ether soluble fraction, and chloroform soluble fraction of *C. diffusa*. All the doses were administered orally at zero hour. After 30 min, all

animals were treated with a 10% glucose solution (2 mg/kg body weight; Eskayef Pharmaceuticals Ltd., Bangladesh). After 30, 90, and 150 min, blood was collected by pricking the tail vein, and the glucose level was measured using a glucometer (Get Well Limited, Bangladesh).²⁴

2.8. Statistical analysis

Data were analyzed using one-way analysis of variance followed by Tukey’s *post hoc* test. All statistical analyses were performed using GraphPad Prism version 8.0.2 (GraphPad Software, USA).

3. Results

3.1. Antimicrobial results

The methanol, petroleum ether, and chloroform-soluble fractions of *C.diffusa* exhibited low to moderate antimicrobial activity against most of the tested organisms (Table 1). At a concentration of 200 µg/disc, the zone of inhibition generated by each of these four extracts varied from 7.0 mm to 14.0 mm, while the standard disc of kanamycin (30 µg/disc) produced 17–27 mm zones. The highest antibacterial activity was noted for the chloroform extract against *Bacillus subtilis*.

These results suggest that the chloroform and methanol extracts of *C. diffusa* have antimicrobial activity, supporting its traditional medicinal use. Our result aligns with previous findings on the antimicrobial potential of this plant.²⁵ While the inhibitory effects were not as potent as standard antibiotics, the observed zones of inhibition suggest that the plant possesses bioactive compounds with

Table 1. Zone of inhibition (mm) exhibited by different fractions of *Commelina diffusa*

Test microbes	ME (200 µg/disc)	PEF (200 µg/disc)	CF (200 µg/disc)	Kanamycin (30 µg/disc)
Gram-positive bacteria				
<i>Bacillus cereus</i>	7	11	8	19
<i>Bacillus subtilis</i>	10	11	14	27
<i>Staphylococcus aureus</i>	8	10	9	22
Gram-negative bacteria				
<i>Escherichia coli</i>	7	9	9	19
<i>Pseudomonas aeruginosa</i>	8	8	9	17
Fungi				
<i>Aspergillus niger</i>	9	10	10	22
<i>Candida albicans</i>	-	10	9	17
<i>Saccharomyces cerevisiae</i>	9	8	9	25

Abbreviations: CF: Chloroform fraction; ME: Methanol extract; PEF: Petroleum ether fraction.

antimicrobial properties. However, the relatively lower efficacy compared to conventional antibiotics may be due to variations in extraction efficiency, the concentration of active compounds, or synergistic interactions among phytochemicals that were not fully optimized in this study.

3.2. Central analgesic activity

Tail flick latency was recorded at 30 and 60 min after sample administration, and the results are presented in Table 2.

The petroleum ether and chloroform-soluble fractions of *C. diffusa* significantly increased tail flick latency, indicating central analgesic activity. A consistent increase in latency was observed across the test groups, suggesting dose-dependent analgesic effects. These findings support the hypothesis that *C. diffusa* possesses central analgesic properties, in line with earlier studies on the plant.²⁶ The analgesic effect is likely due to the presence of bioactive constituents such as flavonoids, alkaloids, and terpenoids, which have been previously reported in *C. diffusa*.¹⁴ The time-dependent response implies progressive absorption and distribution of active compounds, resulting in sustained analgesic effects. Further investigations are warranted to elucidate the exact mechanisms of action and therapeutic potential.

3.3. Peripheral analgesic activity

The peripheral analgesic activity of the various extracts was evaluated using the acetic acid-induced writhing test. Test

samples were administered at doses of 100 and 200 mg/kg body weight. Five minutes after acetic acid administration, each mouse was observed for a 10-min period, during which the number of writhes was recorded. The results are shown in Figure 1.

A remarkably strong analgesic effect was demonstrated by all *C. diffusa* extracts tested, with higher inhibition of writhing compared to the standard reference drug, diclofenac sodium. The crude methanol extract and the chloroform-soluble fractions at 200 mg/kg produced maximum analgesic activity, showing inhibition rates of 56.96% and 64.56%, respectively. The petroleum ether fraction also demonstrated analgesic activity comparable to the standard. This finding is particularly significant, as diclofenac is a well-established non-steroidal anti-inflammatory drug (NSAID) used for pain management. The superior analgesic activity of *C. diffusa* suggests the presence of potent bioactive compounds that may act through mechanisms distinct from or complementary to those of conventional NSAIDs. The strong analgesic effect could be mediated through multiple pathways, including inhibition of prostaglandin synthesis, modulation of opioid receptors, and influence on both peripheral and central pain pathways.^{27,28} Similar analgesic effects have been reported for *Commelina benghalensis* using both the tail flicking and writhing methods.^{29,30}

While the methanol extract showed less potency than morphine, its effect was comparable to NSAIDs. This indicates that *C. diffusa* may offer a safer alternative for pain management with fewer side effects, such as gastrointestinal irritation or addiction risk.¹⁰

Table 2. Tail flicking time for evaluating central analgesic activity of *Commelina diffusa* fractions in mice

Group	Treatment	Dose (mg/kg)	Tail flicking time (mean±SEM) (s)	
			After 30 min	After 60 min
CTL	Normal saline	10 mL	0.698±0.039	0.762±0.097
STD	Morphine	2	1.616±0.223*	1.688±0.201**
ME I	Methanol extract	100	1.006±0.120	0.914±0.109
ME II	Methanol extract	200	0.850±0.034	1.064±0.096
PEF I	Petroleum ether soluble fraction	100	1.128±0.195	0.896±0.064
PEF II	Petroleum ether soluble fraction	200	1.878±0.353**	0.888±0.097
CF I	Chloroform soluble fraction	100	1.262±0.228	1.998±0.250***
CF II	Chloroform soluble fraction	200	1.342±0.167	1.320±0.116

Note: Statistical significance was calculated using one-way ANOVA followed by *post-hoc* Tukey's HSD test. **p*<0.05, ***p*<0.01, and ****p*<0.001 compared to CTL; *n*=5 per group.

Abbreviations: BW: Body weight; CF: Chloroform fraction; CTL: Control; ME: Methanol extract; PEF: Petroleum ether fraction; SEM: Standard error of the mean; STD: Standard.

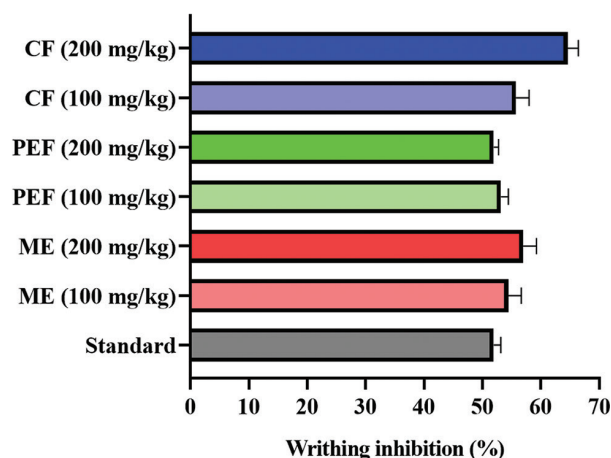


Figure 1. Peripheral analgesic activity of *Commelina diffusa*, expressed as percentage inhibition of writhing in mice. Data presented as mean±SEM (*n* = 5 per group).

Abbreviations: CF: Chloroform fraction; ME: Methanol extract; PEF: Petroleum ether fraction; SEM: Standard error of the mean.

3.4. Hypoglycemic activity

The hypoglycemic efficacy of *C. diffusa* was assessed by measuring blood glucose levels in treated mice following glucose administration. The results are illustrated in Figure 2.

Despite growing interest in natural products for managing metabolic disorders,³¹ the crude methanol extract and the various fractions of *C. diffusa* did not produce a significant reduction in blood glucose levels in this study. This contrasts with reports of *Commelina benghalensis* extracts showing significant hypoglycemic effects in alloxan-induced diabetic rats.³² The discrepancy may be attributed to interspecies variation and differences in phytochemical composition between species. Moreover, the use of a single dose in non-diabetic (normoglycemic) animals limits the interpretation of hypoglycemic efficacy.

4. Discussion

Bangladesh is endowed with rich biodiversity, particularly in medicinal plants that have been used traditionally for centuries. According to the Bangladesh National Herbarium, over 5,000 plant species exist in the country, of which around 500 are recognized as medicinal.^{33,34}

C. diffusa, commonly known as climbing dayflower or spreading dayflower, is a herbaceous plant belonging to the family Commelinaceae. Morphologically, *C. diffusa* is a prostrate or ascending annual herb characterized by succulent stems, ovate to lanceolate leaves, and distinctive blue flowers borne in spathes.³⁵ The plant reproduces both sexually (via seeds) and vegetatively (through rooting at nodes), contributing to its invasive potential. *C. diffusa* has a pantropical distribution and is especially common in Asia (including India, China, Bangladesh, and Southeast Asia), the Caribbean, Africa, and South America. Traditional medicine and animal studies suggest that *C. diffusa* has a relatively low toxicity

profile at therapeutic doses. Comprehensive toxicological evaluations, including chronic toxicity and genotoxicity assessments, are necessary before clinical use. Given its wide geographical distribution, phytochemical richness, and diverse pharmacological activities, *C. diffusa* is a promising candidate for further investigation in natural product drug discovery. With systematic scientific validation, *C. diffusa* could offer sustainable solutions in healthcare and environmental management.^{26,35,36}

Antimicrobial resistance is a growing global health concern characterized by the ability of microorganisms, such as bacteria, viruses, fungi, and parasites, to resist the effects of antimicrobial agents. The World Health Organization has declared antimicrobial resistance as one of the top ten global public health threats.³⁷ The rise in resistance is driven by a complex interplay of factors, including the misuse and overuse of antibiotics, poor infection control practices, and inadequate surveillance and regulation. Natural products have historically served as a primary source of antibiotics.⁶ Many widely used antimicrobials, such as penicillin, streptomycin, and tetracycline, were originally derived from microbial sources. The structural complexity and evolutionary refinement of these compounds allow them to target bacterial cells effectively, often through unique mechanisms. For example, actinomycetes and *Bacillus* species produce a wide array of secondary metabolites, including aminoglycosides, polyketides, and lipopeptides, which exhibit strong antimicrobial activity.^{38,39} Curcumin, a polyphenol from *Curcuma longa*, also exerts antimicrobial effects and has been shown to disrupt bacterial cell membranes and inhibit quorum sensing. Similarly, essential oils (e.g., thymol, carvacrol, eugenol) exhibit bactericidal properties through membrane disruption and inhibition of bacterial enzymes.^{40,41} Importantly, many of these compounds synergize with conventional antibiotics, enhancing their efficacy and reversing resistance mechanisms.

In the present study, the *C. diffusa* extracts showed potent antimicrobial activity, likely due to the presence of bioactive compounds. These results warrant further phytochemical investigation, including the isolation and characterization of the active compounds, for the development of novel antimicrobial agents.⁴² Despite encouraging *in vitro* and *in vivo* findings, the clinical development of natural compounds faces several hurdles. These include poor solubility, low bioavailability, toxicity, and difficulties in standardization and large-scale production. To ensure effective translation, further research must focus on elucidating mechanisms of action, optimizing pharmacokinetics, and conducting rigorous clinical trials. Interdisciplinary collaborations and

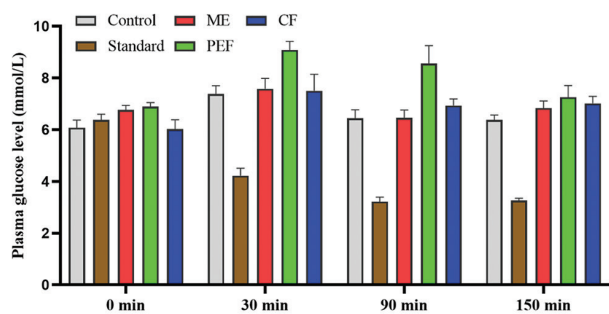


Figure 2. Plasma glucose level of the mice after administration of *Commelina diffusa* extracts at different time intervals. Data are shown as mean±SEM (n = 5 per group). Abbreviations: CF: Chloroform fraction; ME: Methanol extract; PEF: Petroleum ether fraction; SEM: Standard error of the mean.

sustainable sourcing strategies are also vital for realizing the therapeutic potential of natural products, especially in addressing antimicrobial resistance.^{43,44}

Pain management remains a critical concern in modern medicine due to the limitations and adverse effects associated with conventional analgesic drugs, including opioids and NSAIDs. Alkaloids, flavonoids, terpenoids, and polyphenols are the principal classes of plant secondary metabolites investigated for analgesic activity.⁴⁵ In traditional medicine, many plants and their parts are also used for treating itching and rash.⁴⁶

Several clinical and preclinical studies support the analgesic efficacy of flavonoids, such as quercetin, kaempferol, and rutin, which exhibit both central and peripheral analgesic effects by modulating oxidative stress, inflammatory pathways, and opioid receptors.⁴⁷ In our study, the *C. diffusa* exhibited potent analgesic activity in both hot-plate (tail-flick) and acetic acid-induced writhing methods. The observed effect may be mediated, at least in part, through anti-inflammatory pathways. Several phytochemical constituents reported in the genus *Commelina*, such as flavonoids and phenolic compounds, are known to possess anti-inflammatory properties. Further investigations are necessary to isolate and identify the compounds responsible for the analgesic activity.

4.1. Limitations and future research directions

The current study presents preliminary findings on the antimicrobial, analgesic, and hypoglycemic activities of *C. diffusa*. However, the study has some limitations. Notably, detailed phytochemical characterization of the bioactive extract was not conducted due to the lack of advanced analytical instrumentation such as liquid chromatography-tandem mass spectrometry, gas chromatography-mass spectrometry, or nuclear magnetic resonance at our current facility. As a result, the specific compounds responsible for the observed biological activities remain unidentified. This limits our ability to elucidate the underlying mechanisms of action and to propose a standardized phytochemical profile for potential therapeutic use. Future studies should focus on isolating and identifying the key active constituents using advanced chromatographic and spectroscopic techniques. Such investigations will be essential for understanding the mechanisms of action, ensuring batch-to-batch consistency, and progressing toward the development of standardized herbal formulations or drug leads. The study also employed a small sample size for the *in vivo* tests, which may limit the statistical power and generalizability of the findings.

Furthermore, although the antimicrobial activity was assessed using the disc diffusion method, determining the minimum inhibitory concentration is essential for

quantifying the antimicrobial strength and enabling a more precise comparison with standard antibiotics. The poor solubility or limited diffusion of the extract components within the agar medium could contribute to the relatively weak antimicrobial effect observed in our assay. Future studies may include broth microdilution and alternative solvents to better assess the true antimicrobial potential of the extract under conditions that allow more homogeneous dispersion. In addition, the hypoglycemic potential was assessed using a single-dose OGTT in healthy animals. To fully evaluate the antidiabetic potential of *C. diffusa*, future studies should utilize diabetic models.

5. Conclusion

This study investigated the antimicrobial, analgesic, and hypoglycemic activities of the methanol extract of the whole *C. diffusa* plant, along with its petroleum ether- and chloroform-soluble fractions. Strong antimicrobial properties were demonstrated by the methanol and chloroform fractions against a variety of microorganisms. Central analgesic activity, assessed using the tail-flick test in mice, revealed potent efficacy for both fractions. The extracts also showed strong peripheral analgesic efficacy in the acetic acid-induced writhing test. However, the extract did not show significant hypoglycemic effects in a single-dose OGTT conducted in healthy mice. It is important to note that this finding is based on an acute model in normoglycemic animals and does not rule out potential antidiabetic effects under pathological conditions. Further investigations using diabetic animal models are warranted to comprehensively evaluate the plant's hypoglycemic activity and its possible mechanisms of action in metabolically compromised states. Overall, *C. diffusa* shows promising pharmacological potential and warrants further investigation for its possible therapeutic applications.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Tasnia Islam Anika, Mohammad Shawkat Ali,

Investigation: Tasnia Islam Anika, Md. Monirul Islam

Methodology: Tasnia Islam Anika, Mohammad Shawkat Ali

Writing–original draft: Tasnia Islam Anika, Md. Monirul Islam

Writing–review & editing: Md. Monirul Islam, Mohammad Shawkat Ali

Ethic approval and consent to participate

The animal study was reviewed and approved by the ethical review board of Northern University Bangladesh (reference number: DoP/RC/EC/2022/01/01).

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author on reasonable request.

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