



Physiological Role of Salicylic Acid and Potassium Nitrate in Mitigating Salinity Stress in Mung bean (*Vigna radiata* L.)

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ABSTRACT

Background: Salinity is one of the most pervasive abiotic stresses affecting agricultural productivity worldwide. Mung bean, a salt-sensitive crop, is particularly impacted by salt stress, which hinders its development. Salicylic acid (SA), a vital plant hormone, offers a significant agronomic advantage by enhancing crop tolerance to salt. Potassium nitrate, a source of potassium, is an essential nutrient that improves plant resilience against various stresses, including salinity.

Methods: An experiment was conducted at the Student Instructional Farm at ANDUAT, Ayodhya, during *Kharif* 2021 and *Kharif* 2022 to study the physiological role of salicylic acid and potassium nitrate in mitigating salinity stress in mung bean (*V. radiata*). The experiments consist of seven treatments, including untreated control and evaluated biochemical parameters (chlorophyll content, total protein content, peroxidase content, proline content and nitrate reductase activity), morphological parameters (plant height, number of branches per plant), total dry biomass, relative water content (RWC%) and yield components (number of pods per plant, seeds per pod, clusters per plant and seed yield (q per ha).

Result: The results showed that the highest plant height, number of branches per plant, total dry biomass, proline content, nitrate reductase activity, number of pods per plant, number of seeds per pod, number of clusters per plant and seed yield per plant were observed at salicylic acid @150 ppm. The maximum seed yield per ha was observed at salicylic acid @100 ppm. On contrast, maximum plant height, peroxidase activity, total dry biomass, proline content, nitrate reductase activity, number of seeds per pod, number of clusters per plant and seed yield per plant were observed at potassium nitrate @ 400 ppm.

Key words: Abiotic stress, Plant characters, Potassium nitrate, Salicylic acid, *Vigna radiata*.

INTRODUCTION

Pulses have high potential for holding health and also maintain a strategic position in the Indian agricultural economy. They are the best sources for protein, constituting about 27% of total dietary protein in our country. Pulses are the third-important group of crops in the Indian agricultural system after cereals and oilseeds. Among the pulses, mung bean (*Vigna radiata*), native to China and part of the Leguminosae family, has been cultivated for a long time and features various strains. As a traditional legume, it is rich in protein, cellulose, antioxidants and phytonutrients, offering substantial nutritional and medicinal benefits (Madhumitha *et al.*, 2022). In India, the mung bean crop is raised in three seasons, viz., *Kharif*, *Rabi* and *Zaid*, to about 5.5 m ha with production of 3.17 million tonnes and productivity of 570 kg/ha (Anonymous, 2022). This crop can be used for both seed and forage since it produces a large amount of biomass and then recovers after grazing to yield abundant seeds and then can be used in broiler diets as a non-traditional feed.

Salinity is one of the most destructive factors among the abiotic stresses, which limit crop production worldwide. Mineral nutrients and plant growth regulators play a significant role in the mitigation of salinity stress. Potassium nitrate (KNO) is a water-soluble compound composed of two major essential plant nutrients. It's commonly used as a fertilizer for high-value crops that

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benefit from nitrate (NO) nutrition and a source of potassium (K⁺). Salinity in farms that are in arid and semi-arid areas in almost all regions of the world is a major problem. Salt stress is a major abiotic stress that causes detrimental

effects on plant growth and productivity. Salt stress has a triple effect on plant growth. First, it decreases water absorption. Second, it leads to an imbalance ion and third, it results in a plant toxicity ionic effect. Ionic imbalance occurs in the cells due to excessive accumulation of Na⁺ and Cl⁻ ions and reduces uptake of mineral nutrients such as K⁺ and Ca (Yusuf *et al.* (2012).

Potassium (K) is the most abundant inorganic cation and it is important for ensuring optimal plant growth. K is an activator of dozens of important enzymes, such as protein synthesis, sugar transport, N and C metabolism and photosynthesis (Hasanuzzaman *et al.*, 2018). It plays an important role in the formation of yield and quality improvement. K is also very important for cell growth, cell elongation, regulation of stomatal opening and closing and other important physiological processes (Xu *et al.*, 2020). Foliar application of potassium nitrate to the salt-treated plants may reduce toxic ions uptake as well as improve the K and N status of the salt-treated plants, resulting in better plant growth. Potassium gradually increased biomass production in different plant parts. Potassium generally increased the exudation rate significantly under a saline condition. Potassium fertilisation influences Mung bean yield attributes significantly under salinity.

Salicylic acid (SA) and its derivatives, as one of the plant hormones produced naturally by the plant, belong to the phenolic acid group and have a ring-like structure with hydroxyl and carboxyl groups. It is mostly synthesized in the cytoplasmic cell of the plant. This acid was first discovered in *Salix* spp., which comprises a 9.5-11% salicylic component and is found in plants as free phenolic acids or in combination with amino compounds. Exogenous application of SA improved photosynthetic efficiency, metabolism and development of mustard plants at high temperatures, according to research (Husen *et al.*, 2018). Salicylic acid participates in the regulation of various physiological processes in plants, such as stomatal closer, ion uptake, inhibition of ethylene biosynthesis, transpiration and stress tolerance. When applied to the leaves of plants, salicylic acid at a physiological concentration had a big effect on their growth metabolism. It was one of the substances that controlled plant growth (Navya *et al.*, 2021). Salicylic acid is involved in the protection of plants against multiple stresses, including freezing, salinity, ozone, ultra-violet radiation, water stress and drought stress (Patel and Hemantaranjan, 2012). Keeping in view the importance of SA and potassium nitrate in regulating plant defences against abiotic stresses, including salinity, the present study was conducted with the objective of examining the physiological role of salicylic acid and potassium nitrate in mitigating salinity stress in mung bean (*Vigna radiata* L.).

MATERIALS AND METHODS

The present investigations were carried out in the Student Instructional Farm at Acharya Narendra Deva University of Agriculture and Technology's Narendra Nagar, Kumarganj,

Ayodhya (U.P.) during the *Kharif* season of 2021 and 2022 in a randomised block design (R.B.D.) with the three replications. The experiments consist of seven treatments, including three concentrations of potassium nitrate (100, 200 and 400 ppm) and three salicylic acid (50, 100 and 150 ppm) foliar sprays along with an untreated control. Mung bean variety *Shikha* was selected for this study and all the agronomical practices were followed as recommended for the crop in this area to raise the crop in experimental sites.

Plant height and number of tillers per plant

Plant height at various intervals was measured from the soil surface up to the tip of the plant with the help of a meter scale and average plant height was calculated from the replicated data and represented in cm. The number of tillers per plant was recorded by counting total tillers, while the number of ear-bearing tillers per plant was recorded by counting the number of ear-bearing tillers.

Total dry biomass (g per plant)

Five tagged plants were uprooted and separated into their plant parts, *i.e.*, leaf and stem and dried separately in an oven at $\pm 70^{\circ}\text{C}$ for 24 hours and dry weights of all plant parts were recorded at different stages (*i.e.*, 45, 60 DAS and maturity) of observation. The average dry matter of the plant was calculated from five plants.

Photosynthetic pigments

Chlorophyll content of leaf was directly measured in intact leaves with the help of SPAD meter.

Peroxidase content (g⁻¹ fresh weight)

This enzyme was assessed by the calorimetric method given by McCune and Galston (1959). The activity was measured at 430 nm after the formation of purpurogallin change in O.D. was detected at 37°C for the first 5 minutes. Results were expressed as enzyme units per gram of fresh weight.

Proline content (mg/g fresh weight)

Proline content in leaves was estimated according to the procedure of Bates *et al.* (1973).

Nitrate reductase (μg nitrite produces g⁻¹ fresh weight)

Nitrate reductase activity was assayed according to the method of Jaworski (1971) and expressed as g nitrite produced g⁻¹ fresh weight ha.

Relative water content (RWC%)

The relative water content was determined by the method described by Turner (1981). Leaf discs were cut from the leaves, weighed and saturated by floating on distilled water in a petri dish for 4 hours. The discs were surface dried and weighed. After that, discs were kept in the oven at $75 \pm 5^{\circ}\text{C}$ for 24 hours. After drying, the weight of the discs was calculated with the help of an electronic balance. RWC was calculated by the following formula:

$$\text{RWC \%} = \frac{\text{Fresh weight (g)} - \text{Dry weight (g)}}{\text{Saturated weight (g)} - \text{Dry weight (g)}} \times 100$$

Number of pod cluster⁻¹

Mature pod clusters were picked from sampled plants and their numbers were counted and the average was calculated.

Number of pod plants⁻¹

The total number of pods of three tagged plants was counted and the average number of pods per plant was calculated.

Number of seed pods

Three pod seeds were counted for the average number of seeds pod⁻¹ in each treatment.

Seed yield plant⁻¹ (g)

Five plants were harvested and dried after air drying. The produce was meshed and seeds were cleaned. The average value of seed weight was recorded in grams to represent the seed yield plant.

Total number of grains

The total number of grains was worked out by taking 3 panicles randomly selected from each tagged plant and the number of grains was counted and expressed as the average number of grains per panicle. All panicles of individually tagged plants were threshed manually and weighed to obtain grain yield per plant.

Statistical analysis

The experimental data was analysed statistically using the SPSS 27 program and a three-way ANOVA (analysis of variance technique) was performed at $P \leq 0.05$. The mean significance was tested at a 5% probability level.

RESULTS AND DISCUSSION

Plant height (cm)

The data (Table 1) revealed that plant height was significantly influenced by foliar applications of potassium nitrate and salicylic acid at all growth stages. The highest

plant height was observed with the plot treated with potassium nitrate @ 400 ppm (25.14 cm at 45 DAS and 66.01 cm at maturity), while the plot treated with salicylic acid @ 150 ppm showed the tallest plants (47.79 cm at 45 DAS). The minimum plant height was recorded in a plot treated with salicylic acid @ 50 ppm (22.71 cm at 45 DAS and 53.36 cm at maturity) and potassium nitrate @ 100 ppm at 60 DAS (40.86 cm). All the treatments significantly performed over the control. This indicates that higher concentrations of potassium nitrate and salicylic acid promote greater elongation under salinity stress. The findings are consistent with those of (El-Beltagi *et al.*, 2023), who reported similar trends in mung bean under saline conditions. Similar results were found by Ogunsiji *et al.* (2023) that there was an increase in plant height with the application of SA.

Number of branches per plant

The number of branches per plant was also significantly influenced by the foliar treatments (Table 1). The maximum number of branches was recorded with salicylic acid @150 ppm treated plot with 4.51 and 5.45 branches at 45 DAS and 60 DAS, respectively, which was significantly higher than the untreated control (2.73 and 3.39 branches at 45 DAS and 60 DAS, respectively). However, the minimum number of branches was observed in the plot treated with salicylic acid @50 ppm (3.16 and 3.96 branches at 45 DAS and 60 DAS, respectively). These results suggest that salicylic acid at higher concentrations plays a more significant role in enhancing branch development, possibly due to its regulatory effects on growth hormones under stress conditions. Similar findings were reported by El-Yazeid (2011).

Total dry biomass (g per plant)

Significant differences in total dry biomass were observed across treatments as compared to control (Table 1). The highest biomass was recorded in the plot treated with potassium nitrate @ 400 ppm (13.51 g at 45 DAS, 24.45 g at 60 DAS and the salicylic acid @150 ppm treated plot showed high biomass accumulation at maturity (53.51 g). In contrast, the lowest biomass was recorded in the plot

Table 1: Effect of foliar application of potassium nitrate and salicylic acid on plant height, number of branches and total dry biomass at different growth stages in mung bean (pooled data of *kharif* 2021 and 2022).

Treatments	Plant height (cm)			No. of branches/plant		Total dry biomass (g per plant)		
	45 DAS	60 DAS	Maturity	45 DAS	60DAS	45 DAS	60 DAS	Maturity
T ₁ - Potassium nitrate (100 ppm)	23.26	40.86	55.29	3.27	3.89	8.40	15.93	40.99
T ₂ - Potassium nitrate (200 ppm)	23.91	42.44	61.98	3.56	4.19	12.37	21.03	44.53
T ₃ - Potassium nitrate (400 ppm)	25.14	43.46	66.01	3.78	3.93	13.51	24.45	44.77
T ₄ - Salicylic acid (50 ppm)	22.71	41.52	53.36	3.16	3.96	7.34	11.66	37.49
T ₅ - Salicylic acid (100 ppm)	23.45	42.22	57.05	3.63	4.72	9.97	13.25	44.01
T ₆ - Salicylic acid (150 ppm)	24.96	47.79	54.23	4.51	5.45	10.81	20.34	53.51
T ₇ - Control	20.79	32.31	42.78	2.73	3.39	6.74	11.84	34.80
S.E.m±	0.42	0.66	1.26	0.21	0.17	0.45	0.72	0.95
C.D.5%	1.31	2.06	3.95	0.66	0.54	1.41	2.24	2.97

treated with salicylic acid @ 50 ppm (7.34 g at 45 DAS, 11.66 g at 60 DAS and 37.49 g at maturity). The increased biomass in the higher treatments can be attributed to better resource utilization and improved light interception, as suggested by Patel and Hemantranjan (2012). Similar results were found by Ogunsiji *et al.* (2023) that there was an increase in total dry biomass due to the alleviating effect of SA under salinity stress.

Bio-chemical parameters

Chlorophyll content in leaf (SPAD value)

The data in Fig 1 showed maximum chlorophyll content (13.80, 15.51 and 12.87) at 45, 60 DAS and maturity were analyzed with a foliar spray of salicylic acid @150ppm. Whereas, minimum chlorophyll content was recorded with a foliar spray of potassium nitrate @100ppm (11.43, 12.72 and 11.14) at 45, 60 DAS and maturity, respectively and

maximum in control. A similar finding was also recorded by Janmohammadi *et al.* (2017).

Relative water content (RWC %)

The data (Table 2) demonstrated that all foliar treatments significantly increased the relative water content (RWC) in mung bean leaves under salinity stress. The maximum RWC was recorded in the plot treated with potassium nitrate @400 ppm (95.85%), followed by potassium nitrate @200 ppm (94.56%), both of which were statistically superior to the control. However, the plot treated with salicylic acid reduced the RWC content as compared to the untreated control and the lowest RWC content was recorded in the salicylic acid @50 ppm treated plot (85.43%). The effect of single or combined SA and salinity stress showed a significant impact of RWC content. The higher RWC in plants treated with potassium nitrate could be attributed to

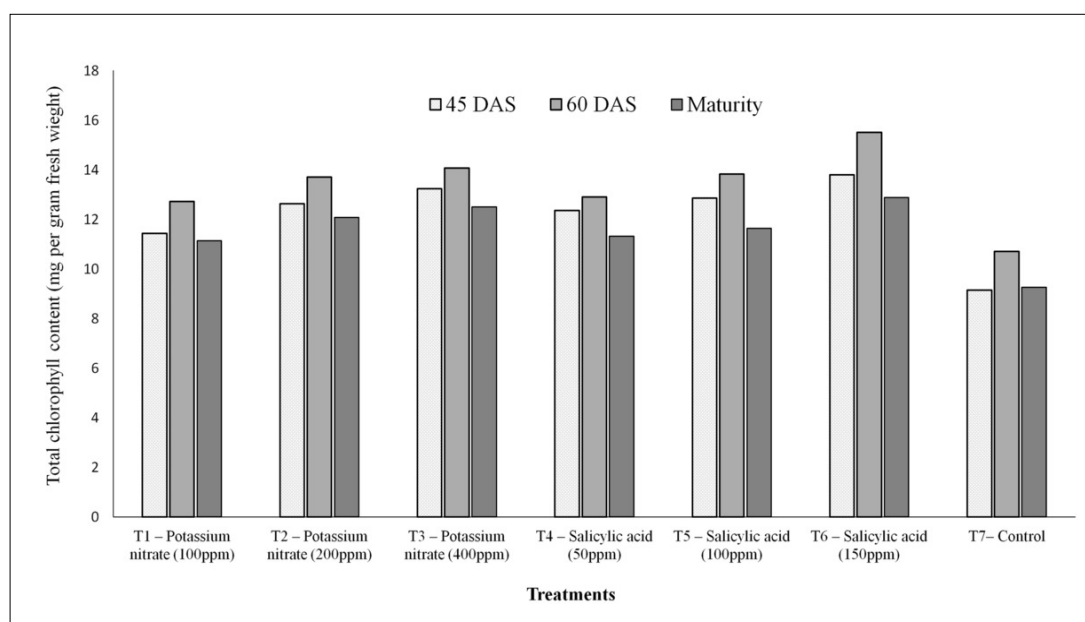


Fig 1: Effect of foliar application of potassium nitrate and salicylic acid on total chlorophyll (SPAD Value) at different growth stages of mungbean (pooled data of *kharif* 2021 and 2022).

Table 2: Effect of foliar application of potassium nitrate and salicylic acid on peroxidase, proline content, nitrate reductase, relative water content of mung bean (pooled data of *kharif* 2021 and 2022).

Treatments	Peroxidase (g^{-1} fresh weight)	Proline (mg/g fresh weight)	Nitrate reductase (μgram /fresh weight)	Relative water content (%)
T ₁ - Potassium nitrate (100 ppm)	72.47	63.00	179.85	93.93
T ₂ - Potassium nitrate (200 ppm)	74.58	64.03	184.58	94.56
T ₃ - Potassium nitrate (400 ppm)	74.71	67.78	244.78	95.85
T ₄ - Salicylic acid (50 ppm)	65.53	67.31	191.67	85.43
T ₅ - Salicylic acid (100 ppm)	72.49	64.70	211.32	92.52
T ₆ - Salicylic acid (150 ppm)	72.82	71.93	265.45	92.84
T ₇ - Control	66.78	61.22	79.34	93.06
S.E.m $\pm$	0.80	1.03	0.513	0.201
C.D.5%	2.49	3.21	1.59	0.62

improved osmotic adjustment and water retention, which are crucial under saline conditions. Similar results were found by Ogunsiji *et al.* (2023) that RWC showed a gradual decrease under progressive salt stress conditions. Potassium-applied plants maintained high leaf water potential over control (Goud *et al.*, 2022). Potassium is reported to improve water relations as well as productivity of different crops under water stress conditions (Islam *et al.*, 2004).

Peroxidase content (g^{-1} fresh weight)

The peroxidase activity in mung bean leaves increased significantly with the foliar treatments over control (Table 2). The highest peroxidase content was observed in the plot treated with potassium nitrate @400 ppm (74.71 g^{-1} fresh weight), while the lowest peroxidase content was recorded with insalicylic acid @50 ppm treated (65.53 g^{-1} fresh weight). Increased peroxidase activity indicates enhanced antioxidant defence, which helps in detoxifying reactive oxygen species (ROS) generated under salinity stress. These findings align with Larkindale and Knight (2002), who observed that peroxidase plays a crucial role in mitigating oxidative damage in plants under stress.

Proline content (mg/g fresh weight)

The proline content, a marker for stress tolerance, showed significant variation among treatments (Table 2). The highest proline accumulation was recorded in the plot treated with salicylic acid @150 ppm (71.93 mg/g fresh weight) and the lowest proline content was observed in the potassium nitrate @100 ppm (63.00 mg/g) treated plot. However, in the untreated control plot, it was observed 61.22 mg/g proline accumulation. The increase in proline levels suggests that these treatments enhance the osmoprotective mechanisms of mung bean under salinity stress. This finding corroborates the results of Sharma *et al.* (2017) and Verslues and Sharma (2010), who showed that SA increased toward proline accumulation to maintain redox buffering and osmoregulation in mungbean development. Teixeira *et al.* (2020) found that proline concentration serves as a crucial physiological indicator for assessing various stress resistance levels in plants.

Nitrate reductase activity (NR Activity, $\mu\text{g/g}$ fresh weight)

Nitrate reductase (NR) activity was significantly enhanced by the foliar application of salicylic acid and potassium nitrate over untreated control (Table 2). The maximum NR activity was recorded in the plot treated with salicylic acid @150 ppm ($265.45 \mu\text{g/g}$ fresh weight) and the lowest NR activity ($184.58 \mu\text{g/g}$) in the potassium nitrate @200 ppm treated plot. These results indicate that salicylic acid plays a critical role in enhancing nitrogen metabolism under salinity stress, as also reported by Jakab *et al.* (2007).

Yield components

Number of clusters per plant

The number of clusters per plant was significantly influenced by foliar treatments. The maximum number of clusters per plant was recorded in the plot treated with salicylic acid @150 ppm (9.97) and the minimum number of clusters was observed in the salicylic acid @150 ppm (5.34) treated plot (Table 3). Munns and Tester (2008) found that plants redirect energy from anabolic processes and biomass accumulation to adapt to stress. Salicylic acid (SA) enhances growth yield, with 50 ppm increasing height and 150 ppm improving biomass.

Number of pods per plant

The highest number of pods per plant was recorded in the plot treated with salicylic acid @150 ppm (39.77) and the lowest number of pods (22.83) was observed in the potassium nitrate @100 ppm treated plot. However, the untreated plot recorded 17.83 pods per plant (Table 3). Similarly, Goud *et al.* (2022) reported that the number of pods per plant was significantly affected by the application of potassium. Furthermore, Khan *et al.* (2015) showed that SA supports vital plant physiological processes, including antioxidant defence system control, nitrogen metabolism, photosynthesis and improved water use efficiency.

Number of seeds per pod

The maximum number of seeds per pod was recorded in the plot treated with potassium nitrate @400 ppm (5.68) and the minimum seeds per pod were observed in the

Table 3: Effect of foliar application of potassium nitrate and salicylic acid on plant growth and yield of mung bean (pooled data of *kharif* 2021 and 2022).

Treatments	No. of clusters/plant	No. of pods/plant	No. of seeds/pod	Seed yield (g plant^{-1})	Grain yield (q ha^{-1})	100 seed weight (g)
T ₁ - Potassium nitrate (100 ppm)	7.82	22.83	3.78	5.34	15.32	3.51
T ₂ - Potassium nitrate (200 ppm)	9.11	27.37	5.25	6.68	20.05	3.69
T ₃ - Potassium nitrate (400 ppm)	9.32	28.98	5.68	6.76	22.03	3.70
T ₄ - Salicylic acid (50 ppm)	5.34	24.25	5.18	3.98	11.96	3.37
T ₅ - Salicylic acid (100 ppm)	9.12	35.86	4.80	5.88	22.80	3.58
T ₆ - Salicylic acid (150 ppm)	9.97	39.77	5.40	6.70	21.30	3.89
T ₇ - Control	6.41	17.83	2.99	4.94	14.43	3.57
S.E.m $\pm$	0.366	2.956	0.198	0.18	0.76	0.14
C.D.5%	1.13	9.209	0.615	0.56	2.36	0.25

potassium nitrate @ 100 ppm (3.78) treated plot (Table 3). The lowest growth and yield contributing parameters were recorded in no potassium, which might be due to the fact that a high shoot root ratio is associated with potassium uptake (Yang *et al.*, 2004). Khan *et al.* (2015) reported that salicylic acid protects plants from environmental stresses like salinity and drought by maintaining photosynthesis.

Seed yield (q/ha)

The seed yield per hectare and per plant were significantly enhanced by the foliar treatments. The highest seed yield per hectare was observed in the plot treated with salicylic acid @100 ppm (22.80 q/ha) and the lowest seed yield (14.43 q/ha) was recorded in the salicylic acid @50 ppm treated plot. While the 11.96 q/ha grain yield was observed in the untreated control plot. The maximum test weight of the seed grain was recorded in salicylic acid @150 ppm (3.89 g) and the minimum test weight of the seed grain was recorded in salicylic acid 50 ppm (3.50 g) (Table 3). The significant increase in seed yield in the higher treatment groups can be attributed to enhanced physiological performance and better nutrient uptake, as reported by Navya *et al.* (2021) observed seed yield of soyabean increase 35.6% over control with application of 49.8 kg K/ha. Similar findings were observed by Patil and Dhonde (2009) in green gram, Salve and Gunjal (2011) in groundnut, Balai *et al.* (2005), Asghar (1994) in black gram and Saxena *et al.* (1996) in green gram.

CONCLUSION

Salicylic acid-induced modulations assist plants in maintaining balance and adapting to stress. It enhances the metabolic and physiological processes that help mungbean seedlings acclimatise and survive salt stress, improving their growth and yield potential. Understanding how plants regulate SA levels in response to salt stress and identifying specific promoters in the SA synthesis pathway will optimise SA treatment. Future research should focus on SA's role in mitigating salt stress to enhance yield in challenging environments.

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Disclaimers

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Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling

techniques were approved by the University of Animal Care Committee.

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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