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# Activation Of Metabolic Pathways Associated With Phenolic Biosynthesis In Garden Cress Leaves Under Lead Stress.

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**ABSTRACT**

In the present study the effect of lead stress on the primary (shikimic acid) and secondary (phenylpropanoids) metabolic pathways was investigated in leaves of garden cress (*Lepidium sativum* L.). The production of hydrogen peroxide ($H_2O_2$) and malondialdehyde (MDA) increased under lead stress in leaves of garden cress. The activity of NADH-oxidase (EC*:* 1.6. 3.3) is elevated continuously and correlated with the increase of both $H_2O_2$ and MDA. Shikimic acid, the total phenolics, the total flavonoids and anthocyanin accumulated in response to lead stress. The activity of NADP-shikimate dehydrogenase (SKDH, EC 1.1.1.25) increased and correlated with the enhancement of shikimic acid production. The activities of phenylalanine ammonia lyase (PAL, EC*:* 4.3. 1.24), chalcone synthase (CHS, EC*:* 2.3.1.74) and chalcone isomerase (CHI, EC: 5.5.1.6) raised under the same treatment and correlated with the increase of total phenolics, total flavonoids and anthocyanin. The antioxidant activity in leaves was induced by lead stress in a concentration-dependent manner as judged by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and ferric reducing antioxidant power (FRAP) assay. Thus, it seems likely that the application of abiotic stresses is a valid strategy to enhance the primary and secondary metabolism of plants. If certain specific primary or secondary metabolites were required, it would be suggested to apply stresses to hasten their biosynthesis.

**Keywords:** Lead, *Lipidium sativum*, Hydrogen peroxide, Malondialdehyde, Shikimic acid, Phenolics, Flavonoids, Anthocyanin, Enzymes.

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## INTRODUCTION

Medicinal plants are used as raw materials for pharmacological industries and home therapies [1]. In developing countries pollution of irrigation water as well as contamination of soil with heavy metals play an important part in the contamination of medicinal plants [2, 3,4].

Exposure of plants to heavy metals causes accumulation of dangerous reactive oxygen species (ROS). Plants can counteract the toxic effects of heavy metal stress through alteration of physiological processes and certain metabolic activities. These defend the plant against ROS and avoid damage to plant molecules including proteins, lipids and nucleic acids. Among these alterations is accumulation of secondary metabolites including alkaloids, saponins, tannins, phenolic compounds, flavonoids, anthocyanins and terpenoids [5].

The shikimate pathway in plants initiates the synthesis of the three aromatic amino acids phenylalanine, tyrosine and tryptophan [6,7]**.** This pathway is essential for biosynthesis of protein and for secondary metabolism. Plant phenolic and their precursors are synthesized through biosynthetic pathway of shikimate and its various branch points [7, 8].

Plant secondary metabolites are regular sources of active compounds consumed in traditional medicine and in a broad array of industrial purposes [9]. The content of secondary metabolites is altered by environmental, genetic and agronomic factors [10]**.** Treatment of plants with biotic and abiotic stresses is the most applicable means to improve production of secondary metabolite [11].

Heavy metal may stimulate changes in the phenolic compounds that may alter their functions in plant cells. The phenolic compounds are commonly involved in responses to various types of biotic and abiotic stresses. Exposure of humans to lead occurs mostly in lead related activities with various sources including industrial processes such as lead combustion, grids, printing of books**,** boat building, lead smelting process, arm industry, pottery, recycling of battery and pigments [12].

Garden cress or *Lepidium sativum* L. belongs to family Cruciferae, edible and is fast-growing. The seeds, roots and leaves of this plant are therapeutic in nature and applied in cooking since they are tremendously nutritious. Garden cress aids to purify the blood, promote appetite and relieve the anemia because it contains elevated content of iron. Therefore, The present research aimed to investigate the effects of lead stress on both primary and secondary metabolism in garden cress leaves, which were evaluated by the accumulation of primary (shikimic acid) and secondary metabolites (phenolic compounds) and the enzymes involved in their biosynthetic pathways.

## MATERIALS AND METHODS

### Experimental plant

Seeds of garden cress (*Lepidium sativum* L.) were obtained from the medicinal and aromatic Plants Department, Horticulture Research Institute, Agriculture Research Center, Egypt.

### Treatment of garden cress plants with $PbCl_2$

The effect of $PbCl_2$ toxicity on garden cress metabolites and enzymes was assessed using different concentrations of $PbCl_2$ (25, 50, 75, 100 and 125 µM). $PbCl_2$ was obtained from Sigma, St. Louis, MO, USA and prepared for a stock solution. The working concentrations were made from the standard stock solution. The plant seeds were surface sterilized with 2% sodium hypochlorite (NaOCl) for 10 min and then washed several times with distilled water. Seeds were treated with the different concentrations of $PbCl_2$ for 5 days. The control samples were made using distilled water in Petri plates (9 cm) covered with filter paper. All the treatments were carried out in triplicates. After the 5 days uniformly seedlings were chosen and moved to hydroponic culture with half strength Hoagland's solution. The seedlings were grown under light/dark regime of 16/8 h at 25/30 °C for 10 days. The 15-day old plants were collected and analyzed.

**Determination of hydrogen peroxide ($H_2O_2$) content**

The $H_2O_2$ content was determined by the method of [23]. Leaf sample (1g) was extracted in 0.2% trichloroacetic acid (TCA) solution. The resulting homogenate was filtered and 1 ml of 150 mM potassium phosphate buffer (pH 6.5) and 1.5 ml reagent (1M KI dissolved in distilled water) were mixed with 1 ml of leaf extract. The blank sample composed of 0.2% TCA without leaf extract. The mixture was left in darkness for 1h and then the absorbance was recorded at 390 nm. The standard curve was prepared with various concentrations of $H_2O_2$. The content of $H_2O_2$ was expressed as µmol $g^{-1}$ fresh weight.

**Determination of malondialdehyde (MDA) content**

Determination of MDA was carried out by the method adopted by [24]. Fresh leaves (5 g) were homogenized in 5 ml of 5% TCA. The homogenate was centrifuged at 12,000 g for 15 min. The reaction medium contained 0.5 ml of the resulted supernatant, 1 ml 15% TCA and 1 ml 0.5% TBA (thiobarbituric acid). The mixture was then incubated in a water bath at 95 °C for 30 min, cooled, centrifuged followed by recording the absorbance at 450, 532 and 600 nm and then MDA content was calculated according to[24].

**Determination of shikimic acid content**

Shikimic acid content was determined according to the method of [13]. Leaf samples (5g) were homogenized in 300 mM HCl (1 ml per 100 mg of plant leaves). The homogenate was centrifuged at 12,000 g for 20 min. A supernatant (100 µl) was added to 0.5 ml of 2% periodic acid and left for 2h at room temperature. Aliquot of 1 ml of 1 M NaOH and 0.5 ml glycine (100 mM) were added per sample. The samples were then centrifuged at 12,000 g for 20 min and the absorbance was recorded at 380 nm. The content of shikimic acid was expressed as µg $g^{-1}$ dry weight of plant leaves.

**Determination of total phenolic content**

Total phenolic content was determined in garden cress leaf following the method of [14]. Leaf sample (5 g) was pulverized in liquid nitrogen and extracted by shaking with methanol solution (100%) at room temperature for 4 h. The extract was then centrifuged for 20 min at 12,000 g and 4 °C. The reaction medium consisted of 0.5 ml of leaf extract, 1ml distilled water, 400 mM $Na_2CO_3$ and 1 ml of phenol reagent. The reaction mixture was incubated for 10 min at 40 °C, followed by cooling and leaving in dark for 120 min at room temperature. The absorbance was recorded at 765 nm spectrophotometrically. The standard curve for the total phenolic content was carried out using gallic acid. The total phenol content was calculated in mg gallic acid equivalent $g^{-1}$ dry weight of plant leaves.

**Determination of total flavonoid content**

The content of total flavonoids was determined by the method of [15]. Determination of total flavonoids content with $AlCl_3$ reagent is dependent on appearance of yellow color complex between $Al^{3+}$, hydroxyl and carbonyl groups from flavonoids. Leaf samples (0.5 g) were pulverized in liquid nitrogen followed by 80% methanol. The reaction mixture contained 100 µl aliquot of the extract, 2.9 ml methanol solution, 1ml of 10% sodium potassium tartrate, 1 ml of 10% $AlCl_3$ and 1 ml distilled $H_2O$ and the mixture was robustly shaken. The absorbance was recorded at 415 nm spectrophotometrically. The standard curve for total flavonoids was prepared by quercetin. The results were expressed as mg quercetin equivalent $g^{-1}$ of dry weight of plant leaves.

**Determination of anthocyanin content**

Anthocyanin was extracted in 5 ml of methanol-HCl (1% v/v HCl). The extract was kept at in the refrigerator 4 °C for 48 h, filtered and the total anthocyanin content was determined spectrophotometrically as the difference between OD530 and OD657 nm then applied in the formula of A530-A657 for elimination of chlorophyll content and defined as OD 530 $g^{-1}$ fresh weight [16].

**Preparation of enzymes extract**

Plant leaves (5 g) were homogenized in 20 ml of 50 mM phosphate buffer (pH 7.0) contained 1 mM cysteine and 1% polyvinylpolypirrolidone (PVP). The obtained homogenate was centrifuged at 10,000g for 20 min at 4°C. The supernatant was used as crude enzymes extract and used for determination of the enzymes activities [17].

**Assay of NADH oxidase (EC*:* 1.6. 3.3) activity**

The activity of NADH oxidase was determined in a mixture that contained 150 mM sodium acetate buffer (pH 6.5), 1 mM *p*-coumaric acid, 5 mM $MnCl_2$, 5 mM NADH and enzyme preparation. The decrease in absorbance at 340 nm was recorded with extinction coefficient of 6.22 $mM^{-1}$ $cm^{-1}$ according to [25].

**Assay of NADP- shikimate dehydrogenase (SKDH, EC 1.1.1.25) activity**

The assay of SKDH activity was done spectrophotometrically by the method of [18] at 340 nm through production of NADPH due to shikimic acid oxidation to dehydroshikimic. The reaction mixture consisted of 0.1 mol $L^{-1}$ Tris-HCl (pH 9.0), 4 mmol $L^{-1}$ shikimic acid and 2 mmol $L^{-1}$ NADP.

**Assay of phenylalanine Ammonia-Lyase (PAL, EC: 4.3. 1.24) activity**

PAL activity was measured according to [19]. The PAL activity was assayed spectrophotometrically by measuring trans-cinnamic acid produced from L-phenylalanine at 40 °C. Enzyme extract (0.1 ml) was incubated with 0.1 ml of 10 mM L-phenylalanine prepared in 100 mM of Tris-HCl, (pH 8.0). After 10 min the trans-cinnamic acid produced was determined by measuring the increase in absorbance at 290 nm. The standard curve was done using a trans-cinnamic acid standard. The activity of PAL was expressed as units (U) $mg^{-1}$ protein.

**Assay of chalcone synthase (CHS, EC: 2.3.1.74) activity**

The CHS activity was determined spectrophotometrically according to [20]. Leaf sample (5 g) was homogenized in 100 mM borate buffer (pH 8.5) containing 2 mM mercaptoethanol. The resulting homogenate was treated with 0.2 g of Dowex for 15 min and then centrifuged for 10 min at 10,000 g. Dowex resin (0.2 g) was added to the resulting supernatant and left for 15 min. The resin was separated by centrifugation at 15,000 g for 15 min. The obtained supernatant represented crude extract for CHS assay. The assay medium contained 0.1 ml enzyme extract, 2 ml of 100 mM Tris-HCl buffer (pH 7.5) and 15 mM KCN. CHS reaction was left to advance at 30°C for 1 min after the addition of chalcone (10 mg in 10 µl ethylene glycol monomethyl ether). The CHS activity was estimated through measuring absorbance at 370 nm.

**Assay of chalcone isomerase (CHI, EC: 5.5.1.6)**

The activity of CHI was determined by the adopted method of [21]. Leaf sample (5 gm) was extracted in 100 mM Tris-HCl (pH 7.5) at 4°C. Aliquot of the extract (0.1 ml ) was added to a solution contained 100 mM Tris-HCl (pH 7.5), 15 mM KCN, and 0.02 ml of alcoholic solution of iso-liquiritigenin (1 mg $ml^{-1}$ of 70% ethanol). CHS activity was estimated by recording the decrease in absorbance at 395 nm at 27°C for 15 min.

**Protein determination**

Protein content of the extracts was determined by the method of [22] and bovine serum albumin was used as standard.

**2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity**

The DPPH scavenging activity was determined by the method of [26]. DPPH (0.1 mM) was prepared in methanol and the absorbance was recoded at 515 nm. An aliquot (0.05 ml) of leaf extract was added to 3 ml of DPPH solution. The change in absorbance at 515 nm was recorded after 30 min. The antioxidant activity was determined according to the following equation:

% Antioxidant activity = 100 − [(Abs of sample – Abs of empty sample)] × 100)/Abs of control. The optic density of the sample, the control and the empty sample were determined in comparison with the methanol. *α*-tocopherol (vitamin E) and butylated hydroxytoluene (BHT) were used as positive controls.

**Ferric reducing antioxidant power assay (FRAP)**

FRAP assay was done according to [27]. The FRAP reagent was prepared by mixing 200 mM acetate buffer, 10 mM 2,4,6-tripyridyl-s- triazine (TPTZ ) in 50 mM HCl and 30 mM $FeCl_3$ solution, respectively. Aliquot of 3 ml of FRAP reagent was mixed with 0.025 ml of leaf extract and incubated at 37 °C for 15 min in water bath. The absorbance was recorded at 593 nm. Calibration curve was done using $FeSO_4$ and the antioxidant activity was calculated.

**Statistical analysis**

All values are the means of three determinations ± S.E.

## RESULTS AND DISCUSSION

Plants exhibit a number of physiological alterations under heavy metal stress [3]. The reaction of the heavy metal with sulfhydryl group (-SH)-containing amino acids or proteins plays an important part in their biochemical and environmental behavior. The plants vary in their capability to alter their metabolism to endure or store heavy metals. The alterations include biosynthesis of certain organic compounds which can detoxify the heavy metal or sequestration of the metal in their vacuoles [28].

The results in the present investigation indicate that $H_2O_2$ as one of reactive oxygen species (ROS) increased incessantly in a concentration dependent way (Fig. 1). $H_2O_2$ is a harmful but it has signaling parts in plant cells [29]. Experimental attention was focused on $H_2O_2$ and revealed to be tightly implicated in cell death and immunity [29,30] responses to environmental stress [31] and controlling of the stomatal aperture [32]. The main outcome of heavy metal toxicity is production of ROS because of impairing the photosynthesis by the heavy metal. In addition, ROS are harmful to plants because they cause oxidation of large macromolecules and cell membranes [32]. ROS are reported as regulators for development of plants and involved in signaling for the changes in the environmental [33, 34].

Production of MDA increased in leaves of garden cress with the rise of lead concentration (Fig. 2). MDA presents a cytotoxic product of lipid peroxidation and is a marker for production of free radicals and tissue damage. Lipid peroxidation is associated with production of ROS particularly superoxide radical ($O_2^{\cdot-}$) [35].

NADH-oxidase has been reported to play an important role in the production and accumulation of ROS in plants under stress conditions. The activity of this enzyme increased in leaves of garden cress under lead treatment (Fig. 3) in a concentration-dependent manner and correlated with the increase in $H_2O_2$.

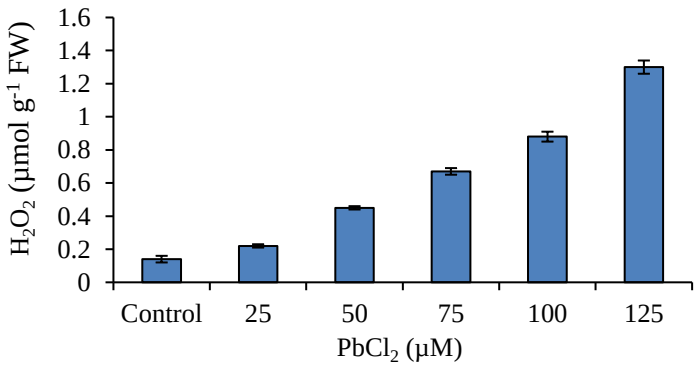


**Fig.1: Effect of lead stress on $H_2O_2$ content in garden cress leaves.**

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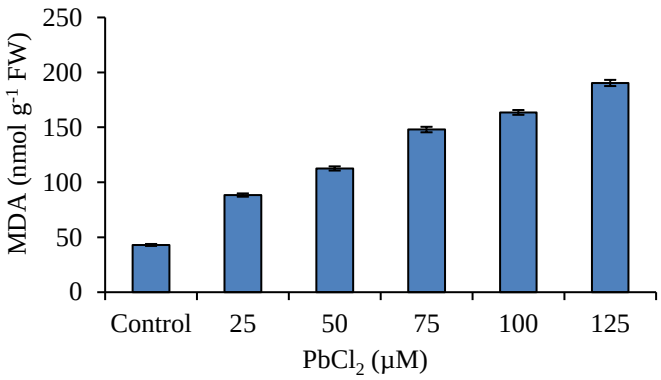


**Fig.2: Effect of lead stress on MDA content in garden cress leaves.**

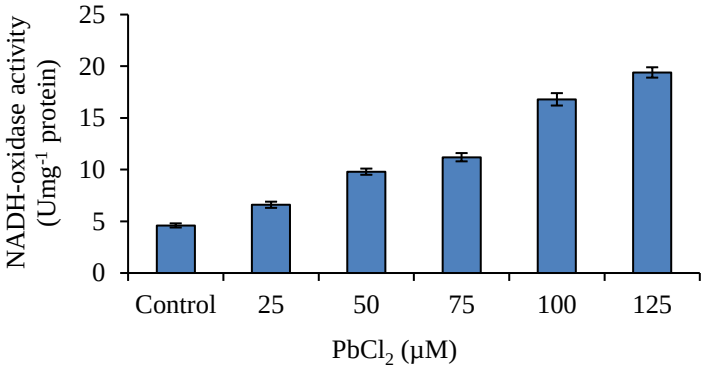


**Fig.3: Effect of lead stress on NADH-oxidase activity in garden cress leaves.**

The level of shikimic acid in garden cress leaves increased as shown in Fig. 4 and this increase was dependent on lead concentration. This is in agreement with the results reported by [45]**.** Other researchers reported similar results for the increase of shikimic acid for maize [46] and *Eucalyptus* [47] where plants were grown under water stress. SKDH an enzyme catalyzes the reversible reaction of 3-dehydroshikimate to shikimate, which is NADPH-dependent. The present results revealed the rise in SKDH activity under lead stress (Fig. 5). Induction of SKDH activity in lead-stressed leaves of garden cress was associated with the phenolic accumulation. It seems likely that the plant can reacts to lead stress through enhancing SKDH biosynthesis to alleviate the enzyme inhibition in vivo by lead. The present results are in agreement with those reported by [48] for the elevation in SKDH activity in pepper treated with cupper.

Plants possess defense mechanisms which persuade defensive responses to heavy metal stress, which defend plants from stress damage [36]. Plants have a strong antioxidant system to reduce oxidative stress. This system includes different enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), ascorbate peroxidase (APX). The non-enzymatic antioxidants comprise ascorbic acid, reduced glutathione, phenolics, carotenoids, flavonoids, alkaloids, non-protein amino acids and anthocyanin, which scavenge ROS production in plants under various stress conditions [37]. Sustaining an optimal ROS level in plant cells allows suitable reactions of redox biology and the control of several processes necessary for plants such as development and plant growth. This intermediate point is retained by the equilibrium between production of ROS and their scavenging.

The accumulation of antioxidant defense system with ROS sustains a steady-state balance in plant cells. However, throughout stress conditions, over-production of ROS destroys the equilibrium, triggers cellular damage and causes cell death in addition to reduction of the plant productivity. The antioxidant system enables plants to carry out their metabolism whether primary or secondary one and improve their metabolic capability to progress the adaptableness to stress [38]. In such case the accumulation of metabolites whether primary or secondary may improve plant adaptation to stress condition.

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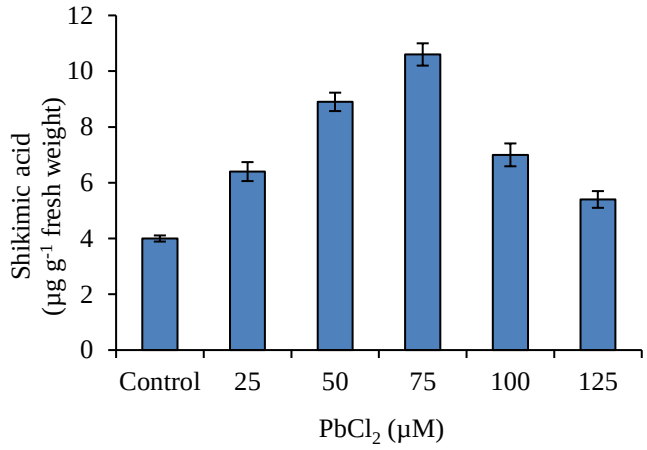


**Fig.4: Effect of lead stress on shikimic acid content in garden cress leaves.**

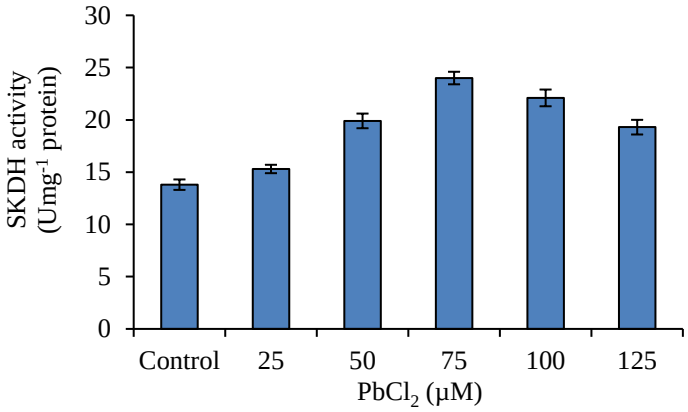


**Fig.5: Effect of lead stress on SKDH activity in garden cress leaves.**

The term phenolic or polyphenol is well-defined chemically as substances that contain an aromatic ring with one or more hydroxyl groups. Polyphenols have many various biological activities such as regulation of enzymes and antioxidant behavior. Numerous studies have reported the association between the antioxidant activity and the total phenolics content. The phenolics in plant cells are believed the most descriptive of the bioactive compounds with this activity [39]. Radical scavenging is a probable mechanism for reduction of oxidative stress by these compounds. In the present work the phenolic compounds as antioxidants increased progressively with rising of lead concentration (Fig. 6). Plants that grow under stressful conditions have the capability to biosynthesize extra phenolic compounds if compared to their counterparts growing under regular conditions. Phenolic compounds are synthesized via shikimate pathway in the higher plants. Such compounds have antioxidant capability to scavenge the produced free radicals under stress leading to altering the kinetics of lipid peroxidation through modification of lipid packing order to reduce membranes fluidity and consequently protect plant cells from oxidative stress [39].

The increase of phenolic compounds was reported to be forced by enhanced expression of enzymes, accountable for biosynthesis of phenylpropanoids, such as shikimate dehydrogenase (SKDH), phenylalanine ammonia-lyase (PAL), chalcone isomerase (CHI), chalcone synthase (CHS) and cinnamyl alcohol dehydrogenase (CAD) [40]. Biosynthesis of phenolic compounds depends on the availability of the amino acid L- phenylalanine which is the precursor of phenylpropanoids biosynthesis [41]. Biosynthesis of L-phenylalanine occurs through the shikimic acid pathway, which represents a part of plant primary metabolism. The antioxidant capacity of phenolics is attributed to their power as electron donors, their property to delocalize the unpaired electrons and their ability to chelate transition metal ions by terminating the Fenton reaction. Radical scavenging activity of phenolics as antioxidants is largely manipulated by their structural and environmental features in vivo. The antioxidant phenolics acids are mainly composed of hydroxybenzoic and hydroxycinnamic acids; those show antioxidant activity as chelators and scavengers of free radicals [42].

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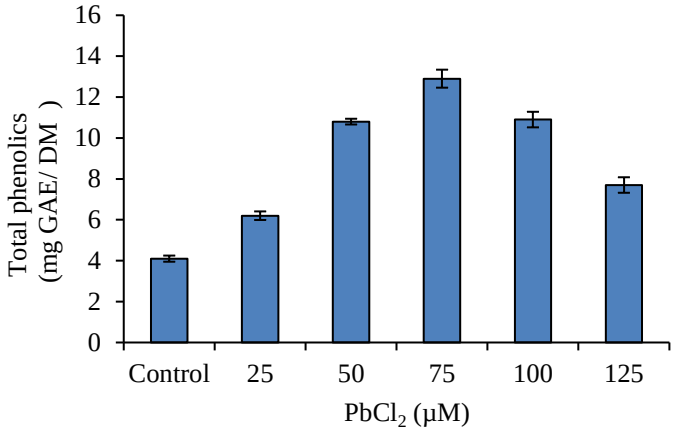


**Fig.6: Effect of lead stress on total phenolics in garden cress leaves.**

Flavonoids include a class of plentiful secondary metabolites, which participate by many ways in growth and survival of plants. Flavonoids possess positive results on human health. Studies on derivatives of flavonoids revealed a wide range of antioxidant, anticancer, antibacterial, anti-inflammatory and antiviral activities. They have been demonstrated to be very efficient scavengers of the main oxidizing agents such as singlet oxygen and different free radicals [43]. Flavonoids are well known by their scavenging capacity of $H_2O_2$ as one of ROS and playing a vital role in the phenolic/ascorbate-peroxidase cycle [44]. The flavonoids as antioxidant compounds rose with the increase of lead concentration (Fig. 7). Furthermore, abiotic stresses control genes expressions related to biosynthesis of flavonoids and stimulate antioxidant defense procedures. Low-molecular-weight flavonoids especially dihydroxy *β*-ring-substituted flavones as well as flavonols have important potential in scavenging the free radicals and reduction of cell destruction by lipid peroxidation [43]. Comparable with the reported results for other plant extracts it seems likely that that phenolics and flavonoids may be the two major contributors for free radical scavenging activity.

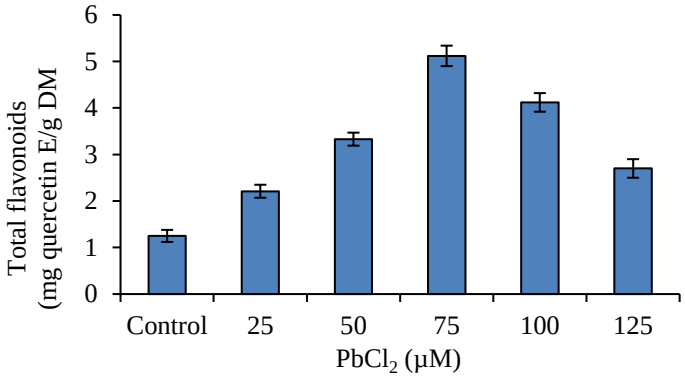


**Fig.7: Effect of lead stress on total flavonoids in garden cress leaves.**

Anthocyanins pigments represent a group of polyphenolic secondary products that are universally discovered in plant kingdom. Anthocyanins are discovered in cell vacuole, regularly in fruits, flowers, roots, stems and leaves [44]. Anthocyanin content increased under lead stress in a concentration-dependent manner (Fig. 8). However, the reduction of anthocyanin in treated plants at higher lead concentration might be due to their active obstruction to toxic effects of lead through mass deposition of metal complex in the vacuoles [44]. The biosynthesis of anthocyanin is split from the pathway of phenylpropanoid by the roles of different enzymes with considerable capacity in biotechnological applications [45]. Anthocyanin is a secondary metabolite developed from primary metabolites [46]. Thus, the rise in the accumulation of anthocyanin exhausts primary metabolites. Anthocyanin was reported to block the oxidation processes that occur naturally in plants [47]. Among the other functions of anthocyanin in plants are the defense against biotic as well as abiotic stresses and attracting insect for pollination [49]. The enhancement of anthocyanin under lead stress is reliable with the remarkable high activity of PAL.

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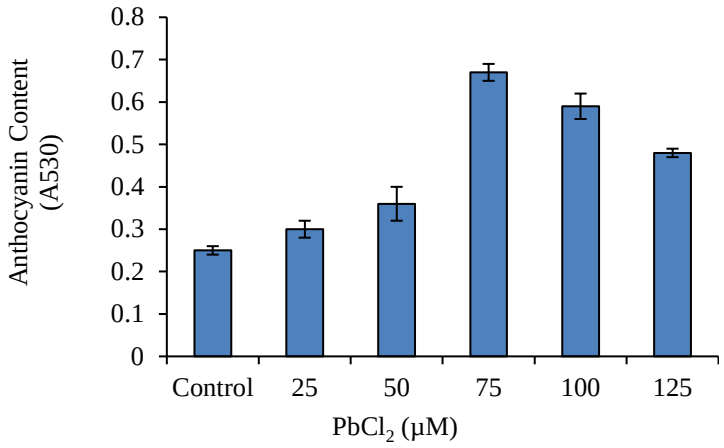


**Fig.8: Effect of lead stress on anthocyanin content in garden cress leaves.**

PAL activity (Fig. 9) was enhanced under lead stress at various concentrations and there was a correlation between PAL and the accumulation of total phenol. PAL is broadly distributed in plants, ferns, algae, and microorganisms. In plants PAL is the chief dedicated enzyme of the cinnamate-linked metabolisms. PAL catalyzes the conversion reaction of L-phenylalanine to trans-cinnamic acid and ammonia. PAL represents one of the few enzymes that transform amino acids and which do not comprise pyridoxal 5'-phosphate as the cofactor. PAL is involved in the biosynthesis of phenylpropanoids, flavonoids, and lignin in plants [50]. The results show that CHS activity was enhanced with an increase in lead concentration as demonstrated in Fig. 10.

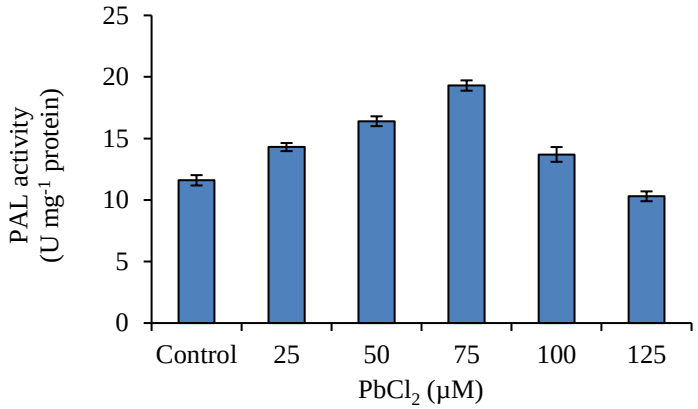


**Fig.9: Effect of lead stress on PAL activity in garden cress leaves.**

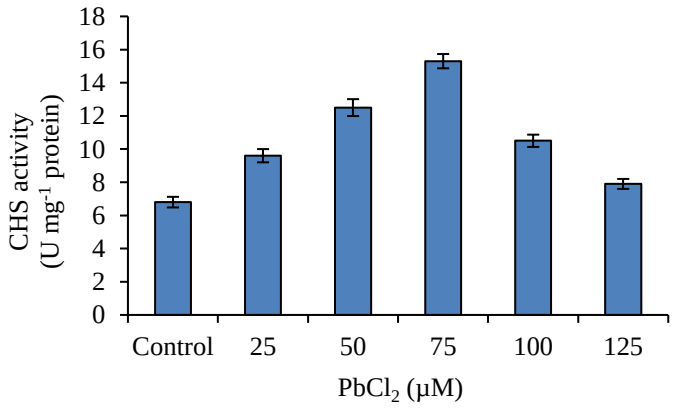


**Fig.10: Effect of lead stress on CHS activity in garden cress leaves.**

CHS is a key enzyme involved in biosynthetic pathway of flavonoid/iso-flavonoid. CHS gene expression is enhanced in plants under stress conditions. CHS expression leads to accumulation of flavonoid as well as iso-flavonoid phytoalexins and is participating in the salicylic acid defense pathway [51,52]. CHS in the present results expressed a remarkable correlation with the pattern of total flavonoids accumulation. Enhancement of PAL and CHS activities has been found in various plants subjected to lead and cadmium [53]. Thus, enhancement production of flavonoids and anthocyanin in the present work might be attributed to the increase in CHS activity. Ozeki et al [54] reported correlation of enhancing CHS activity with the accumulation of anthocyanin under various stress conditions. The second key enzyme in biosynthesis of flavonoids is CHI that catalyzes the isomerization of chalcone to (2S)-flavanones [55]. The CHI activity increased under led stress in a concentration-dependent manner and is in correlation with accumulation of anthocyanin (Fig. 11).

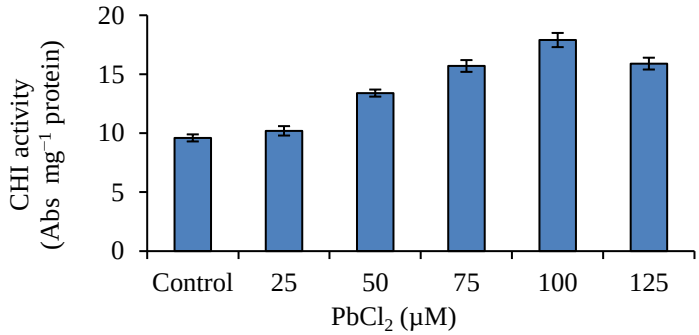


**Fig.11: Effect of lead stress on CHI activity in garden cress leaves.**

Numerous techniques have been managed to determine the antioxidant capacity in vitro to permit rapid selection of substances since compounds which possess low antioxidant capacity in vitro can possibly show slight activity in vivo. The DPPH method is built on scavenging of DPPH throughout the addition of a free radical or antioxidant that can decolorize DPPH solution. The intensity of the color change is related to the concentration as well as the effectiveness of the antioxidants. A reduction in the absorbance of the reaction mixture implies considerable scavenging capacity of the free radical by the substance under test [26]. The FRAP assay is a rapid, simple and somewhat and was developed as an inexpensive assay to estimate the antioxidant capacity of the plant extract. The present antioxidants in the extract reduce $Fe^3$-TPTZ complex to a blue colored complex of $Fe^2$-TPTZ. The variation in the absorbance exactly reveals the antioxidant capacity of the extract [27].

The results revealed induction of the antioxidant activity in garden cress leaves by lead treatment in a concentration-dependent manner as judged by DPPH scavenging activity (Fig. 12) and ferric reducing antioxidant power (FRAP) assays (Fig. 13). This might be attributed to the enhancement of secondary metabolites production predominantly, flavonoids, phenols and anthocyanin under the same treatment. The increase in antioxidant activity in leaves of garden cress under lead stress might be due to accumulation of phenols, flavonoids and anthocyanins and enhancing the activities of their biosynthetic enzymes.

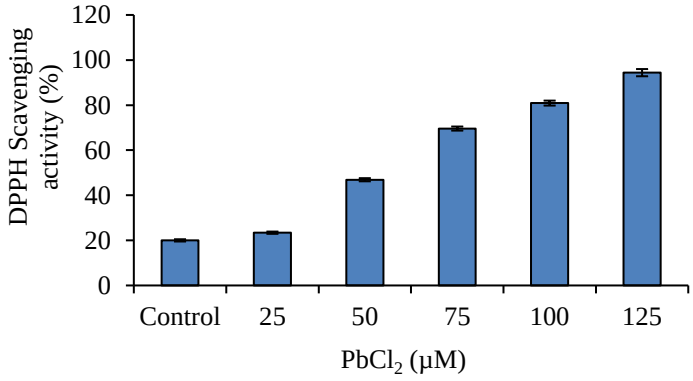


**Fig.12: DPPH scavenging activity of garden cress leaf extract under lead stress.**

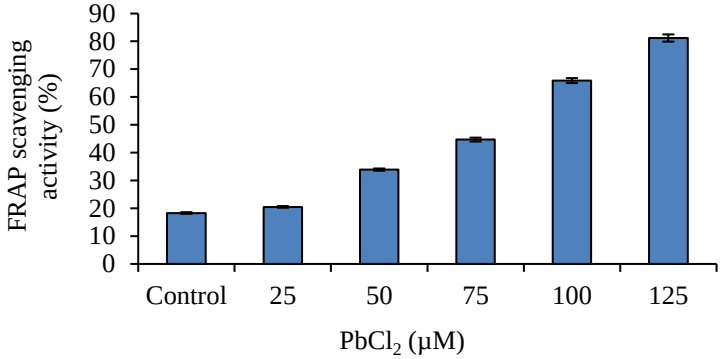


**Fig.13: FRAP scavenging activity of garden cress leaf extract under lead stress.**

## CONCLUSION

Lead stress enhanced both primary and secondary metabolism in garden cress leaves and this enhancement led to accumulation of shikimic acid, phenolics, flavonoids and anthocyanins. If the accumulation of a specific primary or secondary metabolite was desirable, it could be recommended to treat plants with lead stress to accelerate their biosynthesis.

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