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Mechanism of sodium nitroprusside regulating ginseng quality

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The roots of *Panax ginseng* C. A. Meyer (ginseng) are one of the traditional medicinal herbs in Asian countries and is known as the "king of all herbs". The most important active components of ginseng are the secondary metabolite saponins, which are closely related to ecological stress. Unsuitable ecological stress can generate a large amount of reactive oxygen species (ROS), by which the secondary metabolism is regulated, and the quality of herbs can be significantly improved. The purpose of this study was to investigate the effect of sodium nitroprusside (SNP) treatment on the quality of fresh ginseng roots. In this study, 5-year-old fresh ginseng was exposed to 0.1, 0.5, and 2 mmol/L SNP, a nitric oxide (NO) donor for five consecutive days. SNP significantly increased the levels of $O_2^{\cdot-}$, H_2O_2, malondialdehyde (MDA), NADPH oxidase (NOX), superoxide dismutase (SOD), catalase (CAT), peroxides (POD), ascorbate peroxidase (APX), glutathione reductase (GR), ascorbate (AsA) and GSH/GSSG. The main root treated by 0.5 mmol/L SNP for three days was the best, with the activities of the key enzymes of the ginsenoside synthesis pathway, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), farnesyl pyrophosphate synthase (FPS), squalene synthase (SS), squalene epoxidase (SE), and dammarane diol-II synthase (DS) activities increased markedly; the ginsenosides Rg_1 + Re, Rb_1, Rf, Rc, Rg_2 + Rh_1 and the total ginsenoside contents increased by 51.0%, 77.7%, 44.6%, 26.8%, 63.2% and 48.2%, respectively, but only a trace amount of the ginsenoside monomer Rb_2 decreased 23.4%. The fibrous roots treated by 0.1 mmol/L SNP for four days showed the best effect, HMGCR, FPS, SS, SE, and DS also increased significantly; ginsenosides Rg_1 + Re, Rb_1, Ro, Rc, Rf, Rb_3, Rb_2, and total saponin contents increased 37.6%, 47.8%, 34.2%, 75.1%, 51.0%, 49.4%, 28.3%, and 20.4%, respectively. The 1,3-diphosphoglycerate (1,3-DPG) and phosphoenolpyruvate carboxylase (PEPC), related to primary metabolism, were also significantly elevated. The Morris water maze (MWM), histopathological analysis and oxidative stress indexes in brain tissues were used to evaluate the anti-aging effect, indicating that the SNP-treated ginseng further ameliorated D-gal-induced the impaired memory function and oxidative stress in mice, implying the efficacy of SNP-treated ginseng was better than untreated ginseng's. SNP can build the physiological state of ginseng under ecological stress, stimulate the antioxidant protection mechanism, increase the secondary metabolites, and improve the quality of ginseng.

Keywords Ginsenosides, Sodium nitroprusside, Reactive oxygen species, Secondary metabolite, Anti-aging

Contrary to animals, the immovability of plants prevents them from avoiding ecological stress or seeking out suitable environments. Ecological stress activated the NADPH oxidase (NOX) at the plasma membrane, promoting the transfer of electrons from cytoplasmic NADPH to extracellular O_2 and forming $O_2^{\cdot-}$[1]. Declined photosynthesis due to ecological stress leads to the accumulation of electrons in the chloroplasts' electron transport chain, producing photo-oxidative stress and excess ROS; ROS are also produced when electron transfer in the mitochondria exceeds the elimination of the alternative oxidase (AOX) and cytochrome oxidase (COX) under ecological stress[2]. Ecological stress also stimulates peroxisome metabolism to promote $O_2^{\cdot-}$ and H_2O_2 production[3]; therefore, the burst of ROS is an inherent feature of ecological stress acting on plants[4,5]. ROS can alter the structure of proteins by promoting -S-S- bonds formation and inevitably alter metabolism; the biosynthesis of secondary metabolites is usually induced by various ecological stress, pathogen attacks, and nutritional deficiencies[6], which in this case usually produce excess ROS. Antioxidant enzymes such as SOD, CAT, POD, and APX are essential to eliminate ROS, but antioxidant enzymes are also proteins that can be destroyed by too much ROS, making it difficult for plants to resist severe adversity stress. Plant can adapt to severe ecological

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stress because it has evolved secondary metabolism systems that act as additional supplements[7]. However, the production of secondary metabolites inevitably consumes a lot of energy and material, and therefore, they are produced in large quantities only when plants are under ecological stress. Plant secondary metabolites, such as phenols, saponins, alkaloids, and others, can eliminate excess ROS or mitigate their damage. The -OH of flavonoids has a strong electron-losing capacity and can scavenge ROS[8]. Ginsenosides contain a rigid steroidal backbone with four anticyclic rings[9], similar in structure to sterols, therefore, ginsenosides can fill the membrane voids to improve cell membrane fluidity and the adaptability of ginseng to the external environment. Some ginsenosides also can directly eliminate ROS, e.g., the double bond on the side chain of ginsenoside Rb_1 can eliminate ·OH and HOCl[10]. Thus, ROS is the mediator of adversity stress and secondary metabolite biosynthesis. Secondary metabolites are usually the medicinal components in herbal medicines[11]. Numerous studies have found that ROS can stimulate the production of plant secondary metabolites[12], e.g., the contents of most major secondary metabolites increased by more than 60% in both *Antrodia cinnamomea* and *Scutellaria baicalensis*[13,14].

The dried roots and rhizomes of *Panax ginseng* C.A. Mey (ginseng) is one of the traditional medicinal herbs in Asian countries, known as the "king of all herbs". Ginsenosides are the main active components, mainly including ginsenosides Rg_1, Re, Rb_1, Rc, Rb_2, Rd, and so on[15], with anti-aging, scavenging free radicals, anti-diabetic and neuroprotective effects[16]. The ginsenosides were synthesized via the mevalonate (MVA) and the pyruvate (MEP) pathway, involving several enzymes such as 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), farnesyl pyrophosphate synthase (FPS), squalene synthase (SS), squalene epoxidase (SE), dammarane diol-II synthase (DS), cytochrome P450 (CY P450) and glycosyltransferase (GT)[17]. As a perennial herbaceous plant, the biosynthesis of ginsenosides is closely related to environmental factors: strong light, water stress, and suitable low temperatures all promote the synthesis and accumulation of ginsenosides[18–20]. And ginseng grows mostly in the hillside, afraid of waterlogging, so excessive water is also a kind of stress for ginseng.

Nitric oxide (NO) is a highly active gaseous small molecule nitrogen oxide with high lipid-solubility and slight water-solubility and readily diffuses between protoplasmic and lipid-soluble membrane systems[21]. NOX on the plasma membrane is an important $O_2^{\cdot-}$ production pathway. NO promotes intracellular Ca^{2+} influx, increases Ca-ATPase activity, activates calcium-dependent protein kinase, and phosphorylates NOX, forming $O_2^{\cdot-}$[22,23]. NO interacts with ROS and is involved in regulating intracellular metabolism and signal transduction. Nitric oxide synthase (NOS) inhibitors inhibited cadmium-induced NO production, H_2O_2 production and apoptotic processes, suggesting that NOS-dependent NO is located upstream of H_2O_2[24]. In postharvest tomatoes, exogenous NO bi-directionally regulate the level of H_2O_2, increasing the H_2O_2 when H_2O_2 level was low, inhibiting the synthesis and accumulation of H_2O_2 when H_2O_2 level was high, which are regulated by SOD, CAT and APX[25]. NO can remove Fe^{2+} in the Fenton reaction and avoid the production of ·OH, which is highly destructive[26]. The appropriate amount of NO also protects the organism from oxidative stress[27]. NO induces ROS elimination and repairs damage[28–30]. In summary, NO initiated intact physiological metabolism under adverse conditions without causing more significant cellular damage.

The ecological role of secondary metabolites is mainly to eliminate ROS originating from adversity or to minimize the damage caused by ROS[31]. ROS can modulate the structure of enzymes through the formation of disulfide bonds between different protein subunits and thus may regulate metabolism[32]. Natural selection has led plants to develop a biological mechanism to eliminate excess ROS. Once ROS are produced in excess, it triggers an increase in the content or activity of antioxidant substances, which in turn eliminate excess ROS, i.e., a negative feedback process, and keeps ROS at a relatively stable level. Secondary metabolites are the most important antioxidant substances in plants and usually the active ingredients of herbal medicine, therefore, the biological mechanisms of plant adaptation to adversity stress are also the mechanisms of quality formation of herbal medicine. NO can generate physiological states under ecological stress, regulate secondary metabolism through the produced ROS, promote the biosynthesis and accumulation of ginsenosides, and enhance the adaptive capacity of plants. Plants display remarkable totipotency. So, sodium nitroprusside was directly applied to ginseng fresh root in this study, which could reduce the stress damage to the maximum extent without affecting the plant growth. Our primary objective was to elucidate the relationship between ROS, antioxidant system and changes in ginsenoside content under NO, to elucidate the possible mechanism of ginsenoside accumulation, and to provide a new way to produce high quality ginseng.

Results

Effect of SNP on ROS

NO level

As shown in Fig. 1A,B, the NO levels in the control (ctrl) group were basically unchanged, while those in the 0.1, 0.5, and 2.0 mmol/L SNP groups showed a dose-dependent and continuously increasing trend compared with 0 day. In the main root, the 0.5 mmol/L and 2.0 mmol/L groups showed the greatest elevation of more than 3-fold higher than that on day 0 ($p < 0.01$) on day 4. In the fibrous root, the 2 mmol/L group was 4-fold as large as the 0-day group ($p < 0.01$).

$O_2^{\cdot-}$ and H_2O_2 levels

Compared to the 0 day, the levels of $O_2^{\cdot-}$ and H_2O_2 in the ctrl group remained relatively stable. In contrast, the $O_2^{\cdot-}$ and H_2O_2 levels of 0.1, 0.5, and 2 mmol/L SNP groups tended to increase initially and then decreased (Fig. 2). In the main root, $O_2^{\cdot-}$ and H_2O_2 in the 0.1 mmol/L group peaked on the 4-day and the 3-day, respectively, while the 2 mmol/L group peaked on days 4 and 5. The 0.5 mmol/L group resulted in the highest elevations of $O_2^{\cdot-}$ and H_2O_2, peaked on days 3 and 4, with a increase of 68.6% and 138.0%, respectively ($p < 0.01$). In the fibrous root, the 0.5 mmol/L group exhibited peak levels of $O_2^{\cdot-}$ and H_2O_2 on day 1, while the 2.0 mmol/L group peaked on day 4. The 0.1 mmol/L group had the most significant increase, with $O_2^{\cdot-}$ and H_2O_2 levels rising by 32.0% and 113.0%, respectively, compared to day 0 ($p < 0.01$), and peaked on the day 4.

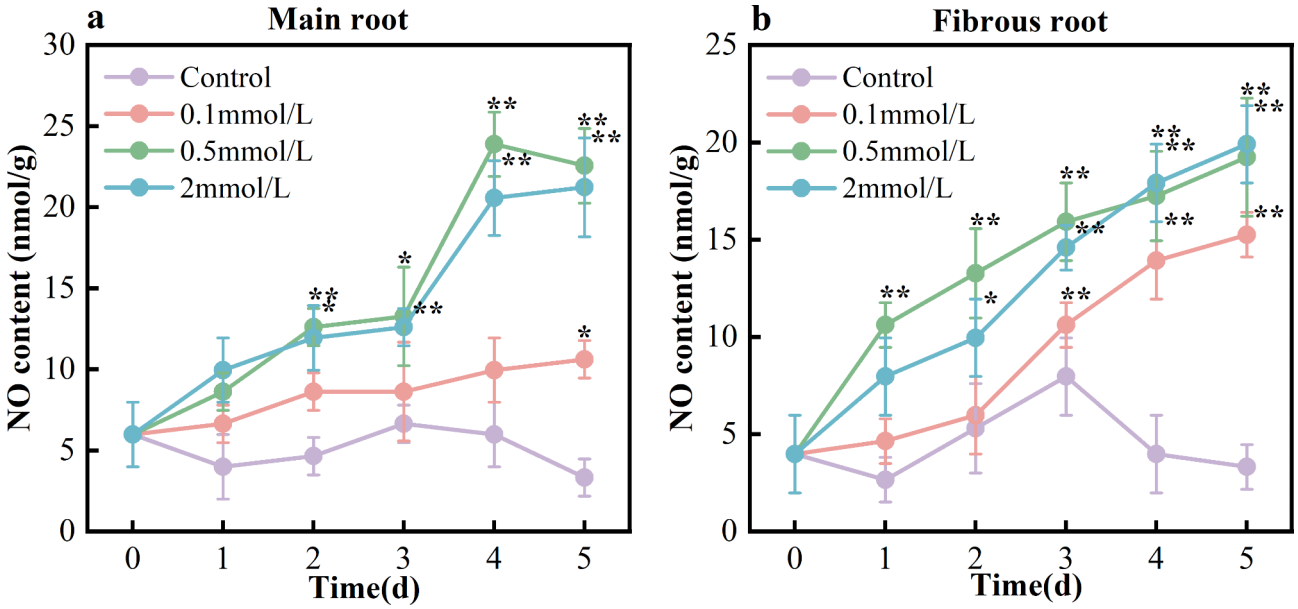

Fig. 1. Effect of SNP on NO. Changes in NO content were analyzed by spraying 0.1, 0.5 and 2 mmol/L SNP on the fresh roots of *P.ginseng* for 5 days (**A**) main root (**B**) fibrous root. All values were presented as Mean ± SD ($n = 3$). * and **: Significant at the 5% and 1% probability levels, respectively.

MDA content

Figure 3A,B showed that with the SNP, MDA contents in 0.1, 0.5, and 2 mmol/L SNP groups increased and then stabilized essentially. It also shows a small accumulation in the ctrl group. In the main root, both the 0.1 and 0.5 mmol/L groups peaked on day 3, and the 2.0 mmol/L group had the greatest elevation of 75.5% ($p < 0.01$), peaked on day 4. In the fibrous root, the 0.1 and 0.5 mmol/L treatment groups peaked on day 3 and day 4, respectively, and the 2.0 mmol/L group had the greatest elevation of 166.5% ($p < 0.01$), peaked on day 2 (Fig. 3).

NOX activity

NOX activities showed an increasing-decreasing trend in the 0.1, 0.5, and 2 mmol/L SNP groups comparing to 0 day, and it also displayed a small increase in the ctrl group. In the main root, the 0.1 and 2.0 mmol/L groups peaked on the 2-day and the 3-day, respectively. The highest NOX activity was recorded in the 0.5 mmol/L group on day 3 which was 125.6% higher than day 0 (Fig. 4). In the fibrous root, the 0.5 and 2 mmol/L treatment groups peaked on the 3-day and the 1-day, respectively, and the 0.1 mmol/L group had the greatest elevation of 160.4% ($p < 0.01$), peaked on day 3 (Fig. 4).

Antioxidant enzyme activity

The activities of each antioxidant enzyme showed an increasing and then decreasing trend in the 0.1, 0.5, and 2.0 mmol/L SNP groups. These trends were more pronounced compared to the ctrl group. In the main root, SOD and CAT peaked on day 4, and POD on day 3 in the 0.1 mmol/L group. In the 2.0 mmol/L group, SOD peaked on day 5, and both CAT and POD peaked on day 3. The 0.5 mmol/L group showed the greatest elevation of SOD, CAT, and POD, with both SOD and CAT peaking on the 3-day and POD on the 4-day, showing increases of 29.0%, 60.2%, and 66.3%, respectively ($p < 0.01$). In the fibrous root, SOD and POD peaked on day 1 and CAT peaked day 4 in the 0.5 mmol/L group. In the 2.0 mmol/L group, SOD and CAT peaked on day 4 and POD on day 2. SOD, CAT, and POD of the 0.1 mmol/L group showed the greatest elevation at 48.3%, 53.3%, and 114.7% ($p < 0.01$), respectively, all peaked on day 4 (Fig. 5).

AsA-GSH cycle

Compared with 0 days, there were little changes in the parameters in the AsA-GSH cycle in the ctrl group but most of them did not show a significant difference. The SNP groups showed a tendency to increase and then decrease. In the main root, AsA and GSH/GSSG peaked on day 2, and APX and GR peaked on day 3 in the 0.1 mmol/L group, respectively. In the 2.0 mmol/L group, GSH/GSSG and APX both peaked on the 4-day, and AsA and GR peaked on the 5-day and the 1-day, respectively. The greatest increase in all parameters was observed in the 0.5 mmol/L group, peaking on day 3; AsA, GSH/GSSG, APX, and GR elevated by 100.0%, 26.9%, 56.8%, and 62.6% compared with day 0 ($p < 0.01$), respectively. In the fibrous root, AsA and GSH/GSSG peaked on day 2 and APX and GR on the day 1 in the 0.5 mmol/L group. In the 2.0 mmol/L group, AsA and GR peaked on day 4 and APX on day 2, while the GSH/GSSG ratio continued to decline and reached its lowest on day 4. The greatest elevation of all parameters was observed in the 0.1 mmol/L group, AsA, APX, and GR peaked on day 4, GSH/GSSG on day 1, with an increase of 76.9%, 34.1%, 94.4%, and 22.6% compared with 0 days ($p < 0.05$), respectively (Fig. 6).

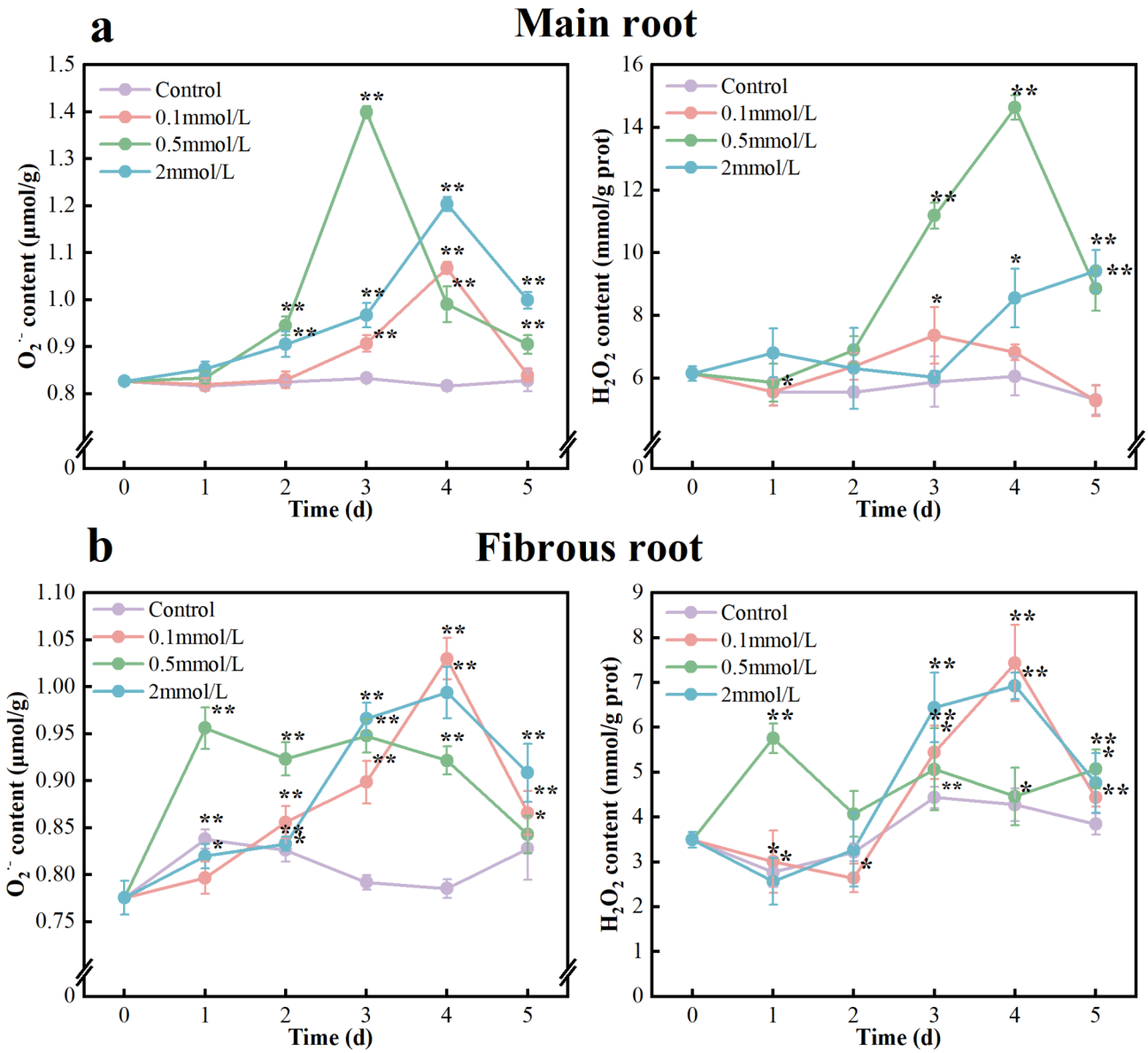

Fig. 2. Effect of SNP on $O_2^{\cdot-}$ and H_2O_2. Changes in $O_2^{\cdot-}$ and H_2O_2 contents were analyzed by spraying 0.1, 0.5 and 2 mmol/L SNP on the fresh roots of *P. ginseng* for 5 days (**A**) main root (**B**) fibrous root. All values were presented as Mean ± SD ($n = 3$). * and **: Significant at the 5% and 1% probability levels, respectively.

Effect of SNP on the key enzymes of secondary metabolites

Compared with 0 days, the 0.1, 0.5, and 2.0 mmol/L SNP showed a tendency of increasing and then decreasing. And there was little change in the ctrl group, but most of them did not show a significant difference compared to the 0 day. In the main root, HMGCR, SS, and SE peaked on day 2, FPS on day 4, and DS on day 1 in the 0.1 mmol/L group. The 2.0 mmol/L group peaked on day 1 for HMGCR, on the 3 days for FPS and SS, and on day 4 for SE and DS. The 0.5 mmol/L group showed the greatest increase in all parameters, with an increase of 49.6%, 34.0%, 119.7%, 93.3%, and 106.5% compared with that on the 0 days ($p < 0.01$), respectively, HMGCR and FPS peaking on day 2, SS, SE, and DS on 3 days. In the fibrous root, HMGCR, SS, and SE peaked on day 1, and FPS and DS on day 2 and day 4 in the 0.5 mmol/L group, respectively. HMGCR and SS peaked on day 2, FPS, SE, and DS on day 3 in the 2.0 mmol/L group, and the SS demonstrated the most significant increases than that of the other treatments, with an increase of 72.1%. The HMGCR, FPS, SE, and DS of the 0.1 mmol/L group elevated more than the other groups. Among them, HMGCR peaked on day 2, with a 67.4% increase, FPS, SS, and SE all peaked on day 4, FPS and SE being elevated by 69.4% and 82.0%, respectively, DS peaked on the 3-day, by 90.1% (Fig. 7).

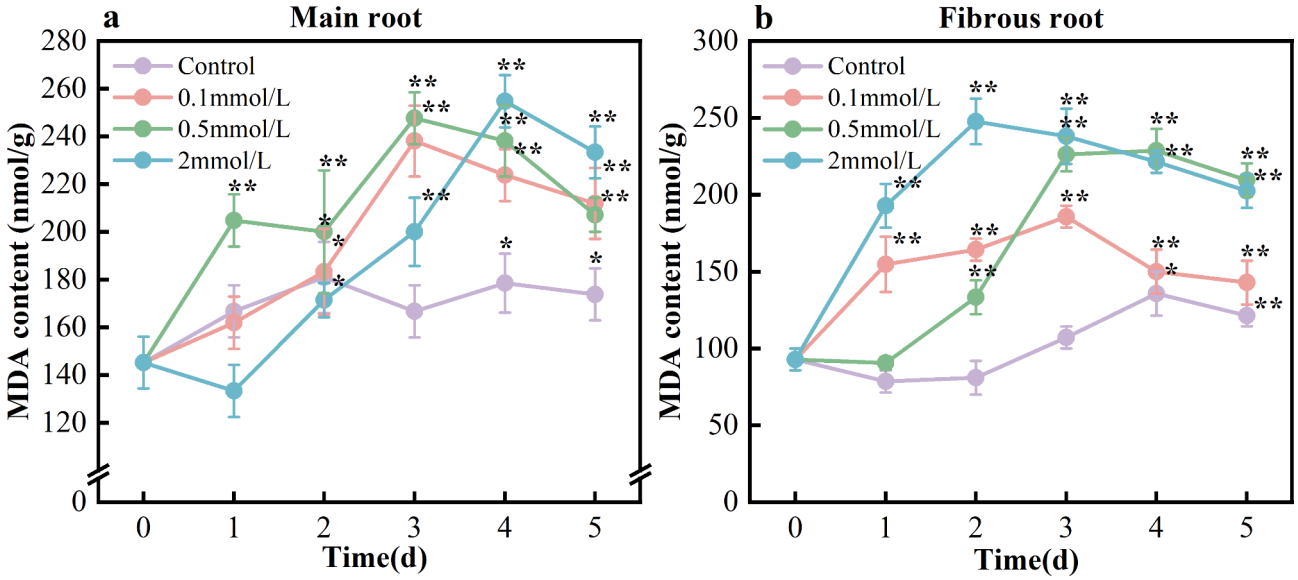

Fig. 3. Effect of SNP on MDA. Changes in MDA contents were analyzed by spraying 0.1, 0.5 and 2 mmol/L SNP on the fresh roots of *P. ginseng* for 5 days (**A**) main root (**B**) fibrous root. All values were presented as Mean ± SD ($n = 3$). * and **: Significant at the 5% and 1% probability levels, respectively.

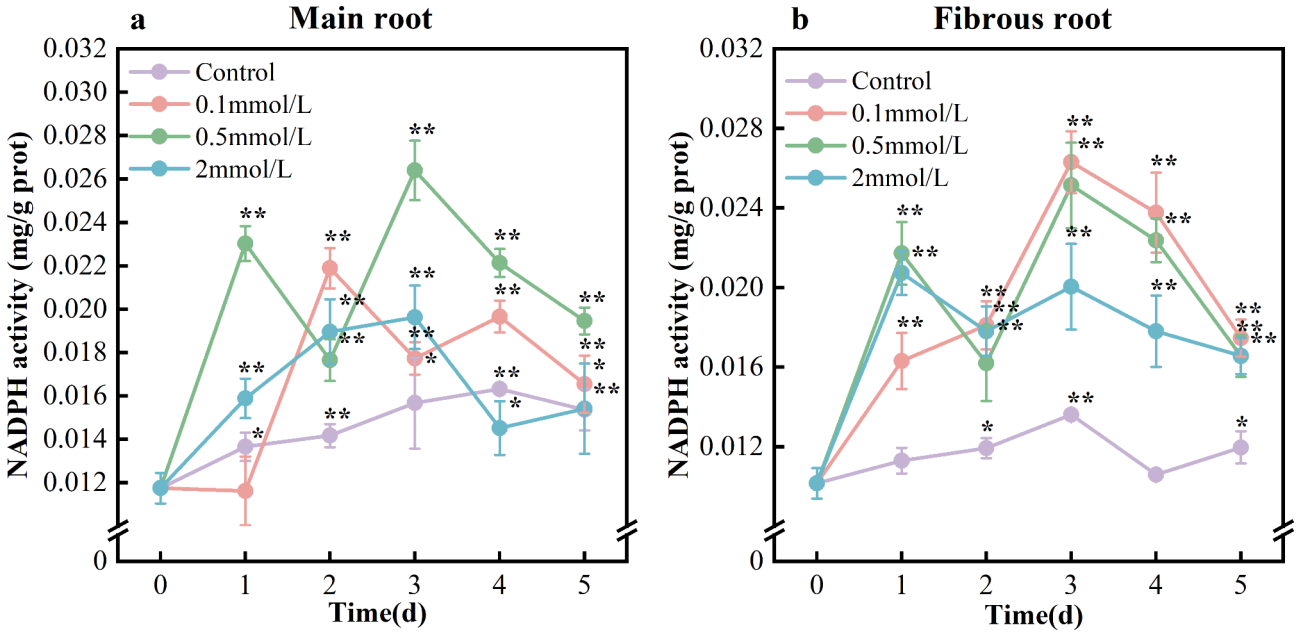

Fig. 4. Effect of SNP on NOX. Changes in NOX activity were analyzed by spraying 0.1, 0.5 and 2 mmol/L SNP on the fresh roots of *P.ginseng* for 5 days (**A**) main root (**B**) fibrous root. All values were presented as Mean ± SD ($n = 3$). * and **: Significant at the 5% and 1% probability levels, respectively.

Effect of SNP on the ginsenosides

Monomer ginsenosides

As shown in Fig. 8, in the main root in the ctrl group, except for ginsenoside Rb_2 had a little increase on day 1 and then decreased rapidly, the other saponins did not show pronounced trends like SNP groups. In the fibrous root, the contents of Rg_1, Rc, Rf, and Rb_2 in the ctrl group peaked on the 3, 4, 2, and 5 days, respectively, with an increase of 43.2%, 29.9%, 65.4%, and 6.0%, but were not as high as those in the SNP-treated groups. These results indicated that SNP could increase the ginsenosides contents, but the trend of each individual saponin was not consistent.

In the main root, except ginsenoside Rb_2, the Rg_1, Re, Rf, $Rg_2 + Rh_1$, Rb_1, Rc, Ro and Rb_3 of SNP treatment were markedly higher than that of the ctrl group ($p < 0.01$), Rg_1, Re, Rb_1 and Rf peaked on the day 3 in 0.5

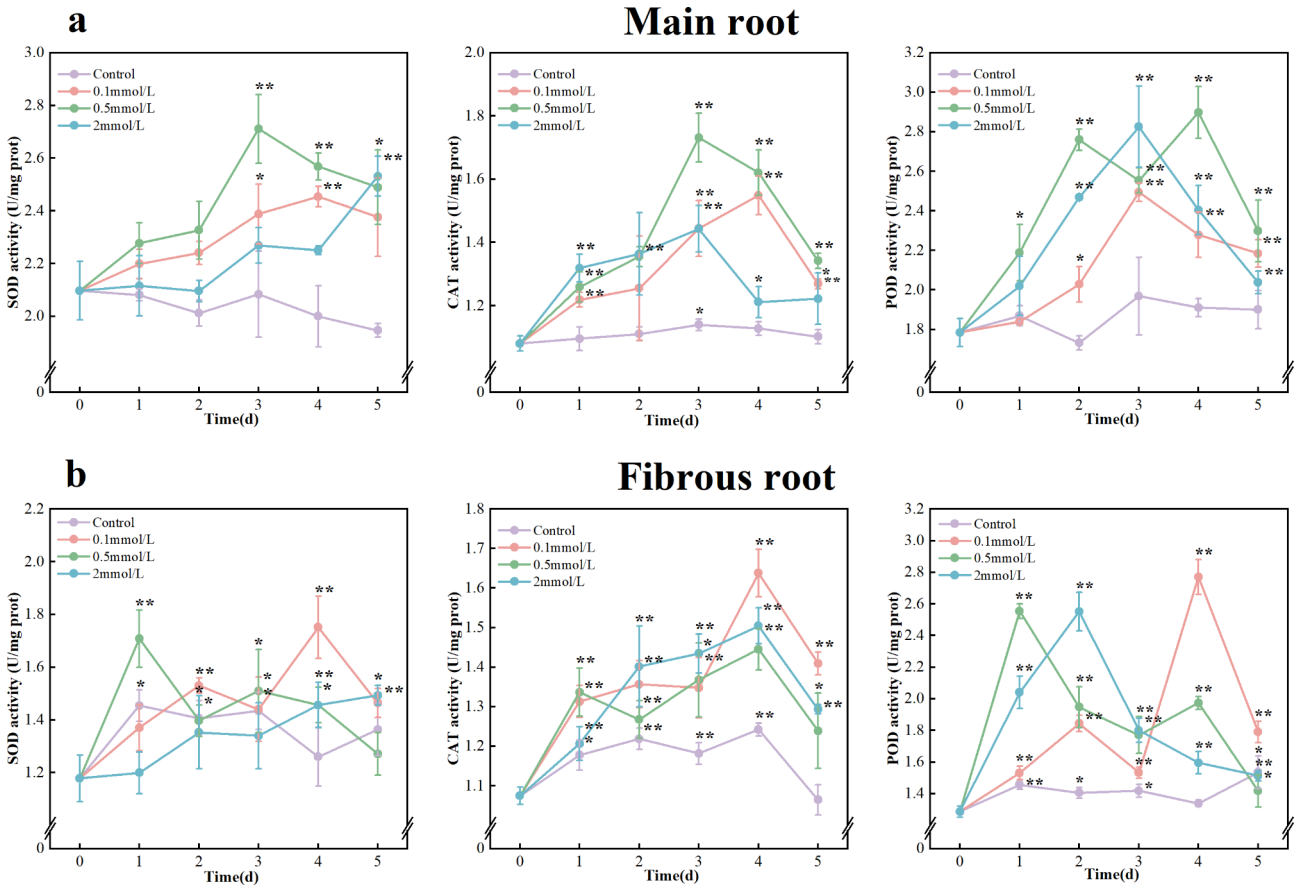

Fig. 5. Effect of SNP on SOD, CAT, and POD. Changes in SOD, CAT, and POD activities were analyzed by spraying 0.1, 0.5 and 2 mmol/L SNP on the fresh roots of *P. ginseng* for 5 days (**A**) main root (**B**) fibrous root. All values were presented as Mean ± SD ($n = 3$). * and **: Significant at the 5% and 1% probability levels, respectively.

mmol/L group, and $Rg_2 + Rh_1$ peaked on day 4, the contents were 6.17 mg/g, 2.24 mg/g, 2.79 mg/g, 1.20 mg/g, and 0.49 mg/g, respectively, with an increase of 73.3%, 11.4%, 77.7%, 44.6%, and 157.9%, respectively. The Ro, Rb_3, and Rc peaked at 1.63 mg/g, 1.30 mg/g, and 1.03 mg/g, respectively, on day 5 in the 2.0 mmol/L group, with an increase of 24.4%, 15.0%, and 45.1%, respectively. (Fig. 8A).

In the fibrous root, all ten monomer ginsenosides peaked at different times. Rg_1, Rb_1, Ro, Rc, Rb_3 and Rb_2 peaked on day 4, Re on day 3 in the 0.1 mmol/L group, the contents were 6.50 mg/g, 9.59 mg/g, 8.63 mg/g, 7.32 mg/g, 1.15 mg/g, 0.94 mg/g and 9.57 mg/g, respectively, with an increase of 81.1%, 47.8%, 34.2%, 75.1%, 49.4%, 42.4% and 28.3%, respectively. Rf reached a peak at 3.07 mg/g on day 5 in the 2.0 mmol/L group, with an increase of 100.7%. $Rg_2 + Rh_1$ reached a peak at 2.68 mg/g on day 1 in the 0.5 mmol/L group, which increased by 52.3% (Fig. 8B).

In the main root, the 0.5 mmol/L group on day 3 presented the best results: $Rg_1 + Re$, Rb_1, Rf, Rc, and $Rg_2 + Rh_1$ increased 51.0%, 77.7%, 44.6%, 26.8%, and 63.2%, respectively. In the fibrous root, the 0.1 mmol/L group had the best effect on the 4 days, in which $Rg_1 + Re$, Rb_1, Ro, Rc, Rf, Rb_3, and Rb_2 increased by 37.6%, 47.8%, 34.2%, 75.1%, 51.0%, 49.4%, and 28.3%, respectively. Rg_1, Re and Rb_1, with high contents, are important indicators for evaluating the quality of ginseng.

Total ginsenosides

Figure 9 displayed the total ginsenosides contents in the ctrl group had little change, while they changed markedly in the SNP-treated groups compared to 0 day. In the main root, the total ginsenosides peaked at day 4 in the 0.1 mmol/L group, peaked at day 5 in the 2.0 mmol/L group. The highest total ginsenoside content was recorded in the 0.5 mmol/L group on day 3 as 37.35 mg/g, which was 48.2% higher than day 0. In the fibrous root, the total ginsenosides of both 0.5 and 2 mmol/L groups peaked on day 1, showing the greatest increase of 20.4%, peaking at 82.88 mg/g on day 4.

Effects of SNP on 1,3-DPG and PEPC

Compared with the 0 days, 1,3-DPG and PEPC in the main root in the ctrl group had little change, but did not show pronounced trends like SNP groups. While in the 0.1, 0.5, and 2 mmol/LSNP-treated groups tended to increase. In the main root, 1,3-DPG and PEPC peaked on day 3 in both the 0.1 and 2.0 mmol/L groups. The

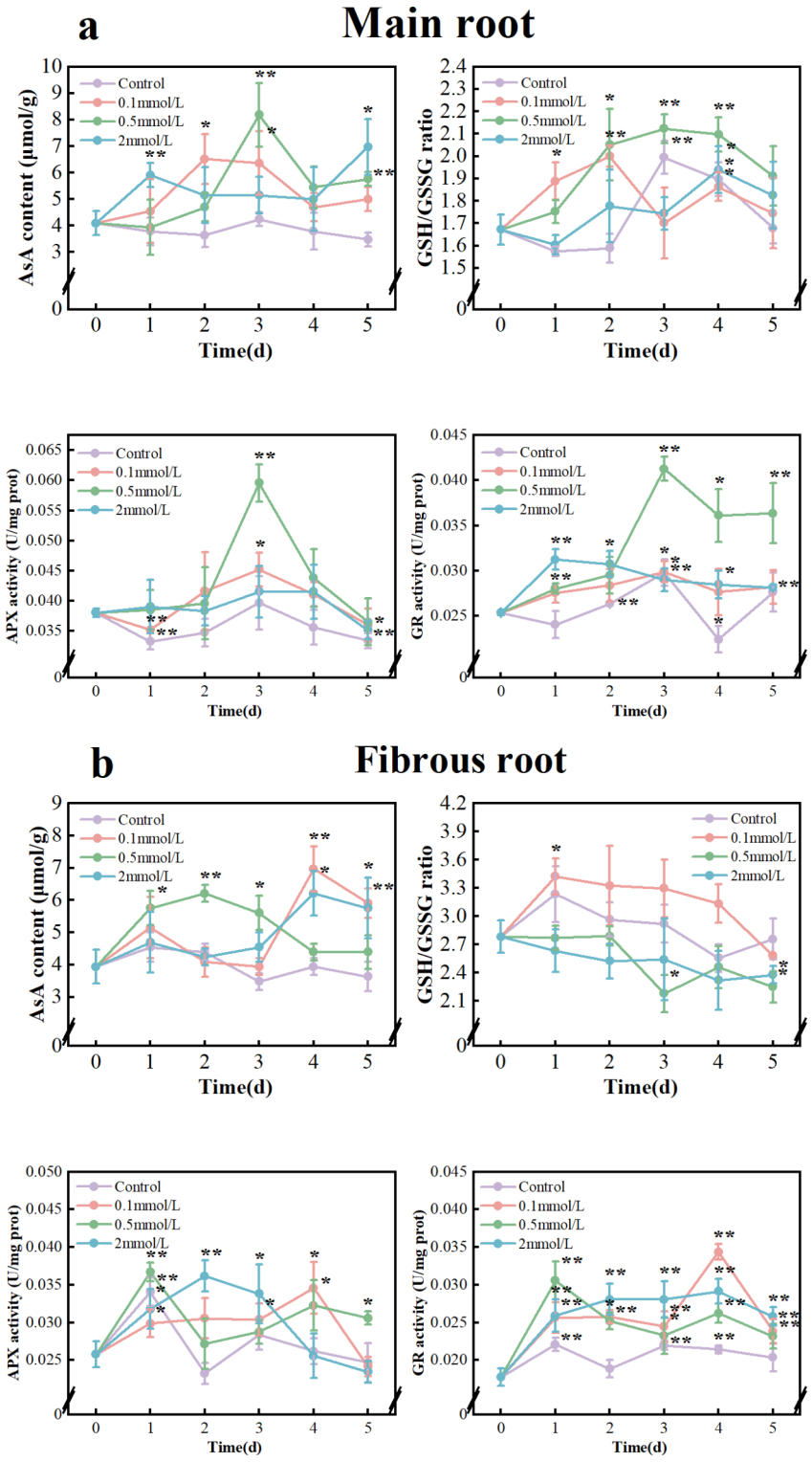

Fig. 6. Effect of SNP on AsA, GSH/GSSG ratio, APX, and GR. Changes in AsA, GSH/GSSG ratio, APX, and GR were analyzed by spraying 0.1, 0.5 and 2 mmol/L SNP on the fresh roots of *P. ginseng* for 5 days (**A**) main root (**B**) fibrous root. All values were presented as Mean ± SD ($n = 3$). * and **: Significant at the 5% and 1% probability levels, respectively.

greatest elevation of 1,3-DPG and PEPC was observed in the 0.5 mmol/L group, peaked on day 3, with elevations of 33.2% and 63.2%, respectively ($p < 0.01$). In the fibrous root, 1,3-DPG and PEPC both peaked on day 1 in the 0.5 mmol/L group and peaked on the 4 and 3 days, respectively, in the 2.0 mmol/L group. The 0.1 mmol/L group showed the greatest elevation of 32.6 and 68.0%, respectively ($p < 0.01$), peaked on day 4 (Fig. 10).

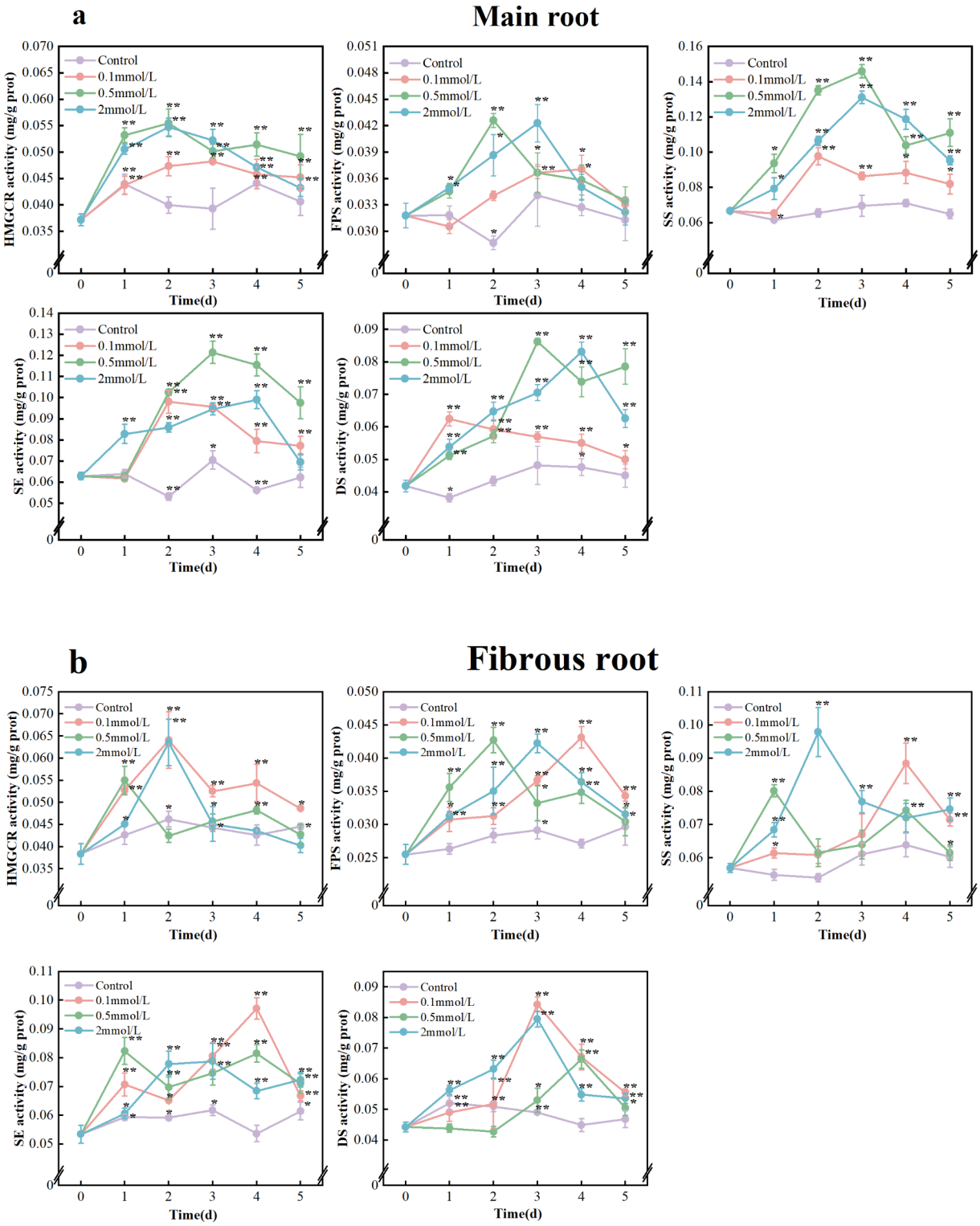

Fig. 7. Effect of SNP on HMGCR, FPS, SS, SE and DS. The changes in HMGCR, FPS, SS, SE and DS activities were analyzed by spraying 0.1, 0.5 and 2 mmol/L SNP on the fresh roots of *P. ginseng* for 5 days (**A**) main root (**B**) fibrous root. All values were presented as Mean ± SD ($n = 3$). * and **: Significant at the 5% and 1% probability levels, respectively.

Pharmacological effects verification

Morris water maze experiment

Compared with the blank group, the model group traversed the original platform an average of 3.1 fewer times and had a 54.7% shorter dwell time in the target area. Compared with the model group, groups C, D, and E

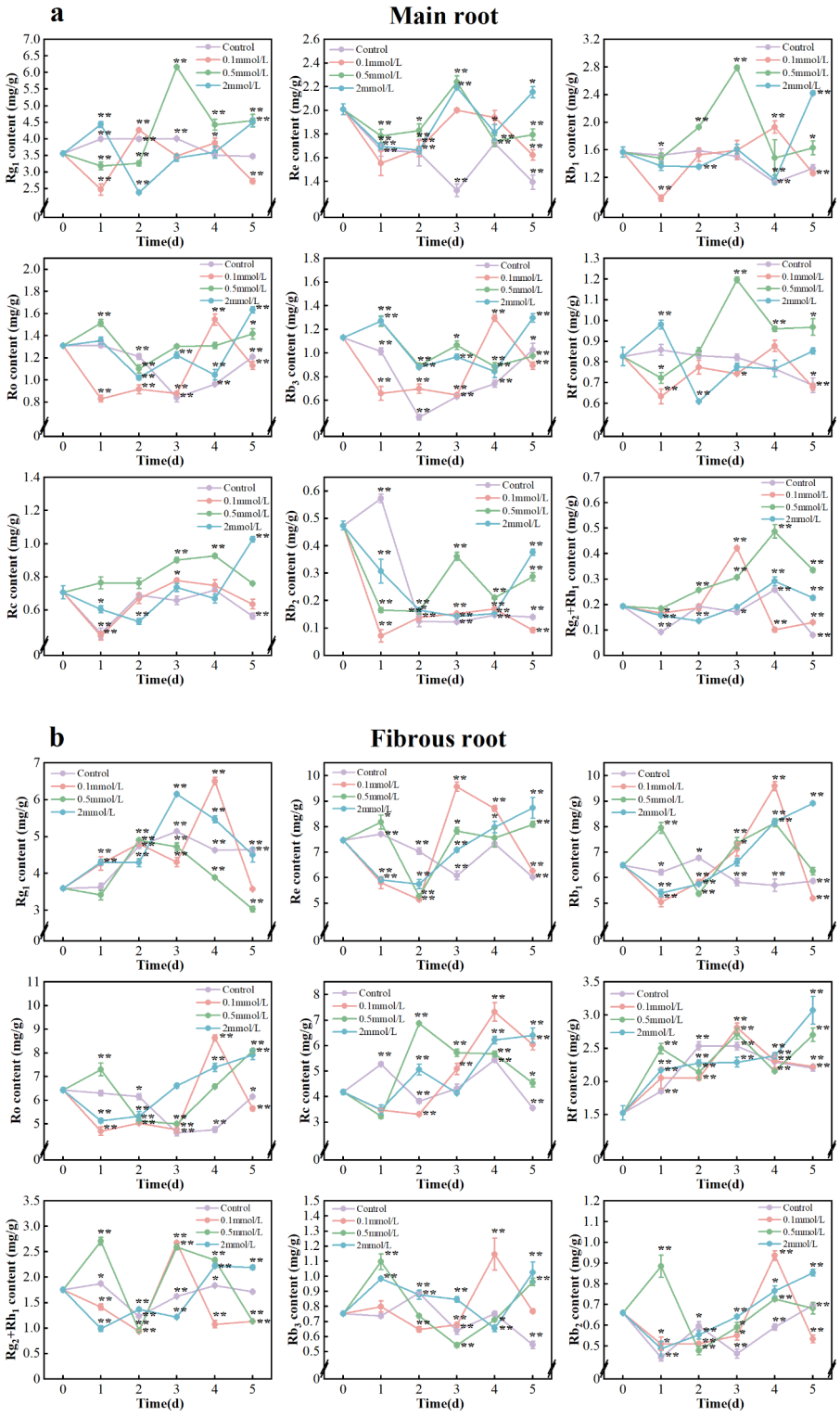

Fig. 8. Effect of SNP on monomer ginsenosides. Changes in monomer ginsenosides contents were analyzed by spraying 0.1, 0.5 and 2 mmol/L SNP on the fresh roots of *P. ginseng* for 5 days (**A**) main root (**B**) fibrous root. All values were presented as Mean ± SD ($n = 3$). * and **: Significant at the 5% and 1% probability levels, respectively.

showed a 39.2%, 14.6%, and 37.4% shortening of the evasion latency, a 2.5, 1, and 1.1 increase in the number of crossings of the original plateau, and an increase in the residence time in the target area by 85.7%, 44.3%, and 69.4%, respectively. Compared with group D, group E showed a 26.7% ($p = 0.046 < 0.05$) reduction in avoidance latency, a 1.1 increase in the number of traversals across the original platform ($p = 0.031 < 0.05$), and 17.4%

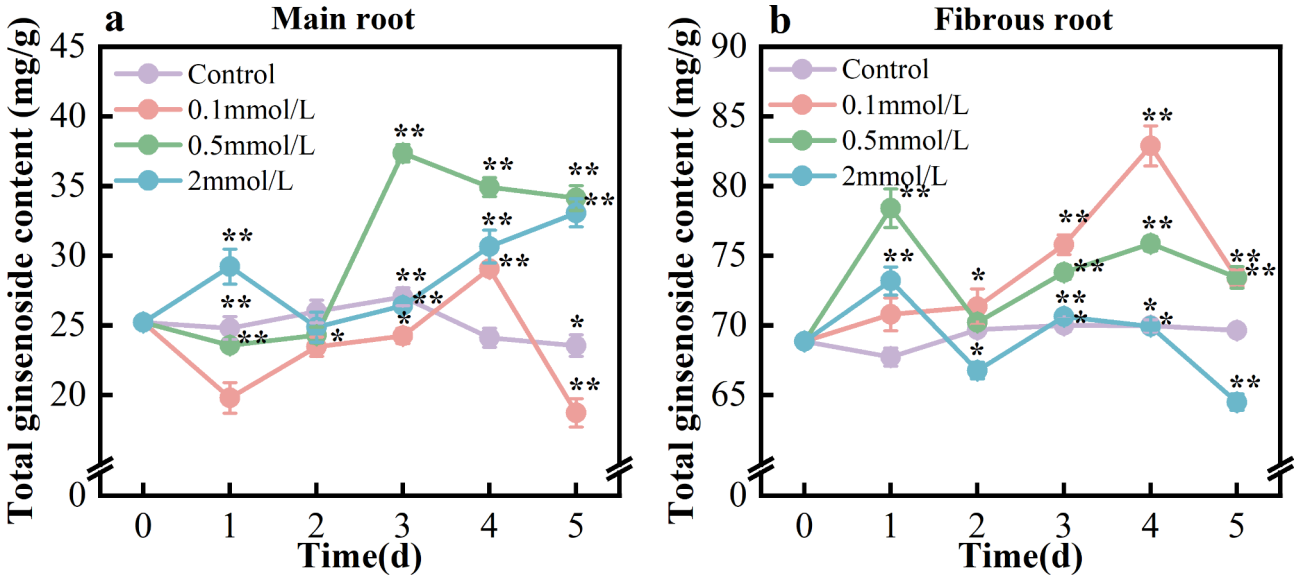

Fig. 9. Effect of SNP on total ginsenosides. Changes in total ginsenosides contents were analyzed by spraying 0.1, 0.5 and 2 mmol/L SNP on the fresh roots of *P. ginseng* for 5 days (**A**) main root (**B**) fibrous root. All values were presented as Mean ± SD ($n = 3$). * and **: Significant at the 5% and 1% probability levels, respectively.

increase in the duration of stay in the target area ($p = 0.139$) (Fig. 11), suggesting that the high-quality ginseng alleviated the memory deficits of the mice.

Histopathological staining

In the blank group, the neurons in each region of the hippocampus were regularly arranged, with normal morphology and structure, clear demarcation of cytoplasmic nuclei and obvious nucleoli, and no obvious inflammatory cell infiltration was seen. More crumpled neurons were seen in the CA3 region of the hippocampus in the model group, with deepened cellular staining and unclear cytoplasmic demarcation of the nucleus. Compared with the model group, the degree of lesions in the hippocampal region of the mice in Groups C, D, and E was significantly reduced; the E was less severe than that in the D (Fig. 12).

Oxidation-associated indexes in mice

Compared with the blank group, MDA in the brain tissue of the model group increased by 187.1%, and the activities of GSH-PX and SOD decreased by 58.5% and 50.1%, respectively. Compared with the model group, the GSH-PX activity was elevated by 114.4%, 39.5%, and 77.6%, the SOD was elevated by 73.0%, 35.0%, and 66.5%, and the MDA was reduced by 52.7%, 33.3%, and 47.7% in groups C, D, and E, respectively. In the E group, the GSH-PX and SOD were elevated by 27.3% ($p = 0.012 < 0.05$) and 23.4% ($p = 0.007 < 0.05$), respectively, and the content of MDA was reduced by 21.6% ($p = 0.016 < 0.05$) compared with that of the D group, with a significant difference (Fig. 13).

Discussion

Effects of SNP on ROS and MDA

Sodium nitroprusside (SNP) as NO donor[33] had been used to regulate plant metabolism[34,35]. Since a single cell of a plant has a complete metabolism, secondary metabolites can be produced by suspension cell culture, and therefore can also be obtained by treating plant tissues and organs. SNP consists of Fe^{2+}, NO, and five cyanide anions, capable of releasing NO in aqueous solution[36], and its ability to produce NO depends on the presence of molecules containing sulfur groups[37]. NO-activated calcium-dependent protein kinase phosphorylates NOX and generates ROS[23]. Compared with the 0 days, SNP increased NO by more than 3-fold in fresh roots (Fig. 1), and the levels of $O_2^{\cdot-}$ and H_2O_2 were elevated by 68.6% and 138.0%, respectively (Fig. 2). $O_2^{\cdot-}$ in the main root did not change markedly from day 1 to day 2 but peaked rapidly on day 3; in the fibrous root, it increased gradually and peaked on day 4 (Fig. 2). The slight change in $O_2^{\cdot-}$ in the main root at the beginning of the stress may due to the inhibition of respiratory metabolism by NO at this time, which reduced the rate of ROS production[38]. In addition, NO also inhibits the NOX activity[39]. In the late stage of stress, more $O_2^{\cdot-}$ produced could combine with NO and formed the low active $ONOO^-$, when the SOD was also higher, so the $O_2^{\cdot-}$ decreased and the MDA remained stable.

To summarize, NO produces large amounts of ROS and MDA, which are physiological markers of ecological stress, indicating that SNP are capable of inducing physiological states of ecological stress.

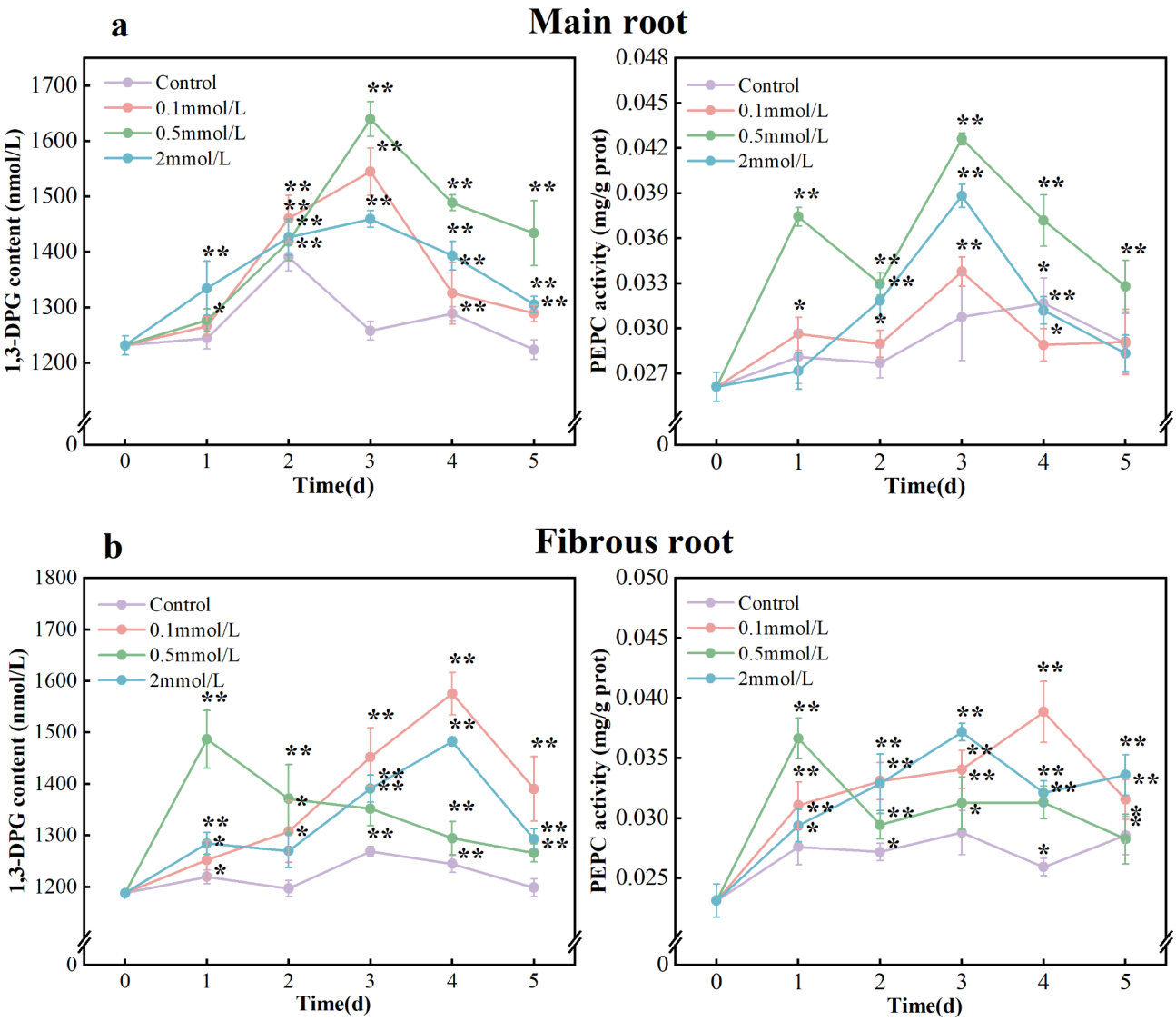

Fig. 10. Effect of SNP on 1,3-DPG and PEPC. Changes in 1,3-DPG and PEPC were analyzed by spraying 0.1, 0.5 and 2 mmol/L SNP on the fresh roots of *P. ginseng* for 5 days (**A**) main root (**B**) fibrous root. All values were presented as Mean ± SD ($n = 3$). * and **: Significant at the 5% and 1% probability levels, respectively.

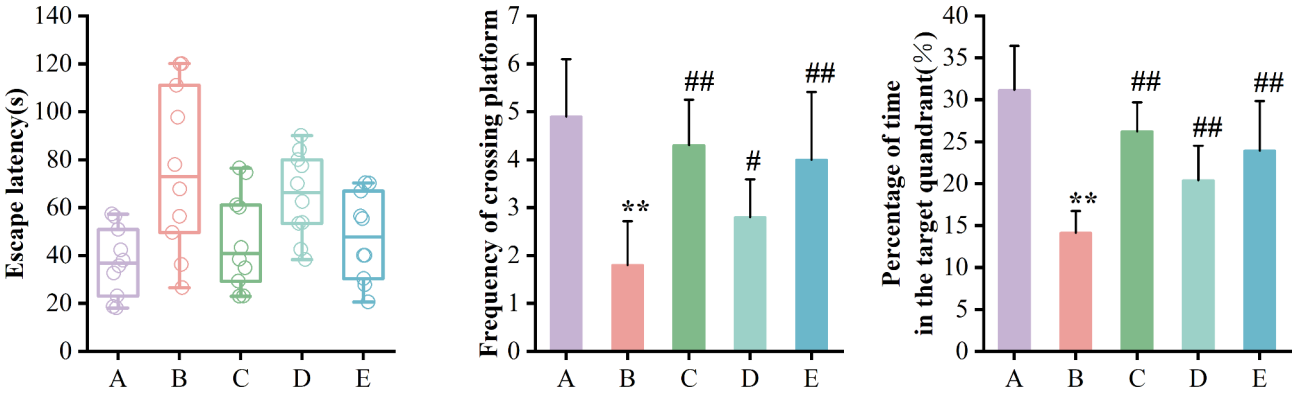

Fig. 11. Results of Morris water maze experiment in mice. (**A**) Blank ctrl group. (**B**) Model group. (**C**) Positive ctrl group. (**D**) Regular ginseng. (**E**) High-quality ginseng. All values were expressed as Mean ± S.D. ($n = 10$). $^{**}p < 0.01$ vs. blank ctrl group (**A**); $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. model group (**B**).

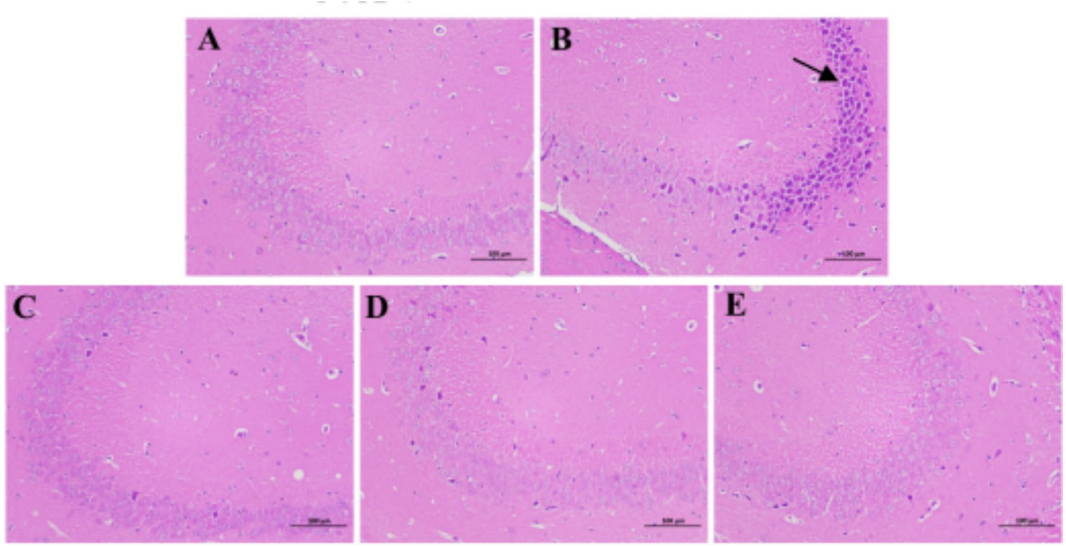

Fig. 12. Mice brain tissues stained with H&E dye kits (×200). (**A**) Blank ctrl group. (**B**) Model group. (**C**) Positive ctrl group. (**D**) Regular ginseng. (**E**) high-quality ginseng.

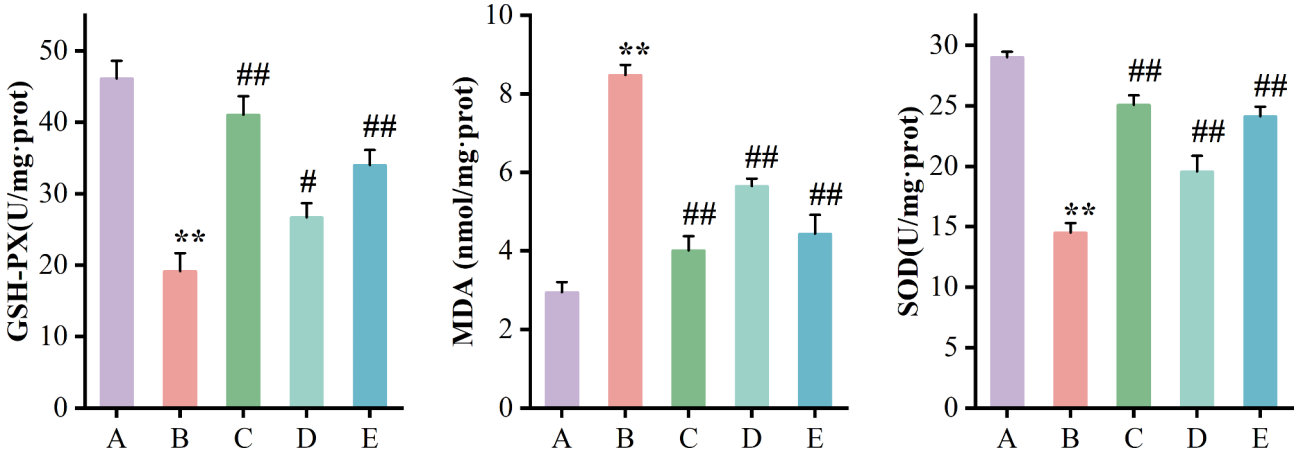

Fig. 13. GSH-PX, SOD activities, and MDA contents in mice brain tissues. (**A**) Blank ctrl group. (**B**) Model group. (**C**) Positive ctrl group. (**D**) Regular ginseng. (**E**) High-quality ginseng. All values were expressed as Mean ± S.D. ($n = 10$). $^{**}p < 0.01$ vs. blank ctrl group (**A**); $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. model group (**B**).

Effects of SNP on main antioxidant enzymes

The basic substances that scavenge ROS are antioxidant enzymes such as SOD, CAT, POD, APX, and glutathione transsulfurase (GST). The activities of antioxidant enzymes were enhanced when ROS were elevated in plants.

ROS plays an important role in –S-S– formation within protein molecules[32], thereby affecting gene expression, transcription, translation, and regulation of enzyme activity, so appropriate levels of ROS are also indispensable for life[40]. In this study, SNP increased the activities of various antioxidant enzymes with similar trends, implying that the antioxidant enzymes enhance the scavenging of ROS. Among them, the 0.5 mmol/L group showed the most significant enhancement ($p < 0.01$) in the main root (Fig. 5). However, excessive NO and ROS can also disrupt protein structure[41,42]. CAT has an active nitroxidative metabolism and continued NO accumulation leads to tyrosine nitrification and S-nitrification and affects enzyme activity[43]. In this study, the SOD and POD decreased slowly after reaching their peak, the CAT activity decreased at a faster rate, indicating that the continuously accumulated ROS leads to a general decrease of the antioxidant enzymes activities as the duration of stress increases. The higher enzyme activities in the main roots of the 0.5 mmol/L group were mainly because the dose of SNP at 0.1 mmol/L was insufficient to induce more ROS, while 2.0 mmol/L SNP led to the over-production of ROS, destructed antioxidant enzymes. The application of exogenous SNPs is continuous, and therefore, the NO content in ginseng is continuously increased; NO has not only oxidizing but also reducing properties. In the early stage of stress, NO promoted the generation of ROS and increased the activity of antioxidant enzymes. In the late stage of stress, the increased antioxidant enzymes and NO together eliminated the overproduced ROS, and NO played an antioxidant role, therefore, ROS tended to decrease in

the late stage of stress. As shown in Fig. 2. In addition, changed antioxidant enzyme activity is also an essential feature of ecological stress, which indicates that SNP can induce a physiological state of ecological stress.

Effects of SNP on AsA-GSH cycle

AsA-GSH is a ubiquitous antioxidant system in organisms and plays an important role in scavenging H_2O_2 and alleviating oxidative stress. In this cycle, APX uses AsA as an electron donor to decompose H_2O_2 into H_2O, and GR catalyzes the reduction of GSSG to GSH using NADPH as an electron donor. Higher AsA, GSH content, APX, GR activity, and lower GSSG content contribute to maintaining redox homeostasis in the cell, allowing the free radical scavenging response in the body to be sustained[44].

In this study, SNP promoted AsA-GSH cycling, as evidenced by higher levels of AsA, APX, GR ($p < 0.01$) and the ratio of GSH/GSSG, among them the 0.5 mmol/L group in the main root and 0.1 mmol/L in the fibrous root showed better effects (Fig. 6), indicating that AsA-GSH plays an important role in alleviating oxidative stress. In Ca-stressed tomato plants, exogenous NO increased all enzymatic and non-enzymatic components of the AsA-GSH cycle[45]. High-temperature stress increased maize levels of the AsA-GSH cycle and effectively alleviated the oxidative stress[46]. NO regulates proteins through tyrosine nitration and S nitrosylation, e.g., APX activity is up-regulated by S nitrosylation but down-regulated by tyrosine nitrosylation[47]. In contrast, AsA and GSH play an important role in the neutralization of $ONOO^-$, which seems to explain why, in the main root, the GR- GSH cycle of 0.5 mmol/L group was able to maintain high levels after reaching the peak, whereas AsA and APX declined faster in the later stage (Fig. 6). They both did not show significant differences in the fibrous root, probably due to the lower concentration in the 0.1 mmol/L SNP-treated group and failed to down-regulate the APX.

Effects of SNP treatment on the synthesis of metabolites of ginseng

Ginsenosides are triterpenoids composed of isoprenoid units, consisting of triterpene glycosides and sugars, glyoxylates, and other organic acids. Its synthesis process can be divided into three stages, the synthesis of IPP and IMAPP, the generation of intermediates, and the synthesis of terpenoids and the final glycosylation modification[48]. During which HMGCR, FPS, SS, SE, DS, β-AS, CYP450, and UGT are the key enzymes[49]. Sucrose promotes the biosynthesis of ginsenosides by increasing the activity of HMGCR[50]. Exogenous thymol and carvacrol induced over-expression of FPS, SS, and DS genes in *Panax quinquefolius*, resulting in a significant increase of total saponin and eight saponins monomers[51,52], suggesting that exogenous inducers can promote the synthesis of ginsenosides by enhancing the activity or gene expression of key enzymes of the ginseng. ROS are able to influence –S-S– formation within protein molecules[32], which inevitably affects enzyme activity. In the main root, the greatest elevation of the five enzyme activities was observed in the 0.5 mmol/L-treated group, HMGCR and FPS peaking on day 2, and SS, SE, and DS on day 3. HMGCR, FPS, SE, and DS were elevated more in the 0.1 mmol/L group in fibrous root than in the other groups. Among them, HMGCR peaked on day 2, DS on day 3, FPS, SS, and SE on day 4, and SS showed the greatest enhancement in the 2 mmol/L group, which peaked on day 2 (Fig. 7). It indicates that the five key enzymes showed an increasing trend throughout the SNP-regulated period, thereby promoting the biosynthesis of ginsenosides. According to the glycosidic ligands, the ten components monomers were classified into five tetracyclic triterpenoid protopanaxatriol-type (PPT) ginsenosides, including Rg_1, Re, Rf, Rg_2, and Rh_1; four tetracyclic triterpenoid protopanaxatriol-type (PPD) saponins, including Rb_1, Rc, Rb_2, and Rb_3, and one pentacyclic triterpenoid oleanocardioidane-type saponin, Ro. In the main root, except for Rb_2, all the ginsenosides showed significant peaks during the stress period, with Rb_1 showing the greatest elevation of 77.7% in the main root on day 3 of 0.5 mmol/L SNP, followed by Rg_1 of 73.3% (Fig. 8A); they are all indicators of ginseng quality in national pharmacopeias. All ten individual components in the fibrous root were enhanced ($p < 0.01$) compared with day 0. Rg_1 showed the greatest enhancement of 81.1%, followed by Rc at 75.1% on day 4 of the 0.1 mmol/L treatment (Fig. 8B). Therefore, the biosynthesis and accumulation of these ginsenosides are assumed to be the result of physiological adaption and an effective strategy to protect ginseng from of oxidative stress. The ROS represent a stress signal that triggers the activation of antioxidant systems and accumulation of ginsenosides in this study. 1,3-DPG is an important intermediate product in the process of glucose glycolysis, and since the fresh roots of ginseng were used in this study, the 1,3-DPG in ginseng must be a catabolic product of sugars rather than directly from photosynthesis. PEPC is a key branching point for primary and secondary metabolism[35]; it has been proved that the biosynthesis of ginsenosides shows a significant linear correlation with glycolysis ($R^2 = 0.9562$)[50]. The present study showed that SNP markedly elevated 1,3-DPG content and PEPC activity (Fig. 10), suggesting that ginsenosides are converted from sugars rather than between saponins, thus ensuring a large simultaneous increase in multiple secondary metabolites (Fig. 9).

Studies have shown that the composition of saponins in ginseng fibrous root and ginseng main root is essentially the same, and the content of ginseng saponins in ginseng root is significantly higher than that in the main root. Therefore, research on improving the quality of ginseng fibrous root is also highly significant. Given that the ginseng fibrous root has a small diameter and that sodium nitroprusside can quickly permeate all parts of the tissue, so the main and fibrous roots were treated separately. In this study, 0.5 and 0.1 mmol/L SNP better improved the quality of ginseng in the main root and fibrous root, respectively. The optimum concentration of SNP in the main root was higher than that in the fibrous root, probably because the fibrous root has a larger surface area, making the SNPs more readily available for uptake.

Validation of pharmacodynamics

The content of active ingredients is an important index for the quality evaluation of Chinese herbs, but herbs are a complex system, and it is difficult to fully respond to the quality and therapeutic efficacy of herbs only by virtue of the content indexes of several ingredients, therefore, the quality of herbs should also be evaluated in

more depth by pharmacodynamic indexes. Ginsenosides Rb_1 and Rg_1 are effective in scavenging free radicals and increasing the activity of antioxidant enzymes such as SOD, thus showing strong anti-aging ability[53,54]. In this study, the high-quality ginseng group (Group E) showed a 26.7% ($p=0.046$) shorter escape latency, a 1.1 times ($p=0.031$) increase in the number of times of crossing the original plateau, and a 17.4% ($p=0.139$) increase in the residence time in the target area compare to the regular ginseng group (Group D), suggesting that the SNP-treated ginseng had a good effect on improving memory (Fig. 11). The activities of GSH-PX and SOD were elevated by 27.3% ($p=0.012$) and 23.4% ($p=0.007$), respectively, and the MDA content was reduced by 21.6% ($p=0.016$) (Fig. 13), suggesting that SNP-treated ginseng had a good antioxidant effect. Combined with morphological staining results (Fig. 12), SNP significantly improved ginseng herbs' quality.

Materials and methods

Plant materials

Fresh roots of 5-year-old *Panax ginseng* were collected from Ji'an County, Jilin Province, China, in September 15, 2023, identified by Prof Meng Xiangcai. Preserve the freshness with moss after collection.

Instruments

CP225D Electronic balance (Shanghai Precision instrument Co., Ltd., Shanghai, China), TGL-16LM desktop high-speed freezing centrifuge (Hunan Star Science instrument Co., Ltd., Changsha, China), QL-902 Vortex oscillator (Haimen Qilin Bell instrument Manufacturing Co., Ltd., Haimen, China), SD40 Ice making Machine (Guangzhou Guangkun Electric Appliance Manufacturing Co., Ltd., Guangzhou, China), Thermo enzyme labeling instrument (Thermo Co., Ltd., Waltham, Massachusetts, USA), JulaboTW20 digital display constant temperature water bath pot (Julabo Co., Ltd., Seelbach, Germany); DHG-9015 A blast drying oven (Shanghai-Heng Scientific instrument Co., Ltd., Shanghai, China), LC 2010 A Shimadzu High performance liquid Chromatography (Shimadzu Co., Ltd., Japan); 752 UV-vis Spectrophotometer (Shanghai Jinghua Science and Technology instrument Co., Ltd., Shanghai, China), 98-1-B Electronic temperature regulating Electric heating sleeve (Tianjin Tester instrument Co., Ltd., Tianjin, China), Eclipse Ci-L light microscope (Nikon Co., Ltd., Tokyo, Japan).

Reagents

Protein quantification (TP) kit (Nanjing Jiancheng Bioengineering, Nanjing, China, 20230420), NO kit (Nanjing Jiancheng Bioengineering, Nanjing, China, 20230325), $O_2^{\cdot-}$ free radical kit (Beijing Solarbio Science & Technology, Beijing, China, 2309002), H_2O_2 kit (Nanjing Jiancheng Bioengineering, Nanjing, China, 20230726), MDA kit (Nanjing Jiancheng Bioengineering, Nanjing, China, 20230422), NOX kit (Jiangsu jingmei Science & Technology, Yancheng, China, 202303); SOD kit (Nanjing Jiancheng Bioengineering, Nanjing, China, 20230624), CAT kit (Nanjing Jiancheng Bioengineering, Nanjing, China, 20230503), POD kit (Nanjing Jiancheng Bioengineering, Nanjing, China, 20230616); AsA kit (Beijing Boxbio, Beijing, China, 1012304011), GSH kit (Beijing Boxbio, Beijing, China,1012303305), GSSH kit (Beijing Boxbio, Beijing, China, 1012304041), GR kit ((Beijing Boxbio, Beijing, China, 1012304011), APX kit (Nanjing Jiancheng Bioengineering, Nanjing, China,20230521); HMGCR kit (Jiangsu jingmei Science & Technology, Yancheng, China, 202303), FPS kit (Jiangsu jingmei Science & Technology, Yancheng, China, 202303), SS kit (Jiangsu jingmei Science & Technology, Yancheng, China, 202303), SE kit (Jiangsu jingmei Science & Technology, Yancheng, China, 202303), DS kit (Jiangsu jingmei Science & Technology, Yancheng, China, 202303); 1,3-DPG (Jiangsu jingmei Science & Technology, Yancheng, China, 202307), PEPC kit (Jiangsu jingmei Science & Technology, Yancheng, China, 202306). SNP (Zhengzhou Paini Technology, China), HPLC grade acetonitrile (Loughborough, UK), phosphoric acid chromatograph pure (TEDIA Co., Ltd., Ohio, USA). The ginsenoside standards, Rg_1, Re and Rb_1 were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), ginsenoside standards, Rf, Rg_2, Rh_1, Rc, Ro, Rb_2, Rb_3, were purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China), the purity is more than 98%. D101 macroporous adsorption resin (Tianjin Huida Chemical Co., Ltd., China), vanillin (Tianjin Guangfu Fine Chemical Research Institute, China), perchloric acid (Tianjin Fuyu Fine Chemical Co., Ltd., China), glacial acetic acid (Tianjin Tianli Chemical Reagent Co., Ltd., China), D-galactose (Beijing Biotopped Co., Ltd., China), Donepezil (Zhejiang Huahai Pharmaceutical Co., Ltd., China).

Methods

Treatments

A total of 156 fresh ginseng roots with similar diameter were rinsed with distilled water. Subsequently, they were randomly and equally divided into four groups. Based on the pre-experimental results, 0.1 mmol/L SNP, 0.5 mmol/L SNP and 2.0 mmol/L SNP were selected as treated concentrations. The SNP groups were sprayed the solution evenly on the surface until saturated, and rehydrated once a day for 5 days. The ctrl group was covered with moss to preserve the freshness, the humidity was consistent with the SNP groups. All four ginseng groups were placed in an Artificial Intelligence (AI) incubator at 15 °C/10 °C. Samples were collected at 0, 1, 2, 3, 4, and 5 days, and each sample was collected from several plants ($n \geq 6$). The main roots phloem and fibrous roots were cut into separate pieces of 0.30 g × 30 samples, each for the determination of the activities of various enzymes, including NOX, SOD, CAT, POD, HMGCR, FPS, SS, SE, DS, PEPC and the contents of NO and 1,3-DPG. 0.1 g × 30 samples were taken to determine the APX and GR activities as well as the contents of $O_2^{\cdot-}$, H_2O_2, MDA, AsA, GSH, and GSSG. All samples were sealed with aluminum foil and stored at − 80 °C. Several strains of the main roots and fibrous roots were dried in an oven at 55 °C, pulverized with an ultrafine pulverizer, and sieved through a 60-mesh sieve for the determination of Rg_1, Re, Rf, Rg_2, Rh_1, Rb_1, Rc, Ro, Rb_2 and Rb_3. All samples were repeated three times for each index.

Determination of indexes relating to ROS and antioxidant system

$O_2^{\cdot-}$, NO and H_2O_2 content

The content of homogenate protein and H_2O_2 were determined as described in the instruction manual of the kits. Sample (0.3 g) was ground with 6 ml of NaCl solution and centrifuged to take the supernatant. After mixing with the reaction reagents, the absorbance was measured at 595 nm and 405 nm and expressed in g/L and mmol/g prot, respectively. The $O_2^{\cdot-}$ content was measured as described in the instruction manual of the kit. Sample (0.1 g) was ground with 3 ml of extraction solution and centrifuged to take the supernatant. After mixing with the reaction reagents, the absorbance was measured at 530 nm and expressed in μmol/g. The contents of NO was determined as described in the instruction manual of the NO kit. Sample (0.3 g) was ground with 6 ml of phosphate buffer (pH 7.4) and centrifuged to take the supernatant. After mixing with the reaction reagents, the absorbance was measured at 550 nm and expressed in nmol/g.

MDA content

The MDA contents were measured as described in the kit instruction manual. Sample (0.1 g) was ground with 3 ml of extraction solution and centrifuged to take the supernatant. After mixing with the reaction reagents, the absorbance was measured at 530 nm and expressed in nmol/g.

Antioxidant enzyme activity

The activity of NOX was determined as described in the kit instruction manual. Sample (0.3 g) was ground with 6 ml of phosphate buffer (pH 7.2) and centrifuged to take the supernatant. After mixing with the reaction reagents, the absorbance was measured at 450 nm and expressed in mg/g prot. The activity of SOD was determined as described in the kit instruction mannul. Sample (0.3 g) was ground with 6 ml of phosphate buffer (pH 7.4) and centrifuged to take the supernatant. After mixing with the reaction reagents, the absorbance was measured at 450 nm and expressed in U/mg prot. The content of CAT and POD were determined as described in the instruction manual of the kits. Sample (0.3 g) was ground with 6 ml of NaCl solution and centrifuged to take the supernatant. After mixing with the reaction reagents, the absorbance was measured at 405 nm and 420 nm and both expressed in U/mg prot.

AsA-GSH cycle

The AsA, GSH, and GSSG contents and APX activity were measured as described in the instruction manual of the kits. Sample (0.1 g) was ground with 3 ml of their respective extraction solution and centrifuged to take the supernatant. After mixing with the reaction reagents, the absorbance were measured at 265 nm, 412 nm, 412 nm, 290 nm and expressed as μmol/g, μg/g, μg/g and U/mg prot, respectively. The GR activity was determined as describe in the GR kit protocol. Sample (0.1 g) was ground with 0.9 ml of NaCl solution and centrifuged to take the supernatant. After mixing with the reaction reagents, the absorbance was measured at 340 nm and expressed in U/mg prot.

Secondary metabolism-related enzyme activity

The activities of HMGCR, FPS, SS, SE, and DS were determined as described in the kit instruction manual. Sample (0.3 g) was ground with 6 ml of phosphate buffer (pH 7.4) and centrifuged to take the supernatant. After mixing with the reaction reagents, the absorbance was measured at 450 nm and expressed in mg/g prot.

Ginsenosides contents

Monomer ginsenosides

The contents of ginsenosides Rg_1, Re, Rf, Rg_2, Rh_1, Rb_1, Rc, Ro, Rb_2 and Rb_3 were determined by HPLC. Ginseng main root and fibrous root powder 1.0 g were accurately weighed, placed in a 50 ml cork triangle bottle, ultrasonic extraction with methanol at 100 Hz for 40 min, and filtered by 0.22 μm filter for HPLC analysis. Each sample was repeated three times. Rg_1, Re, Rf, Rg_2, Rh_1, Rb_1, Rc, Ro, Rb_2, and Rb_3 standards were weighed precisely, then placed in a 5 ml centrifuge tube, dissolved with methanol and fixed volume to prepare a ginsenoside mixed standard solution.

The prepared samples were analyzed using a Shimadzu LC-2010 A HPLC system with a C18 column (250 mm × 4.6 mm, ID 5 μm). The column temperature was 30 ℃, the flow rate was 1.0 mL/min, the injection volume was 10 μL, and UV measurements were obtained at 203 nm. The mobile phase was composed of A (acetonitrile) and B (0.1% phosphoric acid); the gradient elution was performed as follows: 0–20 min, 18–20% A; 20–60 min, 20–24% A; 60–80 min, 24–30% A; 80–110 min, 30–34% A.

Total ginsenosides

The total ginsenosides were determined by using the vanillin-glacial acetic acid-perchloric acid colorimetric method reported by Kubo et al.[55]. The standard curve was plotted with ginsenoside Re as standard, absorbance (Y) as the vertical coordinate, and solubility (X) as the horizontal coordinate, and the regression equation was obtained as $Y = 0.0043X + 0.0247$ ($R^2 = 0.999$), which showed an excellent linear relationship within the range of 20–160 μg.

Validation for quantitation

The linear relationship of 10 active components, the precision of the instrument, the stability of the solution within 24 h, the repeatability of the method, and the recovery rate were investigated, respectively.

Determination of primary metabolite content and photosynthetic enzyme activity
1,3-DPG content and PEPC activity were determined as described in the instruction manual of the kits. Sample (0.3 g) was ground with 6 ml of phosphate buffer (pH 7.4) and centrifuged to take the supernatant. After mixing with the reaction reagents, the absorbance was measured at 450 nm and expressed in nmol/L and mg/g prot, respectively.

Pharmacodynamic verification

Drug preparation
The ctrl group (main root on the 0 day) and the 0.5 mmol/L SNP (main root on day 3) were dried to a constant level and crushed into a coarse powder. Took the appropriate amount of ginseng, added 10 times the amount of water, soaked for 12 h, decocted for 1 h, filtered, and repeated the extraction three times; the filtrate was combined and concentrated to contain 0.039 g of raw herb per ml for spare.

Animal experiments
The 8-week-old male Kunming mice (weighing 18–24 g), provided by Liaoning Changsheng Biotechnology Co., Ltd with Certificate of Quality No. SCXK (Liao)-2020-0001 (Benxi, China) were raised in a ventilated and dry environment, with a temperature of 20–23 °C, a relative humidity of 50–60%, and a 12 h light/dark cycle. They had free access to food and water.

After suitable feeding for 7 days, the mice were randomly divided into 6 groups ($n = 10$). The blank ctrl group (A): administrated sterile saline and injected with saline. The model group (B): D-galactose (120 mg/kg/day) was subcutaneously injected with a volume of 0.01 ml/g, qd×42. The positive ctrl group (C): from the 16th day of the establishment of the D-galactose model, the mice were administrated with donepezil (1.3 mg/kg/day) qd×27). The regular ginseng group (D): from the 16th day of the establishment of the D-galactose model, the mice were administrated ginseng decoction (0.39 g/kg/day) qd×27 of the 0-day untreated main root group. The high-quality ginseng (E): from the 16th day of the establishment of the D-galactose model, the mice were administrated ginseng decoction (0.39 g/kg/d) qd×27 of the 3rd day treated with 0.5 mmol/L SNP main root.

Morris water maze
The spatial learning and memory abilities of mice were evaluated by Morris Water Maze. Referring to the method described in the literature by Li et al.[56], the mice were trained to find a circular platform, 10 cm in diameter and 1 cm under cloudy water, located in the middle of any quadrant. The mice in each group were placed into the pool from four fixed points along the pool wall in a random order, and the time and movement track of each mice in searching for the platform within 120s were recorded, and the mice were guided to return to the platform for 10s, for a total of 5 days of training. A single 120 s probe trial was performed without the platform on the 6 day, and the following were recorded to evaluate reference memory: escape latency, frequency of crossing platform, and the percentage of time in the target quadrant. All data were recorded by a digital camera automatically.

Histopathological staining analysis

Histopathological staining was employed to evaluate the pathological changes of brain tissues. After the water maze experiment, mice were anesthetized by intraperitoneal injection of urethane (0.008 ml/g). Brain tissues were perfused with 4% paraformaldehyde and placed for 24 h, rinsed with a slow stream of water for 12 h, dehydrated with gradient ethanol and xylene, then soaked in melted wax and embedded, sliced 5–8 μm. Sections were stained with HE reagent, and the lesions in the hippocampal region of mice were observed with a light microscope.

Analysis of oxidative stress indexes in brain tissues

Took the whole brain of each group of mice after mice dying, put it in pre-cooled saline and washed quickly, absorbed the residual liquid with filter paper, weighed it precisely, prepared 10% brain tissue homogenate, centrifuged it at 3500 rpm for 10 min, collected the supernatant, and then determined the SOD, glutathione peroxidase (GSH-PX) activities and MDA content according to the instructions of the kits, expressed in U/mg, U/mg, and nmol/mg, respectively.

Statistical analysis

Microsoft Office Excel 2007 (Microsoft Corp., Redmond, WA, United States) was used to sort the original data. Origin 2021 (OriginLab, Northampton, MA, United States) was used for graphic illustration. The whole data was expressed in mean ± standard deviation (Mean ± S.D.), and IBM SPSS 27.0 (IBM Corp., Armonk, NY, United States) was used for the independent samples t-test. All results are the mean of each experiment with three replicates except those stated otherwise. The statistically significant differences were thought to be $p < 0.05$ or $p < 0.01$.

Ethics declarations

Ethics approval to conduct animal experiments were conducted in accordance with the guidelines of the National Institutes of Health (NIH guidelines), ARRIVE guidelines and approved by the Animals Laboratory Ethical Committee of Heilongjiang University of Chinese Medicine (Approval Number: SYXK2023-122913).

Conclusions

SNP induced oxidative stress in ginseng fresh roots and stimulated the antioxidant defense system; antioxidant enzyme activities and secondary metabolite contents improved significantly, and they worked together to reduce ROS damage. In the main root, the 0.5 mmol/L SNP significantly increased the content of saponins, $Rg_1 + Re$,

Rb_1, Rf, Rc, and $Rg_2 + Rh_1$ increased by 51.0%, 77.7%, 44.6%, 26.8%, and 63.2%, respectively, and the total saponin increased by 48.2%. In the fibrous root, the 0.1 mmol/L SNP was the best, $Rg_1 + Re$, Rb_1, Ro, Rc, Rf, Rb_3, and Rb_2 increased by 37.6%,47.8%, 34.2%, 75.1%, 51.0%, 49.4%, and 28.3%, respectively, and the total saponins increased by 20.4%. Pharmacodynamics also demonstrated that SNP-treated ginseng was of better quality. SNP can significantly improve the ginseng quality.

Data availability

The data that support the findings of this study are available from the corresponding author upon request.

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Author contributions

W.Z. was responsible for data acquisition, data analysis, figure/table preparation and wrote the main manuscript text. P.C.Y. and W.F.L. carried out the statistical analysis and interpretation of data. L. Y.W., X.W.S. and Y.Y. contributed to the study design, provided technical support, collected and organized data and provided new ways to solve problems. X.B.L. developed practical measures to fix the core issues, controlled the language of the articles, and corrected typos. X.C.M. participated in the conceptualization and design of the experiment, interpretation of data, and revision and approval of the final version of the manuscript. All authors reviewed and agreed to the published version of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

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