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Original Article

28-Homobrassinolide Alters Protein Content and Activities of Glutathione-S-Transferase and Polyphenol Oxidase in *Raphanus Sativus* L. Plants Under Heavy Metal Stress

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ABSTRACT

Objectives: The application of brassinosteroids (BRs), the plant steroidal hormones, results in an increased tolerance toward stress and thus helps improving the yield of crop plants. The present study was carried out to investigate the effect of 28-homobrassinolide (28-HBL) on the protein content as well as activities of antioxidant enzymes viz., glutathione-s-transferase (GST) and polyphenol oxidase (PPO) in radish plants grown under Cadmium (Cd) and Mercury (Hg) metal stress. **Materials and Methods:** Shoots of 60 and 90 days old radish plants, grown under Cd and Hg metal stress (0, 0.5, 1.0, 1.5 mM) and given the presowing treatment of 28-HBL (0, 10^{-7}, 10^{-9}, 10^{-11} M) to seeds for 8 h, were analyzed for protein content and GST and PPO enzyme activities. **Results:** Protein content showed decrease in plants given Cd and Hg metal treatment alone, while treatment with 28-HBL enhanced the protein content, suggesting its stress protective role. An increase in the activity of antioxidative enzymes was also observed in plants stressed with heavy metals as well as in those supplemented with 28-HBL. **Conclusions:** In the present investigation, the activity of antioxidative enzymes was found to increase due to metal stress and a further increase was noticed in plants given both metal and 28-HBL treatment, suggesting the stress protective role of 28-HBL via modulating the antioxidative enzymes.

Key words: 28-HBL, glutathione-s-transferase, polyphenol oxidase, *Raphanus sativus* L.

INTRODUCTION

Raphanus sativus L. (radish) is an important horticulture crop of the Brassicaceae family. The roots are the most used part, but leaves and fruits are also used in cuisines. Roots are consumed either raw or cooked and are also preserved by pickling, canning, or drying.[1] Radish sprouts harbor more concentrations of glucosinolates (3.8-fold) and isothiocyanates (8.2-fold) and greater concentrations of phenolics (on average 6.9-fold) than the mature radish taproot.[2] The leaves are rich source of calcium, iron, and ascorbic acid.[3] However, the presence of heavy metals in agricultural soils as a result of industrial and sewage discharge is posing a threat to the yield of radish crops.

Heavy metals are considered as major environmental pollutants. When these are present in high concentrations in soil, they show potential toxic effects on growth as well as development in plants.[4] Heavy metals cause damage to plant growth in many ways. One of the possible mechanisms is that the heavy metals lead to the production of free radicals in plants which results in oxidative stress which inhibits certain cellular processes at different levels of metabolism.[5]

Access this article online

Quick Response Code:

Website: www.toxicologyinternational.com

DOI: 10.4103/0971-6580.128792

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Mercuric ions in plants are able to induce oxidative stress by triggering generation of reactive oxygen species (ROS), e.g., superoxide anion radical (O_2), H_2O_2, and hydroxyl radical (OH^-) in plants.[6] These ROS generated due to mercury (Hg) contamination can led to disruption of biomembrane lipids and cellular metabolism.[7,8] Cadmium (Cd) also causes oxidative stress in plants as it interacts with functional S-H group of various enzymes and leads to their activation.[9]

Recent reports on exogenous application of brassinosteroids (BRs) to plants strongly suggest that these steroidal hormones have the ability to modify antioxidant enzymes system under stress conditions. BRs are known to confer resistance against various stresses to plant such as temperature, drought, salinity, pesticides, heavy metals, and infections due to fungus, bacteria, and viruses.[10] 28-homobrassinolide (28-HBL) is a highly stable and active steroidal hormone.

Glutathione-*S*-transferases (GSTs, EC.2.5.1.18) are reported to play role in detoxification of endobiotic and xenobiotic compounds. It brings about the conjugation of glutathione (GSH) to a hydrophobic substrate and as a result, water-soluble and less toxic glutathione S-conjugates are formed that are coupled to internal compartmentation due to the lack of effective excretion pathways.[11,12] Polyphenol oxidase (PPO, EC1.14.18.1) is a copper-containing enzyme catalyzing the oxidation of phenols to the respective quinones. PPO is found in the chloroplast in healthy plant cells, although it is synthesized in the cytoplasm under nuclear control.[13]

Keeping in view the importance of radish as an important horticulture crop and the heavy metal stress being faced by the crop, the present study was designed to test the hypothesis that 28-HBL ameliorates the phytotoxicity of Cd and Hg metals on radish plants in terms of modulation of protein content and the activity of anioxidative enzymes *viz.,* GST and PPO.

MATERIALS AND METHODS

A field experiment was conducted in Botanical Garden of Guru Nanak Dev University, Amritsar to study the effects of 28-HBL on radish plants grown under Cd and Hg metal stress. Certified and disease free seeds of *Raphanus sativus* L. var. "Pusa Chetki" were procured from Punjab Agricultural University, Ludhiana, Punjab. The 28-HBL used for the study was purchased from Sigma-Aldrich Ltd., New Delhi. The seeds were surface sterilized with 0.01% sodium hypochlorite and were then soaked in different concentrations (0, 10^{-7}, 10^{-9}, 10^{-11} M) of 28-HBL for 8 h prior to sowing. The plants were raised in a 10 × 11 feet piece of land having soil arranged in form of crests and troughs and supplied with Cd and Hg metals at the concentrations 0, 0.5, 1.0, and 1.5 mM. The shoots of 60- and 90-days-old plants were harvested and then subjected to biochemical analysis.

Biochemical analysis

Extract preparation

The apical leaves (5 g) of both 60- and 90-days-old plants were harvested at respective time period and were homogenized in 50 mM phosphate buffer [pH 7.0, EDTA (1 mM), Triton X-100 (0.5%)] in a prechilled pestle and mortar. The homogenate was centrifuged at 13,000 × *g* for 20 min at 4°C and the supernatant was used for assessing the protein content and activities of GST and PPO enzymes using UV-Visible PC-based Double Beam Spectrophotometer (Systronics 2202).

Protein content

The method of Lowry *et al*.,[14] was followed to estimate total protein content in the shoots using bovine serum albumin as standard. A graph of absorbance versus concentration for standard solutions of protein was plotted and the amount of protein in the sample was calculated from the graph. The amount of protein is expressed as mg/g tissue.

GST activity

The method based on the reaction of the GSTs in a mixture of 1-Chloro-2,4-dinitrobenzene (CDNB; 20 mM) and glutathione (GSH; 100 mM) proposed by Habig *et al*.,[15] was used to estimate GST (EC.2.5.1.18) activity. The change in optical density due to the emergence of complex CDNB-GSH is measured spectrophotometrically every 15 s for 2 min at 340 nm. The assay mixture (2.25 mL) contained 2 mL phosphate buffer (0.2 M, pH 7.4), 100 µL GSH (20 mM), 100 µL CDNB (20 mM), and 50 µL enzyme sample. The concentration of GST was expressed in µmole unit activity (UA) mg^{-1} protein. UA is defined as the change in absorbance by 0.1 min^{-1} mg^{-1} protein.

PPO activity

PPO (EC 1.10.3.1) activity was assayed by the method of Kumar *et al*.[16] The assay mixture for PPO contained 2 mL of 0.1 M phosphate buffer (pH 7.0), 1 mL of 0.1 M catechol, and 0.5 mL of enzyme extract. This was incubated for 5 min at 25°C, after which the reaction was stopped by adding 1 mL of 2.5 N H_2SO_4. The absorbency of the benzoquinone formed was read at 495 nm. To the blank 2.5 N H_2SO_4 was added of the zero time of the same assay mixture. PPO activity is expressed in µmole UA mg^{-1} protein. (UA = change in absorbance by 0.1 min^{-1} mg^{-1} protein).

Statistical analysis

All the experiments were performed in triplicates and the values presented here are the mean of three values ± standard error. The statistical differences between means were assessed with one-way analysis of variance according to the

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methodology proposed by Bailey[17] using Microsoft excel. A significant difference was evaluated at a level of $P \leq 0.05$.

RESULTS

The studies carried on biochemical parameters indicated significant effects of both metal stress and 28-HBL treatment in *Raphanus sativus* L. plants.

Protein content

The protein content was decreased in the plants treated with Cd and Hg metal when compared with untreated plants. However, treatment with 28-HBL enhanced the protein content. Among 60-days old plants, maximum effect was noticed in 10^{-7} M 28-HBL treatment supplemented with 1.5 mM Cd [Table 1]. In case of plants grown under Hg metal stress, seed presoaking treatments with 10^{-7} M and 10^{-11} M 28-HBL were observed to be most effective in increasing the protein content at the 0.5 mM Hg and 1.5 mM Hg, respectively [Table 2]. Similar was the case with 90-days old plants. The protein content was remarkably higher in plants treated with 10^{-7} M of 28-HBL supplemented with 1.5 mM Cd as well as 1.5 mM Hg as compared with plants treated with 1.5 mM Cd/Hg alone.

GST activity

Among 60-days old plants, along with Cd metal concentrations, 10^{-7} 28-HBL had effectively increased the specific activity to maximum except 1.5 mM Cd, where 10^{-9} M 28-HBL was effective. 1.0 mM Cd treatment resulted in maximum increase in GST activity in 90-days old plants and the application of 28-HBL upregulated the activity and maximum activity was anticipated in plants supplemented with 1.0mM Cd and 10^{-7} M 28-HBL [Table 3].

In case of Hg treated 60-days old plants, 0.5 mM concentration of Hg and application of 10^{-7} M 28-HBL to seeds resulted in increased enzyme activity as compared with metal treated control plants. In case of 90-days old plants, maximum activity was found in case where 10^{-11} M 28-HBL pretreated seeds were planted under 1.5 mM Hg metal concentration and minimum was found in case of 10^{-11} M 28-HBL alone treated plants as compared with control plants [Table 4].

PPO activity

Specific activity of PPO was observed to increase considerably under Cd stress in 60-days old plants, with a maximum value observed with 1.0 mM Cd. In plants supplemented with both metal and 28-HBL, maximum increase in specific activity was noticed in 0.5 mM Cd supplemented with 28-HBL as compared to control as well as metal treatments alone. 90-days old plants also had increased PPO activity with the application of different concentrations of Cd alone as well as in supplementation with 28-HBL concentrations [Table 5].

In case of Hg metal, the PPO activity among 60-days old plants increased with increase in the metal concentration and maximum was at the concentration of 1 mM Hg as compared with control plants. At concentration of 1 and 1.5 mM, 10^{-9}

Table 1: Effect of 28-HBL on protein content (mg/g f.w.) in 60 and 90 days old shoots of *Raphanus sativus* L. plants grown under Cd metal stress (mean±S.E.)

Treatments	Protein content (mg/g f.w.)							
	Control (DW)		0.5 mM Cd		1.0 mM Cd		1.5 mM Cd	
	60 days	90 days	60 days	90 days	60 days	90 days	60 days	90 days
Control (DW)	616.7±2.96	298.2±0.882	296.6±3.18	163.6±2.72	305.9±1.0	164.9±2.0	332.6±0.88	122.6±1.528
10^{-11} 28-HBL	530.6±2.848	334.9±2.0	342.6±0.33	353.9±2.082	332.2±0.333	247.2±1.333	325.2±0.667	454.2±2.404
10^{-9} 28-HBL	566.6±0.882	325.2±1.453	376.2±0.333	215.9±1.528	364.9±1.155	295.2±1.453	363.9±0.577	295.9±1.528
10^{-7} 28-HBL	563.2±0.333	268.8±1.667	377.9±0.577	385.6±1.764	388.9±12.49	264.2±2.404	479.9±0.577	316.6±1.453
F value (HSD)	290.55*(10.07)	371.04*(7.43)	0.249 (156.30)	2653.22*(9.91)	144.28*(30.08)	904.55*(8.83)	199.55*(3.28)	9458.79*(6.80)

*statistically significant values at $P \leq 0.05$. DW = Distilled water, HBL = Homobrassinolide, HSD = Honestly significant difference, S.E. = Standard error of the mean

Table 2: Effect of 28-homobrassinolide on protein content (mg/g f.w.) in 60 and 90 days old shoots of *Raphanus sativus* L. plants grown under Hg metal stress (mean±S.E.)

Treatments	Protein content (mg/g f.w.)							
	Control (DW)		0.5 mM Hg		1.0 mM Hg		1.5 mM Hg	
	60 days	90 days	60 days	90 days	60 days	90 days	60 days	90 days
Control (DW)	0.434±0.007	0.239±0.001	0.299±0.08	0.23±0.018	0.378±0.01	0.307±0.016	0.356±0.02	0.265±0.007
10^{-11} 28-HBL	0.372±0.016	0.211±0.005	0.376±0.013	0.248±0.009	0.295±0.007	0.238±0.003	0.449±0.006	0.212±0.007
10^{-9} 28-HBL	0.328±0.01	0.198±0.003	0.396±0.013	0.182±0.002	0.407±0.007	0.209±0.007	0.353±0.01	0.287±0.032
10^{-7} 28-HBL	0.314±0.02	0.216±0.013	0.417±0.002	0.238±0.005	0.408±0.004	0.238±0.008	0.283±0.002	0.32±0.004
F value (HSD)	15.77*(0.064)	6.038* (0.033)	42.17*(0.037)	8.492* (0.048)	70.35*(0.032)	19.41* (0.045)	2.623 (0.141)	7.184* (0.081)

*statistically significant values at $P \leq 0.05$. DW = Distilled water, HBL = Homobrassinolide, HSD = Honestly significant difference, S.E. = Standard error of the mean

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Table 3: Effect of 28-homobrassinolide on glutathione-s-transferase activity (unit activity/mg protein) in 60 and 90 days old shoots of *Raphanus sativus* L. plants grown under Cd metal stress (mean±S.E.)

Treatments	GST activity (unit activity/mg protein)							
	Control (DW)		0.5 mM Cd		1.0 mM Cd		1.5 mM Cd	
	60 days	90 days	60 days	90 days	60 days	90 days	60 days	90 days
Control (DW)	0.058±0.025	0.05±0.013	0.074±0.023	0.20±0.05	0.076±0.023	0.28±0.082	0.113±0.021	0.11±0.24
10^{-11} 28-HBL	0.065±0.014	0.103±0.017	0.066±0.004	0.181±0.023	0.12±0.024	0.114±0.038	0.059±0.01	0.179±0.025
10^{-9} 28-HBL	0.044±0.017	0.077±0.005	0.058±0.017	0.072±0.007	0.121±0.027	0.0858±0.014	0.094±0.031	0.116±0.005
10^{-7} 28-HBL	0.040±0.018	0.07±0.01	0.064±0.024	0.048±0.014	0.090±0.044	1.487±0.257	0.086±0.017	0.138±0.013
F value (HSD)	0.454 (0.088)	0.836 (0.101)	0.040 (0.100)	7.169*(0.071)	0.773 (0.463)	24.117*(0.652)	1.613* (0.962)	11.835*(0.074)

*statistically significant values at $P \leq 0.05$. DW = Distilled water, GST = Glutathione-s-transferase, HBL = Homobrassinolide, HSD = Honestly significant difference, S.E. = Standard error of the mean

Table 4: Effect of 28-homobrassinolide on glutathione-s-transferase activity (unit activity/mg protein) in 60 and 90 days old shoots of *Raphanus sativus* L. plants grown under Hg metal stress (mean±S.E.)

Treatments	GST activity (unit activity/mg protein)							
	Control (DW)		0.5 mM Hg		1.0 mM Hg		1.5 mM Hg	
	60 days	90 days	60 days	90 days	60 days	90 days	60 days	90 days
Control (DW)	0.012±0.003	0.09±0.01	0.144±1.016	0.211±0.014	0.139±0.02	0.113±0.01	0.094±0.023	0.088±0.006
10^{-11} 28-HBL	0.192±0.004	0.029±0.011	0.112±0.032	0.129±0.018	0.035±0.005	0.138±0.026	0.145±0.02	0.303±0.051
10^{-9} 28-HBL	0.166±0.027	0.144±0.008	0.06±0.005	0.071±0.008	0.008±0.002	0.065±0.005	0.1±0.037	0.032±0.009
10^{-7} 28-HBL	0.162±0.027	0.193±0.03	0.198±0.005	0.04±0.01	0.074±0.007	0.103±0.012	0.012±0.003	0.239±0.032
F value (HSD)	18.07* (0.092)	46.97*(0.049)	4.409 (0.137)	32.71*(0.063)	27.18* (0.052)	4.168*(0.071)	6.455* (0.102)	16.51*(0.157)

*statistically significant values at $P \leq 0.05$. DW = Distilled water, GST = Glutathione-s-transferase, HBL = Homobrassinolide, HSD = Honestly significant difference, S.E. = Standard error of the mean

Table 5: Effect of 28-homobrassinolide on polyphenol oxidase activity (unit activity/mg protein) in 60 and 90 days old shoots of *Raphanus sativus* L. plants grown under Cd metal stress (mean±S.E.)

Treatments	PPO activity (unit activity/mg protein)							
	Control (DW)		0.5 mM Cd		1.0 mM Cd		1.5 mM Cd	
	60 days	90 days	60 days	90 days	60 days	90 days	60 days	90 days
Control (DW)	1.562±0.068	1.304±0.028	2.397±0.064	1.956±0.036	2.573±0.065	2.455±0.044	2.152±0.172	1.779±0.046
10^{-11} 28-HBL	0.0641±0.019	2.312±0.022	1.573±0.062	2.356±0.024	1.556±0.115	1.152±0.054	1.528±0.017	2.205±0.025
10^{-9} 28-HBL	0.137±0.025	0.929±0.025	1.686±0.053	1.333±0.04	2.381±0.068	1.457±0.059	1.347±0.047	1.091±0.039
10^{-7} 28-HBL	1.316±0.026	2.686±0.008	2.961±0.05	1.836±0.016	0.869±0.03	1.252±0.052	2.141±0.056	1.466±0.073
F value (HSD)	147.92*(0.189)	1355.58*(0.107)	331.49*(0.27)	830.83*(0.141)	107.454*(0.362)	129.23*(0.251)	1.613 (0.962)	94.268*(0.232)

*statistically significant values at $P \leq 0.05$. DW = Distilled water, HBL = Homobrassinolide, HSD = Honestly significant difference, PPO = Polyphenol oxidase, S.E. = Standard error of the mean

M and 10^{-11} M 28-HBL treated plants showed increased plant activity respectively, while PPO activity decreased in case of 10^{-7} M 28-HBL treatment to these plants as compared with respective metal treated control plants. Similarly, among 90-days old plants also, plants treated with Hg metal concentrations as compared with control had elevated levels of PPO activity. In 1.5 mM Hg treated plants application of 10^{-7} M HBL and 10^{-11} M HBL enhanced the enzyme activity as compared with only metal-treated plants [Table 6].

DISCUSSION

To neutralize the oxidative stress generated under a variety of abiotic and biotic stresses, plants are equipped with several antioxidants which are directly involved in the scavenging and neutralization of ROS.[18] Another strategy of defense to plants includes certain secondary metabolites and plant growth regulators. They include abscisic acid (ABA), ethylene, auxin, jasmonic acid, and plant hormones. Plant hormones such as auxins, ABA, polyamines and BRs regulate key metabolic responses of plant growth and development, and these have also been recently found to work as vital component of stress management. Along with growth-promoting effects, BRs also confer resistance to plants against various abiotic and biotic stresses like heat, drought, heavy metals, infections, pesticides, salt, and even viruses. These modulate the antioxidative defence system of plants facing stress and thus help in stress amelioration.[19]

Toxic heavy metals and metalloids do not biodegrade and are accumulated in organisms leading to certain diseases and

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Table 6: Effect of 28-homobrassinolide on polyphenol oxidase activity (unit activity/mg protein) in 60 and 90 days old shoots of *Raphanus sativus* L. plants grown under Hg metal stress (mean±S.E.)

Treatments	PPO activity (unit activity/mg protein)							
	Control (DW)		0.5 mM Hg		1.0 mM Hg		1.5 mM Hg	
	60 days	90 days	60 days	90 days	60 days	90 days	60 days	90 days
Control (DW)	0.583 ± 0.03	1.898 ± 0.171	0.854 ± 0.08	2.135 ± 0.108	0.967 ± 0.02	2.183 ± 0.191	0.937 ± 0.09	2.051 ± 0.056
10^{-11} 28-HBL	0.8 ± 0.059	3.02 ± 0.066	2.066 ± 0.047	2.495 ± 0.154	1.054 ± 0.043	1.987 ± 0.141	2.291 ± 0.079	2.73 ± 0.347
10^{-9} 28-HBL	0.963 ± 0.036	3.147 ± 0.098	1.315 ± 0.064	2.537 ± 0.185	1.091 ± 0.086	2.255 ± 0.108	0.908 ± 0.157	1.422 ± 0.029
10^{-7} 28-HBL	0.182 ± 0.023	3.527 ± 0.146	0.748 ± 0.048	2.241 ± 0.09	0.586 ± 0.056	1.509 ± 0.056	0.166 ± 0.023	2.69 ± 0.098
F value (HSD)	68.13* (0.196)	30.59* (0.61)	87.07* (0.307)	2.082 (0.648)	16.79* (0.027)	6.349* (0.637)	78* (0.479)	11.42* (0.874)

*statistically significant values at $P \leq 0.05$. DW = Distilled water, HBL = Homobrassinolide, HSD = Honestly significant difference, PPO = Polyphenol oxidase, S.E. = Standard error of the mean

disorders. These impose negative impact on soil microflora, ground cover as well as on plant growth.[20] Heavy metals toxicity in plants have emerged as an important field of research in recent decades as a result of the increased environmental pollution. The results of the present study revealed the toxic effects of both Cd and Hg metal on radish plants and the role of 28-HBL to protect the plants from metal toxicity by modulating the biochemical parameters of plant growth. Heavy metal toxicity induces decreased growth and lowered contents of chlorophyll (Chl). Reduced root and shoot length in radish plants subjected to chromium (Cr) metal toxicity have been reported. Contents of total Chl, Chl A and B have also been observed to lower in Cr metal-treated plants.[21]

Decrease in protein content and increase in enzyme activities in presence of Cd and Hg metal shows the subsequent effects of metal phytotoxicity. Changes in the total soluble protein contents are taken as indicators of the physiological status as well as of the reversible or irreversible metabolic changes of the plant.[22] *Raphanus sativus* grown under copper and lead stress elicited an antioxidative response, measured in terms of lipid peroxidation, protein and proline accumulation and peroxidase and proline accumulation and altered protein content.[23] In the present study, treatment of metal stressed *R. sativus* plants with 28-HBL considerably enhanced the protein content. An increase in protein with the application of BRs has been related to antistress protective mechanism of plants. Similar was the observation in Zn stressed *Brassica juncea* plants, in which 28-HBL application led to an increase in protein levels.[24] It is postulated that BRs alter the activity of proteins and other enzymes within the membrane either by affecting protein conformation or protein activity by direct protein-sterol interactions.[25]

Increase in activities of antioxidative enzymes *viz.,* GST and PPO under Cd and Hg metal stress in the present study is consistent with the study of Vitoria *et al*.,[26] in which four *Raphanus sativus* varieties (cv. Redondo Vermelho, Comprido Vermelho, Grinson Gigante, and ScarlettGlobe) were grown under $CdCl_2$ stress and activities of superoxide dismutase (SOD), glutathione reductase (GR), and catalase (CAT) were found to increase. In another experiment, the effect of different levels of mercury treatments (0, 5, 10, 25, and 50 µM Hg) on Indian mustard (*Brassica juncea* L. Czern and Coss.) cv. Pusa Jai Kisan in a hydroponic system was studied. It was observed that inhibitory effect of mercury on the growth parameters *viz.,* shoot and root length, leaf area, and plant dry mass, increased with increase in the metal concentration and oxidative stress characteristics such as the malondialdehyde (MDA) and H_2O_2 levels also increased. Maximum inhibitory effect was measured at concentration 50 µM of Hg. An increase in activities of antioxidant enzymes such as SOD, CAT, ascorbate peroxidise (APX), and GR was also witnessed.[27]

In an experiment carried out by Mitrovic *et al*.,[28] the effects of different concentrations of anatoxin-a (bicyclic amine alkaloid) on GST in *L. minor* were evaluated and GST activity found to get significantly elevated at concentrations of 5 and 20 µg/mL. Chun-hua and Ying[29] studied the effects of Cd exposure to rice seedlings and reported correlation between increasing Cd concentrations and increased level of GSH and GST. GSH, plays a pivotal role in the processes of detoxification and redox buffering. GSTs, catalyze the conjugation of GSH to an electrophilic substrate.[30] Lead-dependent increase in activities of SOD, CAT, APX, GST and peroxidise (POD) from wheat seedling extracts was observed at 0.15, 0.30, 1.5 and 3.0 mM of lead concentration.[31]

In plants submitted to stress, PPO acts as a defence mechanism which gets activated.[32] In cucumber seedlings, after a stress stimulus, enhanced activity of resistance-related enzymes, including PPO was observed. The defence mechanism activation also improved resistance of cucumber seedlings to pathogen infection.[33] A study was undertaken to estimate the ameliorating effect of triadimefon (TDM) on drought stress in sunflower (*Helianthus annuus*) plants and it was observed that the activity of PPO increased as compared to control.[34]

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The consequent increase in level of GST and PPO activity in plants supplemented with metal and 28-HBL both as compared with plants given metal stress alone signifies the diminution of stress effects of Cd and Hg on *R. sativus* plants via 28-HBL. This is in accordance with the study in which the Cd metal stress protective role of 28-HBL on growth, photosynthesis and activities of carbonic anhydrase, nitate reductase as well as antioxidant enzymes in *B. juncea* plants were studied by Hayat *et al*.[35] It was observed that the toxic effects of Cd were reduced with 28-HBL spraying treatment via increase in plant growth and enhanced enzyme activities. The foliage application of 28-HBL or supply through roots of *Brassica juncea* enhanced the growth, nucleic acid content, ethylene, and seed yield in plants generated from the seeds soaked in NaCl.[36] The plants of *Vigna radiata* were exposed to high temperature and/or NaCl and a significant decline in growth, photosynthetic parameters, and a maximum quantum yield of PSII was observed. Treatment with 28-HBL amended the stress generated by high temperature and/or NaCl and improved the growth parameters.[37]

CONCLUSION

The decrease in protein content and increase in GST and PPO activity under Cd and Hg metal stress in the present investigation signifies the toxic effects of these heavy metals. In plants raised after pretreatment of seeds with 28-HBL, the increase in protein content as well as in activities of antioxidative enzymes, the stress ameliorative role of 28-HBL is strengthened. The harmful effects of heavy metals on *R. sativus* plants, grown as horticulture crop as well as a medicinal plant, thus may be abridged with the application of 28-HBL.

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How to cite this article: Sharma N, Hundal GS, Sharma I, Bhardwaj R. 28-Homobrassinolide alters protein content and activities of glutathione-S-transferase and polyphenol oxidase in *Raphanus sativus* L. plants under heavy metal stress. Toxicol Int 2014;21:44-50.

Source of Support: Nil. **Conflict of Interest:** None declared.

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