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Research Article

Physicochemical Contents, Antioxidant Activities, and Acute Toxicity Assessment of Selenium-Enriched Chinese Kale (*Brassica oleracea* var. *alboglabra* L.) Seedlings

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The oxidative activities of hydroponically produced Chinese kale (*Brassica oleracea* var. *alboglabra* L.) seedlings grown under different concentrations of selenium (Se) biofortification with sodium selenite (Na_2SeO_3) at 0, 5, 10, 15, and 30 mg·Se/L and different ages (5, 10, and 15 days after germination) were determined by the electron transfer method (DPPH and FTC). Highest antioxidant activities of Se-enriched Chinese kale seedlings (Se-KS) were found in seedlings grown on 30 mg·Se/L, 15 days after germination. The physicochemical contents (moisture, crude fibre, ash, crude protein, and carbohydrate), total Se contents, and Se bioaccessibility and antioxidant activities (after treatment with an in vitro simulated gastrointestinal digestion process) between Se-KS and a control of regular Chinese kale seedlings (R-KS) were compared. The crude protein and total Se contents of the air-dried matter in Se-KS were higher (37 ± 1% and 433 ± 22 mg·Se/kg) than those of the R-KS (33 ± 2% and 17 ± 3 mg·Se/kg). Total Se content and antioxidant activities were strongly correlated in the range from 0.93 to 0.99. Testing acute toxicity, a single dose of the Se-KS was orally administrated to rats at 1250, 2500, and 5000 mg/kg·b.w., which consisted of 0.54, 1.08, and 2.16 mg Se/kg·b.w., respectively. These results demonstrated that Se-KS did not create any toxicological signs and mortality. No abnormal pathological changes in the liver, kidney, and heart were found. The results suggested that the Se-KS could be a good source of organo Se for development as a new Se supplementary product.

1. Introduction

Selenium (Se) is an essential trace element which accomplishes important functions for animal and human health that are relevant to several pathophysiological conditions. Its deficit can cause many disorders, but an overdose can also lead to severe consequences [1]. The functions of Se in the human body are mainly associated with essential roles in the function of important antioxidant enzymes, as well as being involved in major metabolic pathway and immune function [2]. Se plays a crucial role in the component of glutathione peroxidases (GSH-Px), thioredoxin reductase (TRx), iodothyronine deiodinases, selenophosphate synthetase, selenoprotein P, and other selenoproteins [1–3]. It helps to control the intracellular redox state via the family of Se-dependent GSH-Px. Benefits of the redox function of Se-dependent GSH-Px mean the protection of cell membranes against oxidation by reduction of the oxidative damage to biomolecules such as lipids, lipoproteins, and DNA [3]. This damage forms the basis of a wide variety of diseases, most notably cardiovascular disease and cancer [2, 3]. Consequently, in recent years, Se research has attracted

tremendous interest because of its important role in antioxidative processes [3]. Several plants and some types of yeast can produce organo Se by converting Se to selenomethionine (Se-Met) and incorporating it into protein and enzyme in place of methionine [4]. Several forms of Se species have been widely studied as a potential antioxidant and anticarcinogenic compounds, such as selenocysteine (Se-Cys), Se-Met, and methylselenocysteine (MSeCys) [5]. However, a narrow concentration range between therapeutic and toxic doses of Se has been reported [4]. Human nutrition societies recommended amounts in a range of 0.025–0.065 mg Se per day [6]. An upper limit (UL) of 0.3 mg Se per day has been suggested [7].

Several Se-enriched plants showed higher antioxidant activity compared with untreated ones, e.g., Se-enriched broccoli [8], brown rice [9], green tea [10], mushroom [11], rice [12], *Spirulina platensis* [13], *Pleurotus ostreatus*, and *Pleurotus eryngii* [14]. The Se biofortification can increase polyphenol and antioxidant capacity/free-radical scavenging activity [15, 16].

Chinese kale (*Brassica oleracea* var. *alboglabra* L.) is a member of the family *Brassicaceae*, and it represents the most popular vegetable consumed in Thailand. Chinese kale can reduce the toxicity of inorganic Se because it transforms inorganic Se to several organic species of Se. In a previous study, we cultivated Se-enriched Chinese kale seedlings with Se supplied at 0, 5, 10, 15, 30, and 45 mg/L Se from sodium selenite in a hydroponic nutrient solution based on Hoagland and Arnon [17] and harvested every 5 days (5, 10, and 15 days). Results indicated that the different Se concentrations and cultivation periods affect the growth rate of Se-enriched Chinese kale seedlings. The concentration 0–30 mg/L Se in solution did not affect the growth rate, and the highest total selenium accumulation was found in 15 days in 30 mg/L [18]. Moreover, Se-Met and MSeCys were the major organic forms of Se-enriched Chinese kale seedlings [18]. The bioavailability of Se from Se-enriched Chinese kale seedlings (Se-KS) is similar to that of Se-enriched yeast but higher than that of Se from sodium selenite in broilers [19] and laying hens [20]. Furthermore, the toxicity of Se from Se-KS was studied in laying hens, and it showed less toxicity than Se from sodium selenite at 5 and 10 mg·Se/kg. The antioxidant property of Se-KS was evaluated by the GSH-Px activity in the red blood cell of laying hens (basal diet plus 5 and 10 mg·Se/kg from Se-KS) [21]. Results showed that the GSH-Px activity markedly increased when compared to the hen receiving the basal diet.

Although the previous studies had reported antioxidant activity and toxicity of Se-KS in poultry, its toxicity has never been studied in mammal models. Thus, in the present study, the physicochemical contents of Se-KS, the Se bioaccesibility after *in vitro*-simulated gastrointestinal digestion, the antioxidant activity, and also the toxicity in a mammal model were evaluated. Data obtained from this study suggested that Se-KS could be beneficially established as a functional food.

2. Materials and Methods

2.1. Materials and Chemicals. Ascorbic acid, 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), enzymes (i.e., α-amylase, pancreatin, and pepsin), gallic acid, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox) rutin, sodium selenite (Na_2SeO_3), 2,4,6-tripyridyl-s-triazine (TPTZ), and quercetin were purchased from Sigma (St. Louis, MO, USA). Folin–Ciocalteu reagent was obtained from Fisher Chemical (Leicestershire, UK). Ferrous chloride and linoleic acid were obtained from Fluka (Germany). Butylated hydroxyanisol (BHA) was obtained from Fluka (France). Ethanol and hydrochloric acid (HCl) were purchased from BDH (UK). Ammonium thiocyanate (NH_4SCN) was obtained from Univar (USA). All chemicals were of analytical grade.

2.2. Cultivation of Chinese Kale Seedlings. Seeds of Chinese kale *(Brassica oleracea* var. *alboglabra* L.) were obtained from Chia Tai Company Limited (Thailand). The Chinese kale seedlings were cultivated according to the growth conditions described by Maneetong et al. [18]. Briefly, the Chinese kale seeds were soaked in tap water for 15 h before planting. After that, the seeds were cultivated on a wet sponge ($35 \times 40 \times 30$ cm) at room temperature in the dark for 3 days until the seeds began to germinate. The covers of the plastic pots were released, and the germinated Chinese kale was subjected to light from a fluorescent lamp (36 W) with a12 h dark/light cycle and watered with tap water daily for 4 days. Afterwards, the germinated seedlings were cultivated in the adapted modified Hoagland's solution containing 0, 5, 10, 15, and 30 mg·Se/L in the form of sodium selenite. After 5, 10, and 15 days of cultivation, the Chinese kale seedlings were harvested, washed thoroughly with deionized water, dehydrated in a hot oven at 60°C, gently ground, and stored in a freezer at −20°C until analysis.

2.3. Antioxidant Activities of Chinese Kale Seedlings. The extraction procedure was adapted from Xu and Hu [12]. An accurate weight of one gram of the dried sample was extracted with 30 mL of 75% ethanol (ethanolic extract) or distilled water (aqueous extract). The extractions were conducted in a water bath at 60°C and shaker 200 rpm for 3 h. Each sample was extracted three times with the same volume of solvents. The extracts were filtered and combined, and the combined filtrate evaporated to dryness in a vacuum and kept frozen at −20°C until analysis of antioxidant activity (DPPH and ferric thiocyanate (FTC) methods).

The DPPH radical scavenging capacities of Se-KS extracts and the reference substance trolox and butylated hydroxyanisol (BHA) were evaluated with a method adapted from Xu et al. [22]. The radical scavenging capacities of samples and reference substances against the DPPH radical were evaluated. Briefly, 100 μL of the standard was extracted and mixed with 1000 μL of 0.1 mM ethanolic solution of DPPH radical, and absorbance was measured at 515 nm after 30 min of reaction in the dark. The percentage inhibition was calculated by the following equation:

$$\%\text{inhibition} = \frac{\text{absorbance of blank} - \text{absorbance of sample}}{\text{absorbance of blank}} \times 100. \quad (1)$$

The FTC method followed Xu and Hu [12]. 2 mL of 1000 μg/mL sample extracts or 2 mL of 200 μg/mL trolox and BHT served as standard solutions. 2 mL of 2.51% w/v linoleic acid in ethanol, 4 mL of 0.05 M phosphate buffer (pH 7.0), and 2 mL of distilled water were mixed in a vial of 10 mL with a screw cap and then kept in a 40°C water bath in the dark. A 100 μL amount of the above mixture was added to 9.7 mL of 75% v/v ethanol and 100 μL of 30% w/v ammonium thiocyanate. After 5 min, 100 μL of 0.02 M ferrous chloride in 3.5% v/v hydrochloric acid was added and then maintained at 40°C in a water bath in the dark. The absorbance of the mixture was measured every 24 h at 500 nm until a steady absorbance value was achieved. All of the tests were performed in triplicate, and the results were averaged. The DPPH and FTC methods were chosen to find out and confirm suitable germination time periods because they are simple, have low cost, and used stable free radicals. The percentage inhibition was calculated by the following equation:

$$\%\text{inhibition} = \frac{\text{absorbance of blank} - \text{absorbance of sample}}{\text{absorbance of blank}} \times 100. \quad (2)$$

2.4. Physicochemical Contents. The following physicochemical contents of the sample were determined: moisture, crude fat, crude fibre, ash, crude protein, and carbohydrate content using the standard Association of Official Analytical Chemists procedures [23].

2.5. Total Se Contents. The dried samples were analyzed by using a hydride generation atomic absorption spectrophotometer (wavelength 196.0 nm, quartz cell temperature 890°C, HG-AAS model VGA-77, Agilent Technologies, Inc., USA). The samples were digested with 1.5 mL of 65% nitric acid, and 1.5 mL deionized water was added and mixed in a glass vessel and then heated at 100°C in a metal bath for 45 min. After cooling, 5 mL of concentrated HCl was added to reduce Se (VI) to Se (IV) for 30 min. The digested solution was transferred and stored in a freezer at 4°C until analysis. The digestion process was accurately validated with the percentage recovery test by spiking 20 μL (20 μg·Se/L) of Se standard solution into the sample. The obtained recovery values were in the range of 90.65–102.49.

2.6. Se Bioaccessibility. Gastrointestinal digestion was simulated using the method described previously [24]. Briefly, accurate weight of one gram of the plant samples was mixed with 7.5 mL of gastric juice (6% w/v pepsin, 0.9% w/v sodium chloride at pH 1.8 (HCl)) and stored in a 37°C incubator for 4 h in the dark. After that, gastric juice was adjusted to pH 6.8 with saturated sodium carbonate solution and then incubated with 5 mL of intestinal juice (1.5% w/v pancreatin, 0.5% w/v α-amylase in 0.9% w/v sodium chloride) at 37°C approximately 4 h in the dark with gentle and continuous shaking. After centrifugation at 5000 g for 30 min, the supernatant was brought up to 100 mL with 0.9% w/v NaCl in a volumetric flask, filtered through filter paper, and used for antioxidant activity tests. As the blank of gastrointestinal digestion, 2.5 mL of deionized water was investigated in a gastrointestinal (GI) simulation test. Total Se of the samples after GI digestion was determined using previously described conditions. Total Se GI digestion and blank (without sample) were measured after the GI process. The percentage of the bioaccessibility was calculated by the following equation:

$$\%\text{bioaccessibility} = \frac{\text{total Se GI digestion} - \text{total Se in blank}}{\text{total Se in sample}} \times 100. \quad (3)$$

2.7. Total Phenolic Compounds, Flavonoid Contents, and Antioxidant Activity after In Vitro Gastrointestinal Digestion. Total phenolic contents were determined according to a modified procedure [25]. Briefly, 100 μL of the digested samples was oxidized with 500 μL of 0.2 N Folin–Ciocalteu's reagent and added to 400 μL of 7.5% w/v sodium carbonate. The absorbance was measured at 765 nm after being mixed and incubated in room temperature for 60 min. The results were calculated as milligram per gram gallic equivalent (mg GE/gDW).

Total flavonoid contents were estimated using the aluminum chloride colorimetric method [26]. Briefly, 100 μL of the digestion samples was mixed with 500 μL of 2.5% w/v sodium nitrite and 400 μL of 5% w/v aluminum chloride. The mixture was allowed to stand at room temperature for 30 min, and the absorbance was read at 415 nm. The results were expressed as milligram per gram quercetin equivalent (mg QE/gDW).

Antioxidant activities methods used DPPH, $ABTS^{+\bullet}$, and the ferric reducing antioxidant power (FRAP). The DPPH method was described in the previous section. For the $ABTS^{+\bullet}$ assay [27], the $ABTS^{+\bullet}$ solution was generated by mixing the two stock solutions of 7 mM ABTS and 2.45 mM potassium persulphate (1 : 1, v/v). The mixture was stored in dark at room temperature for 12 h before use. The $ABTS^{+\bullet}$ solution was diluted to 0.7–0.8 absorbance at 734 nm in deionized water. The 100 μL of different concentrations of the supernatant (0.1–1.0 mg/mL) was continually mixed with 900 μL of $ABTS^{+\bullet}$ solution. The mixed solution was stored at room temperature for 15 min and followed by measuring the absorbance at 734 nm using deionized water as blank. Trolox and ascorbic acid were used as standard solutions. The percentage inhibition was calculated using the following equation:

$$\%\text{inhibition} = \frac{\text{absorbance of blank} - \text{absorbance of sample}}{\text{absorbance of lank}} \times 100. \quad (4)$$

The FRAP method [28] of the extract was measured at low pH. Briefly, 100 μL of Se-KS extracts was mixed with 900 μL of FRAP reagent and incubated in a water bath at 37°C for 15 min. The absorbance at 595 nm was measured. The results were calculated as milligram per gram trolox equivalent antioxidant capacity (mg TEAC/gDW).

2.8. Protocol of Acute Oral Toxicity and Determination of Body Weight, Blood Analysis, and Histopathological Investigation of the Liver and Kidney in Experiment on Rat. This experiment was performed to investigate the acute toxicity of R-KS and Se-KS *in vivo* and to confirm a toxicity for a new dietary supplement. The OECD guideline 423 was used to monitor the effect of oral toxicity test [29].

Healthy female Wistar albino rats, 8–12 weeks old, 180–220 g b.w. were purchased from the National Laboratory Animal Center, Mahidol. Two rats were housed in a stainless-steel cage in a room maintained under environmentally controlled conditions of 22 ± 3°C and 50–60% relative humidity, with 12/12 light/dark cycle. Animals were fed conventional laboratory diets with an unlimited supply of drinking water. The animals were acclimated to the laboratory conditions for 5 days prior to the start of the experiments. All animal procedures conformed to the Animals for Scientific Purpose Act, B.E.2558 (A.D. 2015), Thailand, and approved by the Animal Ethics Committee of Mahasarakham University (Approval No. 005/2558). Animals were randomly divided into seven groups of 4 rats each. All animals were fasted overnight and the R-KS or Se-KS at different amounts was administered orally as a single dose. The first group received corn oil (vehicle control). The 2nd, 3rd, and 4th groups were treated with regular kale seedling (R-KS) in corn oil at doses of 1250, 2500, and 5000 mg/kg·b.w. which consisted of 0.02, 0.04, and 0.08 mg Se/kg·b.w., respectively. Additionally, the 5th, 6th, and 7th groups were treated with Se-enriched kale seedling (Se-KS) in corn oil at the same doses as R-KS and also consisted of 0.54, 1.08, and 2.16 mg Se/kg·b.w., respectively.

Animal behaviors were monitored at 0, 0.5, 1, 3, 6, 12, and 24 h during the first day and daily until 14 days after dosing. The following signs of stress or distress such as labored breathing, discharge from nose or mouth, ruffled fur, hunched posture, minimal responsiveness, diarrhea, immobility, lethargy, labored breathing, minimal responsiveness, and uncontrolled bleeding or discharges were criteria prompting immediate euthanasia. The body weight of all rats was measured daily. The lethal dose of 50% (LD_{50}) was evaluated according to the method described by OECD guidelines 423. After 14 days, all animals were anesthetized with Nembutal 45 mg/kg·b.w. (i.p.), and blood samples were collected by cardiac puncture for hematological analysis into tubes with and without EDTA, respectively. All parameters of blood were analyzed by the Animal Hospital Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, Thailand. The organs including liver, kidney, heart, spleen, and lung were collected and weighed, and the relative organ weight was determined (based on terminal body weight).

Additionally, the heart, liver, and kidney were collected for histopathological examinations; tissues were processed into paraffin blocks, sectioned at a thickness of 5 μm, mounted on glass microscope slides, and stained with hematoxylin and eosin. The histological assessments of the slides were examined with a light microscope.

2.9. Statistical Analysis. The results were performed in triplicate and expressed as mean ± SD. The significance of difference was analyzed using one-way ANOVA. Independent *t*-tests were used to compare test results at $p < 0.01$ and 0.05 between R-KS and Se-KS. Correlations among variables were analyzed with Pearson's correlation test.

3. Results and Discussion

3.1. Antioxidant Activities of Se-KS (0–30 mg·Se/L Hydroponic Solution). The H donation by the kale extracts and by trolox and BHA realizes a transfer of the DPP radical to DPPH with a decolourisation or absorbance decrease. In contrast, the FTC assay leads to an absorbance increase. It is a spectrophotometric method determining electron transfer by measuring the capacity of an antioxidant in its reduction with colour change. The corresponding increase or decrease of the absorbance at given wavelength is related to the concentration of antioxidant in the sample [30]. The results showed that during the first 10 min, the reactions of DPPH radical with ethanolic extracts of the tested antioxidants Se-KS vs. R-KS vs. reference antioxidants (Figures 1(a)–1(c)) was more rapid than those with aqueous extracts (Figures 1(d)–1(f)) and reached a steady state within 15 min. The inhibition percentages of antioxidant activity on DPPH at 30 min were calculated. Se-KS at 30 mg Se/L for 15 days provided the highest inhibition percentage of 77% at 30 min when compared with other Se concentrations, but lower than trolox and BHA. The antioxidant activity of Se-KS increased with increase of Se content, suggesting Se played a positive role in enhancing the antioxidant activity of Se-KS. Moreover, organic Se was more effective than inorganic Se on enhancing the hydroxyl radical scavenging ability [31].

In the FTC method, all the Se-KS extracts inhibited linoleic acid oxidation. High inhibition value exhibited higher activity than control (Figure 2). Also, lipid inhibitory activities of Se-KS ethanolic extracts (Figures 2(a)–2(c)) showed higher antioxidant activity than those of aqueous extracts (Figures 2(d)–2(f)). Se-KS extracts from 30 mg Se/L for 15 days of cultivation displayed significantly higher activity than the control and other Se-supplemented concentrations, but lower than those of trolox and BHA. The antioxidant activities from the FTC method showed almost the same patterns of activities as the DPPH method. From the antioxidant activity results, Se-KS at 30 mg·Se/L and 15 days after cultivation was selected for determination of

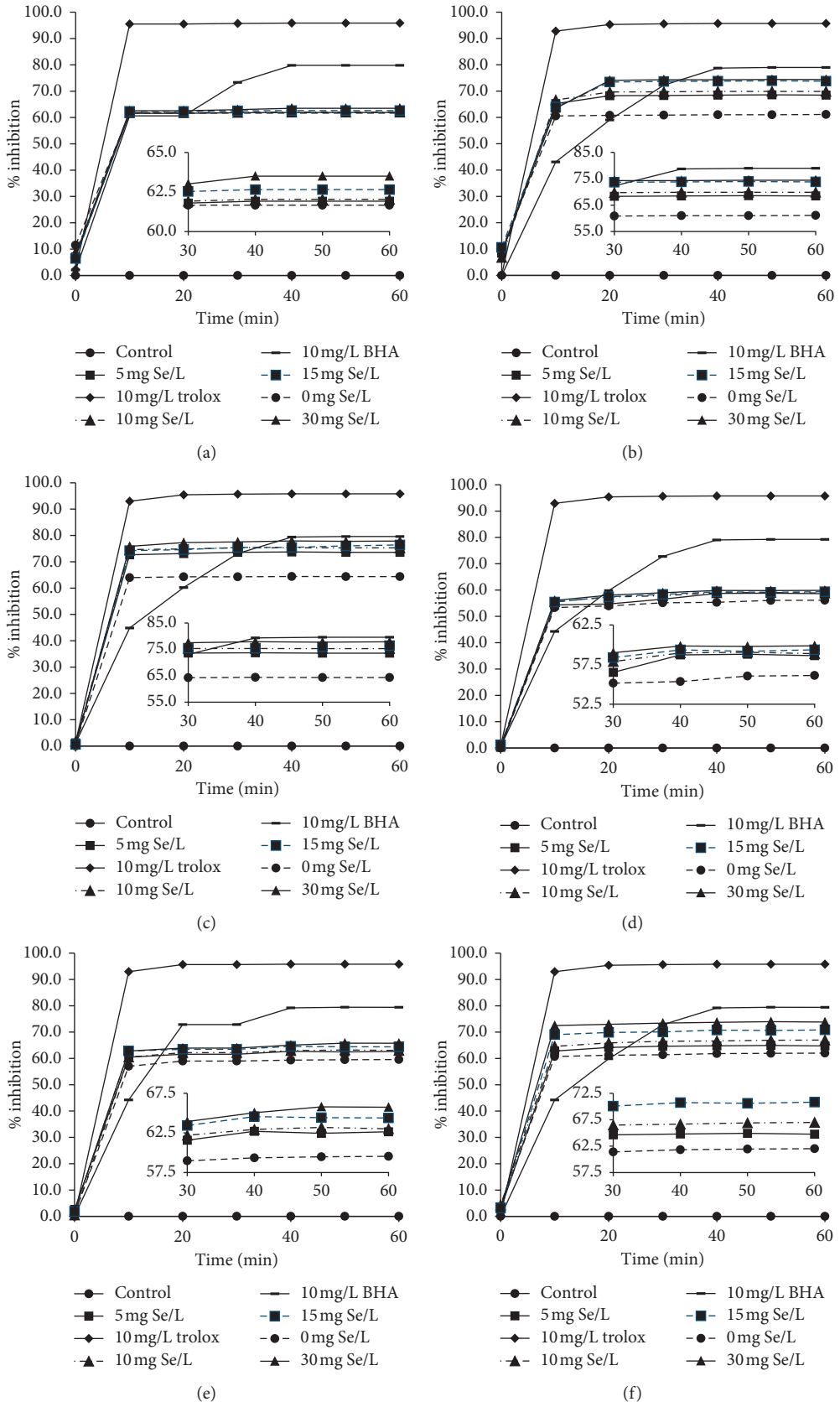

Figure 1: Kinetic behaviors of radical antioxidant activity of Se-enriched kale seedlings. The Se-enriched kale seedling ethanolic extracts (a–c) and aqueous extracts (d–f) with 5 tested Se concentrations and the two reference antioxidants trolox and butylated hydroxyanisol (BHA) in 3 cultivation periods of 5 days (a, d), 10 days (b, e), and 15 days (c, f). The standard represents the blank, the pure ethanol solution and the distilled water.

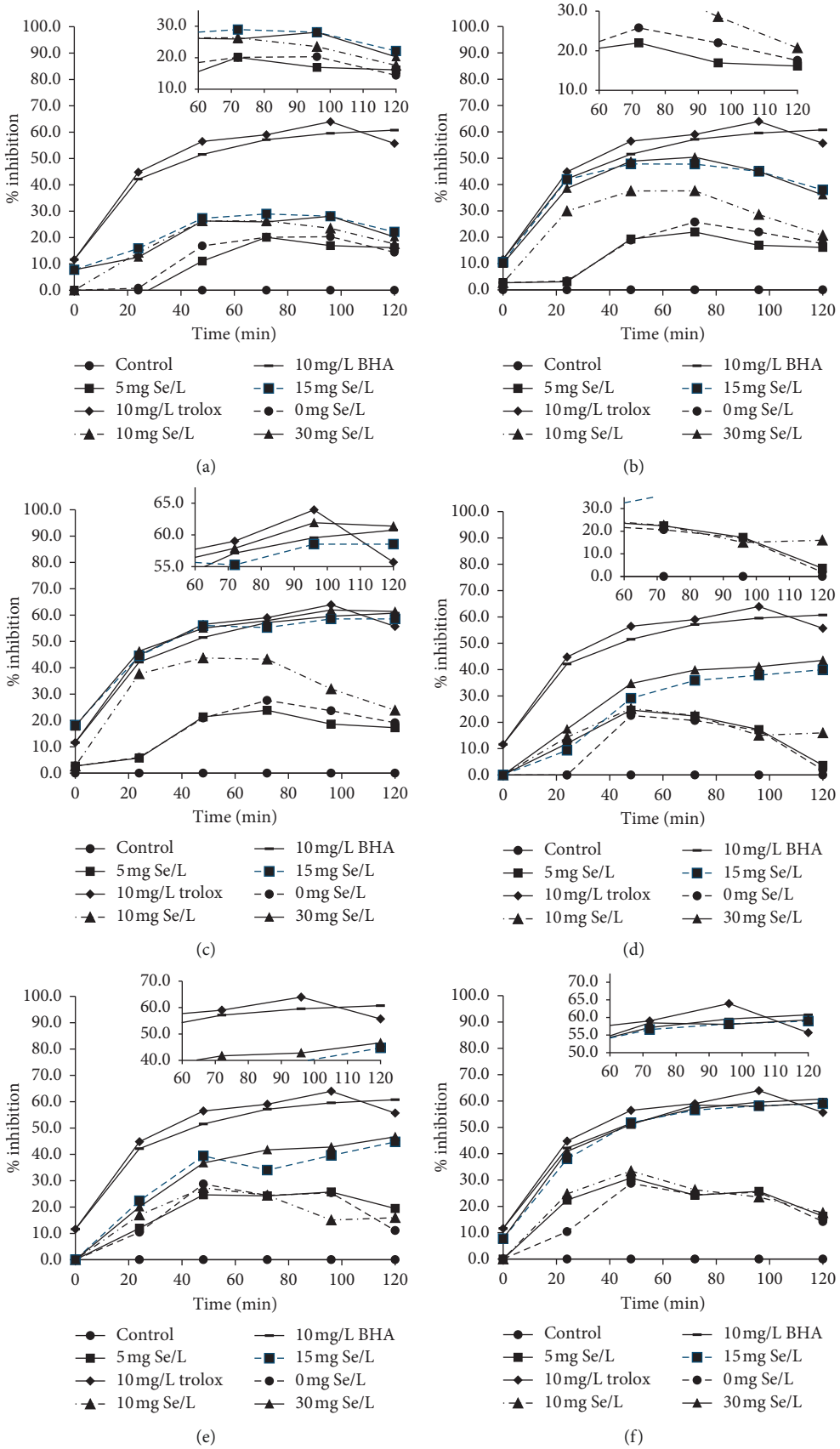

Figure 2: Kinetic behaviors of the ferric thiocyanate method of Se-enriched kale seedlings. The Se-enriched kale seedlings ethanolic extracts (a–c) and aqueous extracts (d–f) with 5 tested Se concentrations and the two reference antioxidants trolox and butylated hydroxyanisol (BHA) in 3 cultivation periods of 5 days (a, d), 10 days (b, e) and 15 days (c, f). The standard represents the blank, the pure ethanol solution and the distilled water.

physicochemical contents, Se bioaccessibility, and antioxidant activities (after passing through the *in vitro* gastrointestinal simulation). Also, in the acute toxicity assessment on rats, the variant R-KS at 0 mg Se/L and 15 days after cultivation as the control was tested in comparison with Se-KS at 30 mg·Se/L, 15 days after cultivation.

3.2. Physicochemical Contents, Total Se Contents, and Se Bioaccessibility and Antioxidant Activities of Se-KS and R-KS. Table 1 shows the physicochemical contents and the total Se content of R-KS (untreated) and Se-KS (30 mg Se/L) each for 15 days after cultivation. In addition, the Se bioaccessibility in the *in vitro* simultaneous gastrointestinal simulation model is presented. The moisture and crude fat content of all Chinese kale seedlings was not significantly different ($p < 0.05$). The results indicated higher ($p < 0.05$) crude protein, total Se, and Se-bioaccessibility contents of the Se-KS when compared to the R-KS. The content of crude protein increased in association with accumulation of Se in plants in line with an increase reported in [32]. Various data revealed that the protein content of Se-enriched plants is higher than that of regular plants [33]. The supplementation of Se increases the synthesis of selenoamino acid and protein content [32]. Regarding the increase of crude fibre contents, a further report suggested that Se accumulation resulted in higher starch concentrations in upper leaves [34]. The mechanism is maybe correlated to change in starch/glucose contents which are important substratess in cellulose synthesis. In this study, Se-KS exhibited 26-fold higher Se accumulations than did R-KS. This finding is in good agreement with Chen et al. [35] who reported an accumulation of Se in rice after Se biofortification with selenate and selenite. Also, Xu et al. [22] reported that the Se accumulation in tea leaves increased by Se fertilization compared with plants not treated with Se.

Se bioaccessibility increased, following *in vitro* simulated gastrointestinal digestion. Fifty percentage of bioavailable capacity in Se-KS showed high absorbtion of Se contents in the gastrointestinal system simulation. These results are in accordance with a previous investigation that reported higher Se bioavailability (64.5%) in Se-KS compared with sodium selenite (54.5%) in laying hens [21]. Conversely, ash mainly consists of the oxide of calcium, zinc, magnesium, and potassium. The R-KS showed a significantly higher ($p < 0.05$) ash content than the Se-KS. Ash mainly consists of the oxide of calcium, magnesium, and potassium. Se biofortification in radish and maize plants can inhibit the absorption of calcium and potassium, copper, and zinc [36]. However, ash contents are unknown in the cited studies, to explain satisfactorily the difference in the mineral content of R-KS and Se-KS.

Antioxidant activities (DPPH, ABTS, and FRAP) of R-KS and Se-KS supernatants after *in vitro* simulated GI digestion are presented in Table 2. The total phenolic content in Se-KS was significantly higher than that in R-KS (30.1 ± 0.1 mg GE/gDW vs. 18.5 ± 0.7 mg GE/gDW). However, there was no significant difference of total flavonoid content between R-KS and Se-KS. Antioxidant activities were determined by DPPH, ABTS, and FRAP assay. Se-KS showed the greatest potential for antioxidant activities on free-radical scavenging in different concentrations of DPPH (0.025–10.00 mg/mL) and ABTS (0.1–1.0 mg/mL) which were expressed as IC_{50} values (the inhibitory concentration of a substance where the scavenging is reduced by half). In addition, free-radical scavenging of the R-KS was significantly less than that of Se-KS ($p < 0.05$). A high potential antioxidant effect of Se-KS was found in both DPPH (80.72%) and ABTS (75.52%) assays. Se-KS had higher total phenolic compound contents and also higher antioxidant activity than R-KS. This underlines that the phenolic compounds are the main source of natural antioxidant in *Brassica* plants. Bachiega et al. [37] confirmed the broccoli biofortification with selenium showed significantly higher total phenolic contents and antioxidant capacity in different maturity stages (sprout and seedling). Se treatment increases phenolic compounds and antioxidant activity, but the mechanism is still not fully understood. It is hypothesized that the Se enhances accumulation of glucose which is an important substrate in many metabolic pathways [34, 38].

The correlation coefficient between protein content, total Se content, Se bioaccessibility, and antioxidant activity has been reported. The relationship between total Se content (TSeC), protein content (PC), and Se bioaccessibility with antioxidant activity measured by DPPH, ABTS, and FRAP assays is shown in Table 3. The results demonstrated that Se

Table 1: Physicochemical contents, total selenium, and Se bioaccessibility of air-dried substance of regular Chinese kale seedlings (R-KS) and Se-enriched Chinese kale seedlings (Se-KS).

Constituents	R-KS	Se-KS
Moisture (%)	4.8 ± 0.1	4.9 ± 0.1
Crude protein (%)	33 ± 2^a	37 ± 1^b
Crude fat (%)	3.6 ± 0.4	3.4 ± 0.2
Crude fibre (%)	5.5 ± 1^a	7.1 ± 1^b
Ash (%)	22.7 ± 0.4^a	15.5 ± 0.1^b
Carbohydrate (%)	30.7 ± 0.9	32.3 ± 0.5
Total selenium before GI digestion (mg/kg)	17 ± 3^a	433 ± 22^b
Total selenium after GI digestion (mg/kg)	4 ± 2^a	219 ± 1^b
Se bioaccessibility (%)	20 ± 6^a	50 ± 0^b

The data are expressed as mean ± SD ($n = 3$); identical superscripts in row denote no significant ($p < 0.05$) difference between mean values according to the independent *t*-test (SPSS 16.0).

Table 2: Antioxidant capacities after passing gastrointestinal system simulation of regular Chinese kale seedling (R-KS) and Se-enriched Chinese kale seedling (Se-KS).

Method	Sample	
	R-KS	Se-KS
IC_{50} DPPH (mg/mL)	0.79 ± 0.00^a	0.74 ± 0.01^b
IC_{50} ABTS (mg/mL)	0.58 ± 0.03^a	0.45 ± 0.03^b
FRAP (mgTE/gDW)	18.8 ± 0.2^a	15.3 ± 0.4^b

The data are expressed as mean ± SD ($n = 3$). The different lowercase letters indicate significant difference between rows at $p < 0.05$. DPPH = 2,2-diphenyl-1-picrylhydrazyl; ABTS = 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), and FRAP = Ferric reducing antioxidant power.

Table 3: Correlation coefficients (r) between difference of Se-bioaccessibility, total Se contents, and antioxidant capacity methods.

	PC	Se-bio	TSeC	TPC	TFC	DPPH	ABTS	FRAP
PC	1	0.89*	0.95**	0.95**	0.79	−0.91**	−0.93**	0.98**
Se-bio	—	1	0.98**	0.98**	0.73	−0.96**	−0.92**	0.95**
TSeC	—	—	1	0.99**	0.72	−0.99**	−0.93**	0.98**
TPC	—	—	—	1	0.73	−0.99**	−0.91*	0.99**
TFC	—	—	—	—	1	−0.71	−0.85*	0.78
DPPH	—	—	—	—	—	1	0.89*	−0.97**
ABTS	—	—	—	—	—	—	1	−0.94**
FRAP	—	—	—	—	—	—	—	1

*Significant model difference at $p < 0.05$. **Significant model difference at $p < 0.01$. PC = protein contents; Se-bio = Se bioaccessibility; TSeC = total Se contents; TPC = total polyphenol contents; TFC = total flavonoid contents.

Table 4: Hematological and biochemical values of rats treated with Chinese kale seedlings for acute toxicity administration.

Dose mg/kg b.w.	HB (g/dL)	Hct (%)	RBC (10^6/cm)	MCV (fL)	MCH (pg)	MCHC (%)	WBC (10^3/cm)	Neu (%)	Lym (%)
Control	16.8 ± 0.3	49.3 ± 0.5	8.2 ± 0.1	60.3 ± 1.0	20.3 ± 0.3	33.7 ± 0.3	2400 ± 594	7.5 ± 0.7	92.3 ± 0.8
R-KS 1250	16.7 ± 0.3	50.5 ± 0.3	8.6 ± 0.1	58.5 ± 1.2	19.4 ± 0.6	33.1 ± 0.4	2475 ± 460	6.8 ± 1.5	91.3 ± 2.4
R-KS 2500	15.8 ± 0.2	48.5 ± 0.3	8.4 ± 0.1	57.5 ± 0.7	18.8 ± 0.2	32.8 ± 0.3	1800 ± 141	10.5 ± 1.3	89.3 ± 1.3
R-KS 5000	16.8 ± 0.1	50.3 ± 0.5	8.2 ± 0.3	57.0 ± 2.9	20.5 ± 0.7	34.0 ± 0.6	2025 ± 322	9.0 ± 4.5	89.5 ± 5.9
Se-KS 1250	16.8 ± 0.3	50.5 ± 0.7	8.5 ± 0.2	59.5 ± 1.8	19.9 ± 0.6	33.4 ± 0.5	1650 ± 206	7.3 ± 1.9	90.5 ± 1.7
Se-KS 2500	16.5 ± 0.5	49.3 ± 1.3	8.4 ± 0.1	58.0 ± 0.8	19.6 ± 0.4	33.7 ± 0.2	1600 ± 339	7.0 ± 1.1	95.8 ± 1.7
Se-KS 5000	16.5 ± 0.3	49.5 ± 1.0	8.6 ± 0.3	57.5 ± 0.7	19.4 ± 0.6	33.4 ± 0.3	2500 ± 635	4.0 ± 1.2	94.0 ± 1.5

Dose (mg/kg b.w.)	BUN (mg/dL)	CREA (mg/dL)	UA (mg/dL)	TP (mg/dL)	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	21 ± 1	0.63 ± 0.02	0.85 ± 0.10	6.15 ± 0.18	74 ± 11	26 ± 1	109 ± 5
R-KS 1250	24 ± 2	0.85 ± 0.12	2.00 ± 0.36	5.83 ± 0.09	94 ± 24	30 ± 2	142 ± 16
R-KS 2500	24 ± 1	0.78 ± 0.05	1.90 ± 0.56	6.38 ± 0.19	101 ± 19	29 ± 2	117 ± 8
R-KS 5000	22 ± 1	0.75 ± 0.06	1.85 ± 0.71	6.38 ± 0.07	104 ± 13	25 ± 3	104 ± 14
Se-KS 1250	21 ± 1	0.80 ± 0.06	1.58 ± 0.25	7.05 ± 0.25	62 ± 12	31 ± 3	144 ± 14
Se-KS 2500	23 ± 1	0.68 ± 0.05	0.73 ± 0.11	6.75 ± 0.21	102 ± 9	24 ± 1	127 ± 11
Se-KS 5000	23 ± 4	0.58 ± 0.11	1.28 ± 0.48	6.50 ± 0.17	105 ± 38	32 ± 3	147 ± 14

Values are mean ± SEM for 4 rats in each group. HB = hemoglobin; Hct = hematocrit; RBC = red blood cell; MCV = mean cell volume; MCH = mean cell hemoglobin; MCHC = mean corpuscular hemoglobin concentration; WBC = white blood cell; Neu = neutrophils; Lym = lymphocytes.
Values are mean ± SEM for 4 rats in each group. BUN = blood urea nitrogen; CREA = creatinine; UA = uric acid; TP = total protein; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase.

bioaccessibility and antioxidant activity were strongly correlated: DPPH, $r = 0.96$; ABTS, $r = 0.92$; and FRAP assay, $r = 0.95$. The PC of all Chinese kale seedlings showed a high correlation coefficient with antioxidant capacities of DPPH and ABTS of 0.91 and 0.94, respectively. Moreover, a positive correlation was found for TSeC/TPC ($r = 0.99$). There were strong internal correlations between the results of the different antioxidant assay methods. Additionally, there was a significant positive correlation coefficient between TSeC, Se bioaccessibility, TPC, and antioxidant activity. In concordance with previous studies, our studies found that there is a positive correlation between TSeC and antioxidant activity in several Se-enriched plants such as brown rice [9], green tea [10], and *Spirulina platenis* T. [13]. In conclusion, the accumulation and transformation of an inorganic Se form to a bioactive organic compound in Se-KS is responsible for higher Se bioaccessibility, total phenolic compounds, and antioxidant activities.

3.3. Acute Toxicity Study in Rats. In the acute toxicity study, oral administration of varying doses of R-KS and Se-KS to rats exhibited no evidence of toxicity over a period of 14 days. No changes in rat behaviour were observed. No mortality or any morbidity was verified in all groups after administration. These results revealed that the LD_{50} of Chinese kale seedlings in rats would be more than 5000 mg/kg b.w.

Daily food and water consumption was found to be normal in both control and treatment groups. No significant changes in body weight of the rats which were given the Se-KS were observed when compared with the control group (data were not shown). Changing body weight is one of the indicators of toxicity effects of chemicals [39]. Therefore, it can be assumed that the ingestion and digestion of kale seedling (R-KS and Se-KS) did not affect the normal growth of rats.

The hematological analyses of blood parameters including hemoglobin content, packed cell volume (hematocrit), red blood cell count, total white blood cell count with neutrophils, and lymphocytes percentages and the derived red blood cell criteria MCV, MCH, and MCHC (for abbreviations, see footnote in Table 4) were exhibited within normal limits compared to the control group, as shown in Table 4. The hematological system is one of the most sensitive targets to toxic compounds [40]. Consequently, the

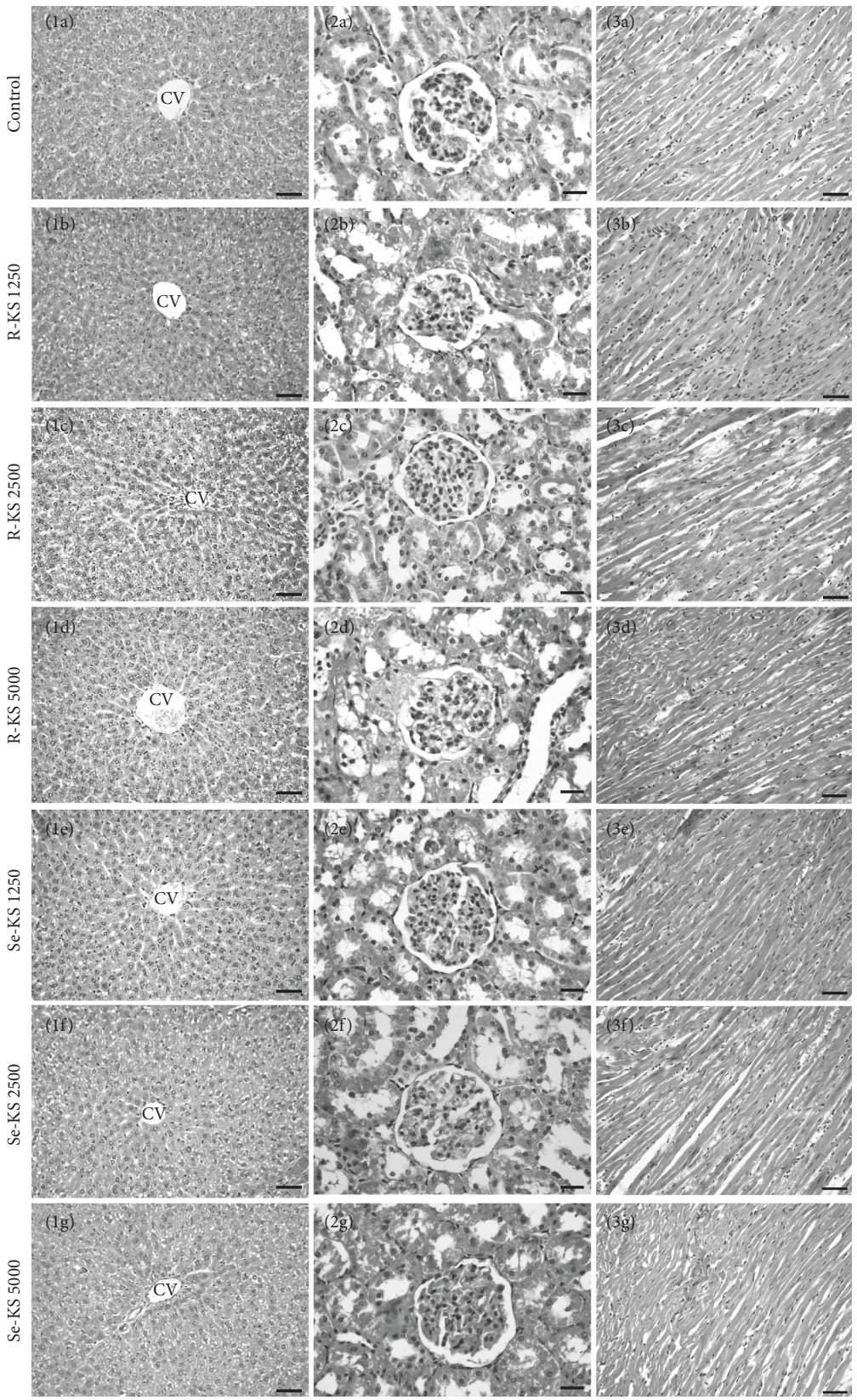

Figure 3: Histopathological analysis of organs treated with control (vehicle), regular Chinese kale seedlings (R-KS), and Se-enriched Chinese kale seedlings (Se-KS) at 1250, 2500, and 5000 mg/kg·b.w. Column 1 (liver), scale bar is 50 μm (CV is central vein); column 2 (kidney) glomerulus, scale bar is 20 μm; and column 3 (heart muscle), scale bar is 50 μm.

results indicate that neither R-KS and Se-KS were toxic to blood cells nor interfered with blood cell production.

Biochemical markers were also evaluated in the blood serum samples, and results are summarized in Table 4. The single oral administration of R-KS and Se-KS at doses of 1250, 2500, and 5000 mg/kg·b.w. did not have any significant adverse effects on liver enzyme activities of serum and further biochemical markers when compared with the control group. High serum activities of aspartate transaminase (AST) and alanine transaminase (ALT) are indicators of a predamaged or damaged liver function and biomarkers. In addition, alkaline phosphatase (ALP) is an enzyme that plays a major function in cell membrane transport in many tissues, including bone, intestine, kidney, liver, placenta, and white blood cells. Damage to these tissues causes the release of ALP into the bloodstream [41, 42]. Elevated ALP in plasma has been found in large bile duct obstruction, intrahepatic cholestasis, or infiltrative diseases of the liver [43]. High TP levels indicate dehydration, inflammation, infections, and bone marrow disorders. A low TP level can suggest a liver disorder, a kidney disorder, or a disorder in which protein is not digested or absorbed properly. Low levels may be seen in severe malnutrition and with conditions that cause malabsorption [43]. Uric acid (UA) is a product of the metabolic breakdown of purine nucleotides [44]. The presence of hyperuricemia should initiate an evaluation in conditions associated with increased purine metabolism or altered renal clearance of uric acid. These conditions have been linked to many disease states such as articular degenerative disorders, vascular inflammation, atherosclerosis, and cardiovascular as well as renal disease [45]. High levels of BUN and creatinine indicate kidney failure or disease, dehydration, shock, high protein diet, certain toxin ingestions, poor circulation to the kidneys, and urinary obstruction [46]. The administrations of all dosages of R-KS and Se-KS did not cause any organ atrophy, hypertrophy, or degenerative effect. The tissues did not show any changes in colour, volume, and texture when compared with the control group (data not shown). No significant difference was noticed in relative weight of primary body organs. Toxic substances induce abnormal metabolic reactions that may affect primary organs such as heart, liver, and kidney [41, 47, 48].

The histopathological examination of several tissues of heart muscle, liver, and kidney is shown in Figure 3; no changes were observed. Therefore, our findings suggest that, in the Chinese kale seedlings, both R-KS and Se-KS at 1250, 2500, and 5000 mg/kg·b.w. did not affect the morphology of the organs. In former investigations, it has been reported that Se compounds such as selenite and MSeCys provided signs of toxicity at 3–5 mg·Se/kg·b.w. with almost no survival at 16 mg·Se/kg b.w. [49]. Moreover, the LD_{50} of Se-Met in rats which were given an intraperitoneal injection was evaluated as 4.25 mg·Se/kg b.w. [50]. In our study, R-KS at doses of 1250, 2500, and 5000 mg/kg·b.w. consisted of 0.02, 0.04, and 0.08 mg·Se/kg·b.w., whereas Se-KS at doses of 1250, 2500, and 5000 mg/kg·b.w. consisted of 0.54, 1.08, and 2.16 mg·Se/kg·b.w., respectively. Moreover, our previous study found that the major organic forms of Se-KS were Se-Met and MSeCys [18]. Therefore, the amount of Se in Se-KS did not exceed the toxicity doses reported; for this reason, no signs of toxicity in animals were seen in the present experiment. Indeed, together with our previous research [18–20], good biological activity was proven for the Se-KS with low acute toxicity when compared with selenite. We suggest 0.05–0.15 g of the air-dried Se-KS corresponded with 0.025–0.065 mg Se per day [6] or a maximum dose of <0.7 g of the air-dried Se-KS corresponded with a tolerable dose 0.3 mg Se per adult and day [7]. However, this hypothetical recommendation does not consider the Se ingestion via plant and animal source in food consumption.

4. Conclusions

Se-KS had a high Se concentration, a higher crude protein content, and a stronger antioxidant activity. In rat experimental, nontoxicity was shown. Therefore, Se-KS has been proved to be a potential source of organic Se for use as a food supplement.

Data Availability

The numerous data used to support the findings of this study are included within the article and additionally are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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