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Experimental Teaching Design of Exploring the Protective Effect of Vegetable Active Components on DNA via Fluorescent Probe Method under the Background of New-Medical-Science Education

Ao Chen[1], Hairong Zhang[2], Qingqing Deng[1], Zonglin Yang[2], Jie Zhang[1*]

[1]Guangzhou Railway Polytechnic, Guangzhou, China
[2]Guangzhou Huashang College, Guangzhou, China
Email: *zhangjie703@gtxy.edu.cn

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Abstract

To meet the requirements of the Healthy China Initiative and the development of new-medical-science education, and implement the health concept of shifting from treatment-oriented mode to whole-life-cycle health management, this paper integrates the concept of clinical preventive medicine into biochemistry experimental teaching, and designs an exploratory experiment themed on the protective effect of vegetable active components on DNA. The ethidium bromide fluorescent probe method was adopted to characterize EB-DNA fluorescence modulation triggered by flavonoids, polyphenols and vitamin C extracts from 11 common vegetables. Through active-component extraction, content determination, fluorescence spectrum analysis and action constant calculation, students are guided to build interdisciplinary thinking integrating medicine and chemistry, understand the role of daily food in disease prevention and treatment, and explore the antioxidant and potential anti-cancer mechanisms of vegetables. Breaking the barriers between basic experiments and clinical medicine, this experiment effectively improves students' scientific-research thinking and clinical practical literacy, and highlights the educational characteristics of new-medical-science education featured with humanistic care and whole-life-cycle health management.

Keywords

New-Medical-Science Education, Fluorescent Probe, Antioxidation, Exploratory Experiment

1. Introduction

In March 2026, the special seminar entitled "Healthy China 2030 and the Development of the Big Health Industry" was held at the Annual Conference of China Development Forum in Beijing. With the continuous implementation of the national "Healthy China 2030" strategy and the vigorous promotion of emerging medical science construction by the Ministry of Education and the Ministry of Science and Technology, modern medical education is undergoing profound transformation, shifting from the traditional disease-centered diagnosis and treatment model to a new talent training system focusing on whole-life-cycle health prevention and refined health management [1]. Centered on innovation and interdisciplinary integration, the New Medical Science requires medical talents to possess not only solid clinical skills but also public health awareness, research initiative, and the capacity to address medical challenges with science and engineering knowledge [2]. Conventional pharmacology or biochemistry experiments generally focus on the detection of single compounds or verification of classic chemical reactions, lacking in-depth connections to disease pathogenesis. The disconnection between theory, practice and real-world scenarios leaves students confined to experimental operations without adequate links to clinical practice and daily life, which hinders the formation of their thinking paradigm for whole-life-cycle health management [3]. Epidemiological and nutritional studies have demonstrated that fresh vegetables constitute the primary dietary source of natural antioxidant substances for residents. The major water-soluble bioactive components in vegetables include flavonoids, polyphenols and vitamin C (Vc), all of which can inhibit DNA oxidation by scavenging oxygen free radicals [4] [5]. Existing domestic and international studies mostly investigate the antioxidant properties of bioactive ingredients from a single vegetable, whereas few systematic studies have compared the DNA-protective effects arising from synergistic interactions among bioactive components across multiple vegetable varieties. Ethidium bromide (EB), a classic nucleic acid fluorescent probe, exhibits variable fluorescence intensity upon binding to DNA, which sensitively reflects the integrity of the DNA double-helix structure as well as the protective or damaging effects of small-molecule agents on DNA [6]-[8].

Guided by the philosophy of the New Medical Science, our teaching team has translated the research achievement "Fluorescent-probe-based investigation on the protective effects of vegetable bioactive components against DNA damage" into an inquiry-oriented experimental teaching project. Driven by the core question "Which vegetables can most effectively mitigate oxidative DNA damage?", this experiment instructs students to quantify active constituents against oxygen-radical-induced DNA oxidative damage in 11 common vegetables, and to understand the micro-interaction mechanisms between vegetable bioactive components and DNA, as well as the potential anti-cancer and health-promoting values of these vegetables. This experiment enables students to master the operation of modern analytical instruments such as fluorescence spectrometers. More im-

portantly, it fosters their medical thinking of disease prevention through rational dietary patterns to maintain physical wellness, and realizes the organic integration of knowledge imparting, competence cultivation and value guidance.

2. Experiments

2.1. Experimental Objectives

On the one hand, students are expected to gain theoretical cognitive understanding of the pathological mechanism of free-radical-induced oxidative DNA damage through theoretical learning, and learn about the putative structure-activity relationship between the molecular structures of flavonoids, polyphenols and vitamin C and their free-radical-scavenging effects (this mechanistic knowledge is acquired from theoretical curriculum rather than verified by the present teaching-experiment dataset). On the other hand, by integrating theories with practical operations, students can proficiently grasp the technical principle of the fluorescent probe method and its application in investigating biomolecule-DNA interactions; note that this teaching assay does not involve real drug-DNA interaction measurement. Meanwhile, students will go through the whole experimental workflow including independently designing experimental schemes (teaching-suggested extended task; the dataset reported in this paper does not include self-designed student schemes), standardly operating fluorescence spectrophotometer and ultraviolet spectrophotometer, and completing sample detection and data analysis. It should be emphasized that the present experiment cannot generate definitive biological mechanistic conclusions; parameter D is only an assay-specific fluorescence-response index. Necessary interference-elimination control groups (extract-only, EB-plus-extract, DNA-plus-extract) are recommended as student extended-practice assignments, while measured data of these controls are absent in the dataset of this manuscript. This process improves their interdisciplinary collaborative ability and cultivates their thinking mode of combining basic experiments with popular-science content on health without over-claiming direct biological evidence for in-vivo health effects. Consequently, the comprehensive teaching objectives of knowledge acquisition, competence improvement and literacy cultivation can be achieved.

2.2. Experimental Materials and Instruments

2.2.1. Experimental Materials

Sodium chloride (Xilong Scientific Co., Ltd.), ascorbic acid (Sinopharm Chemical Reagent Co., Ltd.), tris (hydroxymethyl)aminomethane (Sinopharm Chemical Reagent Co., Ltd.), ferrous sulfate (Sinopharm Chemical Reagent Co., Ltd.), absolute ethanol (Tianjin Fengchuan Chemical Reagent Science and Technology Co., Ltd.), ammonium molybdate (Xilong Scientific Co., Ltd.), potassium sodium tartrate (Sinopharm Chemical Reagent Co., Ltd.), all of analytical grade; herring sperm DNA (Sigma), ethidium bromide (Sigma), quercetin (Shanghai Hengxin Chemical Reagent Co., Ltd.). Eleven kinds of fresh vegetables (see **Ta-**

ble 1) including tomato, cucumber, green pepper, cole, water convolvulus, celery, lotus root, eggplant, pumpkin, cabbage and carrot were purchased from Jinshan Market, Zengcheng District, Guangzhou. All vegetables were washed clean and stored in a refrigerator for subsequent use. All quantitative concentration results of flavonoids, polyphenols, and vitamin C presented in this manuscript were calculated on a fresh-mass basis.

Table 1. Codes and names of the 11 vegetable raw materials.

Code	Name
I	tomato
II	cucumber
III	Green pepper
IV	cole
V	Water convolulus
VI	celery
VII	Lotus root
VIII	eggplant
IX	pumpkin
X	cabbage
XI	carrot

2.2.2. Experimental Instruments

Fluorescence spectrophotometer (F-4500FL, Hitachi, Japan); precision electronic analytical balance (AB 204-N, Mettler-Toledo Instruments (Shanghai) Co., Ltd.); numerical-control ultrasonic cleaner (KQ3200DE, Kunshan Ultrasonic Instrument Co., Ltd.); UV-Vis spectrophotometer (UV-2550, Shimadzu (Suzhou), Japan).

2.3. Experimental Methods

2.3.1. Establishment of Standard Curves

Standard curves for flavonoids, polyphenols and vitamin C were constructed separately in this experiment. For the flavonoid standard curve: 0.1000 g of quercetin was accurately weighed, dissolved with 95% (volume fraction) ethanol, and transferred into a 1000 mL volumetric flask for constant-volume dilution. Aliquots of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mL of the above stock solution were transferred into 10 mL colorimetric tubes, respectively. Each tube was added with 1 mL of 5% aluminum nitrate ethanol solution and diluted to the mark with 95% ethanol. After mixing thoroughly and standing for 15 min, distilled water was used as blank control, and the absorbance was measured at the maximum absorption wavelength of 428 nm. The standard curve was plotted with absorbance A as the ordinate and concentration c (μg/mL) as the abscissa, as shown in Figure 1.

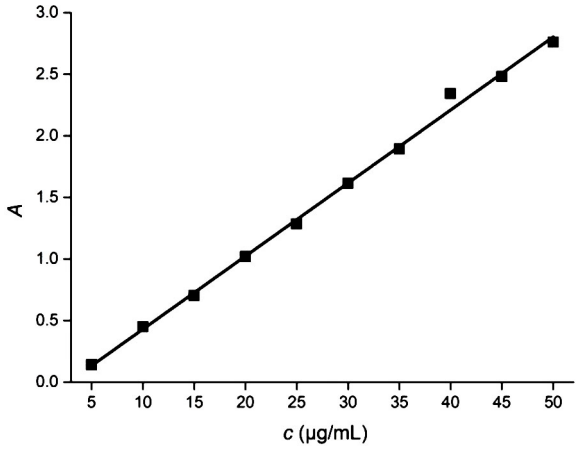

Figure 1. Standard curve of flavonoids.

For the polyphenol standard curve: 0.1000 g of gallic acid was accurately weighed, dissolved in absolute ethanol, and diluted to constant volume in a 100 mL volumetric flask with distilled water. Aliquots of 0, 0.25, 0.50, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25 and 2.5 mL of the above solution were transferred into 25 mL colorimetric tubes. Each tube was supplemented with 4.0 mL of double-distilled water and 5.0 mL of ferrous tartrate solution, then diluted to 25 mL using disodium hydrogen phosphate-sodium dihydrogen phosphate buffer solution. After thorough mixing and standing for 1 h, the absorbance was determined at the maximum absorption wavelength of 549 nm against reagent blank. The standard curve was drawn with absorbance A as the ordinate and concentration c (μg/mL) as the abscissa, as illustrated in Figure 2.

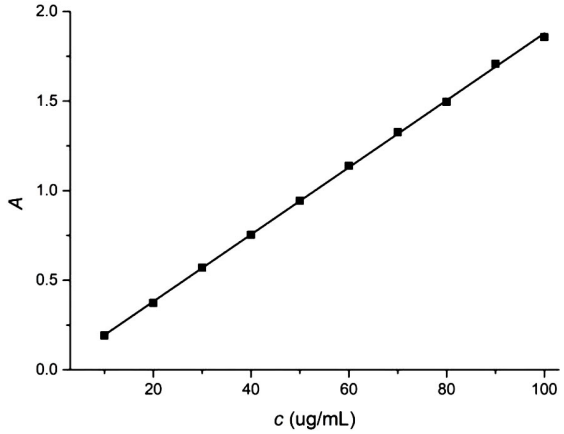

Figure 2. Standard curve of polyphenols.

For the vitamin C standard curve: 0.1000 g of ascorbic acid was accurately weighed, dissolved in double-distilled water and made up to 1000 mL in a volumetric flask. Aliquots of 1.25, 2.5, 3.75, 5.0, 6.25 and 7.5 mL of the above solution were transferred into 25 mL colorimetric tubes. Each tube was added with 2.5 mL of 10% sulfuric acid and 5.0 mL of 10% ammonium molybdate solution, diluted

to volume and mixed well. The mixtures were heated in boiling water for 6 min. After slight cooling, absorbance was measured at the maximum absorption wavelength of 721 nm with reagent blank as reference. The standard curve was plotted with absorbance A as the ordinate and concentration c (μg/mL) as the abscissa, as presented in **Figure 3**.

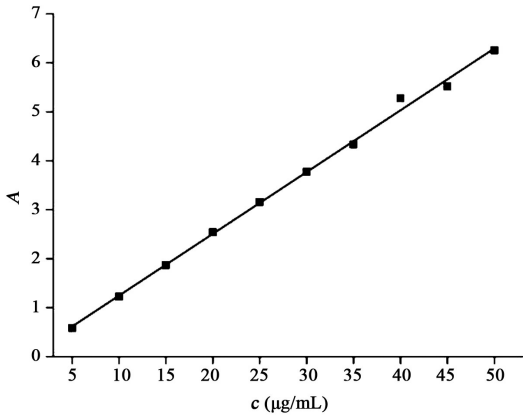

Figure 3. Standard curve of vitamin C.

2.3.2. Extraction of Active Components

For the extraction of flavonoids, polyphenols and vitamin C from the 11 varieties of vegetables, 10.0000 g of washed fresh vegetable sample was accurately weighed and ground in a mortar. The ground sample was transferred into a clean conical flask, followed by the addition of 20 mL of 65% ethanol. Ultrasonic-assisted extraction was performed at 80 W for 30 min. The extract was collected by suction filtration, and the residue was extracted once more under identical conditions. The two filtrates were combined and diluted to the mark with 65% ethanol in a 50 mL volumetric flask. Each sample was measured with 3 replicates. Fluorescence-signal variability was presented as standard deviation (SD). One-way ANOVA was used for inter-group statistical comparisons. The predefined criterion for ND (not detectable): the calculated D value falling within blank-control mean ± 2SD was classified as ND, indicating that measurable effective signal could not be distinguished from background noise.

2.3.3. Determination of Active Components

An appropriate volume of each extract was transferred, and the absorbance was measured under the identical conditions applied for establishing the standard curves, with the corresponding extract solution set as the reference blank. The obtained absorbance values were substituted into the regression equations of standard curves to calculate the contents of flavonoids, polyphenols and vitamin C.

The specific calculation formula for the contents of flavonoids, polyphenols and vitamin C is given as follows:

$$\text{Content}(\mu g/g) = \frac{c(\mu g/mL) \times \text{dilution factor} \times \text{extract volume}(mL)}{\text{dry weight of raw material} \times 10^6} \quad (1)$$

2.3.4. Measurement of Fluorescence Integrated Intensity of Samples

Thirteen 10 mL colorimetric tubes were prepared. DNA, NaCl and buffer solution were added sequentially to each tube, followed by a certain volume of vegetable extract. After mixing and standing for 1 h, ethidium bromide (EB) was added. Double-distilled water was used to adjust the solution to the final volume. The solution was mixed thoroughly and kept standing for another 1 h, and then the fluorescence integrated intensity was determined by fluorescence spectrophotometer. The composition of each sample is listed in Table 2.

Table 2. Composition of each sample.

Reagent	Sample A/mL	Sample B/mL	Sample C/mL
DNA	0	0.5	0.5
EB	0.1	0.1	0.1
Tris-HCl	2.0	2.0	2.0
NaCl	1.0	1.0	1.0
Extract	0	0	3.0
Final volume	10	10	10
Integrated fluorescence intensity	IA	IB	IC

Measurement parameters: scanning speed 2400 nm/min, wavelength range 550 - 700 nm. The wavenumber integration mode was adopted for scanning to obtain the integrated fluorescence intensity values of mixture A, B and C (vegetables No. 1 - 11). The interaction constant D (%) is defined as: D (%) = (1 − (I_C − I_A)/(I_B − I_A)) × 100%, where I_A is the integrated fluorescence intensity of EB solution; I_B is the integrated fluorescence intensity of E-DNA mixed solution; I_C is the integrated fluorescence intensity of the mixture containing EB, DNA and vegetable extract.

3. Results and Discussion

3.1. Antioxidant-Substance Profiles of Different Vegetables and Their Dietary Implications

In this experiment, the contents of three core antioxidant substances (flavonoids, polyphenols and vitamin C) in 11 common edible vegetables were determined. Quantitative data were used to compare and analyze the antioxidant-substance profiles of different vegetables. Meanwhile, students were guided to independently summarize data patterns and explore the biological and nutritional significance of compositional differences. Detailed experimental data are shown in Table 3.

Table 3. Determination results of flavonoid, polyphenol and vitamin C contents in 11 vegetables.

No.	Vegetable	Flavonoids (μg/g)	Polyphenols (μg/g)	Vitamin C (μg/g)
1	Tomato	55.2	25.6	850.8
2	Cucumber	ND	25.6	746.4
3	Green pepper	64.2	21.1	1058.5

Continued

4	Rape	114.9	69.0	517.6
5	Water convolvulus	98.2	119.1	514.8
6	Celery	107.0	82.3	191.7
7	Lotus root	49.3	87.9	92.3
8	Eggplant	79.6	96.8	867.7
9	Pumpkin	47.8	61.2	1011.2
10	Cabbage	ND	27.8	499.0
11	Carrot	ND	38.9	441.9

ND: no valid signal detected.

Based on experimental data, students can draw the following conclusions: polyphenols and vitamin C were detected in all 11 tested vegetables, providing comprehensive baseline antioxidant components. Flavonoids exhibited species-dependent differences; no valid flavonoid signals were detected in cucumber, cabbage and carrot, whereas the remaining eight vegetables contained flavonoid antioxidants. Such substantial compositional differences provide good experimental materials for students to investigate vegetable antioxidant properties. Further quantitative ranking showed that the flavonoid content of the tested vegetables followed the order: rape > celery > water convolvulus > eggplant > green pepper > tomato > lotus root > pumpkin. Rape possessed the highest flavonoid content (114.9 μg/g), while pumpkin had the lowest value (47.8 μg/g), representing typical extreme samples for vegetable flavonoid reserves.

Species-specific differences in polyphenol and vitamin C contents were more pronounced, and no positive synergistic increasing trend was observed between the two components. The polyphenol content ranked as: water convolvulus > eggplant > lotus root > celery > rape > pumpkin > carrot > cabbage > tomato = cucumber > green pepper. Water convolvulus exhibited a prominently high polyphenol content of 119.1 μg/g, while green pepper showed the minimum value of 21.1 μg/g among all tested vegetables. Vitamin C content presented a completely different distribution pattern: green pepper > pumpkin > eggplant > tomato > cucumber > rape > water convolvulus > cabbage > carrot > celery > lotus root. Green pepper reached the peak Vc content of 1058.5 μg/g and served as a typical high-vitamin-C vegetable; lotus root had the weakest Vc storage capacity with only 92.3 μg/g.

Through group comparative exploration, students can directly identify the core experimental finding: a high content of a single antioxidant substance cannot represent the overall antioxidant reserve level of a vegetable. Taking green pepper and water convolvulus as examples, green pepper ranked first in Vc content but had remarkably low polyphenol content, leading to a relatively single antioxidant composition. By contrast, water convolvulus possessed moderate Vc content but optimal polyphenol reserves, conferring advantages in antioxidant component

composition. These experimental results correct the misconception that "higher content of a single nutrient means stronger antioxidant value". Students can thereby deeply understand that the synergistic effect among multiple antioxidants constitutes the key to favorable antioxidant potential of vegetables.

Extended discussions based on the core concepts of "Dietary Guidelines for Chinese Residents" can help students establish scientific dietary cognition: there exists no single "all-round nutritional vegetable" in nature; each vegetable has its own merits and limitations in antioxidant composition. Adhering to dietary diversity, balanced meat-vegetable combinations and complementary food categories in daily diets enables balanced intake of various antioxidant nutrients. Nutrient synergism further enhances human antioxidant and anti-damage capacity, which constitutes the core scientific basis for diverse dietary patterns advocated by dietary guidelines. This realizes an in-depth connection between experimental inquiry and practical health knowledge.

3.2. Differences in Gene-Protective Activity of Vegetables and Practical Educational Significance

In this experiment, the interaction constant D, reflecting the binding between vegetable bioactive components and DNA, was adopted as the core indicator to quantitatively evaluate the gene-protective capacity of vegetable antioxidants. A higher D value reflects reduced EB-DNA fluorescence in this teaching assay. It should be emphasised that elevated D may arise from multiple interfering factors including EB competitive displacement, extract intrinsic fluorescence or fluorescence quenching. D serves merely as an assay-specific fluorescence-response index and cannot be directly interpreted as stronger biological DNA-protective potency. The measured D values of each vegetable are summarized in Table 4 (no valid data were obtained for eggplant and carrot as no measurable signal was detected). According to independent statistical sorting performed by students, the overall ranking of gene-protective capacity among the 11 tested vegetables was: water convolvulus > rape > cucumber > lotus root > pumpkin > tomato > green pepper > cabbage > celery.

Table 4. Interaction constant D for binding between vegetable bioactive components and DNA.

No.	Vegetable	Interaction constant D (%)
1	Tomato	15.2
2	Cucumber	26.9
3	Green pepper	14.6
4	Rape	30.5
5	Water convolvulus	31.1
6	Celery	4.7
7	Lotus root	22.8

Continued

8	Eggplant	ND
9	Pumpkin	18.8
10	Cabbage	7.0
11	Carrot	ND

ND: no valid signal detected.

The ranking clearly demonstrates that the gene-protective activity of vegetables is not absolutely correlated with the content of any single antioxidant substance, further verifying the significance of antioxidant synergistic effects. Although water convolvulus is not a high-Vc vegetable, it achieved a D value of 31.1 % and the highest gene-protective capacity, benefiting from its extremely high polyphenol content and well-balanced antioxidant profile. Rape, with optimal flavonoid content combined with moderate polyphenol and vitamin C levels, exerted synergistic antioxidant effects and ranked second for gene-protective activity (D = 30.5%). In contrast, green pepper, which had the highest Vc content, exhibited a moderately low gene-protective capacity (D = 14.6%) due to imbalanced ratios of polyphenols and flavonoids and a relatively simple antioxidant system. Celery, with generally low reserves of all antioxidants, showed the weakest protective effect with a D value of only 4.7%. This experimental procedure effectively cultivates students' capabilities in data analysis, logical reasoning and scientific critical thinking. Students are guided to break away from one-sided "content-only" thinking and establish the scientific viewpoint of "component matching and synergistic potentiation".

From the perspective of health education and practical life, these results possess important popular-science and educational value. Different vegetables have distinctive nutritional functions; there are no absolutely "good" or "bad" vegetables, and maximum nutrition cannot be achieved by consuming only a single vegetable. Meanwhile, this experiment confirms that the composite system consisting of flavonoids, polyphenols and vitamin C from natural vegetables can effectively protect biological DNA and reduce oxidative damage. It provides experimental evidence for the health-promoting concept that dietary vegetable intake enhances human antioxidant status.

The experimental findings can guide students to form healthy dietary habits: picky eating and partiality for certain foods should be avoided in daily meals. Active consumption of diverse green-leaf and fruit-vegetables enables comprehensive intake and synergistic action of antioxidant nutrients, thereby improving bodily antioxidant capacity, stabilizing cellular genome and reducing health risks induced by oxidative damage. The educational goal is thus realized: experimental inquiry empowers healthy lifestyles and scientific knowledge guides everyday dietary choices.

3.3. Transformation from "Data" to "Dietary Prescription"

Students are organized to compare Table 3 and Table 4, and it can be observed

that a tentative visual co-variation trend can be observed between D index and polyphenol content across samples. An inquiry-driven question is proposed: Why does green pepper, which has the highest vitamin C content (1058.5 μg/g), exhibit weaker DNA-protective activity than water convolvulus with high polyphenol content (119.1 μg/g)? Students are guided to consult relevant literature to understand the structural stability of polyphenols and their unique indirect antioxidant mechanism via metal-ion chelation, so as to deepen their comprehension of the "structure-activity relationship".

4. Conclusions

(1) This experimental design successfully integrates the educational philosophy of New Medical Science into inquiry-oriented chemistry experimental teaching, and constructs an interdisciplinary medical-chemistry experimental module centered on "interactions between vegetable bioactive components and DNA".

(2) Through this experiment, students master the contents of flavonoids, polyphenols and vitamin C in 11 vegetables as well as the ranking of their DNA-protective capacities (water convolvulus shows the strongest protective effect). Meanwhile, students understand the dose-effect relationship between dietary nutrition and gene stability at the molecular level.

(3) This experiment stimulates students' learning interest and fosters their clinical prevention-oriented thinking. It achieves the three-in-one educational objective of "knowledge imparting, competence cultivation and value guidance", and can provide references for the reform of basic experimental teaching for pre-medicine, nursing, medical laboratory and other majors in medical universities and comprehensive universities.

Author Contributions

Ao Chen: Methodology, Investigation, Data analysis, Writing original draft; Zong-Lin Yang: Methodology, Data analysis; Hai-Rong Zhang: Verification, Supervision; Qing-Qing Deng: Resources, Funding acquisition; Jie Zhang: Methodology, Supervision, Review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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