

Effects of Chelidonine on Superoxide Dismutase and Catalase Activity in *Pieris rapae* L

Feng Wang*

Department of Biological and Pharmaceutical Engineering, Jilin Agricultural Science and Technology College,
Jilin 132101, China

86710849@qq.com

*The corresponding author

Keywords: *Chelidonium Majus* L; Single Alkaloid; Sod; Cat; Activation Rate

Abstract: The experiment used chelidonine, berberine, coptisine and sanguinarine as materials, studied the effects of different concentrations of single alkaloid on the activity of superoxide dismutase (SOD) and catalase (CAT) in *Pieris rapae* L. The results showed that the concentrations (2 mg/L) of chelidonine, berberine and sanguinarine had the high activation rate. The activation rates of superoxide dismutase were 181.82%, 109.52% and 789.4% respectively. The activation rates of catalase were 225.54%, 173.54% and 105.9% respectively. The highest activation rate of superoxide dismutase of 1 mg/L coptisine was 70.73%, and 2 mg/L coptisine was -19.11%. The highest activation rate of catalase was 109.91%, and 2 mg/L was -9.33%. These results indicated that high concentration of coptisine had inhibition effect on SOD and CAT.

1. Introduction

Chelidonium majus L. is a genus of Helminthaceae in the family Papaveraceae. It is commonly known as turmeric, and it is a perennial herb. Mainly distributed in Jilin, Liaoning, Heilongjiang and other provinces, the whole plant is toxic and bitter [1-3]. The biologically active components are mainly alkaloids, including berberine, coptisine, chelidonine and sanguinarine. Pharmacological studies have shown that roots, stems, leaves and flowers have antibacterial and insecticidal effects [4-6]. Superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) are important protective enzymes for insects to protect against oxidative damage. Protective enzymes are involved in the metabolism of insecticides in insects. When insects are disturbed by pesticides, their protective enzymes will change [7-9]. In recent years, there have been many reports on the extraction and biological activity of total alkaloids from celandine, and their alkaloids have certain insecticidal effects on adult insects and larvae of different purposes such as cabbage caterpillar, gypsy moth, fly, and cabbage butterfly [10-13]. However, studies on the effects of chelidonine, berberine, coptisine and sanguinarine on the protective enzymes superoxide dismutase (SOD) and catalase (CAT) of *Pieris rapae* L. have not been reported. In this experiment, the main alkaloids of chelidonine, berberine, coptisine and sanguinarine, were used as experimental materials to study the effects of different concentrations on the protective enzymes superoxide dismutase and catalase of *Pieris rapae* L. To explore the insecticidal mechanism of the main alkaloids of celandine, and to provide reference for the development of celandine plant-derived pesticides.

2. Material

The 4th *Pieris rapae* L. are provided by the Institute of Plant Protection of the Chinese Academy of Agricultural Sciences and are in the artificial climate incubator of our school. Chelidonine (CAS: 476-32-4), berberine (CAS: 2086-83-1), coptisine (CAS: 3486-66-6), and sanguinarine (CAS: 5576-73-4) provided by Jilin Province Wuhua Drug Distribution Office.

3. Method

Preparation of alkaloid solution. Different concentrations of gradients (0.1mg/L, 0.5mg/L, 1mg/L, 2mg/L) were prepared by using deionized water to partition chelidonine, berberine, coptisine and sanguinarine.

The method of test insect feeding refers to Tan Shiqiang's method [14]. The healthy 4th *Pieris rapae* L. were selected and placed in a petri dish and starved for 6 hours. The fresh cabbage leaves are soaked in a solution of different concentrations of chelidonine, berberine, coptisine and sanguinarine for 5 s. After the surface water is volatilized, it is placed in a petri dish lined with wet filter paper. Two cabbage leaves were placed in each dish, and 30 4th *Pieris rapae* L. were placed in a petri dish which after starvation, fed at room temperature, repeated 3 times, and treated with distilled water as a control. After 48 hours, 10 specimens of similar size were transferred into a pre-cooled glass homogenizer, and then 1 mL of 0.1 mol/L pH 7.4 PBS was added to the homogenate in an ice bath with a micropipette, and the volume was made up to 2 mL. The homogenate was transferred to a centrifuge tube, then placed at 4°C, centrifuged at 10,000 rpm for 20 min, and the supernatant was centrifuged for use.

Protein content determination is based on Bradford's Coomassie Brilliant Blue method [15].

Superoxide dismutase (SOD) and catalase (CAT) enzyme activity and activation rate were determined by the method of Cha Lichun, activation rate = (treatment enzyme specific activity - control enzyme specific activity) / control enzyme specific activity × 100% [16].

4. Test Results

Effects of different concentrations of chelidonine on SOD activity. It can be seen from Fig. 1 that the activity of superoxide dismutase in the body is higher than that in the control group after treatment with different concentrations of chelidonine for 48h, and the activity of superoxide dismutase is increased with the increase of the concentration of chelidonine. When the concentration of chelidonine reached 2 mg/L, the superoxide dismutase activity reached 31.25 U/mg, which was significantly different from the control and other concentrations. The treatment of chelidonine activates superoxide dismutase, the activation rates of superoxide dismutase treated with 0.1mg/L, 0.5mg/L, 1mg/L, 2 mg/L were 9.11%, 36.36%, 54.54%, and 181.82%, respectively.

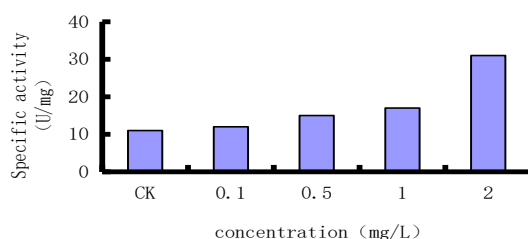


Fig. 1 Effect of chelidonine on superoxide dismutase activity

Effects of different concentrations of berberine on SOD activity. It can be seen from Fig. 2 that the activity of superoxide dismutase in the body of the *Pieris rapae* L. after treatment with different concentrations of berberine for 48h was higher than that of the control group, and the activity of superoxide dismutase increased significantly with the increase of berberine concentration. The difference of 0.1mg/L and 0.5mg/L treatment was not significant, and the activity of superoxide dismutase in 1mg/L and 2mg/L treatment was significantly higher than that in low concentration treatment. The activation rates of superoxide dismutase by different concentrations of berberine were 12.09%, 18.17%, 53.21%, and 109.52%, respectively. The high concentration of 2mg/L was significantly different from the control and other treatments.

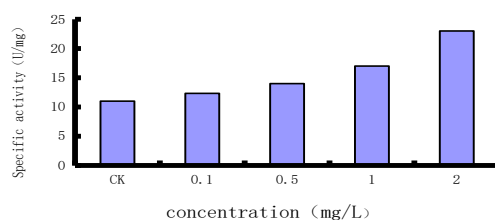


Fig. 2 Effect of berberine on superoxide dismutase activity

Effects of different concentrations of coptisine on SOD activity. It can be seen from Fig. 3 that the activity of superoxide dismutase in the body of the *Pieris rapae* L. after treatment with different concentrations of coptisine for 48 h showed a significant increase with the increase of the concentration of coptisine. The activity of superoxide dismutase was highest at the concentration of 1 mg/L. 2mg/L treatment of superoxide dismutase enzyme activity is the lowest. The activation rates of different concentrations of coptisine treatment were 14.27%, 38.26%, 70.73%, and -19.11%, respectively. The treatment activation rate of 1 mg/L was significantly different from other treatments and controls. The 2 mg/L treatment inhibited the activity of superoxide dismutase.

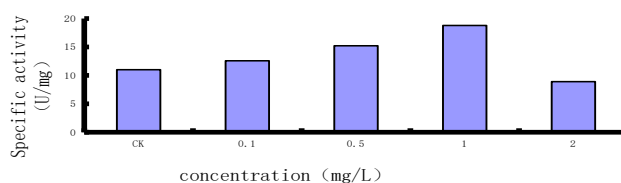


Fig. 3 Effect of coptisine on superoxide dismutase activity

Effects of different concentrations of sanguinarine on SOD activity. It can be seen from Fig. 4 that the activity of superoxide dismutase in the body of the *Pieris rapae* L. after 48 h treatment with different concentrations of sanguinarine was higher than that of the control group, and the activity of superoxide dismutase increased significantly with the increase of sanguinarine concentration. The difference between 0.1 mg/L treatment and control was not significant. The activities of superoxide dismutase (SOD) treatment at 0.5 mg/L, 1 mg/L and 2 mg/L were significantly higher than those at low concentration. The activation rates of superoxide dismutase were 8.72%, 33.81%, 35.52%, and 89.47%, respectively, after treatment with different concentrations of sanguinarine. The difference between 2 mg/L treatment and control and other treatments was extremely significant.

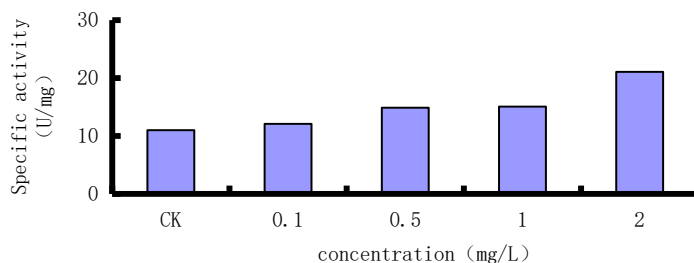


Fig. 4 Effect of sanguinarine on the activity of superoxide dismutase

Effects of different concentrations of chelidonine on CAT activity. It can be seen from Fig. 5 that after treatment with different concentrations of chelidonine for 48h, the activity of catalase in the body was higher than that in the control group, and the activity of catalase increased significantly with the increase of the concentration of chelidonine. When the concentration of chelidonine reached 2 mg/L, the catalase activity reached 38.89 U/mg, which was significantly different from the control and other concentrations. The treatment of chelidonine activated catalase, and the activation rates of

catalase at 0.1 mg/L, 0.5 mg/L, 1 mg/L, and 2 mg/L were 22.27%, 56.91%, 75.45% and 225.54% respectively.

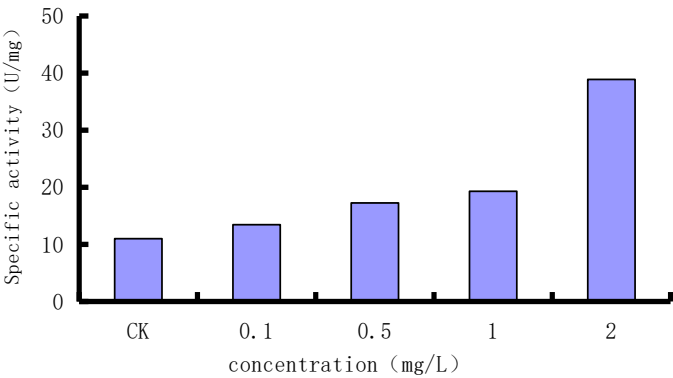


Fig. 5 Effect of chelidonine on catalase activity

Effects of different concentrations of berberine on CAT activity.It can be seen from Fig. 6 that the activity of catalase in the body was higher than that in the control group after treatment with different concentrations of berberine for 48h, and the activity of catalase increased significantly with the increase of berberine concentration.The difference of 0.1mg/L and 0.5mg/L treatment was not significant, and the 2mg/L treatment catalase enzyme activity was significantly higher than the low concentration treatment. The catalase activation rates were 42.45%, 66.72%, 85.31%, and 173.54% after treatment with different concentrations of berberine, and the high concentration of 2 mg/L was significantly different from the control and other treatments.

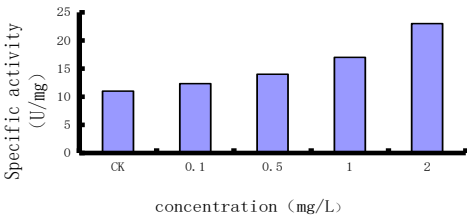


Fig. 6 Effect of berberine on catalase activity

Effects of different concentrations of coptisine on CAT activity.It can be seen from Fig. 7 that after treatment with different concentrations of coptisine for 48h, the activity of catalase in the body increased with the increase of the concentration of coptisine. The activity of catalase was the highest when the concentration was 1mg/L, 2mg. /L treatment of catalase enzyme activity is the lowest.The activation rates of different concentrations of coptisine treatment were 30.41%, 76.64%, 109.91%, and -9.33%, respectively. The treatment activation rate of 1 mg/L was significantly different from other treatments and controls. The 2 mg/L treatment inhibited the activity of catalase.

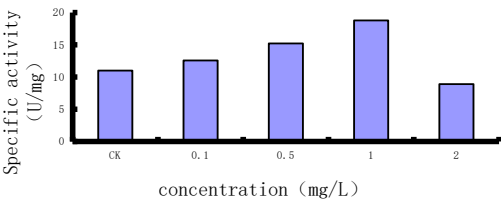


Fig. 7 Effect of coptisine on catalase activity

Effect of different concentrations of sanguinarine on CAT activity. It can be seen from Fig.8 that the catalase activity of the *Pieris rapae* L. after treatment with different concentrations of sanguinarine for 48h was higher than that of the control group, and the activity of catalase increased significantly with the increase of sanguinarine concentration. The difference between 0.1 mg/L treatment and control was not significant. The activities of catalase enzymes at 0.5 mg/L, 1 mg/L and 2 mg/L were significantly higher than those at low concentrations. The catalase activation rates were 32.18%, 47.36%, 76.25%, and 105.9%, respectively, after treatment with different concentrations of sanguinarine. The difference between 2 mg/L treatment are significantly different from control and other treatments.

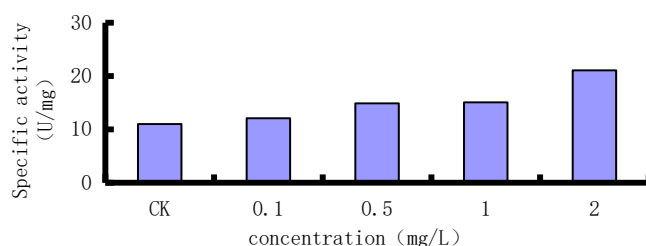


Fig. 8 Effect of sanguinarine on catalase activity

5. Summary

The activation rates of superoxide dismutase treated with chelidonine, berberine and sanguinarine 2 mg/L were 181.82%, 109.52% and 89.47%, respectively. The catalase activation rates were 225.54%, 173.54% and 105.9%, respectively. The activation rate of superoxide dismutase treated with coptisine 1mg/L was 70.73%, while the activation rate of 2mg/L treatment was -19.11%, the highest rate of catalase activation was 109.91%, and the activation rate of 2mg/L treatment was -9.33%. It indicated that high concentration of coptisine inhibited the activities of superoxide dismutase and catalase. The main function of insect protective enzymes is to remove excess oxygen free radicals in insects, and to maintain the generation and elimination of free radicals in cells in a dynamic low level balance to prevent free radicals from being poisoned. Once this balance is broken, it can cause harm. Superoxide dismutase is an important substance for scavenging free radicals in insects. When insects are disturbed by pesticides, drought, low temperature and other external factors, they will increase their enzyme activity and protect themselves. When it encounters superoxide anion radicals, hydroxyl radicals, and carbon tetrachloride, it promotes the decomposition of superoxide radicals, turns it into water molecules and oxygen molecules that are harmless to itself, eliminates lipid peroxidation of tissue cells, and maintains the body. The balance of free radicals improves the immunity of the body.

Acknowledgements

In this paper, the research was sponsored by the 13th Five-Year Scientific and Technological Research Fund of Jilin Provincial Education Department (Project No. JJKH20170342KJ).

References

- [1] X.Y. Yao. Study on the chemical constituents of celandine root and the antibacterial activity of various plants[D]. Qiqihar: Qiqihar University, 2012.47-48.
- [2] X. Zou and so on. Research progress in pharmacological effects of chelerythrine[J]. Drugs & Clinic, 2014, 29(11): 1326-1330.
- [3] P.Q. Wang and so on. Research progress in pharmacological activity of chelerythrine[J]. Chinese Journal of Traditional Chinese Medicine, 2013, 38 (17): 2745-2749.

- [4] W.S. Kang and so on. In vitro antibacterial activity of hematoxylin and chelerythrine ion on compounds[J].Central South Pharmacy,2014,12(5):406-410.
- [5] G.J. Han and so on. Study on the Effect of Quercus sinensis, Journal of Baicheng Normal University[J].2012,26(3):14-18.
- [6] Y.P. He. Anti-lung cancer activity of chelerythrine and its related mechanisms[J].Chinese Journal of Gerontology 2011,31(23):4690-4691.
- [7] C.J. Liang and so on. Two Kinds of Jerusalem Vinegar Pesticides for the Control of Purple Leaf Aphid[J].Pesticide,2000(2):54-55.
- [8] L. Yu. and so on. Biological characteristics and control techniques of A. sinensis[J].Heilongjiang Agricultural Sciences.2004(4):167-168.
- [9] S.J. Liu and so on. Research progress of toxins and their application in biological control[J].Inner Mongolia Agricultural Science and Technology, 2013 (4): 82-83.
- [10] H.H. Zhang. Study on extraction process and insecticidal activity of celandine active substance[D].Harbin: Northeast Forestry University,2007.24-25.
- [11] X.Wang. Study on the extraction and biological activity of celandine alkaloids[D]. Changchun: Jilin Agricultural University, 2016.34-36.
- [12] W.X. Tang and so on. Insecticidal Activity of the Extract of Celandine Extract [J].Journal of Qiqihar University, 2015, 32(5):50-54.
- [13] D.J. Chen and so on. Study on the physical properties and deworming properties of ethanol extract from celandine [J].Northern Horticulture, 2010, (10):52-54.
- [14] S.Q. Tan and so on. Study on the effects of ginseng triterpenoid saponins on two plant-feeding insects [D]. Changchun: Jilin Agricultural University, 2013.
- [15] Bradford M M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dyebinding [J].Analytical Biochemistry, 1976, 72(2):248-254.
- [16] L.C. Zha and so on. Effects of three pesticides on the activity of superoxide dismutase in the caterpillar of the sky caterpillar [J].Journal of Northeast Forestry University, 2011, 39(5):106-108.