into quinones by excess ROS.[36] When highly expressed, UBGAT can convert excess baicalein and wogonin into low-activity baicalin and wogonoside [Figure 5]. However, the observed production of UBGAT was not sufficient to compensate for its consumption; consequently, the content of baicalin and wogonoside decreased from 1.50 to 1.17 mmol/g and from 0.63 to 0.61 mmol/g, respectively. Under ROS stress, reduced GUS expression promotes the accumulation of highly active aglycones although the synthesis of aglycones, such as baicalein, occurs earlier than the synthesis of glycosides. At this point, the aglycone content is already high, and the transformation of aglycones via GUS is unnecessary. Therefore, reducing GUS expression under stress is a favorable mechanism [Figure 5]. In normal cells, flavonoids are stored in the form of glycosides.[37] In response to ROS stress, flavonoid glycosides are rapidly converted into aglycones,[38] and this conversion may represent a stress-response strategy in *S. baicalensis*.

At 0.04 mmol/L, $Na_2S_2O_4$ clearly increased GUS activity on day 3, which is consistent with the change in flavonoids after 3–5 days [Figure 6]. However, the slightly increased PAL expression in response to 0.0004 and 0.04 mmol/L $Na_2S_2O_4$ and greatly enhanced GUS expression in response to 0.04 mmol/L $Na_2S_2O_4$ were not significantly positively correlated with baicalein, baicalin, wogonin, or wogonoside, which may be explained by multiple factors. Transcription, translation, and postmodification delay the increase of flavonoids, and flavonoid contents are continuously reduced by ROS. However, the most important reason is that the sudden artificial increase in ROS disrupts the natural metabolism of *S. baicalensis* and causes an unnatural status that is difficult to quickly restore. Overall, $Na_2S_2O_4$ enhances the biosynthesis of flavonoids, and the flavonoid content reached a peak at day 3. Although baicalin and wogonoside decreased from 6.28% to 5.21% and 3.35% to 2.83%, respectively, baicalein and wogonin increased from 0.28% to 1.96% and 0.14% to 1.24%, respectively. The increased flavonoid contents broke down the redundant ROS, which led to the lowest H_2O_2 levels [Figure 3].

Polyphenol oxidase maintains the biosynthesis of flavonoids

Many studies have shown that the induction of phenolic secondary metabolites reflects a plant response to environmental stress.[18,39] PPO activity was minimally altered after treatment with $Na_2S_2O_4$ for 3 days. A dramatic change occurred after 5 days, which was later than the changes in antioxidant enzymes and flavonoids. Different doses of $Na_2S_2O_4$ had varying effects on PPO activity. The antioxidant capacity of enzymes was replaced by flavonoids in the fresh roots treated with $Na_2S_2O_4$. The sudden addition of $Na_2S_2O_4$, even at low doses of 0.0004 or 0.04 mmol/L, induced excess flavonoids, and the PPO activity increased by 60%–80% to scavenge redundant flavonoids and prevent further reductions of ROS. The high dose of $Na_2S_2O_4$ (4.0 mmol/L) generated large amounts of ROS, and many flavonoids were required to maintain this balance. As a result, PPO activity was reduced by 60% [Figure 3].

CONCLUSION

ROS are produced in plants under stress. $Na_2S_2O_4$ can act as an unfavorable factor that generates $O_2^{\bullet-}$, which is subsequently converted into H_2O_2, $^{\bullet}OH$, and other ROS. Based on this theory, organisms treated with $Na_2S_2O_4$ can be used as models for studying environmental stress. These organisms can produce secondary metabolites that are the same as or similar to those generated under natural conditions. $Na_2S_2O_4$ has strong reducing abilities and transforms into nontoxic $NaHSO_4$; thus, $Na_2S_2O_4$ is widely applied as a tap water dechlorinating agent, a beer bottle disinfectant, a bleaching agent, and a food leavening agent. In recent years, the role of antioxidases under ecological stress has been emphasized, whereas the roles of nonenzymatic components have been undervalued. Reports on the dominant roles of flavonoids in response to ecologic stresses are rare. In the present study, we revealed that secondary metabolites play far greater role, while the antioxidase declines, in response to $Na_2S_2O_4$ stimuli. $Na_2S_2O_4$ significantly enhances the quality of *Radix Scutellariae*.

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Conflicts of interest

There are no conflicts of interest.

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