



Mitigation of Salinity Stress in Soybean [*Glycine max* (L.) Merrill] using Exogenous Soil Drenching with *Stachytarpheta jamaicensis* (L.) Vahl Extract

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ABSTRACT

Background: Salinity stress limits soybean (*Glycine max*) productivity by impairing growth, photosynthesis and cellular balance. This study evaluated *Stachytarpheta jamaicensis* (L.) Vahl leaf extract (SjE) as a natural biostimulant to mitigate salinity stress.

Methods: Soybean plants were exposed to 100 mM NaCl (SS-100) and treated with SjE (10% and 25%) via soil drenching. Growth, photosynthetic pigments, oxidative markers (LPO, H₂O₂) and antioxidant enzymes (SOD, CAT, POD) were assessed.

Result: Salinity reduced growth and pigments while increasing oxidative damage. SjE, especially at 10%, improved growth, restored pigments, reduced LPO and H₂O₂ and enhanced antioxidant enzymes. These effects are linked to phenolics and flavonoids. Overall, 10% SjE showed optimal performance, highlighting its potential as an eco-friendly biostimulant for salinity stress tolerance.

Key words: Antioxidant enzymes, Biostimulant, Salinity stress, Soybean (*Glycine max*), *Stachytarpheta jamaicensis*, Sustainable agriculture.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill; Fabaceae] is a major legume crop valued for its high protein and oil content and its role in improving soil fertility through biological nitrogen fixation (Abd-Alla *et al.*, 2023; Dilawari *et al.*, 2022). Although global production has increased (Hamza *et al.*, 2024), yield is severely constrained by abiotic stresses, particularly salinity (Hasanuzzaman *et al.*, 2022). Salinity, prevalent in arid and semi-arid regions, disrupts plant growth and productivity due to excessive salt accumulation in soils (Hussain *et al.*, 2019; El Sabagh *et al.*, 2020). Field studies have demonstrated that salinity significantly reduces soybean plant establishment, biomass accumulation, nodulation, pod development, seed yield and seed quality, resulting in substantial economic losses (Day and Şahin 2024; Hasanuzzaman *et al.*, 2022; Singh, 2022; Hamwieh *et al.*, 2011). Therefore, developing sustainable approaches to improve salinity tolerance in soybean has become an important research priority.

Several strategies have been developed to mitigate salinity-induced yield losses in soybean (Verma *et al.*, 2024), including soil reclamation (Wang *et al.*, 2024; Abiala *et al.*, 2018), enhanced irrigation (Singh *et al.*, 2023) and breeding for salt-tolerant cultivars (Chen *et al.*, 2024a; Guan *et al.*, 2021; Hamwieh *et al.*, 2011). Advanced approaches such as transgenics and genome editing have also enhanced salt tolerance (Chen *et al.*, 2024b; Wang *et al.*, 2021), but require high cost, regulatory approval and advanced infrastructure. Plant biostimulants have recently emerged as eco-friendly alternatives (Johnson *et al.*, 2024). Derived from plant extracts (Saravanan *et al.*, 2023; Osman *et al.*, 2021), seaweeds (Mannan *et al.*, 2023; Repke *et al.*, 2022), microorganisms (Tran and Hoang 2026;

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Jaybhay *et al.*, 2025; Santos *et al.*, 2023; El-Esawi *et al.*, 2018) and organic compounds (Oliveira *et al.*, 2024; Kim and Sang, 2023; Malekzadeh, 2015) they enhance growth, nutrient uptake, antioxidant defense and hormonal balance, largely due to bioactive compounds such as phenolics and flavonoids (Salam *et al.*, 2023).

Among plant-derived biostimulants, *Stachytarpheta jamaicensis* (L.) Vahl (Verbenaceae) has gained attention due to its diverse phytochemical profile and adaptability

(Chongloi *et al.*, 2025). Widely distributed in tropical and subtropical regions, it is considered an aggressive invasive species (Liew and Yong, 2016). Despite this, it contains bioactive compounds such as phenolics, flavonoids, tannins and antioxidants with significant biological activity (Chongloi *et al.*, 2025; Bliss *et al.*, 2022; Kavitha *et al.*, 2022; Utami *et al.*, 2022). These compounds contribute to stress tolerance by enhancing antioxidant systems, maintaining osmotic balance and protecting cellular structures from oxidative damage (Salam *et al.*, 2023). However, despite its rich phytochemical profile and stress-adaptive characteristics, the potential of *S. jamaicensis* leaf extract as a natural biostimulant for mitigating salinity stress in soybean has not yet been investigated. Therefore, the present study evaluated the effectiveness of exogenous soil drenching with *S. jamaicensis* leaf extract in improving growth, photosynthetic performance, antioxidant defense and oxidative stress tolerance in soybean under saline conditions. This study provides new evidence supporting the utilization of an invasive weed as a sustainable and eco-friendly biostimulant for soybean cultivation in salt-affected soils.

MATERIALS AND METHODS

Treatment procedure

Leaf extract of *Stachytarpheta jamaicensis* was prepared as protocol by Punithavathi *et al.* (2026). Soybean (*Glycine max*) seeds (cv. JS-335) were sown in plastic pots (4-inch; top diameter 13 cm, base 10 cm, height 11 cm) and maintained under controlled conditions (25±2°C; ~80% relative humidity). Five days after germination, plants were divided into six treatment groups:

1. C - Control: Plants received water alone.
2. SJE-10%: Plant received 10% *S. jamaicensis* extract.
3. SJE-25%: Plant received 25% *S. jamaicensis* extract.
4. SS-100: Plants received 100 mM NaCl alone.
5. SJE-10% + SS-100: Plants received combined treatments of 10% *S. jamaicensis* extract along with 100 mM NaCl.
6. SJE-25% + SS-100: Plants received combined treatments of 25% *S. jamaicensis* extract along with 100 mM NaCl.

Treatments were applied as soil drenching at 5-day intervals for a total of six applications. Plants were harvested on the 33rd day after sowing for subsequent morphological (mean number of leaves, shoot length, shoot fresh biomass, number of roots, root length and root fresh weight), physiological and biochemical analyses.

Physiological analysis

Assessment on photosynthetic pigments

Photosynthetic pigments were estimated following the methods of Lichtenthaler (1987) and Arnon (1949).

Biochemical characterization

Preparation of enzyme extract

Soybean tissues were homogenized and centrifuged and the supernatant was used for protein estimation (Bradford, 1976) and future analysis.

Determination of oxidative stress markers

Lipid peroxidation (LPO) and hydrogen peroxide (H₂O₂) contents were determined following Ohkawa *et al.* (1979) and Velikova *et al.* (2000), respectively.

Enzymatic antioxidant activities

Activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were assayed according to Marklund and Marklund (1974); Kar and Mishra (1976) and Aebi (1984), respectively.

Statistical analysis

Data were analysed using IBM SPSS Statistics 20 (IBM Corp., USA). Morphological data (n=5 × 2) and physiological/biochemical data (n = 4) are presented as mean ± SE. Significant differences were determined by DMRT at P≤0.05.

RESULTS AND DISCUSSION

Morphological analysis

Salinity stress (SS-100) significantly reduced soybean growth, with decline in leaf number (42.55%), shoot length (44.76%), shoot fresh weight (45.60%), root number (26.54%), root length (36.58%) and root fresh weight (22.91%) compared to control. SJE improved growth under non-stress conditions, particularly at 10%, increasing leaf number (34.04%), shoot length (19.53%), biomass (42.40%), root number (27.43%), root length (28.90%) and root fresh weight (62.50%). In contrast, SJE-25% reduced growth (23.40-34.92%), indicating dose-dependent inhibition. Under salinity, SJE partially restored growth. SJE-10% + SS-100 increased leaf number (74.1%), shoot length (12.3%), shoot biomass (97.1%), root number (25.3%) and root length (35.5%) over SS-100, though root fresh weight declined (35.1%). SJE-25% + SS-100 showed limited improvement. These results indicate that 10% SJE is optimal for growth enhancement (Table 1 and Fig 1).

Salinity stress markedly inhibited soybean growth by reducing leaf number, shoot and root length and biomass, reflecting the adverse effects of osmotic stress, ion toxicity and nutrient imbalance on plant development. Similar reductions in soybean growth under saline conditions have been reported by Hasanuzzaman *et al.* (2022), Osman *et al.* (2021) and where salinity impaired cell expansion, water uptake and overall biomass accumulation. In the present study, exogenous soil drenching with 10% *Stachytarpheta jamaicensis* extract (SJE) significantly alleviated these growth reductions, indicating its biostimulant potential. Comparable growth-promoting effects have been reported with onion extract in soybean (Saravanan *et al.*, 2023) and maize grain extract in common bean under salinity (Rady *et al.*, 2019). The improved growth observed in SJE-treated plants may be associated with its phenolic- and flavonoid-rich composition, which enhances antioxidant capacity, regulates auxin homeostasis, promotes root development and improves nutrient

acquisition under saline conditions (Singh *et al.*, 2021). Interestingly, the 25% extract was less effective than the 10% treatment, suggesting that excessive concentrations may interfere with normal metabolism, highlighting the importance of optimizing biostimulant dosage.

Physiological analysis

Assessment on photosynthetic pigments

Salinity stress reduced chlorophyll *a* (31.1%), chlorophyll *b* (42.6%), total chlorophyll (38.1%) and carotenoids (27.4%) compared to control. SJE enhanced pigment levels under non-stress conditions, with SJE-10% increasing chlorophyll *a* (61.5%), chlorophyll *b* (76.0%), total chlorophyll (70.4%) and carotenoids (2.26-fold). SJE-25% also improved pigments but to a lesser extent. Under salinity, SJE-10% + SS-100 significantly restored pigments,

increasing chlorophyll *a*, *b* and total chlorophyll (~2.08-2.09-fold) and carotenoids (2.27-fold) compared to SS-100. SjE-25% + SS-100 showed moderate improvements. Overall, 10% SjE effectively alleviated pigment loss under salinity (Fig 2).

Salinity-induced reductions in chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids indicate severe impairment of the photosynthetic apparatus. These observations agree with previous soybean studies demonstrating that salinity accelerates chlorophyll degradation, damages chloroplast ultrastructure and decreases photosynthetic efficiency (Hasanuzzaman *et al.*, 2022; Parvin *et al.*, 2019). Application of SJ-E, particularly at 10%, significantly restored pigment content under salinity stress, suggesting effective protection of the photosynthetic machinery. The phenolic and flavonoid constituents of

Table 1: Influence of *Stachytarpheta jamaicensis* extract treatments on growth attributes of soybean plants under salinity stress conditions.

Treatment	Number of leaves (Plant ⁻¹)	Shoot length (cm plant ⁻¹)	Shoot fresh weight (g plant ⁻¹)	Number of roots (cm plant ⁻¹)	Root length (cm plant ⁻¹)	Root fresh weight (g plant ⁻¹)
C	9.4±0.21 ^b	56.3±0.12 ^b	1.25±0.12 ^b	22.6±0.21 ^b	16.4±0.42 ^b	0.48±0.07 ^b
SjE-10%	12.6±0.23 ^a	67.3±0.41 ^a	1.78±0.04 ^a	28.8±0.27 ^a	21.14±0.21 ^a	0.78±0.06 ^a
SjE-25%	7.2±0.18 ^c	36.64±0.45 ^c	0.92±0.09 ^c	17.6±0.40 ^c	13.86±0.31 ^c	0.45±0.07 ^b
SS-100	5.4±0.68 ^d	31.1±0.71 ^d	0.68±0.56 ^d	16.6±0.57 ^c	10.4±0.53 ^d	0.37±0.05 ^c
SjE-10% + SS-100	9.4±0.27 ^b	34.92±0.18 ^c	1.34±0.13 ^b	20.8±0.29 ^b	14.09±0.43 ^c	0.24±0.05 ^d
SjE-25% + SS-100	5.4±0.29 ^d	20.8±0.39 ^e	0.29±0.06 ^e	12.4±0.28 ^d	14.6±0.30 ^c	0.16±0.04 ^e

Data represent mean values $\pm$ SE of two independent experiments ($n=5 \times 2$). Mean values followed by different letters within a column are significantly different according to Duncan's multiple range test (DMRT) at $P \leq 0.05$.

Note: The data were recorded after 33rd day of sowing.



Fig 1: Effects of *Stachytarpheta jamaicensis* extract (SiE) on soybean under salinity stress.

S. jamaicensis may protect chloroplast membranes against ROS-induced damage while maintaining chlorophyll biosynthesis. Similar improvements in photosynthetic pigments have been reported following application of *Moringa oleifera* leaf extract in faba bean (Eleish *et al.*, 2026), cypress

leaf extract in zucchini (ElSayed *et al.*, 2022) and yeast and carrot root extracts in maize (Abdel Latef *et al.*, 2019). These findings collectively indicate that plant-derived biostimulants preserve photosynthetic efficiency by maintaining chloroplast integrity and reducing oxidative damage.

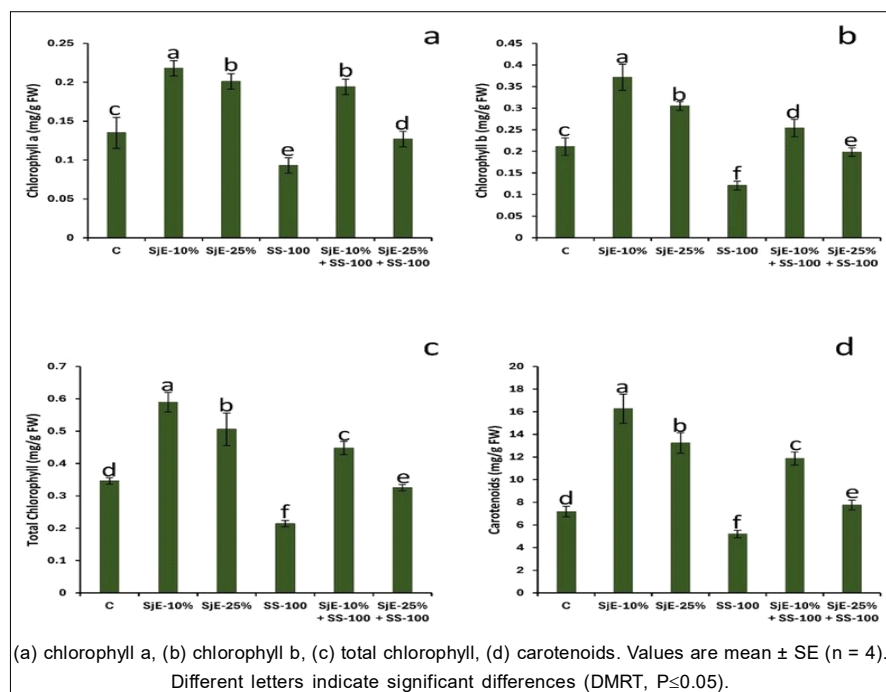


Fig 2: Effect of *Stachytarpheta jamaicensis* extract (SjE) on photosynthetic pigments under salinity stress.

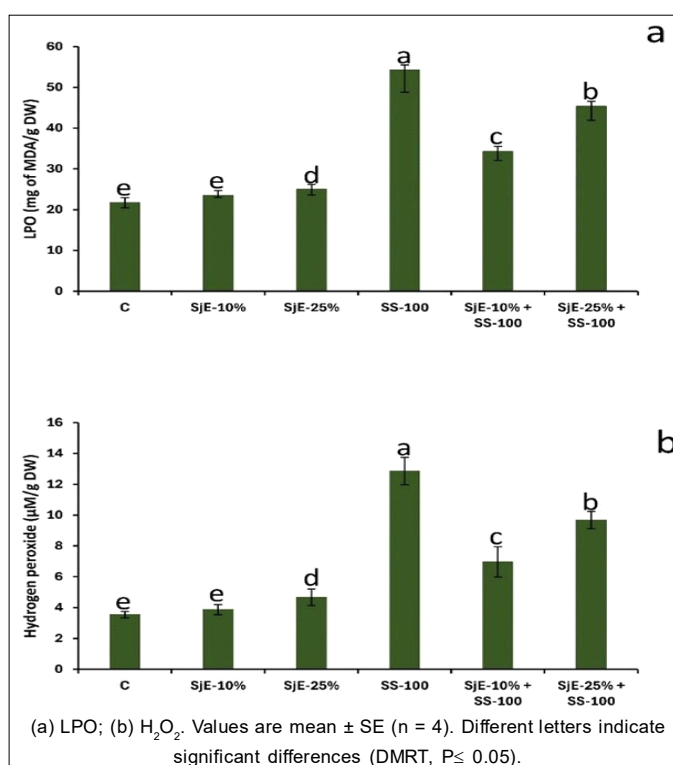


Fig 3: Inhibitory effect of *Stachytarpheta jamaicensis* extract (SjE) on oxidative stress markers under salinity stress.

Biochemical characterization

Lipid peroxidation (LPO) and hydrogen peroxide (H₂O₂) content and enzymatic antioxidant activity

Salinity stress markedly increased oxidative damage, with LPO rising 2.50-fold and H₂O₂ 3.62-fold compared to control (Fig 3). SjE application reduced these effects under salinity. SjE-10% + SS-100 decreased LPO (36.84%) and H₂O₂ (45.82%), while SjE-25% + SS-100 reduced LPO (~16.41%) and H₂O₂ (~25.73%). The 10% treatment showed stronger protection against oxidative stress (Fig 3). Salinity stress reduced SOD (48.22%), CAT (64.91%) and POD (56.27%) activities compared to control. SjE enhanced enzyme activities under non-stress conditions, with SjE-10% increasing SOD (2.4-fold), CAT (1.68-fold) and POD (2.18-fold), while SjE-25% showed lower stimulation. Under salinity, SjE improved antioxidant responses. SjE-10% + SS-100 increased SOD (2.51-fold), CAT (3.16-fold) and POD (4.56-fold). Overall, SjE, particularly at 10%, effectively restored antioxidant defense and reduced oxidative damage (Fig 4). Overall, these results demonstrate that SjE effectively reinforces the antioxidant defense machinery

under salinity stress, with 10% concentration showing maximum efficiency in restoring enzymatic activities and protecting plants from oxidative damage.

The substantial increase in LPO and H₂O₂ under salinity confirms enhanced ROS generation and membrane lipid peroxidation, which are characteristic responses of soybean exposed to salt stress (Hasanuzzaman *et al.*, 2022). SjE application significantly reduced both oxidative stress markers, indicating effective protection against ROS-mediated cellular damage. This response is likely attributable to the high antioxidant potential of *S. jamaicensis*, particularly its phenolics and flavonoids, which directly scavenge ROS and stabilize membrane integrity. Similar reductions in oxidative stress have been reported following application of plant-derived antioxidants (Ahmad *et al.*, 2022) and licorice root extract in pea (Desoky *et al.*, 2019). The enhanced activities of SOD, POD and CAT further demonstrate that SjE strengthens the soybean antioxidant defense system under salinity stress. SOD catalyzes the conversion of superoxide radicals into H₂O₂, whereas CAT and POD subsequently detoxify H₂O₂ into water and oxygen, thereby limiting oxidative injury. Similar enhancement of

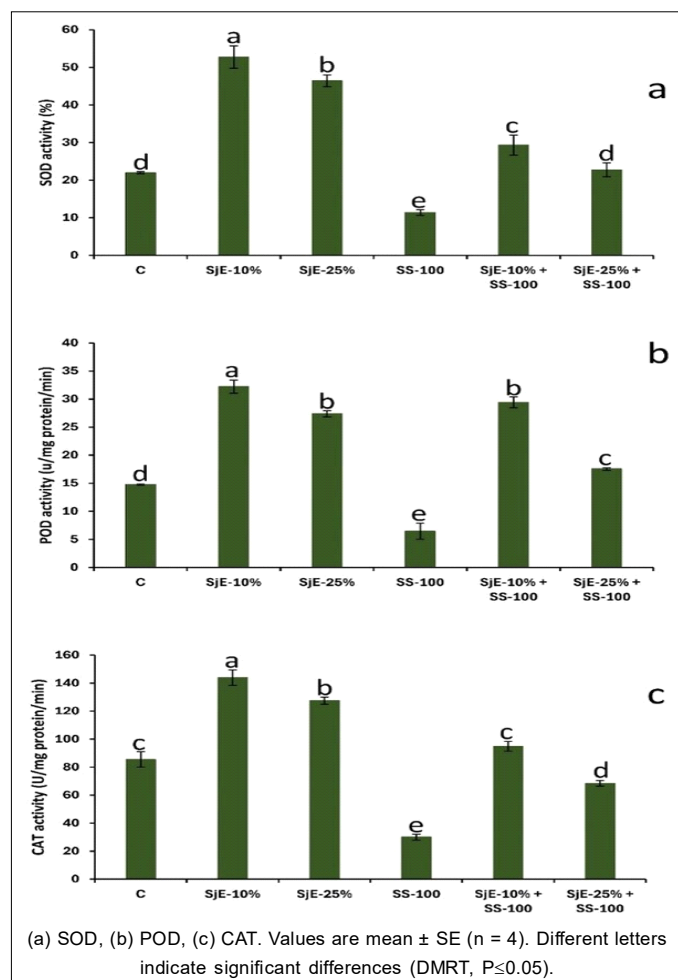


Fig 4: Effect of *Stachytarpheta jamaicensis* extract (SjE) on antioxidant enzymes under salinity.

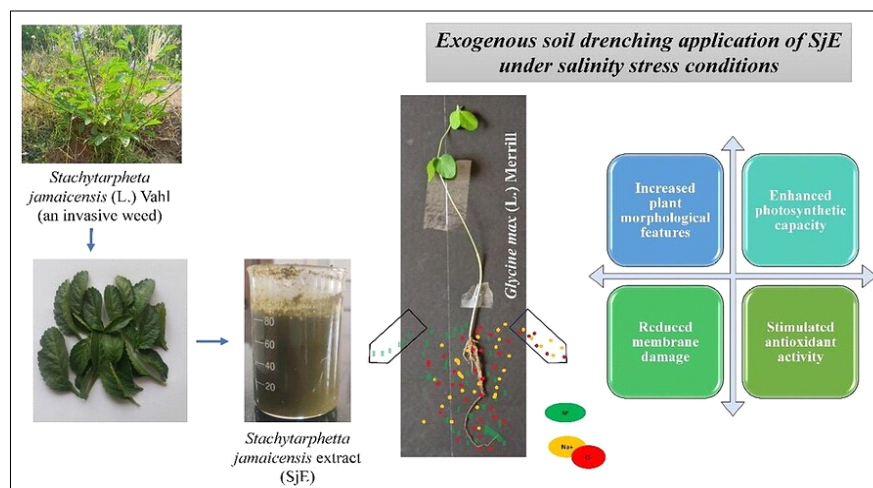


Fig 5: Graphical illustration of *Stachytarpheta jamaicensis* extract (SjE) enhancing morpho-physiological traits and antioxidant activities while reducing oxidative stress in soybean under salinity conditions.

antioxidant enzymes has been reported in soybean inoculated with *Bacillus firmus* (El-Esawi *et al.*, 2018) and tomato supplemented with microalgae-cyanobacteria biostimulants (Mutale-joan *et al.*, 2021). The consistently greater effectiveness of the 10% extract compared with the 25% treatment further indicates that moderate concentrations are sufficient to maximize antioxidant protection without imposing metabolic constraints. Overall, the present study demonstrates that *Stachytarpheta jamaicensis* leaf extract effectively mitigates salinity stress by enhancing growth, preserving photosynthetic pigments, reducing oxidative damage and activating enzymatic antioxidant defenses (Fig 5). Unlike previous studies that mainly evaluated conventional plant extracts or microbial biostimulants, this study provides the first evidence that an invasive weed species, *S. jamaicensis*, can be successfully utilized as a sustainable and eco-friendly biostimulant to improve salinity tolerance in soybean. These findings support its potential application for sustainable soybean cultivation in salt-affected soils and provide a strong basis for future multi-season field validation.

CONCLUSION

Exogenous soil drenching with *Stachytarpheta jamaicensis* extract effectively mitigated salinity stress in soybean (*Glycine max*) by improving growth, restoring photosynthetic pigments and enhancing antioxidant defense. The treatment significantly reduced oxidative damage (LPO and H_2O_2) while stimulating key enzymes (SOD, CAT, POD). Among the tested doses, 10% extract consistently showed superior performance compared to 25%. The findings highlight the potential of *S. jamaicensis* as a cost-effective and eco-friendly biostimulant. This approach offers a promising strategy for improving crop resilience under saline conditions.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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