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Unveiling the Synergistic Effects of Melatonin and Zinc on Enhancing Yield and Yield Components of Mung Bean (*Vigna radiata* L.) under Field Condition

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ABSTRACT

The city of Gotvand in Iran is located in a hot and semi-arid region, where it can be challenging to grow crops. Abiotic stresses such as drought and heat can affect crop productivity during both vegetative and reproductive growth. Melatonin plays a vital role in regulating various mechanisms in plants and acts as a primary defense against environmental stresses. However, its interaction with zinc is not well understood. In the experiment, a randomized complete block design with three repetitions was used. A field experiment was conducted to investigate the interaction effects of zinc fertilizer (ZnSO4) - no application, 1 mg/L, and 2 mg/L and different concentrations of melatonin (0, 25, 50, 100, 200 mg/L. Mung bean plants treated with melatonin and higher levels of zinc fertilizer showed increased vegetative growth and improved yield attributes. The specific combination of 200 mg/L of melatonin and 2 mg/L of zinc fertilizer emerges as a promising strategy to maximize yield components, including pod number, seed number per pod, and seed weight. Notably, this combination proved to be the most effective treatment for enhancing mung bean yield formation which resulted in the highest average seed yield to 1764 kg/ha. It is concluded that melatonin and zinc combination is an effective approach to alleviate environmental challenges such as high temperatures during fluorescence and reproductive growth.

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1. Introduction

As the global population continues to grow, ensuring sufficient food production becomes a paramount challenge. Agriculture must meet this increasing demand while also addressing concerns related to environmental sustainability and resource conservation (Copeland, 2011; Khanna *et al*., 2018). In recent years, research attention has turned towards exploring alternative strategies to increase crop yield and minimize crop risk of agricultural production.

Mung bean (*Vigna radiata* L.) is a warm-season crop belonging to the Phaseoleae family, known for its self-pollinating flowers (Somta *et al*., 2022). Primarily cultivated for food consumption, mung bean holds remarkable nutritional value. In addition, it is cultivated in some regions as a green manure crop to improve soil fertility (Hou *et al*., 2019; Jaya and Sudika, 2021). Mung beans are a resilient crop that can withstand the challenges of summer, such as high temperatures and water scarcity. Moreover, the benefits extend beyond its resilience, as mung bean enriches the soil by improving nitrogen (N) and carbon (C) levels through biological nitrogen fixation (Zang *et al*., 2015). These nutritional attributes make mung bean an advantageous choice for enhancing diets and promoting sustainable agriculture.

Growing crops in summer is challenging practice, mainly due to high temperatures and heat stress. It is even more pronounced when it comes to regions where there is limited precipitation and extreme temperatures. Pervious studies have shown that combined drought and high-temperature stress can significantly decline

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crop yield by more than 50% of crops's potential yield (Annadurai *et al*., 2023; Kamatchi *et al*., 2023).

Melatonin (Mel, N-acetyl-5-methoxytryptamine) has been found to play a role in the regulation of plant developmental processes such as flowering, and seed formation (Golovatskaya and Laptev, 2023). In recent years, numerous studies have revealed beneficial effects of melatonin foliar application in enhancing plant stress tolerance (Li *et al*., 2015; Antoniou *et al*., 2017; Sharma and Zheng, 2019; Mohamadi Esboei *et al*., 2022). One of the key findings in the literature is that melatonin application effectively increases plant stress tolerance by enhancing various physiological and biochemical processes. Melatonin has been found to delay leaf senescence (Tan *et al*., 2020; Zhao *et al*., 2021b), a natural process that occurs during drought stress and leads to decreased photosynthetic capacity and overall plant vitality. By delaying leaf senescence, melatonin helps to maintain a higher level of photosynthesis, contributing to enhanced plant growth and ultimately improving crop yield. Moreover, several studies have suggested that melatonin can modulate auxin biosynthesis and at lower concentrations, melatonin promotes lateral root formation (Chen *et al*., 2009; Wen *et al*., 2016), a critical adaptation response that allows plants to explore a larger soil volume for water uptake (Arnao and Hernández-Ruiz, 2007; Zhang *et al*., 2013). Increased lateral root formation provides plants with an extended root system, enabling better access to water resources in drought conditions. This improved water uptake capacity contributes to maintaining the water balance within the plant and helps to alleviate drought-induced water stress.

It appears that melatonin may act as a regulator of auxin-related genes and promote auxin-induced processes. For example, melatonin can upregulate the expression of PIN-FORMED (PIN) proteins, which are auxin efflux carriers. This can lead to increased auxin transport to certain parts of the plant, such as the root tip and shoot apices (Arnao and Hernández-Ruiz, 2018; Arnao and Hernández-Ruiz, 2021; Sun *et al*., 2021; Zhang *et al*., 2022).

Another important mechanism through which melatonin enhances plant stress tolerance is by modulating oxidative stress and proline metabolism. Reactive oxygen species (ROS) within plant cells can cause oxidative damage to cellular components (Zafar *et al*., 2019). Melatonin acts as a potent antioxidant, scavenging ROS and reducing oxidative stress (Gu *et al*., 2022). Additionally, melatonin has been found to regulate proline metabolism, which plays a crucial role in osmotic adjustment and cellular protection during drought stress (Antoniou *et al*., 2017; Khalid *et al*., 2022). By modulating these processes, melatonin helps plants maintain cellular integrity and function even under water-deficient conditions. Furthermore, melatonin has been shown to regulate water balance in plants by influencing stomatal behavior (Khan *et al*., 2022). Stomata are small openings on the leaf surface that regulate gas exchange and water loss through transpiration. Under drought stress, melatonin has been found to promote stomatal closure, reducing water loss and improving water use efficiency. This regulatory effect on stomatal behavior contributes to maintaining leaf and chloroplast integrity, ensuring optimal photosynthesis and energy production even under water-limited conditions (Moreno and Campos, 2022; Yang *et al*., 2022a).

The studies conducted by Meng et al. (2014), Li et al. (2015), and Antoniou et al. (2017) have provided valuable insights into the mechanisms through which melatonin foliar application enhances plant stress tolerance and improves crop yield under drought conditions. These findings highlight the potential of melatonin as a sustainable and environmentally friendly approach to enhance agricultural productivity, particularly in regions prone to drought stress. Further research in this field will undoubtedly contribute to the development of effective melatonin application strategies and the adoption of sustainable farming practices that address the challenges of water scarcity and climate change (Meng *et al*., 2014; Li *et al*., 2015; Antoniou *et al*., 2017).

Zinc (Zn) is a key element for many enzymatic reactions and metabolic processes in plants, including, enzyme structure, hormone synthesis, protein synthesis, carbohydrate metabolism, and pollen formation. Zinc is also essential for maintaining the integrity and function of cell membranes and genetic materials. In addition, many researchers suggested that foliar application of zinc could enhance crop growth and development (Cakmak, 2009; Yuan *et al*., 2013; Ahmed *et al*., 2023).

The objective of this study was to determine the optimal combination of melatonin and zinc concentrations that would enhance the growth and

productivity of mung bean plants under field conditions.

2. Materials and methods

2.1. Plant material

Seeds of mung bean cultivar Seimit-1 were obtained from Safiabad Agriculture Research Center, Dezful, Iran in 2021.

2.2. Study location and climate conditions

The experiment was conducted in a local field in Gotvand, Iran (coordinates: 32°15′58″N 48°56′09″E) during the summer season of 2021 (From Jun to October). The soil type was Silty loam (Table 1), and the climatic conditions during the experimental period were hot and dry (Fig. 1).

Seedbed preparations were conducted by plowing the soil to break up any compacted layers and create a loose and friable seedbed. All existing weeds, debris, or crop residues were removed from the field to minimize competition and potential disease or pest problems. Moreover, seedbed was carefully leveled to ensure uniform seed placement and irrigation.

Table 1. Physiochemical properties of the experimental soil site

Micro Nutrient				Texture				Ntot. (ppm)	T.N.V (%)	Kava (ppm)	Pava (ppm)	O.C (%)	pH	E.C (ds.cm^{-1})	Depth (cm)
Zn (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)	Texture	Sand	Silt	Clay								
2.3	8.8	10.8	3.0	Si.L	20	64	16	650	43.25	159	15.0	0.58	7.40	3.5	0-30

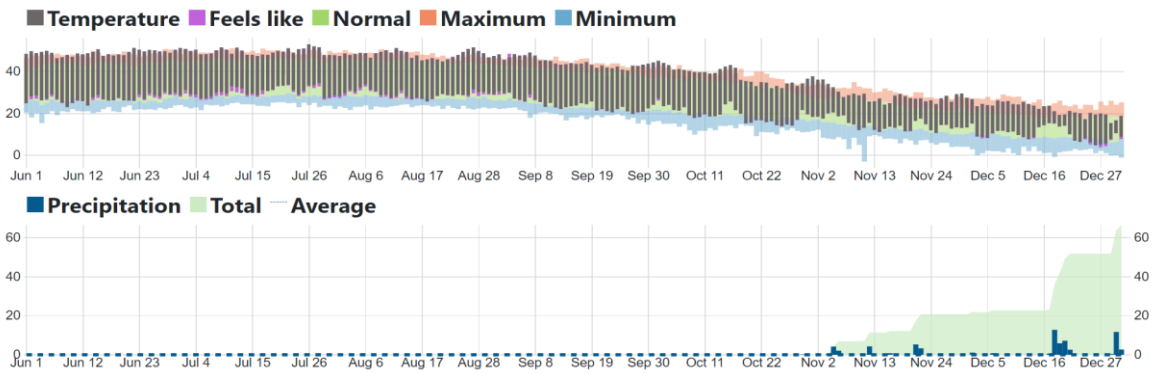

Figure 1. Meteorological data of Gotvand city during experiment (https://www.visualcrossing.com/weather-history/Gotvand)

2.3. Experimental procedure

2.3.1. Desing of experiment and measurements

On July 20, 2021, a factorial experiment was carried out to examine the to investigate the combined effect of melatonin and zinc on different yield attributes of mung bean as complete block design arranged in three replications. The experiment was conducted in individual plots of 3m x 3m. The plots were prepared by plowing and harrowing the soil to a depth of 30 cm. The mung bean seeds were sown in rows with a spacing of 50 cm between rows and 10 cm between plants.

Zinc fertilizer was purchased in the form of ZnSO4 from Tetaco Co. and melatonin was supplied from by Sigma-Aldrich. Mung bean plants were subjected to foliar application of zinc fertilizer at concentrations of (0, 1, and 2 mg/L) on Aug, 24th and subsequently, melatonin was applied seven days after the zinc treatments at varying concentrations of (0, 25, 50, 100, and 200 mg/L). Two spraying frequencies were applied: one time at 30 days after sowing (DAS) and two times at 35 and 45 DAS). The study focused on measuring the yield and yield components of mung bean. Thus, at harvest five plants from center of each plot were randomly taken to yield component measurements: including plant height, number of pods per plant, number of seeds per pod, and 1000-seed weight. To determine yield, the plants from each plot were harvested, dried, and weighed to obtain the total seed yield. All data were collected at the end of the growing season.

2.4. Data analysis

The data were analyzed using analysis of variance (ANOVA) to determine the significance of the treatments and their interactions. Mean separation test (Tukey) was performed to compare the treatment means. The statistical analysis was performed using Minitab ver, 16 software.

3. Results and discussion

The results of the study indicate that the application of Melatonin and Zinc fertilizer had a significant effect on mung bean plant height. The interaction effect of Melatonin and Zinc fertilizer on plant height was also significant ($p<0.05$). The maximum plant height observed was 55 cm in the treatment with 200 mgL-1 of Melatonin and 2 mgL-1 of Zinc fertilizer, while the minimum height was 25 cm in the control group. The findings suggest that the combination of Melatonin and Zinc fertilizer has a synergistic effect on plant growth (Fig. 2).

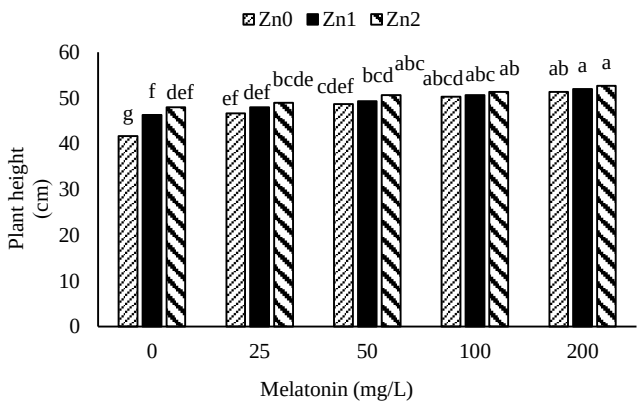

Figure 2. Effect of melatonin and zinc interaction on the mung bean plant height

The analysis of variance showed that there was significant effect for the interaction effect of zinc and melatonin mung bean stem dry weight. The statistical significance was determined at a 1% error probability level. The mean comparison table (Table 2), indicates that increasing the application of zinc fertilizer and melatonin resulted in higher stem dry weight in mung bean crops. The treatment involving 200 mg/liter of melatonin and 2 mg/L of zinc fertilizer exhibited the highest stem dry weight (5.29 grams). In contrast, the control treatment showed the lowest stem dry weight (3.29 grams) (Fig. 3). A significant increase in leaf dry weight was obtained by foliar spraying higher concentrations of melatonin along with higher concentrations of zinc fertilizer. Among the various treatments, the addition of 2 mg/liter of zinc fertilizer and 200 mg/liter of the melatonin hormone resulted in the highest average leaf dry weight of 6.92 g (Fig. 4). Due to the lack of zinc fertilizer or melatonin enhancement, the control treatment had the lowest average leaf dry weight (4.4 g). The maximum pod dry weight was observed in the treatment with 200 mg/L melatonin (22.6 g/plant) while the minimum pod dry weight was recorded in the control treatment without melatonin or zinc fertilizer, averaging 17 grams per plant. These results highlight the importance of melatonin and zinc fertilizer incorporation in influencing the growth and development of mung bean plants. By increasing the concentrations of these substances, the total dry weight of mung bean was significantly enhanced.

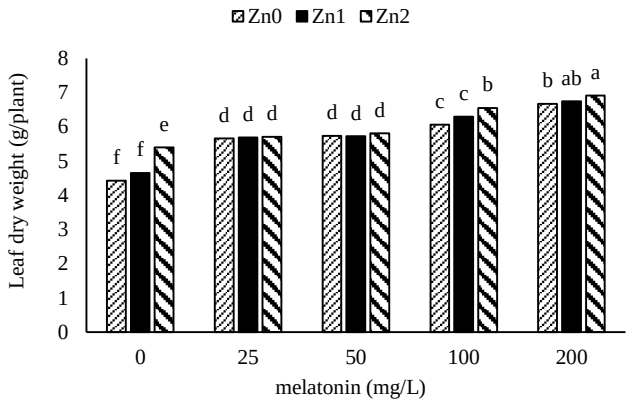

Figure 3. Effect of melatonin and zinc interaction on the mung bean leaf dry weight

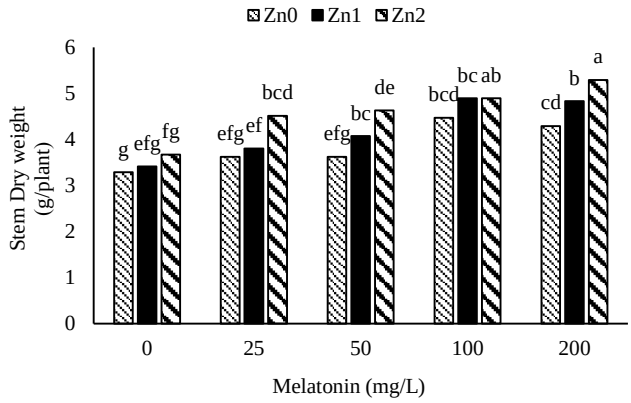

Figure 4. Effect of melatonin and zinc interaction on the mung bean stem leaf weight

Our results are consistent with previous reports, which have studied the effects of zinc fertilizer and melatonin on plant development, suggesting their potential to enhance plant growth. A study was conducted to observe the effects of zinc oxide nanoparticles (ZnONPs) applied to the soil and melatonin (MT) applied to the foliage on wheat growth, cadmium (Cd) uptake, and translocation in wheat that was grown in soil polluted with Cd. The findings indicated that ZnONPs promoted wheat growth, increased chlorophyll content and yield. When combined with MT, these positive effects were

enhanced even further (Chen *et al*., 2023). Such findings underline the potential benefits of combining melatonin and zinc in crop cultivation, which aligns with our findings in mung bean plants. A study by Zhang et al. (2024) showed that melatonin improved the growth and waterlogging tolerance of cotton plants.

The combination of 200 mg/L of melatonin and 2 mg/L of zinc fertilizer exhibited the maximum number of 18 pods per plant. According to higher number of pods per plant, it indicates the potential for increased seed production and overall yield. Furthermore, the same treatment exhibited the highest number of seeds per pod, reaching a maximum of 10 seeds. This finding suggests that the application of melatonin and zinc fertilizer at these specific concentrations promotes pod development and seed set, enhancing the reproductive capacity of the mung bean plants. In addition to the increased pod and seed production, the treatment involving 200 mg/L of melatonin and 2 mg/L of zinc fertilizer resulted in the highest 1000 seed weight of 49 grams. The 1000 seed weight is a crucial determinant of seed quality, as it reflects the size and weight of individual seeds. These findings underscore the significance of optimizing the concentrations of melatonin and zinc fertilizer in mung bean cultivation.

Table 2. The results of the experiment investigating the effects of different concentrations of melatonin and zinc fertilizer on mung bean yield components

Melatonin (mg/L)	Zn (mg/L)	Pod/Plant	Seed/Pod	1000 Seed weight (g)
0	0	14^{e}	7^{f}	46.23^{j}
0	1	14^{e}	7^{f}	46.43^{i}
0	2	14^{e}	7^{ef}	46.63^{h}
25	0	14^{e}	8^{de}	46.77^{h}
25	1	15^{d}	8^{de}	47.27^{g}
25	2	15^{d}	8^{de}	47.6^{f}
50	0	15^{d}	8^{de}	47.6^{f}
50	1	15^{d}	8^{cd}	47.87^{e}
50	2	16^{c}	9^{bc}	48.37^{d}
100	0	16^{c}	9^{bc}	48.5^{cd}
100	1	16^{c}	9^{bc}	48.6^{bc}
100	2	17^{b}	9^{ab}	48.67^{bc}
200	0	17^{b}	10^{a}	48.7^{b}
200	1	17^{b}	10^{a}	49^{a}
200	2	18^{a}	10^{a}	49^{a}

When using 200 mg/liter of melatonin hormone along with 2 mg/liter of zinc fertilizer, with an average yield of 1764 kg. In contrast, the control treatment, which did not involve any melatonin or zinc fertilizer application, exhibited the lowest seed yield with an average of 906.17 kg (Fig. 5). Mung bean yield linearly increased as both melatonin and zinc concentrations increased (Fig. 6).

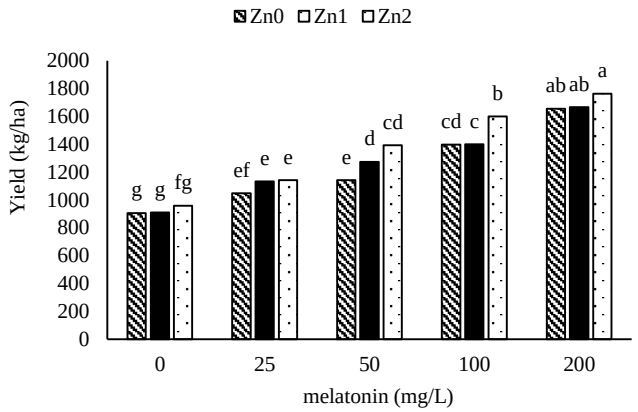

Figure 5. Effect of melatonin and zinc interaction on the mung bean seed yield

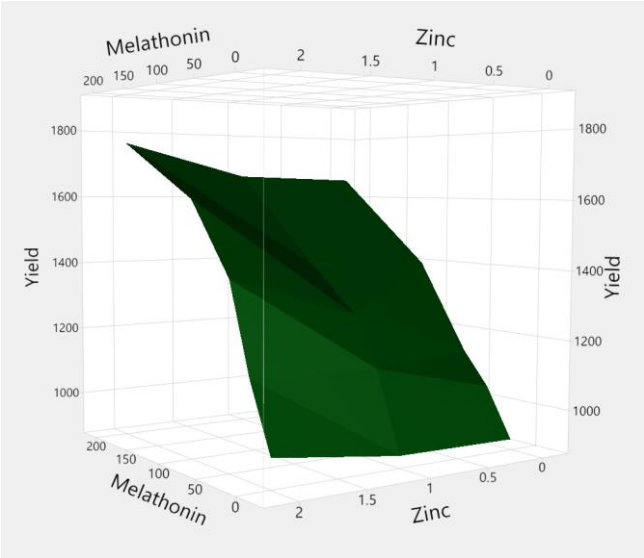

Figure 6. Surface plot showing three-dimensional interaction effects of melatonin and zinc application to improve mung bean seed yield

In the current study, was strong positive correlation existed between seed yield and number of pods per plant (0.98), suggesting that an increase in the number of pods per plant leads to an increase in yield (Fig. 7). This could be due to the fact that more pods per plant result in more seeds, leading to a higher yield. Similarly, a strong positive correlation was observed between yield and seed per pod (0.96), suggesting that an increase in the number of seeds per pod leads to an increase in yield. This is because more seeds per pod result in a higher overall seed production, leading to a higher yield. Additionally, leaf and stem dry weight, 1000 seed weight, and seed yield were positively correlated. This suggests that higher seed yield is associated with heavier seeds, which may be due to

increased photosynthetic assimilates during the seed filling period of mung bean plants, which in turn is a result of increased leaf and stem dry weight. This finding is consistent with research by Yang et al. (2022b), which reported that melatonin application significantly enhanced leaf growth in barley. In another study, Zhao et al. (2021a) reported that a positive effect on leaf development in maize.

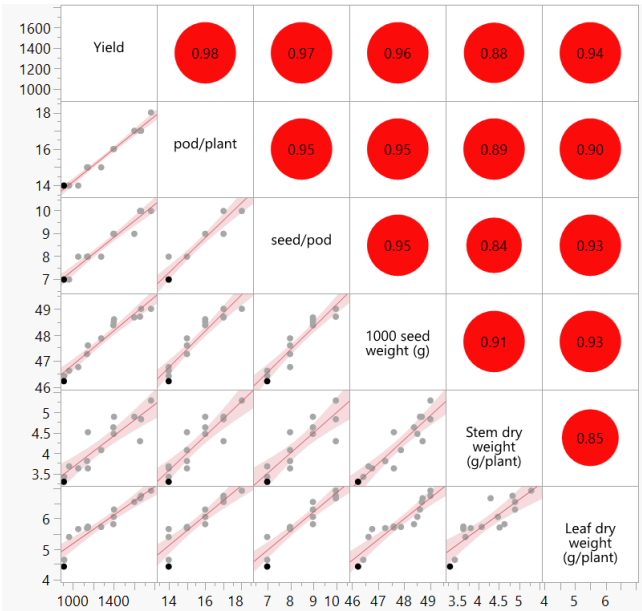

Figure 7. Correlation among mung bean seed yield and yield attributes

The results of a study revealed that melatonin treatment of soybean resulted in higher pod dry weight and yield over control crops (Wei *et al*., 2015). Similarly, Ahmad et al. (2020) demonstrated that the application of melatonin enhanced ear dry weight in maize crops. Furthermore, a study by Han et al. (2011) showed that zinc fertilizer significantly increased soybean pod dry weight. In line with these previous reports, our study contributes to the existing body of knowledge by examining the combined impact of melatonin and zinc fertilizer on mung bean growth. By utilizing both substances, we observed significant increases in stem, leaf, and pod dry weight, further supporting the potential of melatonin and zinc fertilizer as growth-enhancing agents in agricultural practices.

4. Conclusion

In conclusion, this study has suggested an optimal combination of melatonin and zinc concentrations that would enhance the growth and productivity of mung bean plants under field conditions. The plants treated with melatonin and zinc had higher leaf and stem dry weight, as well as more pods, seeds, seed weight, and seed yield. Among the different concentrations of melatonin, 200 mg/L and zinc concentrations of 2mg/L had the most positive effect on seed yield.

Conflict of interests

The authors have no conflict of interest to declare.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All co-authors have read and agree with the contents of the manuscript.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed consent

The authors declare not to use any patients in this research.

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