


Biphasic Dose–Response of Components From *Coptis chinensis* on Feeding and Detoxification Enzymes of *Spodoptera litura* Larvae

Dose-Response:
An International Journal
July-September 2020:1-8
© The Author(s) 2020
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/1559325820916345
journals.sagepub.com/home/dos



Houhui Jiang¹, Yin Chen¹, Juan Ni¹, Jia Song¹, Li Li¹, Zanyang Yu¹, Lei Pang¹, and Hongyi Qi¹ 

Abstract

Due to long-term coevolution, secondary metabolites present in plants apparently function as chemical defense against insect feeding, while various detoxification enzymes in insects are adaptively induced as a prosurvival mechanism. *Coptis chinensis*, a medicinal plant used in traditional Chinese medicine for a thousand years, was found to be less prey to insects in our earlier field observations. Herein, 4 crude extracts obtained from sequential partition of aqueous extract of *Rhizoma coptidis* with petroleum ether, ethyl acetate, and n-butanol exhibited antifeedant activity against *Spodoptera litura* (Fabricius) larvae at high doses and inducing activity at low doses. Furthermore, a similar biphasic dose–response of the antifeedant activity against *S litura* larvae was also observed for jateorhizine, palmatine, and obakunone in *Coptis chinensis*. Notably, the enzyme activities of glutathione-S-transferase and carboxyl esterase in *S litura* larvae affected by the different components (jateorhizine, palmatine, obakunone, berberine, and coptisine) of *C chinensis* also showed a biphasic dose–response with an increasing trend at low doses and a decreasing trend at high doses. Together, our study suggests that the components of *C chinensis* may play a chemical defensive role against *S litura* larvae in a hormetic manner.

Keywords

Coptis chinensis, hormesis, *Spodoptera litura* (Fabricius) larvae, traditional Chinese medicine, mode of action

Introduction

In the natural environment, plants are subjected to various stresses such as insect feeding. Although plants cannot simply change location to escape from disadvantages, they have evolved to synthesize a large number of phytochemicals to play a chemical defense role.¹ Insects have evolved to feed on plants at least 4 billion years ago, thereby establishing a close coevolutionary relationship.² As an adaptive stress response, insects have accordingly evolved diverse metabolic enzymes to detoxify the phytochemicals.³ Accumulating evidence indicates that the effect of phytochemicals on insects may fit into a general concept known as hormesis,^{4,5} which refers to a process in which exposure to a low-dose stressor that is damaging at higher doses induces an adaptive beneficial effect on the cell or organism.^{6,7} Hormesis is characterized by biphasic dose responses (stimulation at low dose and inhibition at high dose) with an inverted U-shaped or J-shaped dose–response curve.^{7,8}

Rhizoma coptidis, also known as Huang Lian, is the dried rhizome of *Coptis chinensis* Franch, *C deltoidea* C. Y. Cheng et Hsiao, or *C teeta* Wall (Ranunculaceae). In traditional Chinese medicine, *R coptidis* has been used to clear heat, eliminate dampness, and purge fire and detoxification for a 1000 years.^{9,10} Currently, there are over 100 chemical constituents isolated and structurally identified from *R coptidis*, which can be assigned to several classes: alkaloids, organic acids,

¹ College of Pharmaceutical Sciences and College of Chinese Medicine, Southwest University, Chongqing, China

Received 12 January 2020; received revised 24 February 2020; accepted 25 February 2020

Corresponding Author:

Hongyi Qi, College of Pharmaceutical Sciences and College of Chinese Medicine, Southwest University, 2 Tiansheng Road, Beibei District, Chongqing 400716, China.
Email: hongyiqi@swu.edu.cn



coumarins, phenylpropanoids, quinones, and other chemical components. The major bioactive component in *R. Coptidis* is alkaloids, such as berberine, palmatine, coptisine, jatrorrhizine, and epiberberine.⁹⁻¹¹ Botanical insecticides, that is, extracts or compounds derived from Chinese herbal medicine, have long been touted as potential alternatives to conventional synthetic insecticides.^{12,13} In the screening studies of agrochemicals, the chloroform fraction isolated from *C. chinensis* exhibited the insecticidal activity against *Myzus persicae* (Sulzer), and 2 active compounds were identified as 2-methoxy-4-vinylphenol and aniline.¹⁴ The methanol extract of *C. chinensis* roots showed potent antifeedant activity against the third instar larvae of *Ostrinia furnacalis*.¹⁵ In the preliminary field observation of *C. chinensis* in Shizhu County of Chongqing City in China, we found that *C. chinensis* was less affected by pests, such as feeding of *Spodoptera litura* (Fabricius) larvae, compared to the plants in the same area. There was no serious insect infestation observed in the field of *C. chinensis*. Thus, it is extremely interesting for us to explore the chemical defense role and the mode of action of the components derived from *C. chinensis* against *S. litura* larvae.

In this study, the antifeedant activity of the components derived from *C. chinensis* against *S. litura* larvae was evaluated. Moreover, influence of these components on the activities of detoxification enzymes in *S. litura* larvae was further examined.

Materials and Methods

Plant Material and Chemicals

Rhizoma coptidis were purchased from Wanglong Coptis Company (Chongqing, China). Palmatine, obakunone, jateorhizine, coptisine, and berberine with a purity greater than 98% were purchased from Chroma-Biotechnology Co (Chengdu, China), dissolved in 50% ethanol (Sigma-Aldrich, St Louis, Missouri), and stored at -20°C .

Preparation of Crude Extracts

The dried slices of *R. Coptidis* were pulverized to a fine powder (granule size $20\ \mu\text{m}$). One hundred twenty grams of the powder was subjected to reflux extraction with 95% ethanol. Then, the extract was concentrated to remove ethanol using a rotary evaporator (Büchi, Essen, Germany) under vacuum and dissolved in distilled water. Subsequently, the aqueous extract was extracted with petroleum ether (B.R. 40°C - 60°C) followed by ethyl acetate and water saturated n-butanol. The solvents were finally removed using a rotary evaporator (Büchi) under vacuum to get the petroleum ether extract (RC-PE) 1.16 g, the ethyl acetate extract (RC-EA) 1.1 g, n-butanol extract (RC-B) 6.56 g, and aqueous phase (RC-A) 6.38 g.

Insect Rearing

The rearing was carried out in our laboratory under constant conditions: $27^{\circ}\text{C} \pm 1^{\circ}\text{C}$, relative humidity 60%-70%, and 12 light:12 dark photoperiod. To maintain the generation of

insects, female and male moths with the same number were caged in a 10-cm-diameter beaker supplied with honey solution (10%) and permitted to copulate for 48 hours. Eggs were then laid by female moths on wax paper. Upon egg hatching, *S. litura* larvae were fed on standard artificial diet.¹⁶

Antifeedant Activity

The selective antifeedant activity was determined with a leaf disk choice test with the third instar *S. litura* larvae. Disks (1 cm in diameter) were punched from cabbage leaves and soaked in the crude extracts of different concentrations for 5 seconds. The control was soaked in distilled water containing equal volume of ethanol. The leaf disks were air-dried before feeding the larvae. The treated leaf disks and the control leaf disks were simultaneously placed into each Petri dish with 3-cm distance. Each larva that already starved for 4 hours was introduced into a Petri dish with 3 leaf disks and allowed to feed. Each treatment used 10 larvae with 3 replicates. The number of larvae within 1-cm range of the leaf disks was counted within 72 hours after the introduction of larvae. The selective antifeedant rate (%) is calculated as: $(C - T)/(C + T) \times 100$, where *C* refers to the average larvae number of the control group and *T* refers to the average larvae number of the treated group.

Determination of Glutathione-S-Transferase and Carboxyl Esterase Activities

The fifth instar larvae were exposed to the treated cabbage leaf disks or the control leaf disks for 48 hours. Midguts separated from larvae of each treatment were homogenized in liquid nitrogen and then added with 5 volumes of 0.1 M phosphate buffer (pH 7.0). Each sample was centrifuged at 8000g for 10 minutes at 4°C . Then, the activities of glutathione-S-transferase (GST) and carboxyl esterase (CarE) were further determined with the respective commercial kits (Nanjing Jiancheng Biotechnology, Nanjing, China), following the instructions provided by the manufacturer.

Statistical Analysis

The data in this study were reported as means \pm standard deviation for triplicate. A *t* test or 1-way analysis of variance was applied to evaluate for significant differences between the 2 groups. A *P* value of $<.05$ was considered to be statistically significant.

Results

Antifeedant Activity of Crude Extracts of *R. Coptidis*

To determine the antifeedant activity of the crude extracts derived from *R. Coptidis*, *S. litura* larvae were provided with the leaf disks treated with different crude extracts and the leaf disks treated with vehicle only. As shown in Figure 1A, *S. litura* larvae exhibited even higher selectivity to leaf disks treated with $0.1\ \text{mg}\cdot\text{mL}^{-1}$ of RC-PE than those with vehicle. Similar

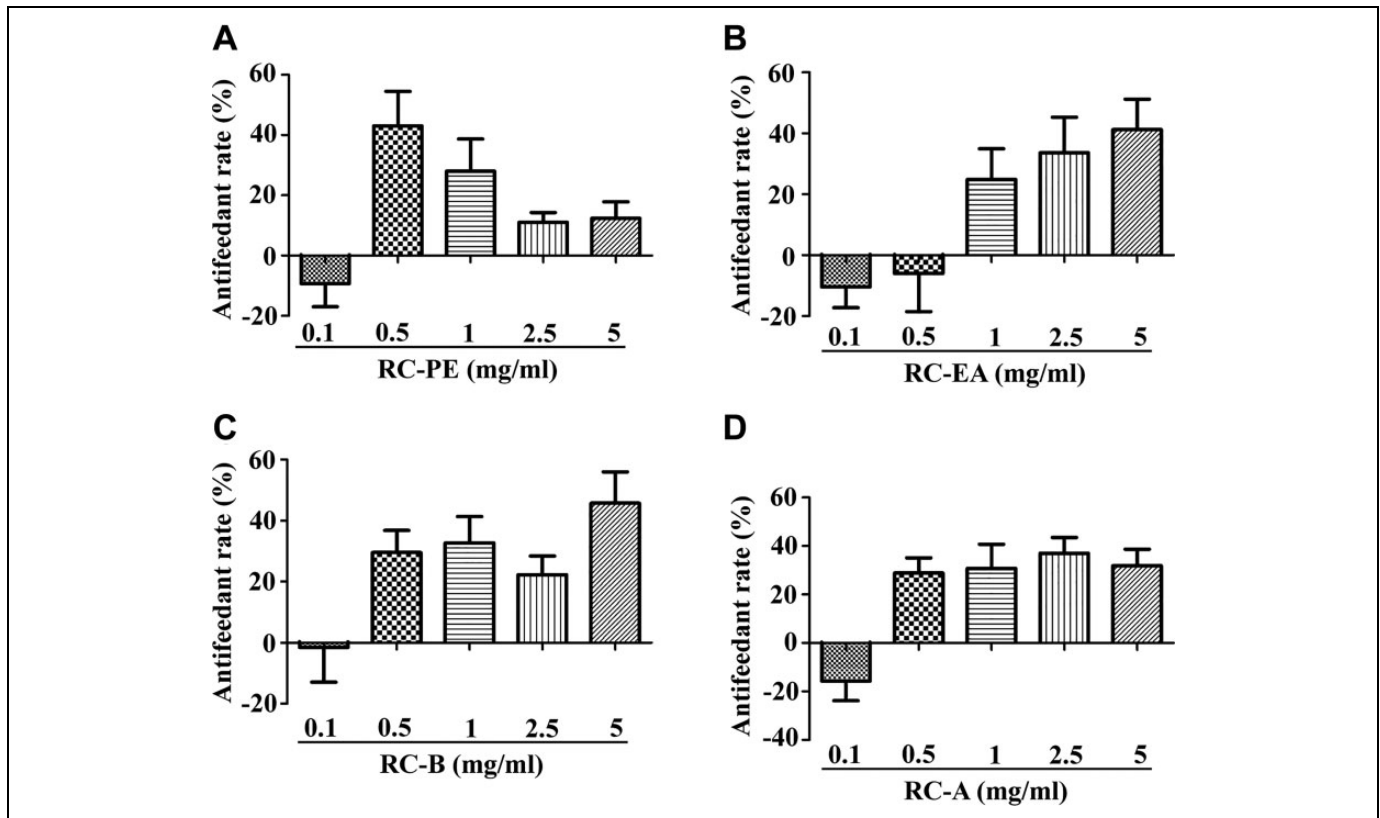


Figure 1. The selective antifeedant rate of crude extracts derived from *Rhizoma coptidis* against *Spodoptera litura* larvae. (A) Petroleum ether extract (RC-PE); (B) ethyl acetate extract (RC-EA); (C) n-butanol extract (RC-B); (D) aqueous phase (RC-A). Replicates = 3 and n = 10 larvae in each replicate. Values are presented as means \pm standard deviation (SD).

observation was also obtained in Figure 1B to D, where *S litura* larvae showed higher selectivity to leaf disks treated with 0.1 and 0.5 mg·mL⁻¹ of RC-EA, 0.1 mg·mL⁻¹ of RC-B, and 0.1 mg·mL⁻¹ of RC-A. However, the selective antifeedant rate remarkably increased to 43.02% in *S litura* larvae exposed to 0.5 mg·mL⁻¹ of RC-PE and then showed a decreased trend from 1.0 to 5 mg·mL⁻¹ of RC-PE. Figure 1B to D shows that *S litura* larvae exhibited an obvious selective antifeedant behavior from 0.5 to 5 mg·mL⁻¹ of RC-B and RC-A and 1 to 5 mg·mL⁻¹ of RC-EA. These results indicate that high doses of crude extracts of *R. Coptidis* may exhibit antifeedant activity to *S litura* larvae, while low dose may show an inducing activity to *S litura* larvae.

Antifeedant Activity of Representative Compounds in *C chinensis*

To further evaluate the antifeedant activity of the representative compounds derived from *C chinensis*, *S litura* larvae were provided with the leaf disks treated with vehicle and the leaf disks treated with one of the representative compounds (jateorhizine, palmatine, obakunone, coptisine, and berberine). As shown in Figure 2A, 0.05 mg·mL⁻¹ of jateorhizine exhibited a little inducing activity to *S litura* larvae, while a dose-dependent increase in antifeedant activity to *S litura* larvae was

observed in 0.1 to 10 mg·mL⁻¹ of jateorhizine. Notably, 10 mg·mL⁻¹ of jateorhizine showed an antifeedant activity of 67.9%. Figure 2B showed that both 0.05 and 0.1 mg·mL⁻¹ of palmatine exhibited the inducing activity to *S litura* larvae, and almost 30% of the inducing activity was observed in 0.1 mg·mL⁻¹ of palmatine. However, 0.5 to 10 mg·mL⁻¹ palmatine showed obvious antifeedant activity to *S litura* larvae. Similar trend was observed in Figure 2C, where 0.05 and 0.1 mg·mL⁻¹ of obakunone showed the inducing activity to *S litura* larvae and 0.5 to 10 mg·mL⁻¹ obakunone showed the antifeedant activity to *S litura* larvae. There was irregular inducing or antifeedant activity to *S litura* larvae for coptisine and berberine (data not shown).

Effect of Representative Compounds in *Coptis chinensis* on GST Activity

To determine the effect on GST activity, the fifth instar larvae were exposed to the leaf disks treated with the representative compounds derived from *C chinensis* or vehicle for 48 hours and then the enzyme activity of GST in the midgut was evaluated. As shown in Figure 3A, 0.05 to 0.5 mg·g⁻¹ of berberine significantly increased the GST activity compared to vehicle ($P < .05$). However, 1 to 10 mg·g⁻¹ of berberine remarkably decreased the GST activity compared with vehicle ($P < .01$ for

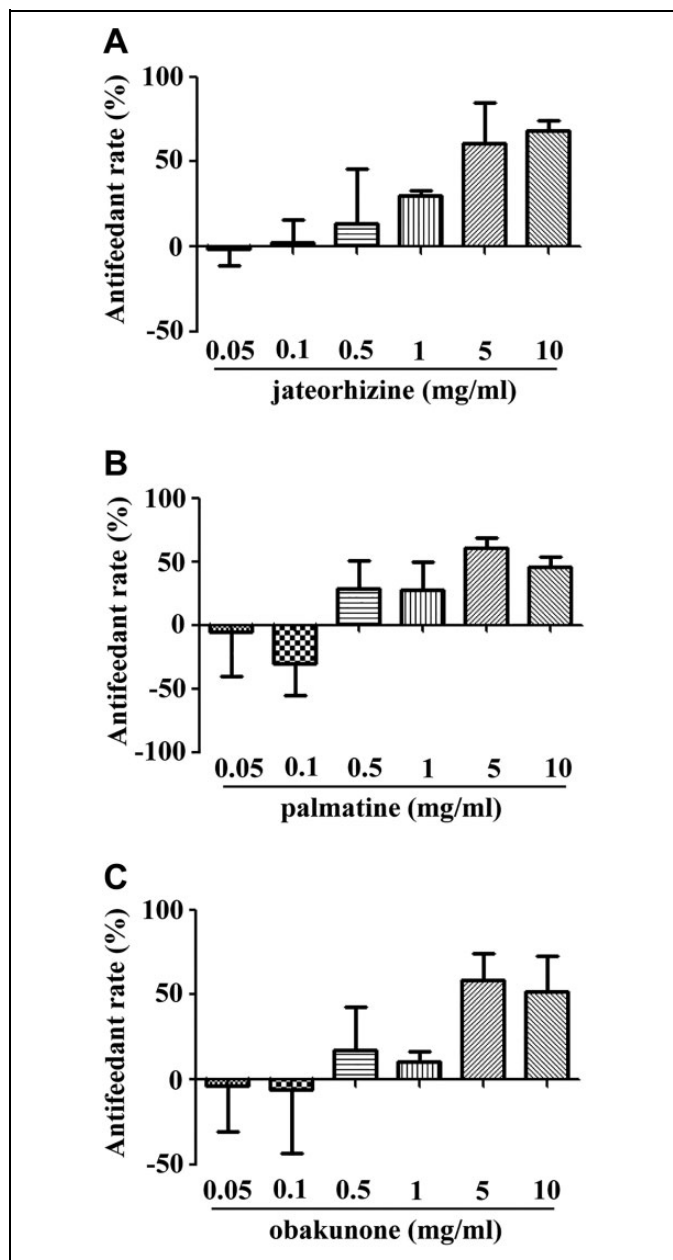


Figure 2. The selective antifeedant rate of the different compounds in *Coptis chinensis* against *Spodoptera litura* larvae. (A) Jateorhizine; (B) palmatine; (C) obakunone. Replicates = 3 and $n = 10$ larvae in each replicate. Values are presented as means \pm standard deviation (SD).

1 mg·g⁻¹ of berberine and $P < .001$ for 5 to 10 mg·g⁻¹ of berberine). Figure 3B demonstrates that 0.05 and 0.5 mg·g⁻¹ of coptisine significantly enhanced the GST activity compared to vehicle ($P < .05$), while 5 and 10 mg·g⁻¹ of coptisine dramatically reduced the GST activity ($P < .001$). Similar trend of phasic dose-response in the change of GST activity was also observed in *S litura* larvae exposed to other compounds derived from *R coptidis*. There was an increasing trend of GST activity in *S litura* larvae, respectively, exposed to 0.05 to 0.5 mg·g⁻¹ of jateorhizine, palmatine, and obakunone, although only each 0.5 mg·g⁻¹ significantly enhanced the GST activity in *S litura*

larvae compared to the vehicle ($P < .05$). However, GST activity dramatically decreased for 1 or 5 to 10 mg·g⁻¹ of these 3 compounds compared to vehicle ($P < .001$; Figure 3C-E).

Effect of Representative Compounds in *Coptis chinensis* on CarE Activity

The CarE activity in the midgut of *S litura* larvae was also determined after exposed to the representative compounds derived from *C chinensis* or vehicle for 48 hours. Interestingly, the effect of all these compounds on CarE activity exhibited biphasic dose responses with an increasing trend from 0.05 or 0.1 to 0.5 or 1 mg·g⁻¹ and a decreasing trend from 1 or 5 to 10 mg·g⁻¹ (Figure 4A-E). Among them, berberine exhibited the greatest impact on the CarE activity. As shown in Figure 4A, all 0.05 to 1 mg·g⁻¹ of berberine significantly increased the CarE activity ($P < .05$), while both 5 and 10 mg·g⁻¹ of berberine remarkably decreased the CarE activity ($P < .01$). Figure 4B shows that 0.1 to 1 mg·g⁻¹ of coptisine significantly increased the CarE activity ($P < .05$) and only 10 mg·g⁻¹ of coptisine remarkably decreased the CarE activity ($P < .01$). Figure 4C and D shows that only 0.5 mg·g⁻¹ jateorhizine and 1 mg·g⁻¹ palmatine significantly enhanced the CarE activity ($P < .05$), while 10 mg·g⁻¹ jateorhizine and 5 and 10 mg·g⁻¹ palmatine remarkably decreased the CarE activity. There was no dose of obakunone exhibited statistical significance on increasing the CarE activity, although an increasing trend was observed in 0.05 to 1 mg·g⁻¹ of obakunone. However, 5 ($P < .05$) and 10 mg·g⁻¹ ($P < .01$) of obakunone decreased the CarE activity ($P < .01$; Figure 4E).

Discussion

Botanical pesticides have emerged as attractive alternatives to synthetic pesticides due to the environmentally friendly and safe characteristics.¹² There is growing evidence indicates that phytochemicals derived from stressed natural plants may interact with insects in a hormesis way owing to the long period of coevolution.³ In our preliminary field observation, we found that *C chinensis* was less fed by *S litura* larvae, one of regular polyphagous and damaging insects, while other plants in the same area suffered serious insect infestation. Thus, the current study aimed to figure out whether the components derived from *R coptidis* play a defense role against *S litura* larvae.

In our study, we first evaluated the antifeedant activity of the crude extracts (RC-PE, RC-EA, RC-B, and RC-A) isolated from *R coptidis* with systematic solvent extraction. Our results demonstrated that high doses (0.5-5 mg·mL⁻¹) of all these crude extracts of *R coptidis* exhibited antifeedant activity to *S litura* larvae, while low dose (0.1 mg·mL⁻¹) may show an inducing activity to *S litura* larvae. It is suggested from this biphasic dose-response that the effect of the crude extracts derived from *R coptidis* may follow the hormesis model. Recent studies have highlighted that many ingredients from natural plants exhibited bifunction in model organisms did so by mediating hormesis. For example, rutin, which is rich in

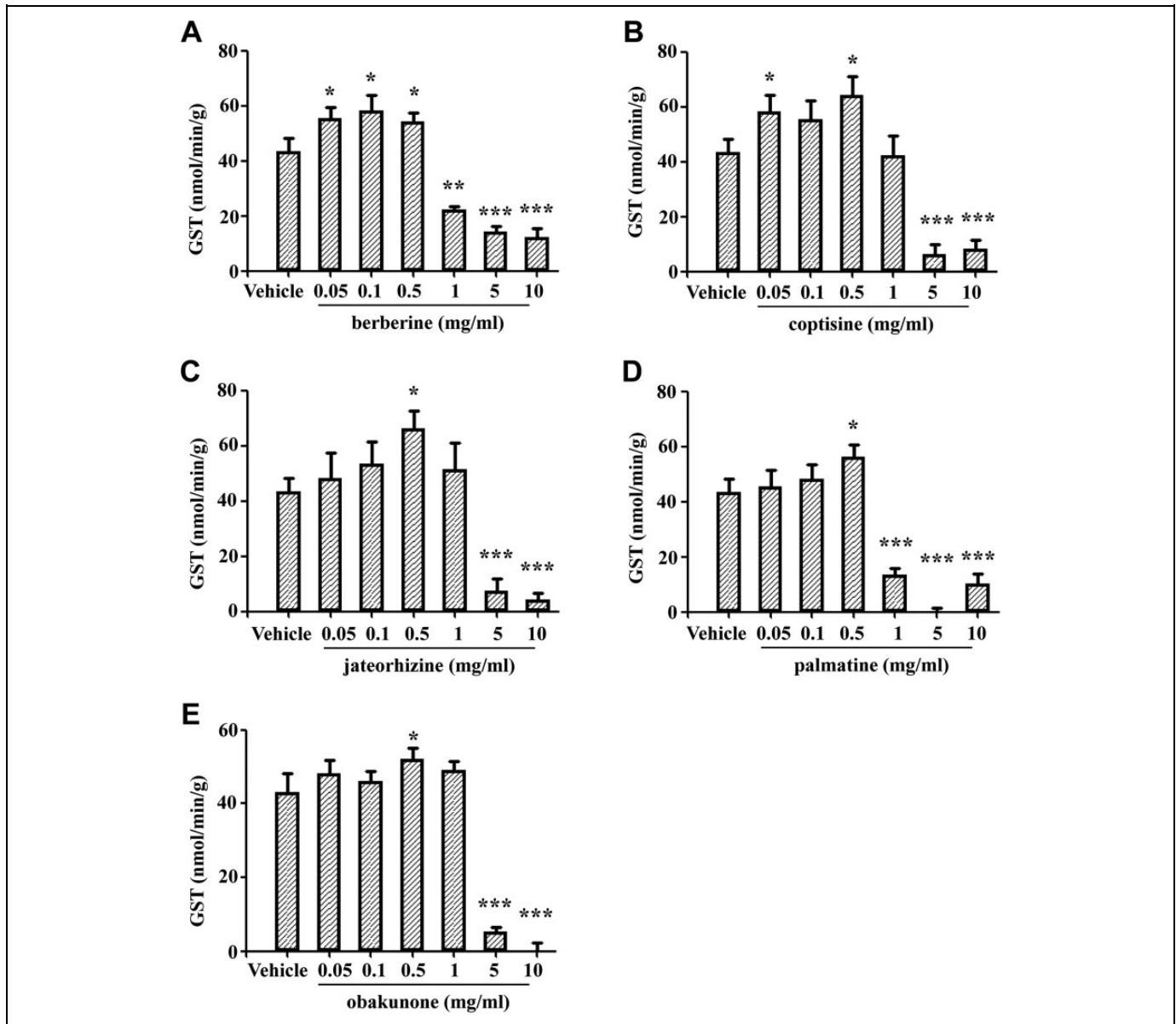


Figure 3. Effect of the different compounds in *Coptis chinensis* on the activity of detoxification enzyme glutathione-S-transferase (GST). The GST activity was determined as mentioned under Materials and Methods section. (A) berberine; (B) coptisine; (C) jateorhizine; (D) palmatine; (E) obakunone. Replicates = 3 and n = 10 larvae in each replicate. Values are presented as means \pm standard deviation (SD). * $P < .05$. ** $P < .01$. *** $P < .001$.

citrus fruits and buckwheat seeds, at 200 and 400 μM significantly extended median lifespan of *Drosophila melanogaster*, while rutin with a dose beyond 400 μM dramatically reduced longevity.⁸ In our previous study, Z-ligustilide in *Ligusticum chuanxiong* Hort. exhibited potent antifeedant activity against *S litura* larvae within the dose range of 0.25 to 5 $\text{mg}\cdot\text{g}^{-1}$ and 0.1 $\text{mg}\cdot\text{g}^{-1}$ of Z-ligustilide showed an inducing activity to *S litura* larvae.¹⁷ Furthermore, we also examined the larvicidal activity of these crude extracts against *S litura* larvae. However, only weak larvicidal activity (<20%) was observed for all the crude extracts of *R coptidis*. Moreover, there was almost no statistical difference for the

larvicidal activity among the different groups (data not shown). Consistent with our results, Zhou et al reported that methanol extract of *R coptidis* exhibited potent antifeedant activity and weak larvicidal activity against the third instar larvae of *Ostrinia furnacalis*.¹⁵ It is believed that the mixture of phytochemicals may be deterrent to insects and herbivores for longer period than single compound.¹² Our study revealed that all the 4 different crude extracts exhibited antifeedant activity against *S litura* larvae under certain doses, indicating that the antifeedant activity of *C chinensis* may be derived from the mixture of different kinds of chemical components and not just 1 or 2 single compounds.

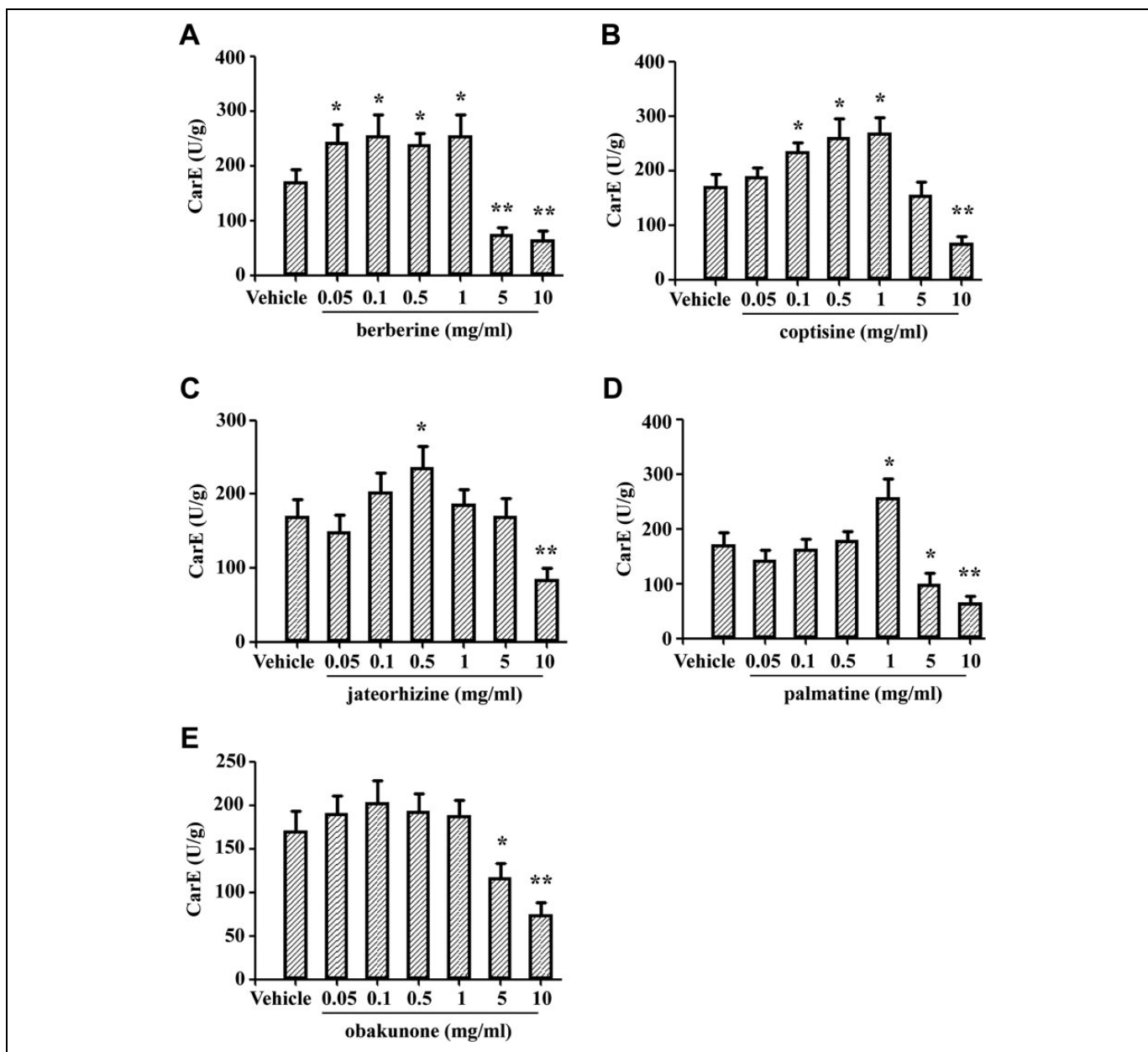


Figure 4. Effect of the different compounds in *Coptis chinensis* on the activity of detoxification enzyme carboxyl esterase (CarE). The CarE activity was determined as mentioned under Materials and Methods section. (A) berberine; (B) coptisine; (C) jateorrhizine; (D) palmatine; (E) obakunone. Replicates = 3 and n = 10 larvae in each replicate. Values are presented as means \pm standard deviation (SD). * $P < .05$. ** $P < .01$.

Palmitine, coptisine, berberine, and jatrorrhizine belong to protoberberine-type alkaloids, which are the most abundant components and considered as the main active ingredients in *C chinensis*, and obakunone is a representative triterpenoid in *C chinensis*.⁹⁻¹¹ Then, we used these 5 compounds as an example to determine the antifeedant activity and the mode of action of the single compound derived from *C chinensis*. Similar with crude extracts of *R coptidis*, our results revealed that the biphasic dose-response of the antifeedant activity was observed for jateorrhizine, palmatine, and obakunone to *S litura* larvae. Moreover, there was also only weak and unregular larvicidal activity against *S litura* larvae observed for these components

(data not shown). Previous study showed that the methanol extract of *Phellodendron amurense* bark exhibited a strong antifeedant activity against *Reticulitermes speratus* and further bioguided fractionation led to the identification of obakunone and kihadanin A in the chloroform fraction and berberine and palmatine iodide as the bioactive components.¹⁸ Berberine, palmatine, and coptisine identified in *Coptis japonica* roots exhibited strong antifeedant activity against *Hyphantria cunea* (Lepidoptera: Arctiidae) and *Agelastica coerulea* (Coleoptera: Galerucinae).¹⁹

Insect GSTs and CarE belong to a superfamily of detoxification enzymes. It is reported that 40, 35, 13, 23, and 32

isoforms of GSTs were identified in *Drosophila*, *Anopheles*, *Apis*, *Bombyx*, and *Acyrtosiphon*, respectively.⁸ Glutathione-S-transferases play a protective role in detoxification through catalyzing the conjugation of reduced glutathione with electrophilic endogenous and xenobiotic compounds, as well as end products of lipid peroxidation, such as 4-hydroxynonenal and malonaldehyde.^{20,21} CarE is a polyprotein belonging to the serine hydrolase family. It hydrolyzes endogenous and exogenous substances such as esters and thioesters, participates in the metabolic detoxification of many toxic substances, and reduces toxicity.²² Notably, our study demonstrated that the enzyme activities of GST and CarE in *S litura* larvae affected by the different components of *C chinensis* also showed a biphasic dose–response with an increase at low dose and decrease at high dose. These results suggest that detoxification enzymes may be adaptively activated in *S litura* larvae as a prosurvival mechanism in response to low dose of the components in *C chinensis*, whereas they were directly inhibited under the insult of high dose of these components, which further confirms the hormetic role of the components in *C chinensis*. Similarly, our previous report indicates that detoxification enzymes in *S litura* larvae was also modulated by *Z*-ligustilide in *Ligusticum chuanxiong* Hort. in a hormesis way.¹⁷ Although berberine and coptisine showed greater impact on GST and CarE enzyme activities compared to other 3 compounds (palmatine, jatrorrhizine, and obakunone), no typical biphasic dose–response for the antifeedant activity of berberine and coptisine was observed in our study. This suggests that there may be other unknown mechanisms, which deserves further in-depth research in our future study, in addition to these 2 enzymes.

Conclusion

Our study demonstrated that a biphasic dose–response was observed in the antifeedant activity of the crude extracts and bioactive compounds in *C chinensis* as well as the modulation of detoxification enzymes of GST and CarE by those bioactive compounds. These results suggest that the components in *C chinensis* may function via a hormesis way in the chemical defense against *S litura* larvae.

Acknowledgments

The authors are thankful to National Demonstration Center for Experimental Pharmacy Education (Southwest University) for providing partial experimental instruments and conditions.


Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by Capacity Building Project for the Sustainable Utilization of Valuable Traditional Chinese Medicine Resources (no.2060302).

ORCID iD

Hongyi Qi  <https://orcid.org/0000-0003-0564-3892>

References

1. Harborne JB. Role of secondary metabolites in chemical defence mechanisms in plants. *Ciba Found Symp.* 1990;154:126-134.
2. Ehrlich PR, Raven PH. Butterflies and plants: a study in coevolution. *Evolution.* 1964;18(4):586-608.
3. Padró J, De Panis DN, Vrdoljak J, et al. Experimental evolution of alkaloid tolerance in sibling drosophila species with different degrees of specialization. *Evol Biol.* 2018;45(2):170-181.
4. Chattopadhyay D, Chitnis A, Talekar A, et al. Hormetic efficacy of rutin to promote longevity in drosophila melanogaster. *Biogerontology.* 2017;18(3):397-411.
5. Leow S-S, Luu A, Shrestha S, Hayes KC, Sambanthamurthi R. *Drosophila* larvae fed palm fruit juice (PFJ) delay pupation via expression regulation of hormetic stress response genes linked to ageing and longevity. *Exp Gerontol.* 2018;106:198-221.
6. Calabrese EJ, Dhawan G, Kapoor R, Iavicoli I, Calabrese V. HORMESIS: a fundamental concept with widespread biological and biomedical applications. *Gerontology.* 2016;62(5):530-535.
7. Mattson MP. Hormesis defined. *Ageing Res Rev.* 2008;7(1):1-7.
8. Agathokleous E, Calabrese EJ. Hormesis: the dose response for the 21st century: the future has arrived. *Toxicology.* 2019;425:152249.
9. Wang J, Ran Q, Zeng H-R, Wang L, Hu C-J, Huang Q-W. Cellular stress response mechanisms of *Rhizoma coptidis*: a systematic review. *Chin Med.* 2018;13:27-27.
10. Wang J, Wang L, Lou G-H, et al. *Coptidis Rhizoma*: a comprehensive review of its traditional uses, botany, phytochemistry, pharmacology and toxicology. *Pharm Biol.* 2019;57(1):193-225.
11. Meng F-C, Wu Z-F, Yin Z-Q, Lin L-G, Wang R, Zhang Q-W. *Coptidis rhizoma* and its main bioactive components: recent advances in chemical investigation, quality evaluation and pharmacological activity. *Chin Med.* 2018;13:13-13.
12. Rattan RS. Mechanism of action of insecticidal secondary metabolites of plant origin. *Crop Prot.* 2010;29(9):913-920.
13. Xu H, Zhang N, Casida JE. Insecticides in Chinese medicinal plants: survey leading to jacaranone, a neurotoxicant and glutathione-reactive quinol. *J Agric Food Chem.* 2003;51(9):2544-2547.
14. Jung JY, Lee HC, Yang J-K. Insecticidal activity of coptis chinensis extract against *Myzus persicae* (Sulzer). *J Korean Wood Sci Technol.* 2015;43(2):274-285.
15. Sunday A, Orjiekwe C, Ehiagbonare JE, Arimah BD. Preliminary phytochemical analysis and insecticidal activity of ethanolic extracts of four tropical plants (*Vernonia amygdalina*, *Sida acuta*, *Ocimum gratissimum* and *Telfaria occidentalis*) against beans weevil (*Acanthscelides obtectus*). *Int J Phys Sci.* 2010;5(6):753-792.
16. Chen QJ, Li J, Pang H. A simple artificial diet for mass rearing of some noctuid species. *Entomol Knowl.* 2000;37(6):325-327.

17. Yi Y, Dou G, Yu Z, et al. Z-ligustilide exerted hormetic effect on growth and detoxification enzymes of *Spodoptera litura* larvae. *Evid Based Complement Alternat Med*. 2018;2018: 7104513.
18. Kawaguchi H, Kim M, Ishida M, et al. Several antifeedants from *Phellodendron amurense* against *Reticulitermes speratus*. *Agric Biol Chem*. 1989;53(10):2635-2640.
19. Park IK, Lee HS, Lee SG, Park JD, Ahn YJ. Antifeeding activity of isoquinoline alkaloids identified in *Coptis japonica* roots against *Hyphantria cunea* (Lepidoptera: Arctiidae) and *Agelastica coerulea* (Coleoptera: Galerucinae). *J Econ Entomol*. 2000; 93(2):331-335.
20. Singh SP, Coronella JA, Benes H, Cochrane BJ, Zimniak P. Catalytic function of *Drosophila melanogaster* glutathione S-transferase DmGSTS1-1 (GST-2) in conjugation of lipid peroxidation end products. *Eur J Biochem*. 2001;268(10):2912-2923.
21. Zhang N, Liu J, Chen S-N, Huang L-H, Feng Q-L, Zheng S-C. Expression profiles of glutathione S-transferase superfamily in *Spodoptera litura* tolerated to sublethal doses of chlorpyrifos. *Insect Sci*. 2016;23(5):675-687.
22. Rane RV, Ghodke AB, Hoffmann AA, Edwards OR, Walsh TK, Oakeshott JG. Detoxifying enzyme complements and host use phenotypes in 160 insect species. *Curr Opin Insect Sci*. 2019; 31:131-138.