ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF THE ETHANOLIC EXTRACT OF SEAWEED *ASPARAGOPSIS* SPP. ISOLATED FROM THE BAY OF BENGAL, BANGLADESH

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ABSTRACT

The marine zone of Bangladesh covers a great diversity of natural ecosystems including a marine environment rich in commercially important seaweeds. Seaweeds are widely used in the pharmaceutical, cosmetics, chemical, antibacterial, and food industries globally. According to the Food and Agriculture Organization of the United Nations (FAO), China, Indonesia, Korea, the Philippines, and Japan are the top five nations in the world for seaweed production. In recent times, Bangladesh has initiated comprehensive research endeavours focused on seaweed, recognizing its immense potential across diverse sectors. Seaweed in Bangladesh is primarily harvested for various purposes, including food, medicine, and industrial applications. In this study, red seaweed *Asparagopsis* collected from the coast of Saint Martin's island of the Bay of Bengal was tested for its bioactive properties like anti-inflammatory and analgesic activity. Anti-inflammatory test was done using carrageenan suspension and three analgesic tests e.g. hot plate test, acetic acid-induced writhing test, and formic acid-induced test were performed in mice model in the laboratory with 50% ethanol extract of *Asparagopsis*. Following *in vivo* testing, no significant activity was observed. However, *Asparagopsis* was found to be rich in phytochemicals like phenols, flavonoids, saponin, glycoside, steroids, tannins, and alkaloids suggesting its importance for future scientific study. This may create a door to a new world in the Blue Economy sector of Bangladesh.

KEYWORDS: seaweed, *Asparagopsis*, analgesic activity, anti-inflammatory, phytochemicals

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Introduction

Seaweed, or macroalgae, is a significant, benthic marine algae that inhabits the intertidal zone. Their fast growth and autotrophic diet distinguish them; they grow more quickly than terrestrial plants and do not require cultivating land. Based on their pigment composition and storage food, they are divided into three distinct groups: Phaeophyta, Rhodophyta, and Chlorophyta (Ismail et al., 2020; Wan et al., 2019).

Marine macroalgae are becoming increasingly popular as a healthy dietary source because they are high in fibre, essential amino acids, minerals, and other bioactive substances like proteins, lipids, and polysaccharides. Among approximately 9200 species, only around 221 of the existing seaweed species are commercially important from a commercial perspective. (Chowdhury et al., 2023; Syakilla et al., 2022). Numerous studies have reported that marine algal polysaccharides exhibit anti-inflammatory, anti-tumor, anti-cancer, antiviral, and antifungal activity. (Chaiklahan et al., 2020).

The coastal region of Bangladesh covers 19 districts and an area of 47,201 km^2 (32% of the country's total geographical area) (Islam et al., 2009). Seaweeds are abundant in the south of the Bay of Bengal, from the Sundarbans mangrove forest to St. Martin's Island, mostly from October to April (Ahmad, 2019).

Marine habitats are the most biodiversity-rich ecosystems compared to terrestrial ecosystems. (Beaumont et al., 2007; Palumbi et al., 2009). At present, a total of 193 different varieties of seaweed have been identified in the Bangladesh coastline. Out of them, 19 have been found to have potential for economic uses (Shirajul et al., 2016).

Seaweed serves various purposes for human consumption, including food, medicine, hydrocolloid production, cosmetics, fish and animal feed, fertilizers, soil conditioners, and more (Rao et al., 2018). Recent studies also indicate that algal extract possesses the ability to scavenge free radicals and active oxygen, potentially inhibiting cancer cell proliferation, making it a suitable candidate for antioxidant-based drug therapies to combat Reactive oxygen species (ROS)-induced oxidative damage (Neethu et al., 2017).

A particular focus has been drawn to red seaweed (Red algae or Rhodophyta) as a potential renewable resource in the marine environment. The Commonwealth Scientific and Industrial Research Organization (CSIRO) reported that the

red algae *Asparagopsis* can reduce methane emissions in ruminant livestock by more than 90 percent (Kinley et al., 2020)Compared to the other two groups of green and brown seaweed, they are also a good source of numerous bioactive chemicals with a wide range of biological and industrial uses. (Akter et al., 2022; Arulkumar et al., 2020). These include polysaccharides like Floridian starch and sulfated galactans, such as carrageenans or agars, minerals, unsaturated fatty acids, amino acids, vitamins, phycobiliproteins, antioxidants, pigments, phycolectins, and mycosporine-like amino acids (Ismail et al., 2016; Torres et al., 2019). Red seaweed has a greater protein concentration than various dietary items and macroalgal categories, ranging from 10 to 50 percent of its dry weight. (Syakilla et al., 2022). Additionally, like other protein sources like leguminous plants, red seaweeds contain essential amino acids at about 25–50% of the total amino acids (Paiva et al., 2014; Vieira et al., 2018).

Asparagopsis is a genus of red seaweed that includes genetically homogenous invasive species. *Asparagopsis armata* is a cryptic species complex distributed in the warm temperate region, and *Asparagopsis taxiformis* is found throughout the tropical and subtropical regions (Zanolla et al., 2015). The term *Asparagopsis* was originally used to demonstrate the gametophyte stage of a triphasic heteromorphic diploplontic life cycle. These incredibly branching pink to reddish-colored gametophytes are often found on rocky substrates or as epiphytes and can grow to a height of 40 cm (Zanolla et al., 2014). *Asparagopsis* is used as food for human consumption and has medicinal applications such as antibacterial, antimicrobial, antibiotic, goiter, and cosmetics (Neethu et al., 2017b). A significant amount of analgesic, anti-tumor, and anti-inflammatory compounds can also be found in these red seaweeds, which are high in phytonutrients (Akter et al., 2022b; Araújo et al., 2017; Shirajul et al., 2016). Previous research demonstrated that the greatest concentration of phenolic compounds, such as flavonoids, phenolic acids, and bromophenols are found in red seaweed having different medical applications due to their reactions with proteins, such as enzymes or cellular receptors. Numerous studies have determined the potential application of marine red algae and their bioactive metabolites for therapeutic advances. Red algae like *Gracilaria verrucosa* has been reported to contain potential phenolic compounds, steroids, saponin, and quinines; *Kappaphycus alvarezii* is known to contain alkaloids, carbohydrates, terpenoids, and *Pyropia orbicularis* is rich in phycocyanin, etc.(Khairinisa et al., 2023; Shojaii et al., 2015).

The study of bioactive metabolites in red algae shows a promising hope for developing novel therapeutics for various diseases. The rich diversity of marine ecosystems and the multifaceted pharmacological actions of these compounds provide a robust foundation for the further exploration and development of marine algae in pharmacognosy and pharmacology. The present study was conducted to evaluate the anti-inflammatory and analgesic activities of ethanol extract from the red alga *Asparagopsis* collected from the Saint. Martin's island of the Bay of Bengal. Further research on *Asparagopsis* isolates and extracts must be thoroughly examined for pharmaceutical and nutraceutical applications.

Materials and Methods

Sample collection and processing

The red algae Asparagopsis was collected in December 2021 at low tide from the shore of CheraDwip on St. Martin's Island in Bangladesh. After the sample was thoroughly cleansed with fresh water, any traces of epiphyte, such as animal castings or sticking sand particles, were carefully brushed off. The sample was washed out with distilled water to get rid of excess salt, and it was then preserved in 50% ethanol in a container that blocks light.

Extract preparation

A shade drawer was used to dry the ethanol-soaked sample at 37°C. The dried samples were then crushed into a fine powder using a mortar and pestle. Next, 20 grams of the weighed algal powder was soaked in 200 ml of 50% ethanol with a concentration of 1g/10mL (1:10) using a shaking incubator at 150 rpm for seven days. Samples were macerated and then filtered via double-layer filter paper. 5 g of ethanol extract was extracted after the suspensions evaporated.

Phytochemical Screening

Established qualitative phytochemical methods were used to identify any potential bioactive compounds in a sample. Several approaches, including the led acetate test, Mayer's test, Salkowski's test, ferric chloride test, alkaline reagent test, and foaming test, were performed to find phytoconstituents such as Phenolic compounds, tannins, alkaloids, steroids, glycosides, flavonoids, and saponins (Chowdhury et al., 2023).

In Vivo Analysis

Test Animal

Female Swiss albino mice (age 4-5 weeks, average weight 25-30 grams) were used in this experiment. Mice were collected from the International Center for Diarrheal Disease Research, Bangladesh (ICDDR, B). A suitable environment for mice was maintained in the biotech lab of Bangabandhu Sheikh Mujibur Rahman Maritime University, Department of Genetic Engineering and Biotechnology. They were kept at a temperature of 25°C, humidity of 55-60%, and a light-dark cycle of 12 hours. They were fed a regular pellet diet and were given unlimited access to purified water. (Akter et al., 2022c; Basit et al., 2022; Chowdhury et al., 2023; Gómez-Guzmán et al., 2018).

Anti-inflammatory Test

This test was performed to detect the activity of the extract against inflammation. The anti-inflammatory test was designed based on previous scientific procedures with some modifications (Akter et al., 2022c; Alim et al., 2022; Chowdhury et al., 2023b; Gómez-Guzmán et al., 2018). Five groups of mice were used during the experiment, and each group had five mice. One group is used as a control group, labeled group 1. Group 2 labeled positive control treated with diclofenac (10mg/kg body weight), and Groups 3, 4, and 5 were test groups. Group 3 and group 4 were treated with 500 mg of 50% ethanol/kg body weight and 250 mg of 50% ethanol/kg body weight, respectively. Group 5 was used as negative control and treated with only 50% ethanol. 1% Carrageenan suspension was injected into the left hind paw of each mouse of all groups. A plethysmometer measured paw volume after being treated with carrageenan in ½ hour, 1 hour,

2 hours, 3 hours, 4 hours, 5 hours, 6 hours, and 8 hours intervals. The % increase in paw volume was measured and compared to the control group. The following formula determined the proportion of inflammation:
% inhibition of paw edema = [(Vc–Vt)/Vc]x100
Where Vc and Vt represent the average paw volume of the control and treated animals respectively.

Analgesic activity
Hot plate test in mice
Modified method (Bin Emran, 2015a and Karimzadeh et al., 2020) was used to conduct the hot-plate test. There were four groups of five mice in each group. To monitor the mice's reaction to electrical heat-induced pain stimuli, a beaker containing the mice was put on a hot plate. Jumping out of the beaker or licking its paws was recorded as an indication of the animal's reaction to the heat-induced pain stimuli.
The response time was measured as the time spent licking paws or leaping out of the beaker. Initially, the reaction time of the test mice was assessed once before treatment, and the average of these measurements represented the baseline reaction time. The mice were then divided into groups and administered treatments orally. A negative control (50% ethanol), a positive control (diclofenac sodium at 10 mg/kg body weight), and ethanol extract at dosages of 250 mg/kg and 500 mg/kg body weight were given orally. Thirty minutes post-treatment, the response time for each group was recorded five times at one-hour intervals. The analgesic score was computed as a percentage (%) as follows:
Percentage (%) of analgesic effect = (Tb-Ta/Tb) × 100
Where Tb= Reaction time (in second) before drug administration
Ta = Reaction time (in seconds) after drug administration.

Analgesic test with formic acid
To evaluate the analgesic activity of the sample extract, the formic acid-induced method was applied with some modifications to this report (Beaumont et al., 2007).30 minutes after treatment, 50% ethanol extract was used in 500 mg/kg body weight and 250 mg/kg body weight dosages of two test groups, 0.5%v/v formic acid was injected into all mice into the sub plantar region of their right hind paw. Water was applied as a control, and Diclofenac (10mg/kg body weight) was considered a positive control. The negative control group takes away from the test group or extract group. The analgesic effect of the extract was compared to the negative group. Observe the pain reaction of the injected paw by counting biting and licking during the neurogenic phase (between 0 to 5 minutes) and inflammatory phase (15 to 30 minutes).
The percentage of inhibition was calculated as –
% of inhibition = [(Nb– Na) ÷Nb] ×100

Here, Nb is the number of responses before the drug administration, and Na is the number of responses after the drug administration.

Acetic acid-induced writhing test in mice
The pain was induced in test animals by intraperitoneal acetic acid injection to explore the analgesic activity of the sample extract. A modified procedure was used from previous experiments (Alim et al., 2022; Bin Emran, 2015b; Gómez-Guzmán et al., 2018).
Two dosages (500 mg and 250 mg/kg body weight) of 50 % ethanol extract were given to two test groups of mice after overnight fasting.10mg/kg body weight dose of sodium diclofenac was given to the positive control group. After 30 minutes, 0.7% acetic acid (10ml/kg body weight) was injected intraperitoneally into all groups of mice. After 5 minutes writhes were recorded for 20 minutes. 15 minutes before acetic acid injection sample extract was given to the test animal. The experiment was conducted in appropriate laboratory conditions at 25° C.

% inhibition= (1-VT/VC) ×100
Where VT = number of writhing motions in drug-treated mice, VC =number of writhing motions in the control group of mice.

Statistical analysis
The data obtained from the anti-inflammatory test and analgesic tests were statistically analyzed. The mean ±SD was utilized for triplicate data, and p-values of 0.05 were used to determine significance. The data were analyzed using one-way analysis of variance (ANOVA) and then Dunnett's t-test as the significance test.

Results
Phytochemical analysis
During the preliminary phytochemical screening of the 50% ethanol extract of Asparagopsis, several bioactive compounds, such as Steroids, Glycosides, Flavonoids, and Saponin, were found to be present in significant amounts (Table 1).

Table 1. 50% ethanolic extract of *Asparagopsis sp was* qualitatively tested for the presence of phytochemicals. The table indicates (-): not detectable, (+): low quantities, (++): moderate quantities, (+++): high quantities.

50% ethanolic Sample extract	Phenol	Flavonoid	Saponin	Glycoside	Steroids	Tannins	Alkaloid
	-	+	+++	++	++	+	+

In vivo study

Anti-inflammatory activity using carrageenan-induced paw edema method

The sub-plantar injection of carrageenan was used to induce edema in the test animals of each group. In the positive control group edema grew progressively to a maximum severity of 5 hours after the administration of carrageenan. *Asparagopsis* spp in 50% ethanolic extract of 500 mg/kg body weight exhibited better anti-inflammatory activity than 250 mg/kg 50% ethanolic extract/kg body weight.

Table 2. The ethanolic extract showed anti-inflammatory activity on carrageenan-induced mice paw oedema on the plethysmometer. (n= 5 animals per group) Values were given as mean ±SD.* p value <0.05, **p value <0.01, ***p value <0.001. *t-test* was used to compare the mean of the extract with the control group.

Anti-inflammatory effect of 50% ethanolic extract				
Time	Control	Diclofenac	500mg/kg	250mg/kg
0H	0.27±0.56	0.28±0.056	0.32 ± 0.068	0.32 ± 0.079
1/2H	0.38±0.065	0.36±0.075	0.36 ± 0.040	0.37 ±00.076
1H	0.37±0.009	0.32±0.051	0.36 ± 0.013	0.34 ± 0.016
2H	0.37±0.009	0.29±0.046	0.26 ± 0.010	5.62 ± 11.94
3H	0.33±0.036	0.26±0.034	0.26 ± 0.042	0.31 ± 0.047
4H	0.35±0.021	0.30±0.033	0.21 ± 0.035	0.26 ± 0.049
6H	0.48±0.098	0.25±0.074	0.19 ± 0.045	0.22 ± 0.060
8H	0.4±0.066	0.21±0.063	0.18 ± 0.076	0.20 ± 0.021

Percent Inhibition								
	0 hour	1/2 hour	1 hour	2 hour	3 hour	4 hour	6 hour	8 hour
diclofenac	0.274	0.384	0.376	0.366	0.334	0.352	0.266	0.482
Extract	0	104.6875	107.4468	116.3934	122.7545	131.25	113.5338	155.6017
control	0	6.25	14.3617	20.76503	19.76048	14.20455	7.518797	48.13278

Analgesic tests: A. Hot Plate Method

In this study, a significant analgesic effect on the same concentration in the same group was found in the Hot plate method. The findings of this experiment of 50% ethanol extract at 500mg/kg and 250 mg/kg body weight, are mentioned in Tables 03 and 04.

Table 3. The analgesic impact of the 50% ethanolic extract of *Asparagopsis sp* in hotplate assay. (n=5 animals per group) Values were given as mean±SD.*p value<0.05,**p value<0.01,***p value<0.001.—a‖=*t*-test was used to compare the extract with the control, and —b‖= extract with diclofenac.

The average response of the Hotplate test						
Time	0hour	½Hour	1 Hour	2 Hours	4 Hours	6 Hours
Control	41±13.2	20.6±0.89	21.6±13.52	22.8±3.033	22.8±3.34	17±10.77
Diclofenac	36.6±4.33	25.8±8.25	23±2	23.8±7.42	20±4.24	21.4±14.99
Extract 500 mg/kg bw	36.8±12.77	29.2±7.32	39.4±19.60	23.2±11.30	15.8±8.49	13.4±9.93
Extract 250 mg/kg bw	22.5±10.41	21.5±12.16	19.5±7.70	10±11.80	12.5±5.40	13±14.53

Table 4. Table 4 compares the percentage of inhibition of the positive control (diclofenac) and two different concentrations of the extract (500mg/kg and 250 mg/kg).

% Inhibition						
Group	0 hour	½ Hour	1 Hour	2 Hours	4 Hours	6 Hours
Diclofenac	3.93	9.89	5.21	20.06	15.86	19.23
Extract 500 mg/kg bw	-0.98	1.98	3.54	14.23	11.87	16.32
Extract 250 mg/kg bw	-0.26	-0.85	0.63	12.96	10.15	11.96

Formic acid-induced method

Various chemical compounds (acetic acid, carrageenan, formalin, kaolin, platelet-activating factor, mustard oil, serotonin, and yeast) are used to stimulate nociceptive behaviour in test animals (Ruslin et al., 2018).

In this study, diluted formic acid was injected into mice to produce nociceptive action. The nociceptive response was observed in two phases. The first phase lasted 0-5 minutes (the neurogenic pain response), and the second phase lasted 15- 30 minutes (the inflammatory response).

Table 5. Effects of *Asparagopsis* extract on the formic acid-induced pain method.

Effects of extraction the formic acid-induced pain.		
	Early phase (0-5min)	Late Phase (15-30 min)
Control	40.6±39.25	116.6±74.07
Diclofenac	52±19.44	147±92.89
Extract(50mg/kg)	59.2±21.42	186±58.52
Extract(25mg/kg)	35.2±28.50	140.6±53.49

Table 6. % Inhibition of formic acid test. (n=5 animals per group). Values were given as mean± SD.*p value<0.05, **p value<0.01, ***p value<0.001.*t*-test was used to compare the mean of the extract with diclofenac.

% Inhibition			
	Diclofenac	Extract (500mg/kg)	Extract (250mg/kg)
Early Phase	20.25	22.56	20.36
Late phase	85.32	88.53	60.86

Acetic acid induced writhing test

The acetic acid-induced writhing test showed that the ethanol extract of *Asparagopsis* spp is more effective at numbing pain than the control. Ethanol extract and positive control diclofenac suppressed the writhing responses caused by acetic acid. Two doses of extract at 500 mg/kg and 250 mg/kg body weight were applied on test animals, and when compared to control, there was no significant reduction in the number of abdominal constrictions due to intraperitoneal injection of 0.7% acetic acid (Table 07).

Table 7. The analgesic activity of an ethanolic extract was tested using the acetic acid-induced writhing test method in mice. (n=5 animals per group) Values were given as mean± SD. *p value<0.05, **p value<0.01, ***p value<0.001. A *t-test* was used to compare the mean of an extract with that of diclofenac.

Treatment	**Mean±SD**
Control	169.2±118.97
Diclofenac	125.25±18.21 -
Extract 500 mg/kg	286.6±196.62
Extract 250 mg/kg	184.2±60.84

Discussion

The oceans harbor a vast array of marine organisms, many of which have unique biochemical properties that could lead to the discovery of novel drugs.

Among all marine organisms, marine macroalgae have been reported to have active ingredients responsible for diverse biological activities, such as antifungal. (Guedes et al., 2012), antimicrobial (Berri et al., 2016), antiviral (Hardouin et al., 2016) and antioxidant activities (Wang et al., 2013). Hence, the present study was designed to evaluate the Anti-inflammatory and analgesic activity of ethanolic extract of *Asparagopsis* spp. from the Bay of Bengal, Bangladesh.

The purpose of this study is to analyze *in vitro* and *in vivo* the phytochemicals, anti-inflammatory and anti-analgesic effects of a 50% ethanolic extract of *Asparagopsis* spp.

Phytochemicals are substances found in many plants and generally help them to resist fungi, bacteria, and plant virus infections. Phytochemicals such as polyphenols, flavonoids, polyphenols, terpenoids, carotenoids, phytosterols, glucosinolates, etc., have great antioxidant potential and are of great interest due to their beneficial effects on the health of human beings. In the present study, the extract of *Asparagopsis* spp. was tested qualitatively to identify bioactive compounds by phytochemical testing. Following investigation, it was found that *Asparagopsis* spp. contained alkaloids, steroids, glycosides, tannins, saponins, and flavonoids. Saponins were detected in large amounts and formed strong foam. (Table 1). Previous studies reported that *Asparagopsis* is an excellent source of alkaloids. Steroids found in marine algae show low anti-inflammatory activity and less cytotoxicity in the treatment of several diseases (Akter et al., 2022). Phenolic compounds could not be detected in the extract of *Asparagopsis* spp.

After the phytochemical screening, the anti-inflammatory and algetic assay were performed. Red algae are rich in potential therapeutic compounds such as polysaccharides, lipids, sterols, alkaloids, terpenoids, and flavonoids conferring analgesic and anti-inflammatory activity.

This study evaluates the anti-inflammatory effects of 50% ethanol extract of algae *Asparagopsis* spp. in carrageenan-induced paw oedema. Further study is required to prove its

potent role as an anti-inflammatory compound. The analgesic impact of an ethanol extract of *Asparagopsis* spp. was assessed using the hot plate method, which is one of the most commonly used procedures for determining drug or compound analgesic effectiveness. Mice's paws are susceptible to heat at temperatures that do not harm the skin, eliciting reactions such as shaking, leaping, paw withdrawal, and paw licking. When centrally active analgesics are administered, the time before these reactions occur is extended (Chowdhury et al., 2023).The extract of *Asparagopsis* spp. at 500 and 250 mg/kg doses do not show persistent results in increasing delay time.

Formic acid-induced paw pain is a well-established in-vivo pain model to study analgesics (Akter et al., 2022). Distinct analgesics may have different effects in the early and late stages of the formic acid test; therefore, both phases must be performed so that the test may be used to understand how a possible analgesic might operate to relieve pain. The anti-nociceptive effect of the extract of *Asparagopsis* spp showed some positive results in 500 mg/kg body weight dose.

The search for analgesic medicines has focused significantly on the acetic acid-induced writhing method in mice. This method and the visceral pain model trigger prostaglandin production through the cyclooxygenase pathway, contributing to the nociceptive process. The response is believed to be mediated by the prostaglandin pathway and acid-sensing ion channels in peritoneal mast cells. Acetic acid-induced writhing is associated with elevated levels of PGE2 and PGF2 in peritoneal fluids and products from the lipoxygenase pathway. Increased prostaglandin levels in the peritoneal cavity lead to heightened capillary permeability, intensifying inflammatory pain. According to the findings of the current studies, the significant analgesic effect of the ethanol extract of *Asparagopsis* may have resulted from the inhibition of the synthesis of the arachidonic acid metabolite (Chowdhury et al., 2023) through the anti-nociceptive effect of ethanolic extracts *Asparagopsis* (500 & 250 mg/kg body weight) on five Swiss Albino mice did not show conclusive results in *in-vivo* analysis.

Conclusion

Today, in the period of advanced biotechnology, the blue economy is the priority for natural drug development, offering the potential to discover new therapeutic compounds from ocean resources while promoting sustainability and environmental stewardship. Various scientific research mentioned that marine algae is a potential source for the pharmaceutical industry. A study on the phytochemical analysis of *Asparagopsis* revealed the presence of bioactive compounds like flavonoids, alkaloids, glycosides, steroids, tannin, etc responsible for biological activities such as antioxidant and cytotoxic activities. These phytochemicals found in *Asparagopsis* spp. from the Bay of Bengal can also benefit drug development.

However, *in vivo*, screening for anti-inflammatory and analgesic activity did not show consistent and effective results for the 50% ethanol extract of *Asparagopsis* spp. Further advanced study is needed to explore the potentiality of these red algae.

Authors Contribution

MNH made the hypothesis, designed the experiments, supervised the work, analyzed the data, and wrote and revised the final version of the manuscript; AN, MA, KRC, and MA contributed equally to conducting experiments and analyzing the data. NRZ wrote and edited the manuscript.

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