



RESEARCH ARTICLE

# Exogenous gibberellin improves the yield and quality of basil (*Ocimum basilicum* L.) and chervil (*Anthriscus cerefolium* L.) plants grown under salinity stress conditions

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## Abstract

Gibberellins play a crucial role as plant hormones in the regulation of various aspects of plant growth and development. They are involved in processes such as seed germination, breaking plant and bud dormancy, and counteracting the effects of auxin. Additionally, gibberellins promote leaf expansion, stimulate stem elongation, and contribute to flower development and fruit set. The objective of this study was to investigate the effects of gibberellic acid (GA3) treatments (T0: 0 ppm, T1: 1 ppm, and T2: 10 ppm) on the growth regulation and physiological parameters of basil and chervil plants under salinity stress conditions (150 mM NaCl). The study explored various growth outcomes and biochemical parameters, including chlorophyll, proteins, soluble sugars, proline, and nitrate. The results indicate that the application of gibberellic acid alleviated the adverse effects of high salinity and resulted in enhanced biomass production. In comparison to the control treatment, foliar surface values for basil and chervil increased by 15% and 35%, respectively, in T2. Moreover, root lengths of basil and chervil reached their highest values in T2, showing a 16% increase for basil and a 33% increase for chervil. Carotenoid levels were positively influenced by GA3 treatments, reaching high concentrations in T2, exceeding T0 levels by 41% for basil and 83% for chervil. Additionally, under T2 treatment, protein and glucose levels increased by factors of 2.7 and 1.7, respectively, in basil plants and by factors of 2.1 and 1.7, respectively, in chervil plants. The application of gibberellic acid led to a 33% reduction in proline content for basil and a 27% reduction for chervil compared to the T0 treatment.

## Keywords

Basil, chervil; phytohormone; salinity; osmoprotectant

## Introduction

Currently, salinity and drought stand as two primary abiotic constraints globally, exerting a substantial impact on plant cultivation outcomes, particularly in arid and semi-arid regions. These areas are marked by elevated concentrations of sodium chloride, magnesium, calcium, and other salinity-related elements, leading to significant challenges in terms of plant growth and productivity (1-4). The degradation of arable land due to soil salinization is a widespread environmental problem with adverse effects on food security and nutrition, particularly impacting vulnerable countries and populations.

Extensive agricultural lands in coastal zones have experienced elevated soil salinity levels, attributed to marine intrusion and climate change, resulting in decreased crop production rates (5). The ongoing rise in population will inevitably lead to increased water demands to support the growing populace, consequently expanding the agricultural sector. However, salinity and drought pose significant threats by diminishing crop yields and directly jeopardizing the food supply required to meet the escalating needs of the entire population (6). In essence, it becomes imperative to implement a desalination protocol before applying irrigation methods to non-tolerant plant cultures. Alternatively, direct drainage is recommended for high-tolerance crops (7). The adoption of modern biotechnological tools and the utilization of nanoparticles and nanomaterials emerge as strategies to enhance plant performance, efficiency, and stress tolerance (6, 8).

A diverse array of potential medicinal plants exists globally, playing a crucial role in traditional and complementary medicines with a history spanning thousands of years. Preserving and sustainably utilizing these plants contributes significantly to biodiversity conservation. However, drought and salinity emerge as the two primary abiotic stresses that lead to the dramatic degradation of natural resources, influencing biodiversity, agricultural production, and food security outcomes (9).

On the contrary, gibberellin (GA3), a plant hormone, plays a crucial role in regulating various aspects of plant growth and development, spanning from seed germination to flower formation. Indeed, faster germination allows plants to develop a robust root system that captures more soil moisture (10). Moreover, there is increasing evidence in the literature highlighting the dynamic involvement of plant growth regulators and phytohormones like gibberellins in responding to and tolerating abiotic stresses (11-14). Gibberellic acid plays a pivotal role in regulating gene expression levels that govern plant physiological responses to stressors such as drought and salinity (15). Under drought and salt stress conditions, the biosynthesis, accumulation, and metabolism of various phytohormones, including gibberellins, can be affected (16). When NaCl-exposed okra plants received a foliar application of exogenous gibberellic acid, this mitigated the detrimental impacts of salinity by promoting growth, increasing chlorophyll and carotenoid concentrations, and enhancing the activities of superoxide dismutase, catalase, and peroxidase (17).

Medicinal and aromatic plants have garnered growing scientific and commercial interest in recent years, primarily because of their natural antioxidants, which find applications in the food, cosmetics, beverage, pharmaceutical, and feed industries (18). These plants serve as sources of anti-inflammatory and antimicrobial components, contributing to the prevention of conditions such as hyperglycemia, hypertension, and hepatic disorders (19, 20).

Basil species, belonging to the genus *Ocimum* and the family Lamiaceae, are renowned aromatic herbs and spices, highly valued for their culinary applications and associated medicinal benefits (21). Within this genus, *Ocimum basilicum* and *Ocimum tenuiflorum* stand out and are widely recognized for their medicinal properties, including anti-inflammatory, antimicrobial, and cardioprotective effects (22). Chervil (*Anthriscus cerefolium* L.), an aromatic and delicate annual herb from the Apiaceae family, serves predominantly as a culinary flavoring agent but also finds applications in traditional medicine. This intriguing herb is known for its distinctive and potent flavor compounds, offering essential nutrients that can enhance the nutritional value of the consumer's diet in specific cases (23).

The primary objective of any investment is to recover funds and maximize benefits (24). As an illustrative example, a study on biochar (25) revealed that large-scale applications were hindered due to insufficient profitability. In our case, the challenge is to reduce the cost of water treatment by applying phytohormones to plants under saline stress. Drawing from our field experience, basil thrives in warm, sunny climates and is cultivated under glass using black mulch. Conversely, chervil prefers a mild coastal climate and can be grown in open fields, preferably in semi-shaded areas. Water requirements vary depending on the crop stage and season. For a basil cycle with four cuts intended for fresh consumption (cooking), 2000 m<sup>3</sup>/ha of water is needed to achieve a target yield of 1 kg/m<sup>2</sup> net. Chervil requires slightly more water than basil, reaching 2800 m<sup>3</sup>/ha for a target yield of 0.6 kg/m<sup>2</sup> net. In this context, the present study aims to investigate the effects of applying various concentrations of gibberellins (GA3) on the growth, osmoprotectant levels, and salinity tolerance of basil (*Ocimum basilicum* L.) and chervil (*Anthriscus cerefolium* L.) plants cultivated under salt stress conditions.

## Materials and Methods

The plants utilized in this study include basil (*Ocimum basilicum* L.) of the Aromix variety sourced from MARALDI SEMENTI, Italy, and chervil (*Anthriscus cerefolium* L.) of the Green Fennel variety obtained from CN seeds, UK.

### Crop Design and Plant Sampling

The experiment was carried out in a plastic greenhouse at Mohammed 1st University in Nador, Morocco. This location is designated as an open-field space and is characterized by experiments designed by researchers from the institution in a semi-arid climate.

The temperature in the greenhouse was maintained within the range of 25-30 °C, with humidity levels between 75-85%. The plants received natural sunlight. The characteristics of the irrigation water used were as follows: pH: 7.7; electrical conductivity: 1.3 ds.m<sup>-1</sup>, Cl<sup>-</sup>: 172 mg L<sup>-1</sup>, Na<sup>+</sup>: 101.4 mg L<sup>-1</sup>, K<sup>+</sup>: 5.14 mg L<sup>-1</sup>, HCO<sub>3</sub><sup>-</sup>: 119 mg L<sup>-1</sup>, Mg<sup>2+</sup>: 55.57 mg L<sup>-1</sup>, Ca<sup>2+</sup>: 99.03 mg L<sup>-1</sup>, NH<sub>4</sub><sup>+</sup>: <0.02 mg L<sup>-1</sup>, SO<sub>4</sub><sup>2-</sup>: 341.80 mg L<sup>-1</sup> and NO<sub>3</sub><sup>-</sup>: 2.30 mg L<sup>-1</sup>.

The study commenced with the sowing of four seeds of each species per cell in dimpled plates with 150 black holes, each with a volume of 33 cm<sup>3</sup> containing peat. After four weeks, the seedlings were transplanted into 2 L pots with peat as a substrate. One week later, salt stress (150mM NaCl) was applied, using a water volume of 50 mL/plant twice a week.

The plants underwent foliar gibberellic acid treatments (T0: 0 ppm, T1: 1 ppm, and T2: 10 ppm) with a series of ten plants for each treatment, administered for 4 weeks at a rate of 10 mL/plant each week. Subsequently, the plants were sampled and transported to the laboratory for further analysis. Plant organs, including the roots, stems and leaves, were washed and thoroughly dried using filter paper. These dried organs were then placed in an oven at a temperature of 70 °C to determine their final constant weights.

### Measurement of Chlorophyll Content and Carotene

As per the methodology outlined by reference (26), leaf sections used for measuring chlorophyll and carotenoids were extracted using 5 mL of pure methanol for subsequent spectrophotometric determination.

and stored at 4 °C for glucose and proline determinations (28). The glucose concentration was determined spectrophotometrically at 650 nm using a colorimetric assay with an anthrone reagent (28). The free proline content was measured spectrophotometrically at 515 nm following the method described by (29).

### Statistics

The data presented in this report were obtained from a minimum of 3 independent experiments, each comprising three or 4 replicates. Analysis of variance was utilized to assess variations between treatments, and significance was attributed to plant genotypes at  $P \leq 0.05$ . In the figures and tables, distinct letters indicate significant differences based on Duncan's multiple range test (DMRT).

## Results

Gibberellic acid plays a crucial role in enhancing plant tolerance to salinity by positively impacting various aspects, including growth (roots, stems, leaf area), physiological parameters (water content, and pigments), and biochemical parameters (proline, sugars, and proteins) (Table 1).

**Table 1.** The application of gibberellic acid improves the agricultural yield of basil and chervil

Parameters	Root dry biomass (g)			Root fresh biomass (g)			Shoot dry biomass (g)			Shoot fresh biomass (g)		
	0 ppm GA3	1 ppm GA3	10 ppm GA3	0 ppm GA3	1 ppm GA3	10 ppm GA3	0 ppm GA3	1 ppm GA3	10 ppm GA3	0 ppm GA3	1 ppm GA3	10 ppm GA3
Basil	6,01± 0,2a <sup>y</sup> A <sup>z</sup>	6,04± 0,4aA	6,23± 0,4aA	11,57± 0,9aA	13,37± 0,8aB	14,97± 0,9aC	13,92± 0,5bA	14,94± 0,5bB	16,98± 0,7bC	26,04± 0,9bA	27,84± 1,2bAB	29,78± 1,6bB
Chervil	6,16± 0,5aA	6,76± 0,7aA	7,88± 0,4bB	11,98± 0,7aA	13,2± 1aA	18,84± 1bB	7,78± 0,8aA	8,16± 0,7aAB	8,98± 0,6aB	16,23± 0,5aA	18,86± 0,6aAB	20,06± 0,9aB

<sup>y</sup> Different lowercase letters indicate significant differences between plant species. <sup>z</sup> Different capital letters indicate differences between gibberellic acid treatments

### Determination of Nitrate Content

The plant juice was obtained through plant grinding and subsequently stored in 2 mL microtubes at 20-22 °C. For the analysis, appropriate aliquots of the juice were introduced to the device sensor. The LAQUAtwin NO3<sup>-</sup> device (HORIBA®, Kyoto, Japan) was used to measure the nitrate content, with each sample measured nine times and the results were averaged.

### Measurement of Soluble Protein Content

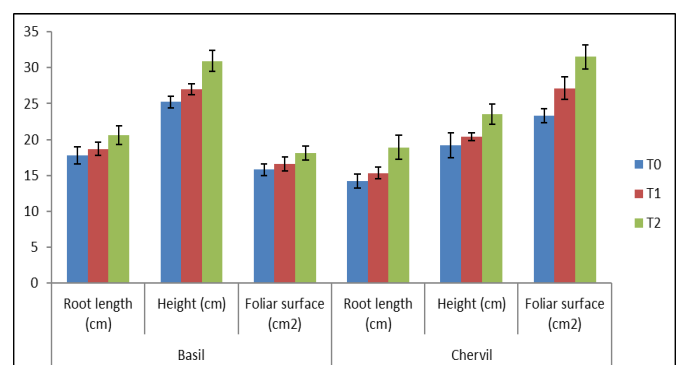
Fresh plant samples (0.5 g) were homogenized with cold phosphate buffer (50 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.0) and then subjected to centrifugation at 12000 × g for 15 min. The resulting supernatant was used to measure soluble proteins with the Bradford G-250 reagent (27). The results are expressed as milligrams of bovine serum albumin per gram of fresh weight.

### Glucose and Proline Determinations for Basil and Chervil plants

Plant tissue samples weighing 0.5 g were crushed in 5 mL of 95% (v/v) ethanol. The insoluble fraction of the extract was then washed twice with 5 mL of 70% ethanol. Subsequently, all soluble fractions were centrifuged at 3500 × g for 10 min, and the resulting supernatants were collected

### Growth Parameters

Fig. 1 illustrates that the foliar application of gibberellic acid has a positive impact on the vegetative growth of basil and chervil plants. In terms of root lengths, T2 exhibited the highest values, surpassing T0 by 16% for basil and 33% for chervil. Similarly, plant height significantly increased in treatments with 10 ppm GA3 for both basil (30.94±1.5) and chervil (23.58±1.4). The foliar surface values of basil and chervil increased in T2 by 15% and 35%, respectively, compared to the control plants.



**Fig. 1.** Positive effect of foliar application of gibberellic acid on the morphological parameters (root length, plant height and aerial surface) of basil and chervil plants. T0: 0 ppm GA3, T1: 1 ppm GA3 and T2: 10 ppm GA3.

### Physiological Parameters

The dry and fresh weights of the roots and shoots increased with the rise in the concentration of exogenous gibberellic acid, reaching a peak in T2 (Table 1). However, treatments with 1 ppm of gibberellins did not significantly affect the root and aerial biomass levels of both plants.

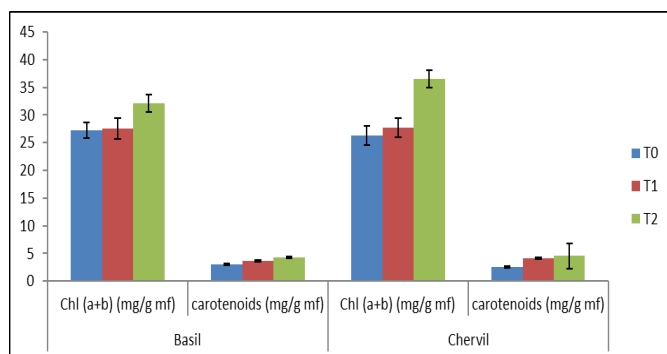
The dry biomass of basil root increased by 0.5 % and 3.7 %, for treatments T1 and T2, respectively. The dry biomass of chervil root increased by 9.7 % and 11.7 % for treatments T1 and T2, respectively. Additionally, the fresh biomass of basil root increased by 15.6% and 28.9% for treatments T1 and T2, respectively. The fresh biomass of chervil root increased by 10.2% and 57.3%, respectively for treatments T1 and T2.

The dry biomass of basil shoots increased by 7.3 % and 28.9 % for treatments T1 and T2, respectively. The dry biomass of chervil shoots increased by 4.9 % and 15.4 % for treatments T1 and T2, respectively.

The fresh biomass of basil shoots increased by 6.9 % and 14.4 % for treatments T1 and T2, respectively. The fresh biomass of chervil shoots increased by 16.2 % and 23.6 % for treatments T1 and T2, respectively.

These results have significant economic and agronomic implications, particularly in terms of job creation and increased agricultural profitability.

In relation to pigment concentrations (Fig. 2), the total chlorophyll content showed a slight effect with the T1 treatment, while the T2 treatment significantly increased this pigment in both species. Carotenoid levels were positively affected by GA3 treatments, reaching high concentrations in T2, surpassing T0 by 41% and 83%, respectively, for basil and chervil.



**Fig. 2.** Foliar application of gibberellic acid reducing the detrimental effect of salinity on chlorophylls and carotenoids in basil and chervil plants. **T0** : 0 ppm GA3, **T1** : 1 ppm GA3 and **T2** : 10 ppm GA3.

### Biochemical Parameters

Table 2 illustrates the impact of exogenous GA3 applications on nitrate, proline, glucose, and protein contents in

**Table 2.** Role of gibberellic acid in mitigating the severe negative effects of salt stress on vegetative growth and biochemical components of basil and chervil plants

Parameters	Nitrate (mg/100g mf)			Proteins (mg/g mf)			Glucose (mg/g mf)			Proline ( $\mu$ g/g mf)		
	0 ppm GA3	1 ppm GA3	10 ppm GA3	0 ppm GA3	1 ppm GA3	10 ppm GA3	0 ppm GA3	1 ppm GA3	10 ppm GA3	0 ppm GA3	1 ppm GA3	10 ppm GA3
Basil	100,4 $\pm$ 9,2a <sup>Y</sup> A <sup>Z</sup>	115,2 $\pm$ 10aB	128,2 $\pm$ 8,6aC	16,6 $\pm$ 0,9aA	27,5 $\pm$ 1aB	44,3 $\pm$ 1,7aC	5,7 $\pm$ 0,2aA	6,1 $\pm$ 0,6aA	9,8 $\pm$ 0,5aB	160,5 $\pm$ 12aA	129,4 $\pm$ 9,8aB	120,7 $\pm$ 9aC
	Chervil	108,3 $\pm$ 8,7bA	124,6 $\pm$ 9,4bB	138,8 $\pm$ 10,4bC	25,4 $\pm$ 1,9bA	38,3 $\pm$ 1,6bB	52,8 $\pm$ 2bC	6,2 $\pm$ 0,9aA	6,5 $\pm$ 0,9aA	10,3 $\pm$ 0,7aB	180,6 $\pm$ 14,6bA	150,9 $\pm$ 16,4bA

basil and chervil plants cultivated under salinity stress conditions. Nitrate content significantly increased with exogenous GA3 application, reaching high levels in T2, exceeding those of the control (T0) by 27.7% and 28.2%, respectively. In basil plants, protein and glucose content levels increased by 1.7- and 0.7-fold under T2 treatment, respectively, compared to the control. Similar trends were observed for chervil, with both GA treatments significantly increasing protein and glucose content levels, where T2 was 2.1- and 1.7-fold higher than T0. Finally, exogenous gibberellins significantly reduced proline content by 33% in basil and 27% in chervil.

### Discussion

Salt stress exerts a detrimental influence on plant development and disrupts plant physiology by impeding photosynthesis. The surplus of salt results in sodium accumulation in the leaves, hindering the plant's capacity to harness energy from sunlight and carbon dioxide, thereby impacting biochemical reactions and physiological processes like mineral nutrient homeostasis, osmolyte accumulation, and hormonal signaling (30, 31). Despite these challenges, plant hormones, particularly gibberellins, can play a pivotal role in facilitating the plant's response to abiotic stresses, including salinity (1, 12). Gibberellins regulate various stages of plant growth and development, such as seed germination, leaf expansion, stem elongation, and flowering, all closely linked to GA (32). The phytohormone GA also engages in the plant's adaptive response to diverse abiotic stresses, including cold, salinity, heat, flooding, and drought (33-35). Many plant species employ gibberellins to contend with a range of abiotic challenges in nature, thereby minimizing the impact of salt stress by enhancing seed germination and other growth, physiological, and biochemical parameters.

In this study, the application of exogenous gibberellin yielded positive results in enhancing growth parameters. From a commercial perspective, it could serve as a valuable agricultural input for boosting agricultural yields and enhancing the overall profitability of agricultural production. In the case of the aromatic plants under investigation, exogenous gibberellin was observed to enhance the salt tolerance of basil and chervil plants, manifested through increased plant height, root length, and foliar area. Similarly, in the case of *Spondias tuberosa* grown under normal conditions (without salinity stress), the application of exogenous gibberellin significantly increased parameters such as plant height, root length, stem diameter, and leaf area (36). Hormones generally

function in cellular communication, serving as chemical messengers produced in a cell or tissue that modulate cellular processes in other cells. By interacting with receptors, hormones contribute to metabolism—a process vital for maintaining and promoting the growth of primary organs (37). Gibberellin's chemical signaling likely occurs synergistically and additively with the action of auxins, activating H<sup>+</sup>-ATPases and creating a conducive environment for expansin activity (38).

Regarding plant biomass, the rise in gibberellin (GA3) concentrations correlated with increased fresh and dry biomass in both roots and shoots (Table 1). Similarly, a study by (39) indicated that the application of GA3 significantly enhanced the shoot dry weight of control and salt-stressed pea plants. This effect was attributed to the improved allocation of assimilates to the root system, resulting in more robust and efficient nutrient absorption. Additionally, the application of exogenous gibberellins not only alleviated the adverse impact of salinity but also stimulated cell division and elongation behaviors, reduced water loss rates, leading to increased leaf water potential and carbon gain rates. These positive changes in physiological processes may contribute to an overall increase in biomass production (40).

Chlorophyll and carotenoids, the primary pigments involved in plant photosynthesis, play a crucial role in various physiological processes (41, 42). Abiotic stresses, including salinity, can inflict damage on the photosynthetic machinery and chlorophyll content, impacting plant health (43). In this study, the application of exogenous GA significantly increased both chlorophyll and carotenoid contents in both species (Fig. 2). These findings align with previous studies, such as those by (36, 43), indicating that GA3 application enhances the accumulation of chlorophyll pigments, including chlorophylls a and b, as well as carotenoids, particularly in leaves. The observed increase in chlorophyll pigments (Chl a and Chl b) and carotenoids in gibberellin-treated plants under saline stress induced by sodium chloride (NaCl) can be attributed to reduced Na<sup>+</sup> accumulation, diminished oxidative damage, and an augmented antioxidant defense system (43). Gibberellin's positive impact on the concentration of chlorophyll pigments and carotenoids, essential components of photosynthesis, underscores its potential as a promising phytohormone for broader commercial and agronomic applications.

Proline, glucose, and proteins play a crucial role in enhancing plant tolerance to various abiotic stresses, including drought and salinity (1, 44). The heightened synthesis of these parameters represents a crucial adaptive mechanism for drought and salt tolerance, playing a pivotal role in osmotic adjustment, enhanced water absorption, scavenging of reactive oxygen species, and protection of subcellular structures in plants (45).

Proline is preferentially accumulated in plant leaves to uphold chlorophyll levels and cell turgor, thereby safeguarding photosynthetic activity under saline stress conditions (46). The accumulation of proline in response to

unfavorable conditions reflects a stress-resistant nature in many plant species. This accumulation of compatible solutes during salinity stress serves as a metabolic adaptation for osmotic balance between the vacuole and cytosol, with higher concentrations generally observed in plants exhibiting greater tolerance to salinity stress conditions (47-49). In the current study, the application of gibberellin led to a reduction in proline accumulation, suggesting its role in alleviating salt stress levels (Table 2). Similar findings were reported by (39), indicating a relative decrease in proline levels in saline-treated plants after GA application. In contrast to proline, the increased application of exogenous GA significantly elevated glucose and protein contents in the leaves of both species. Previous studies have highlighted that external GA application can alleviate the severe negative effects of salt stress by activating specific enzymes involved in RNA and protein synthesis (8). Our results align with other research demonstrating GA3's promotion of protein biosynthesis in maize plants by enhancing soil nitrogen absorption (43). More recently, we observed a significant increase in glucose content in both wild-type and transgenic tomato plants overexpressing the LeNHX4 gene following the exogenous application of GA3. The substantial rise in soluble protein content with GA3 application could be attributed to its positive impact on protein synthesis or a decrease in protein degradation.

Moreover, the exogenous application of gibberellic acid resulted in an increase in nitrate concentration (Table 2). This aligns with similar findings reported by (50), who observed that salt stress mitigation in wheat was associated with enhanced nitrogen status and nitrate reductase activity following treatment with plant growth regulators like gibberellic acid. The study by (51) highlighted that gibberellin (GA) hormones influence nitrogen (N) uptake activity by regulating the transcription of nitrate transporters (NRTs). Furthermore, external supplementation of GA3 stimulated plant growth, creating an elevated demand for nitrogen during tissue development (52).

These results suggest that the application of exogenous gibberellin to the two species studied and potentially to plants in general could serve as a highly effective approach for agricultural production in areas facing salinity-related challenges.

## Conclusion

The exogenous application of GA3 demonstrated beneficial effects in alleviating the negative impacts of high salinity levels by improving various growth parameters. This hormone emerges as a crucial element in enhancing agricultural yields, economic profitability, and the availability of plant material in arid and semi-arid areas that face salinity-related challenges.

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## Authors contributions

HE carried out the experiments, wrote the manuscript. MA, AS, AM and SB and participated in the design of the study, reviewed, advised and supervised the work. MM prepared the manuscript, organized and formatted the article, MB and AR revised and improved the manuscript. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None.

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