Determination and evaluation of acteoside content of *Scrophularia striata* Boiss. under Lead stress

Reyhaneh Danaeipour[1], Mohsen Sharifi[1*]

[1]Department of Plant Biology, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran, R.danaeipour@ modares.ac.ir, Msharifi@modares.ac.ir.

Introduction

Global industrialization and anthropogenic sources increase the release of heavy metals into the environment. lead (Pb) is known as the second most toxic metal among heavy metals [1–3]. Pb absorption through plants is one of the most well-known paths, which enters into the food chain of various organisms such as humans, and finally threatens their health [4,5].

Ilam province, located in the west of Iran, is an area exposed to various metal mines and refineries, and the human health risk caused by heavy metals is increasing [6]. Ilam is a city rich in medicinal plants, and native inhabitants have traditionally used these plants to treat various diseases for many years. Heavy metal pollution, especially Pb, in the region and its absorption by plants can greatly hazard biosystems and human society. Among the plants of this region, which are consumed abundantly, we can mention *Scrophularia striata* Boiss. species from the Scrophulariaceae family with the local name of Teshnehdari. *S. striata* has been prepared in different forms, including infusion and compress, to treat several diseases such as pain and digestive disorders, colds, and infectious wounds. For preparing tea, aerial parts of Teshnedari plants have often been infused in boiling water and used for therapeutic purposes [7–9]. Because of its beneficial properties, Teshnehdari is sent to other areas of Iran. The *S. striata* plant was reported that has a remarkable bio-accumulation of Pb [10,11]. This herb is also known for its phenolic compounds, especially phenylethanoid glycosides, and its medicinal and therapeutic properties refer to these compounds [12,13].

In addition to Pb having destructive effects on human health, it can also have undesirable effects on plants, including the effect on the growth and metabolism, and force the plants to respond to this pollution [1,14,15]. In response to Pb toxicity, plants change metabolite biosynthesis pathways, and this causes a loss of plant energy. The studies demonstrated that the biosynthetic pathway of phenylpropanoid compounds is an important pathway that changes in different stresses such as lead pollution [16]. This change can move towards the production of other medicinal compounds. However, this plant grows in habitats exposed to lead pollution, but there is not adequate information about its metabolic pathways under Pb pollution and stress on this plant. Considering the mentioned cases, we decided to investigate the biosynthetic pathway of phenolic compounds in response to Pb stress in the *S. striata* plant.

Materials and Methods

Growth condition

The Teshnehdari seeds were collected from northern Ilam Province, Iran. the mature seeds were rinsed with water for 48h, then disinfected with 2% sodium hypochlorite, and then immersed in gibberellin (500 ppm, 24h). The seeds were washed and moved in perlite. After 37 days, the plants were moved on hydroponic and grown for 32 days. The growth chamber conditions were 16h light photoperiod (220 µmol photons m^{-2} s^{-1} light irradiance), 25ºC temperature, 70% air humidity. The plants were prepared to treat with adding 250 ppm Pb $(NO_3)_2$ in a fresh half-strength Hoagland's nutrient solution (PH 5.8) for three collecting times (24, 48 and 72 hours, respectively). The shoots were gathered and prepared for future analysis.

Metabolite extraction and quantitative analysis of phenolic compounds

0.1 g of dried shoots were extracted within 4 mL methanol. after evaporation of solvent, the sediment was dissolved within acetonitrile, and extracted with n-hexane, the acetonitrile solvent dried. The sediment was dissolved in methanol (0.5 ml). The extracts were analyzed by HPLC (Agilent Technologies 1260 Infinity, USA) with a C18 column (Perfectsil Target ODS-3 (5 µm), 250×4.6 mm; MZ Analysentechnik, Mainz, Germany). The mobile phases consisted solvent A: 2% acetic acid in water and solvent B: methanol. The gradient program was used by Zafari et al (2016) method. Phenolic acids were detected at the 278-300 nm wavelength range by DAD detector (Agilent Technologies 1260 infinity, USA).

Statistical analysis

Data, performed in three independent repeats, were analyzed by using SPSS-16 software. A one-way ANOVA test was obtained to represent significant differences between groups ($P < 0.05$), then measured using the Duncan test. The correlation coefficient was accomplished using R software v.3.6.1 (http://www.r-project.org).

Results and Discussion

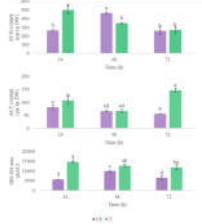

Figure 2. Effect of Pb stress on phenylethanoid glycosides compounds in *Scrophularia striata*. X+Y: Scroside D and scrophuloside B.

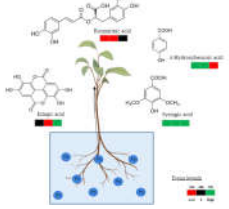

Figure 2. Pb element modulates phenylpropanoid compounds in *Scrophularia striata*. and changes the biosynthetic pathway of them.

Results and Discussion

S. striata was introduced as a phenylethanoid glycosides (PhGs) source. *S. striata* functions as a resistant plant under various stresses. The essential mechanism in response to abiotic and biotic stress is the production of phenolic compound precursors, which eventually leads to PhG compound accumulation, especially acteoside, in this plant. Acteoside operates as a powerful antioxidant that scavenges excess ROS content in biological systems. The study purposed to evaluate the acteoside content in response to Pb stress of *S. striata*. HPLC analysis was employed to identify PhGs between the untreated and Pb-treated shoots plants with three gathering times (24, 48, and 72 hours). Our results indicated that acteoside increased significantly after 72h under Pb stress, and no significant difference was observed in other time courses. In general, *S. striata* is a good source of PhGs, especially acteoside, with application in the pharmaceutical industry.

Pb element modulates phenylpropanoid compounds in *Scrophularia striata*. and changes the biosynthetic pathway of them.

HPLC analysis of four phenolic compounds, including syringic, ellagic, 4-hydroxybenzoic, and rosmarinic acid, were examined between two groups, untreated (CK) and Pb-treated shoots samples. Syringic acid, ellagic acid, and 4-hydroxybenzoic acid are located among benzoic acid derivatives, and Rosmarinic acid is a common cinnamic acid derivative. As shown in Figure 1, syringic acid meaningfully increased in all Pb-treated plants against CK samples. At 72h after Pb treatment, ellagic and 4-hydroxybenzoic acid showed increase and decrease, respectively. Rosmarinic acid was approximately 4 mg g^{-1} DW in the control samples while significantly decreased in response to Pb stress.

Rosmarinic acid (RA) is known as the indicator of the Lamiaceae family that has been reported with different contents. This compound was analyzed in *Scrophularia* species by a few researches. On the base of our research, RA amounts were remarkably demonstrated and were similar to some of the Lamiaceae family species. The behavior of RA affected by heavy metal stress was differently illustrated by different plant species. In this study, our results showed that RA was decreased in *S. striata* under Pb stress and a strongly negative correlation with the syringic acid. Therefore, it can be stated that the defensive response changes of the plant are not in favor of the biosynthetic pathway of rosmarinic acid in the Pb stress and the biosynthesis pathway shifted toward the syringic acid biosynthesis pathway.

The dimeric derivative of gallic acid, or in other words ellagic acid, is abundantly found in different types of berries and pomegranate. In these plants, gallic and ellagic acid increased exposure to various stresses. Likewise, the *Scrophularia* genus showed not only remarkable amounts but was also comparable to the dominant plants. However, few studies have been shown on the content of ellagic acid in this genus. The amount of ellagic acid of this species has been observed in lower amounts than other species of this genus. Considering that the behavior of ellagic acid in three time periods is not known, it is better to investigate its contents in the more time course under Pb treatment.

Conclusions

The phenylpropanoid biosynthesis pathways shift toward modulation of their compounds, resulting in the change of their profiles in stresses. Overall, the observations suggest that aerial parts of the *S. striata* (Teshnehdari) Due to notably amounts of rosmarinic acid can be used in the food and pharmaceutical industries.

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