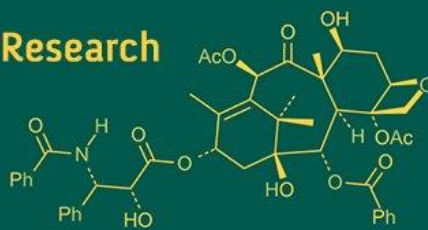


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(9): 1263-1268

www.biochemjournal.com

Received: 07-07-2026

Accepted: 10-08-2026

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Effect of iron nanoparticles on seed germination and early seedling growth of finger millet (*Eleusine coracana* L.)

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i9Sp.9993>

Abstract

Iron is a vital element for plant growth and plays a significant role in various physiological and biochemical processes. In recent years, iron nanoparticles (INPs) have gained attention as a potential approach for enhancing nutrient availability and boosting early plant growth. However, their effects can vary with concentration and excessive application may negatively affect seed germination and seedling development. The present study aimed to evaluate the effect of various concentrations of INPs on seed germination and early seedling growth of finger millet (*Eleusine coracana* L.) seeds. Seeds were exposed to INPs at concentrations of 0.05, 0.10, 1.0, 2.0, 3.0 and 4.0 mg/10 mL, with untreated seeds used as the control. The experiment was conducted with three replications, with 10 seeds per Petri plate and observations were recorded for seven days. Germination percentage, root length, shoot length and total seedling growth were evaluated. The results indicate that the different concentrations of INPs respond on finger millet seeds. Highest germination percentage at lower concentration, with 93.33 % at 0.1 mg/10 mL and 90 % at 0.05 mg/10 mL compared with control (100 %). Whereas, germination percent decreased with an increase in INP concentration and reached only 23.33%. Concentration-dependent response showed in seedling root and shoot length. The highest root length (3.58 cm) and shoot length (1.74 cm) were recorded in control. Among the INPs treatments, the 0.1 mg/ 10mL concentration showed 3.52 cm root length and 1.70 cm shoot length. At higher INP concentrations, seedling growth and vigour decreased. Among all the treatments, 0.1 mg/10 mL showed the most effective response with a 93.33 % germination and a seed vigour index of 487.18. The findings suggest that a suitable concentration of INPs may support early establishment of finger millet, whereas higher concentrations may adversely affect seed germination and seedling vigour.

Keywords: Finger millet, iron nanoparticles (INPs), vigour index, seed germination, seedling

Introduction

Iron is the fourth most abundant element in the Earth's crust. However, its actual availability to plants remains limited because it readily precipitates into insoluble hydroxides under natural soil conditions. Aeration and drainage conditions have a significant influence on the chemical speciation of iron in soils. Iron is an essential micronutrient for better growth and development of plants. In the plants it is required not only for energy production but also for sulfate and nitrate reduction. In chlorophyll, iron acts as a key precursor, iron is required as an active site of glutamyl-tRNA reductase, an enzyme for the formation of 5-aminolevulinic acid. Due to limited mobility of iron within plant tissue, the younger leaves show interveinal chlorosis. Iron deficiency affects physiological functions of plants and thus normal growth and development. Not only for plants, iron plays an important role in human health, as it's essential for hemoglobin synthesis, biochemical pathways, and electron transport reactions (Olivares *et al.*, 1999) [4]. Plants get their iron needs from the soil and the beneficial bacteria in it. In recent years, application of nanotechnology in the agriculture area has been highlighted as a possible alternative to conventional fertilizers. Nanoparticles (NPs) are particles with sizes typically ranging from 1 to 100 nm. NPs help in plant growth and development due to their physiochemical properties, like high surface area to volume ratio (Reddy and Chhabra, 2022) [7]. Several nanofertilizers, like nano urea, nano DAP, nano zinc, nano nitrogen, etc., are available in the market (Mondal, 2022) [3].

Iron Nanoparticles (INPs) help in increasing nutrient availability, uptake, translocation and physiological response in plants. INPs also help in enzyme activity, metabolic activation, water uptake and nutrient availability. NPs also enhance micronutrient uptake, stimulating the biosynthesis of antioxidant and secondary metabolites.

Finger millet (*Eleusine coracana* L.), which is commonly known as ragi or nagli, is a nutritionally rich millet crop commonly cultivated in semi-arid and tropical regions. Finger millet is the fourth largest crop among millets and the sixth largest crop among cereals. Due to finger millet's nutritional composition and capability to grow under drought conditions and marginal soil conditions, it is highly valuable. Finger millet is particularly notable for its significantly high iron content, and it serves as an important dietary source. Apart from that, it is also a rich source of calcium, protein, fiber and other minerals that contribute to its nutritional and health-promoting properties. For diabetic patients, finger millet helps to regulate blood sugar level due to its low glycemic index. Its anti-inflammatory and antioxidant compounds help in improving digestive and metabolic health. Finger millet is referred to as "Nutri-cereal" due to its superior nutritional quality, and finger millet plays an important role in preventing malnutrition and micronutrient deficiencies, especially iron deficiency in anaemia, which may contribute to improving public health by helping address micronutrient deficiencies. In particular, understanding the response of finger millet seeds to different INP concentrations can help identify concentrations that promote germination and early seedling vigour while avoiding potential inhibitory effects. Therefore, the present study was undertaken to evaluate the effects of different concentrations of iron nanoparticles on seed germination and early seedling growth of finger millet (*Eleusine coracana* L.) and to identify a concentration range favourable for seedling establishment.

Materials and methods

Plant material and experimental conditions

For the seed germination experiment, finger millet seeds were used (variety: gira). Healthy and undamaged seeds were used for further study. The experiment was conducted at laboratory-controlled conditions to check the response of different concentrations of INPs on finger millet seeds.

Iron nanoparticle preparation

INPs were synthesized by the co-precipitation method as described by Palaniswamy *et al.* (2013) [5]. To begin with, equal volumes of 1M ferric chloride (FeCl_3) and 1.5 M ferrous sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) solutions were mixed in a clean glass beaker and placed on a magnetic stirrer under continuous and vigorous stirring. To the prepared mixture, 3 M sodium hydroxide (NaOH) solution was added at the rate of one drop per second. The addition of NaOH was continued until the solution reached a pH between 10 and 11. At that point a visible color change occurred and a formation of a black precipitate. The precipitate obtained during synthesis was carefully washed with deionized water to remove any residual salts and impurities from the solution. Using the magnetic decantation method, separation of the black precipitate was achieved, which ensured effective collection of the nanoparticles. The collection material was subsequently dried in a hot air oven at 150 °C for 2-3 hr. The resulting dried product was identified as iron nanoparticles, which were subsequently utilized for further experimental investigations.

Experimental design and INPs treatments

The experiment was conducted in a completely randomized design (CRD) with seven treatments and three replications (T1-T7), including the untreated control. Each treatment was maintained in three replications, with 10 seeds per Petri plate. Thus, each treatment consisted of 30 seeds.

Table 1: Treatments details

Treatment	INPs concentrations	Replication	Seeds
T1	Control	3	10
T2	0.05 mg/10 mL	3	10
T3	0.10 mg/10 mL	3	10
T4	1.00 mg/10 mL	3	10
T5	2.00 mg/10 mL	3	10
T6	3.00 mg/10 mL	3	10
T7	4.00 mg/10 mL	3	10

Germination test

To study the effect of INPs on finger millet seeds (variety Gira) germination. For the germination test, seeds were collected from Hill Millet Research Station, Waghai Agricultural University, Navsari, Gujarat. For the germination test germination plates were used. LD_{50} was determined as described by Sutariya *et al.*, (2024) [11]. To find LD_{50} , seeds were grown at different INP concentrations (0.05, 0.1, 1, 2, 3 and 4 mg/10ml) in sterile germination plates and % seed germination was calculated. The plates were incubated under the experimental laboratory conditions used for the study. Moisture was maintained throughout the experimental period, and Hoagland solution was added to maintain nutrition to ensure adequate conditions for seed germination. Germination observations were recorded for 7 days. Seed germination was recorded when visible radicle emergence was observed.

Determination of germination percentage

Germination percentage was calculated based on numbers of seeds germinated relative to the numbers of total seeds (Akshitha *et al.* 2026) [1]. Ten seeds were used per replication, with three replications for each treatment, giving a total of 30 seeds per treatment. Germination percentage was calculated using the formula:

$$\text{Germination (\%)} = (\text{Number of germinated seeds} / \text{Total number of seeds}) \times 100$$

Measurement of seedling growth

Seedlings were carefully removed from Petri plates after the experimental period. For measurement of root and shoot length of each seedling using a scale and expression in centimeters (cm). Root length was measured from the point of root emergence to the tip of the primary root, while shoot length was measured from the seed base to the tip of the shoot. The recorded root and shoot measurements were used to determine the effect of INP treatment on early seedling development.

Seed vigour Index

Seed Vigour Index (SVI) is a key indicator of overall seedling performance, as it integrates germination percentage with seedling length (Akshitha *et al.* 2026) [1]. The seed Vigour Index was calculated using the formula:

$$\text{Seed Vigour Index} = \text{Germination (\%)} \times \text{Seedling length (cm)}$$

Results and discussion

Effect of iron nanoparticles on seed germination

Effect of INPs on seed germination of finger millet (*Eleusine coracana* L.) in a concentrations-dependent manner. Three replications of each treatment, giving a total 30 of seeds per treatment. The untreated control (T1) recorded the highest germination rate, 100%. T2 and T3 recorded 90 % and 93.33 % germination rates, respectively. Germination rate decreasing with increasing INP concentration from T4 (66.67 %) to T7 (23.33 %). The highest INP concentration T7 resulted in the lowest germination (7/30 seeds), representing a 76.67 percentage-point reduction compared with the control. The study demonstrates that the effect of INPs on finger millet seeds was dose-dependent. At lower concentrations of INPs, likewise, T2 (90.00 %) and T3 (93.33 %) germination rates were higher as compared to other treated seeds. However, increasing INP concentration, the germination rate was

declining and the strongest inhibition was observed in T7 (23.33 %).

The relatively high germination observed at the lower INP concentrations may be associated with the requirement of iron as an essential micronutrient during early plant development. Iron participates in several important cellular processes, including electron transport, respiration and enzymatic reactions. At an appropriate level, Fe availability may therefore support metabolic processes associated with seed activation and seedling establishment. However, the present results indicate that increasing INP concentration beyond a suitable level can negatively affect germination.

In response to nanoparticles-induced stress, at higher concentrations of INPs, germination was decreased. Alkhatib *et al.* (2021) and Serpoush *et al.* (2022)^[9] reported that the effect of INPs increased germination and early seedling growth at optimal concentrations of INPs. At higher concentrations shows an inhibitory response.

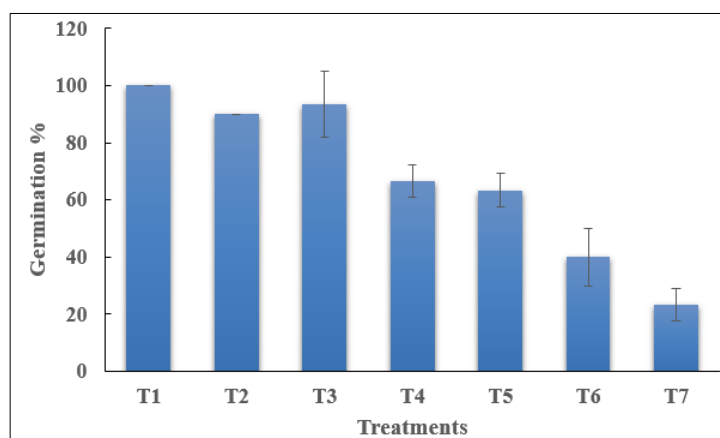


Fig 1: Effect of INPs on finger millet seed germination (Error bar indicates $\pm$ SD)

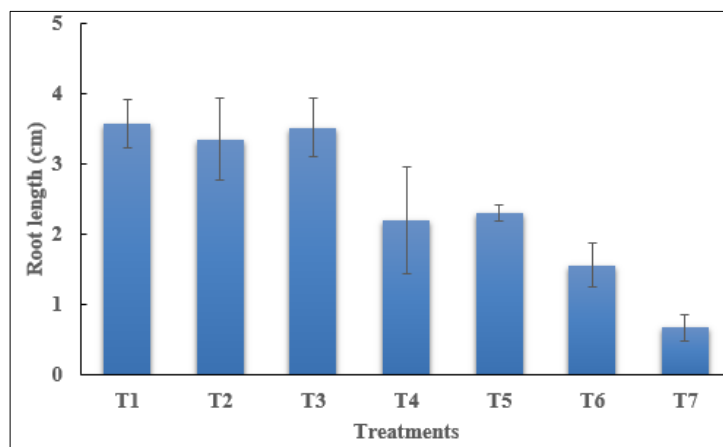


Fig 2: Effect of INPs on root length (cm) (Error bar indicates $\pm$ SD)

Table 2: Effect of different concentrations of iron nanoparticles on seed germination and early seedling growth of finger millet variety Gira

Treatment	Germination (%)	Root length (cm)	Shoot length (cm)	Total seedling length (cm)	Seed vigour index (SVI)
T ₁ (Control)	100.00	3.57667	1.73667	5.31	531.33
T ₂ (0.05 mg/10 mL)	90.00	3.36	1.61167	4.97	447.3
T ₃ (0.10 mg/10 mL)	93.33	3.52333	1.7	5.22	487.18
T ₄ (1.00 mg/10 mL)	66.67	2.20667	1.35	3.56	237.34
T ₅ (2.00 mg/10 mL)	63.33	2.31333	1.37	3.68	233.05
T ₆ (3.00 mg/10 mL)	40.00	1.57	1.1	2.67	106.8
T ₇ (4.00 mg/10 mL)	23.33	0.68	0.357	1.03	24.19
S. Em ($\pm$)	3.98	0.25	0.13	0.34	33.61
CD (5 %)	12.20	0.78	0.41	1.04	102.94
CV (%)	10.13	17.84	17.65	15.49	19.64

Effect of iron nanoparticles on root length

The effect of INPs on root length shows a clear dose-dependent response. The untreated control (T1) recorded a 3.58 cm root length. Among the other treatments treated with different INP concentrations, T3 showed the highest root length (3.52 cm) other than the control, followed by T2 (3.36 cm). When INP concentration increases, with root length decreasing to 2.21 cm in T4, 2.31 cm in T5, and 1.57 in T6 and lowest root length was found in T7 (0.68 cm).

The critical difference (CD) at 5% was 0.78 cm, showing significant variation among the treatments. Root growth was comparatively less affected at lower INP concentrations, while higher concentrations markedly inhibited root elongation. The responses of seed germination and root development exhibited a similar decreasing trend with increasing INP concentration in the present study. Germination decreased from 100.00% in the control T1 to 23.33% at T7, while root length decreased from 3.58 cm in the control to 0.68 cm at the highest concentration. Among the INP treatments, T3 recorded the highest root length (3.52 cm), which was close to the control, followed by T2 with 3.36 cm. At higher concentrations, root length declined to 2.21, 2.31, 1.57 and 0.68 cm in T4, T5, T6 and T7, respectively. Thus, unlike the contrasting response described previously, the present data indicate that increasing INP concentration adversely affected both seed germination and root elongation. The pronounced reduction in root length at T6 and T7, together with the corresponding decline in germination percentage, suggests that higher INP concentrations exerted an inhibitory effect on early seedling development. Overall, T3 appeared to be the most favourable INP concentration among the treated groups for maintaining root growth.

Statistically, root length differed significantly among treatments, with a S.E.m. of ± 0.25 cm, CD (5%) of 0.78 cm, and CV of 17.84%. The differences between treatments exceeding the CD value were considered significant. T1 and T3 recorded comparatively higher root lengths, whereas T6 and T7 showed significantly lower root growth.

Effect of iron nanoparticles on shoot length

The effect of INPs on shoot length shows dose-dependent manner. T1 (untreated control) shows the highest shoot length of 1.74 cm, followed by T3 with 1.70 cm and T2 with 1.61 cm. With increasing INPs concentration, shoot length decreases from T4 (1.35 cm) to T7 (0.357 cm). Thus, higher INP concentrations, particularly T6 and T7 substantially inhibited shoot elongation. Among all the treatments, T3 showed shoot growth closest to the control.

Statistically, shoot length varied significantly among the treatments, with an S.E.m. of ± 0.13 cm, CD (5%) of 0.41 cm, and CV of 17.65%. Treatment differences exceeding the CD value were considered significant. Overall, shoot growth declined with increasing INP concentration, and the highest inhibition was recorded under T7.

Effect of iron nanoparticles on total seedling growth

The effect of INPs on total seedling length shows the dose-dependent manner, with increasing INPs concentration decreasing total seedling length. The highest seedling length was observed in T1 (5.31 cm), followed by T3 (5.22 cm), and T2 (4.97 cm). At higher concentrations, total seedling length decreased in T5 (3.68 cm), T4 (3.56 cm), T6 (2.67 cm) and T7 (1.03 cm). Thus, increasing INP concentration, particularly T6 and T7, substantially reduced overall seedling growth. Among the INP treatments, T3 maintained total seedling length closest to the control.

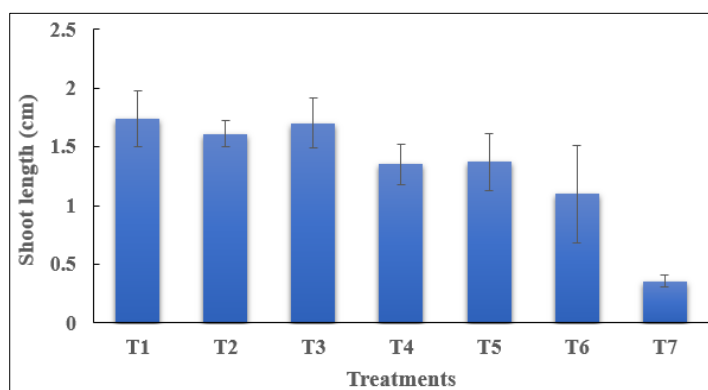


Fig 3: Effect of INPs on shoot length (cm) (Error bar indicates $\pm$ SD)

The response of total seedling length varied with INP concentration and should be interpreted alongside germination percentage. In this study, the highest total seedling length was recorded in the control (T1) at 5.31 cm, followed closely by T3 at 5.22 cm and T2 at 4.97 cm. At the higher concentration of INPs, seedling length decreased from in T4 (3.56 cm), T5 (3.68 cm), T6 (2.67 cm) and T7 (1.03 cm). This decline was followed by a significant decrease in germination from T1 (100 %) to T7 (23.33 %).

Therefore, total seedling length alone should not be considered an indicator of overall treatment performance. The combined response of germination and seedling growth indicates that higher INP concentrations, particularly T6 and T7, adversely affected early seedling establishment and development, whereas T3 maintained seedling growth close

to the control with a relatively high germination percentage of 93.33%. Total seedling length showed a statistically significant difference within all the treatments, with S. Em $\pm$ (0.34 cm), CD at 5% (1.04 cm) and CV (15.49 %). Differences greater than the CD value were considered significant. Overall, results indicate that the higher concentration of INPs showed an inhibitory response to seedling development, with the lowest seedling growth observed at T7. The present finding aligns with Plaksenkova *et al.* (2019) ^[6] and Shankamma *et al.* (2016) ^[10], which shows that INPs increased germination, root length and shoot length and shows dose dependent response. Mohan *et al.* (2025) ^[2] investigated the effect of INPs on okra. After 10 days at 0.5 mM, INPs increased seedling root length by 2.9 cm and shoot length by 9.2 cm.

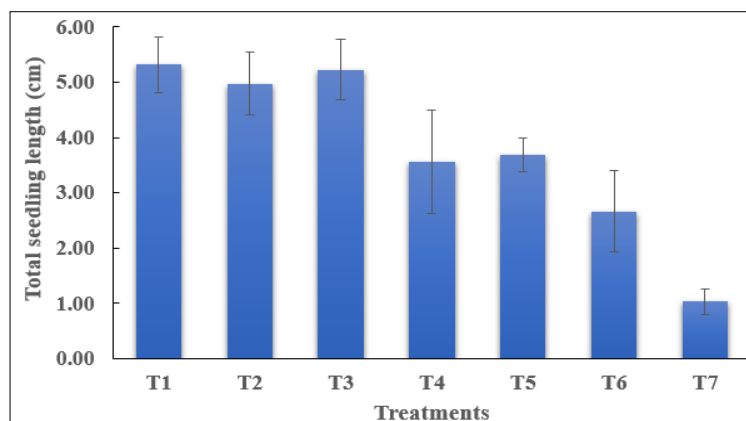


Fig. 4: Effect of INPs on total seedling length (cm) (Error bar indicates $\pm$ SD)

Seedling vigour index (SVI):

SVI shows the decreased with increased INP concentration. The highest SVI observed at T1 (531.33), followed by T3 (487.18) and T2 (447.3). When, INP concentration increased, SVI decreased from T4 (237.34), T5 (233.05), and T6 (106.8) and the lowest SVI was observed in T7 (24.19). At higher concentrations of INPs, SVI declined. Among all the treatments, SVI was highest at T3 and lowest at T7 showing the inhibitory effect of INPs on finger millet SVI. Declined SVI at higher doses was associated with reduced seed germination and total seedling length. T1 (531.33) recorded the highest SVI. Among INP treatments, T3 (487.18) showed the highest SVI, followed by T2 (447.3). At higher concentrations of INPs, SVI declined from T4 (237.34), T5 (233.05), and T6 (106.8) to T7 (24.19). This reduction was due to the decrease in the germination from

T4 (66.67 %) to T7 (23.33 %) along with a decrease in total seedling length from T4 (3.56 cm) to T7 (1.03 cm).

Statistically, SVI showed significant variation among treatments, with a S.E.m. of ± 33.612 , CD (5%) of 102.941, and CV of 19.639%. Differences exceeding the CD value were considered significant. Overall, the results indicate that increasing INP concentration adversely affected seedling vigour, with the greatest reduction observed at T6 and T7.

The findings of the present study are consistent with previous reports demonstrating that the effect of INPs on seed germination, seedling length and SVI. Sarma and Prakash (2019) [8] and Triveni *et al.* (2021) [12] reported INPs, enhance seed germination and root and shoot length, at lower concentration, whereas higher concentrations reduced germination and seedling growth, indicating a dose-dependent response.

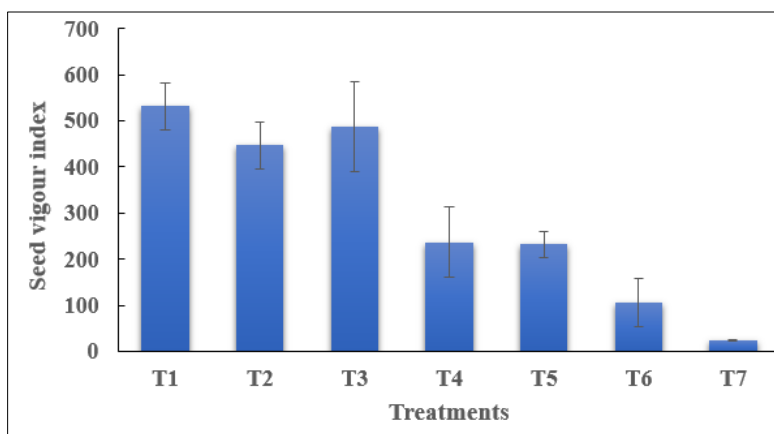


Fig 5: Effect of INPs on seed vigour index (Error bar indicates $\pm$ SD)

Conclusion

The effect of different concentrations of INPs shows a clear dose-dependent response on finger millet seed (variety Gira) germination and early seedling growth. At lower concentrations of INPs, increase in germination and seedling growth, while at higher concentrations of INP reduced germination and seedling growth. Among the INP treatments, T3 performed best, with 93.33% germination and a seed vigour index of 487.18. Root length, shoot length and total seedling length were also maintained close to the control at this concentration. In contrast, T6 and T7 strongly reduced germination and overall seedling vigour. The results indicate that the beneficial effect of INPs depends largely on the concentration applied. The findings suggest that T3 is the most suitable concentration among those tested for

supporting early seedling establishment. Overall, to obtain potential benefits of INPs, the selection of concentration or dose is important for plant growth and development.

Acknowledgement: The authors gratefully acknowledge Navsari Agricultural University for providing the financial support required to conduct this research.

Conflict of Interest: Regarding the publication of this manuscript, there is no conflict of interest among authors.

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