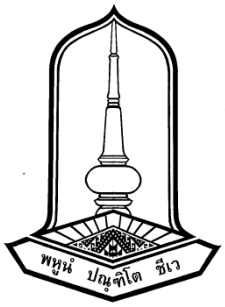

PHYSICOCHEMICAL PROPERTIES, ANTIOXIDATIVE ACTIVITIES AND ACUTE TOXICITY OF SELENIUM-ENRICHED KALE (*Brassica oleracea* var. *alboglabra* L.) SEEDLING

KWANYUEN LEAMSAMRONG

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry at Mahasarakham University

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The examining committee has unanimously approved this dissertation, submitted by Miss Kwanyuen Leamsamrong, as a partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry at Mahasarakham University.

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Kwanyuen Leamsamrong

ชื่อเรื่อง สมบัติทางเคมีกายภาพ ฤทธิ์ต้านอนุมูลอิสระ และความเป็นพิษเฉียบพลันของต้นกล้าคะน้าเสริมซีลีเนียม (***Brassica oleracea*** **var. *alboglabra* L.)**

ผู้วิจัย ขวัญยืน เลี่ยมสำโรง

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บทคัดย่อ

การศึกษาองค์ประกอบทางเคมี และฤทธิ์ต้านอนุมูลอิสระของต้นกล้าคะน้า ซึ่งประกอบด้วยต้นกล้าคะน้าไม่เสริมซีลีเนียม (R-KS) และต้นกล้าคะน้าเสริมซีลีเนียม (Se-KS) โดยมีการปลูกต้นกล้าคะน้าด้วยระบบไฮโดรโปนิกส์ ด้วยการเสริมโซเดียมซีลีไนท์ที่ความเข้มข้น 30 ppm ในครึ่งสูตรของสารละลายฮอกแลนด์ การทดลองนี้ได้มีการทดสอบปริมาณชีวภาพพร้อมใช้ของซีลีเนียม และฤทธิ์ต้านอนุมูลอิสระในต้นกล้าคะน้าหลังจากผ่านแบบจำลองการย่อยในกระเพาะอาหารและลำไส้เล็ก จากการทดลองพบว่าต้นกล้าคะน้าเสริมซีลีเนียมมีปริมาณชีวภาพร้อมใช้ของซีลีเนียม เพิ่มสูงขึ้นจากต้นกล้าคะน้าไม่เสริมซีลีเนียม 2.5 เท่า โดยมีค่าเท่ากับ 50.43±0.27 และ 20.74±5.53ตามลำดับ รวมถึงมีฤทธิ์ต้านอนุมูลอิสระด้วยวิธี DPPH, ABTS และ FRAP มากกว่าคะน้าไม่เสริมซีลีเนียมอย่างมีนัยสำคัญ ($p<0.05$) และพบว่าฤทธิ์ต้านอนุมูลอิสระมีความสัมพันธ์กับปริมาณชีวภาพพร้อมใช้ของซีลีเนียม ปริมาณซีลีเนียมทั้งหมด และปริมาณโพลิฟีนอลรวม นอกจากนี้ได้ประเมินความปลอดภัยในการบริโภคต้นกล้าคะน้า โดยการประยุกต์จากวิธี OECD ซึ่งเป็นวิธีมาตรฐานที่ใช้ในการทดสอบความเป็นพิษของสาร ในการทดลองครั้งนี้ได้มีการทดสอบในหนูสายพันธุ์ Wistar albino ทั้งสองเพศ โดยการทดสอบความเป็นพิษเฉียบพลันของต้นกล้าคะน้า ด้วยการป้อนครั้งเดียวที่ ขนาด 1250 2500 และ 5000 มิลลิกรัมต่อกิโลกรัมของน้ำหนัก พบว่าต้นกล้าคะน้าไม่ทำให้เกิดความเป็นพิษ ไม่ทำให้หนูตาย รวมถึงไม่ทำให้เกิดการเปลี่ยนแปลงลักษณะทางพิษวิทยาในตับ ไต และหัวใจ จึงสามารถประเมินได้ว่าต้นกล้าคะน้ามีปริมาณค่ามัธยฐาน (LD_{50}) มากกว่า 5000 มิลลิกรัมต่อกิโลกรัมน้ำหนัก มากไปกว่านี้จากการศึกษาปริมาณซีลีเนียมที่สะสมตามอวัยวะต่างๆ หลังจากการป้อน พบว่ามีการสะสมของซีลีเนียมที่ไตมากที่สุด ตามมาด้วยที่ พลาสมา ตับ ม้าม กระเพาะ หัวใจ ลำไส้ใหญ่ ปอด สมอง และมีปริมาณต่ำสุดที่ลำไส้เล็กตามลำดับ ซึ่งปริมาณซีลีเนียมที่สะสมได้มีแนวโน้มสูงขึ้นอย่างมีนัยสำคัญ ณ ช่วงเวลา 6 ถึง 24 ชั่วโมงหลังจากได้รับการป้อน และกลับมาสู่สภาวะปกติภายใน 14 วัน ต้นกล้าคะน้าเสริมซีลีเนียมอุดมไปด้วยซีลีเนียมจากสารอินทรีย์ จึงอาจเป็นแหล่งวัตถุดิบใหม่เพื่อนำมาปรับปรุงและพัฒนาให้เป็นผลิตภัณฑ์เสริมสุขภาพได้

คำสำคัญ: ต้นกล้าคะน้าเสริมซีลีเนียม; สมบัติทางเคมีกายภาพ; ปริมาณชีวภาพพร้อมใช้; ฤทธิ์ต้านอนุมูลอิสระ; ความเป็นพิษ

TITLE Physicochemical properties, antioxidative activities and acute toxicity of selenium-enriched kale (*Brassica oleracea* var. *alboglabra* L.) seedling

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ABSTRACT

The chemical composition and antioxidant activities of the kale seedlings including regular kale seedling (R-KS) and selenium enriched kale seedling (Se-KS) was studied. The kale seedlings were hydroponically germinated and supplemented with selenium in form of sodium selenite at 30 ppm in half formula of Hoagland's solution. The selenium bioacessibility (Se-bioacessibility) and antioxidant activities were investigated in kale seedlings after pass through the gastrointesine simulation process. It was found that the Se-KS showed higher percentage of Se-bioaccessibility about 2.5 fold more than the R-KS, which were 50.43±0.27% and 20.47±5.53%, respectively. The Se-KS was found to exhibit scavenging radical activity by DPPH, ABTS and FRAP assay significantly ($p < 0.05$) higher than the R-KS. The power potential of antioxidant activity in Se-KS was good correlated with Se-bioacessibility, total selenium and polyphenol contents. In addition, acute toxicity of kale seedlings was evaluated by using method that modified based on the OECD guidelines as standard toxicity testing. Both sex of Wistar albino rats were used in this experimental. A single dose acute toxicity of kale seedlings was oral administrated at 1250, 2500 and 5000 mg kg^{-1}. These results exhibited the kale seedlings did not cause any toxicological signs as well as there was no mortality seen in animals. Additionally, there were no abnormal pathology changed in the liver, kidney and heart. These results suggested that the median lethal dose (LD_{50}) of the kale seedlings is

more than 5000 mg kg^{-1} b.w. Moreover, selenium content accumulation in several tissues was found highest level in the kidney follow by plasma, liver, spleen, gastric, heart, large intestine, lung, brain and lowest level in small intestine, respectively. The selenium concentration of Se-KS in these tissues were significantly increased in the period of time range from 6 h to 24 h then it decreased and restored to normal condition in 14 days. The results suggested that the Se-KS could be a well source of enhance organic selenium for improving and developing as new source of supplement dietary.

Keywords Selenium-enriched kale seedling; Physicochemical property; Bioaccessibility; Antioxidant activity; Toxicity

CONTENTS

PAGE

PAGE

PAGE

LIST OF TABLES

PAGE

LIST OF FIGURES

PAGE

LIST OF FIGURES

PAGE

LIST OF FIGURES

PAGE

LIST OF ABBREVIATIONS

Dimethyl-selenide	DMSe
DPPH	1,1-diphenyl-2-picryhydrazyl
GI	Gastro-intestine
Glutathione peroxidase	GPx
Glutathione peroxidase-1	GPx-1
Glutathione peroxidase-2	GPx-2
Glutathione peroxidase-3	GPx-3
Glutathione peroxidase-4	GPx-4
Glutathione peroxidase-6	GPx-6
IC_{50}	Fifty percent inhibition concentration
Iodothyronine deiodenases	DIO
Iodothyronine deiodenases-1	DIO-1
Iodothyronine deiodenases-2	DIO-2
Iodothyronine deiodenases-3	DIO-3
R-KS	Regular kale seedling
Selenoprotein-H	Sel-H
Selenoprotein-I	Sel-I
Selenoprotein-K	Sel-K
Selenoprotein-N	Sel-N
Selenoprotein-R	Sel-R
Selenoprotein-S	Sel-S
Selenoprotein-P	Sel-P
Selenoprotein-W	Sel-W
Selenocysteine	SeCys
Selenomethionine	SeMet
Selenomethylselenocystheine	MeSeCys
Selenosugar	Se-sugars

LIST OF ABBREVIATIONS

Se-bioacessibility	Selenium bioacessibility
Se-KS	Selenium enriched kale seedling
Trimethyl-selenide	TMSe
Thioredoxin reductase-1	TrxR-1
Thioredoxin reductase-2	TrxR-2
Thioredoxin reductase-3	TrxR-3
Trolox	6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

CHAPTER 1

INTRODUCTION

1.1 Background

Selenium is a vital universal micronutrient that plays a key role in human and animal health. It is crucial for many cellular processes, and it is an essential element in the formation of glutathione peroxidases (GSHPx), thioredoxin reductase (TRx), iodothyronine deiodinases, selenophosphate synthetase, selenoprotein P and other selenoproteins (Rayman, 2000, 2002; Suhajda *et al*., 2000; Abdulah *et al*., 2005; Letavayova *et al*., 2006; Liu *et al.,* 2011). Several functions of selenoprotein are related with the immune system for the prevention of many diseases such as anti-asthma, anti-diabetes mellitus, anti-cancer, anti-aging, anti-cardiovascular disorders, anti-preeclampsia and anti-HIV/AIDS. (Allan *et al*., 1999; Ganther, 1999; Suhajda *et al*., 2000; Schrauzer, 2003; Tapiero *et al*., 2003).

Selenium is a crucial trace element from environmental and biological sources that can occur in organic and inorganic forms. There can be beneficial or toxic effects of selenium predicated on concentration ingested, chemical forms, method of administration, animal species, exposal time, idiosyncrasy, physiological status and interaction with any metals (Vadhanavikit *et al*., 1993; Fairweather-Tait, 1997; Hoefig *et al*., 2011). Selenium deficiency is found in the People's Republic of China, where acutely low soil levels of the element are detected. Selenium deficiency is associated with several diseases such as Kashan diseases, Kashin-Beck diseases, hypothyroidism, cardiomyopathy, cancer, endemic osteoarthropathy and anemia coronary heart disease etc (Chen *et al*., 1999; Burk, 2000; Tan *et al*., 2002; Flores-Mateo *et al*., 2006; Nawrot *et al*., 2007). To the contrary, high selenium level can result in selenosis. The symptoms of selenium toxicity include gastrointestinal upsets, hair loss, white blotchy nails, garlic breath odor, irritability, fatigue and mild nerve damage (Goldhaber, 2003; Maurice, 2006). Because only a narrow safety range of selenium has been reported (Spallholz *et al*., 2002; Encinar *et al*., 2003; Tapiero *et al*., 2003) it becomes very significant and

interesting to assure the balance of selenium in living humans and animals is correct (Suhajda *et al*., 2000)

Several edible plants and mushrooms have successfully produced a selenium enriched diet. These include alfalfa (*Medicago sativa*), lentil (*Lens culinaris*: Funes-Collado *et al*., 2013), cabbage (*Brassica oleracea*), lettuce (*Lactuca sativa*), chard (*Beta vulgaris*) and parsley (*Petroselinum crispum*: Funes-Collado *et al*., 2013), chives (*Allium schoenoprasum*: Kapolna *et al*., 2006), garlic (*Allium sativum*: Tsuneyoshi *et al*., 2006), green tea (Xu *et al*., 2007 Li *et al*., 2008; Taheri *et al.*, 2011), green onions (*Allium fistulosum*: Wrobel *et al*., 2004; Kapolna *et al*., 2006), carrot (*Daucus catota*), spinach (*Spinaia oleracea*), barley (*Hordeum vulgare*), buck wheat (*Fagopyrumesculentum*: Sugihara, M. *et al*., 2004), radish (*Kaiware*) sprouts (Yoshida *et al*., 2007, soybean (*Glycine max*: Chan *et al*., 2009; Funes-Collado *et al*., 2013) pakchoi (*Brassica chinensis*: Thosaikham *et al*., 2014) kale (*Brassica oleracea*: Meneetong *et al*., 2013), mushroom (Zhao *et al*., 2004; Cremades, *et al*., 2012) etc. It has been reported that there are many types of primary selenium compounds in plants including inorganic species (selenite Se(IV) and selenate Se(VI)), simple organic species (methylselenol, dimethylselenide, diethylselenide and dimethylo-selenooxide), amino acids and low molecular species (selenomethionine, selenocysteine, selenocystine, selenohomo-cysteine, Se-methylselenocysteine, Se-methylselenomethionine, Se-allyl-selenocysteine, Se-propylselenocysteine, ϒ-glutamyl-Se-methylseleno-cysteine, Se-adenosylseleno-homocysteine, selenoglutatione and other compounds (selenoproteins and selenoenzymes: Pyrzynska, 2009; Meneetong *et al*. 2013). Further research has shown that γ-glutamyl-Se-methyl selenocysteine serves primarily as a carrier of Se-methyl selenocysteine (Sugihara *et al*., 2004). Se-methyl selenocysteine is a good precursor for generating methylselenol when enzymes like β-lyase are present. This selenium metabolite seems to be the most active species for cancer reduction. As such, Se-methyl selenocysteine has been widely studied as a potential anti-carcinogenic compound (Montes-Bayon *et al*., 2006, Maneetong *et al*., 2013).

Where human health is considered, an estimation of bioavailability and bio-accessibility is of critical concern. The efficiency of bioavailability differs and depends on the chemical form of the element. Organic forms can be absorbed higher than inorganic forms of selenium by about two-fold. This has been investigated in more than 90 percent

of humans (Moreda-Pineiro *et al.*, 2011). Selenium bio-availability can also be studied using in vitro GI digestion and antioxidant assays (Moon and Shibamoto, 2009; Vendeland, 1992). These approaches are faster and simpler, and do not use laboratory animals thus providing a good alternative to *in vivo* studies.

Additionally, the bioavailability and bioaccessibility of selenium can be investigated by selenium speciation analysis in which the contents of total selenium and selenium species are determined. The methods used for determining total selenium are hydride generation atomic fluorescence spectrometry (HFS-AFS), or hydride generation atomic absorption spectrometry (HFS-AAS: Wang *et al*., 2012). The methods used for selenium species analysis, HPLC inductively coupled plasma mass spectrometry (HPLC-ICP-MS) and HPLC electrospray ionization mass spectrometry (HPLC-ESI-MS) (Dumont *et al*., 2006) have been modified. Fourier transformation cyclotron resonance mass spectrometry (FT-ICR-MS) is distinguished from other MS technologies by its superior sensitivity and ultrahigh resolution power (Ohta *et al*., 2010), making it an appropriate instrumental method to identify and quantify the selenium species. To improve the separation efficiency of organic selenium, ion-pair reversed-phase chromatography is often included in the HPLC system (Tsopelas *et al*, 2005; Zheng *et al*., 2003; Wang *et al.*, 2013).

Selenium toxicity depends on its chemical form. The general toxicity of most forms of selenium is low. Exposure to selenious acid or selenium dioxide can cause serious toxicity (Koppel *et al*., 1986). In animal studies, inorganic selenite is more toxic compared with selenate and elemental forms of selenium. Selenocysteine possesses similar toxicity in animals compared with selenite, while selenomethionine is somewhat less toxic (McAdam and Levander, 1987). In the 1970s, Schwarz and Pathak (1975) spent a great deal of effort to find nontoxic organoselenium compounds that prevented dietary liver necrosis in rats. However, almost none of the organoselenium compounds were really much better than the nontoxic levels of selenite in preventing dietary liver necrosis.

Previous studies showed that chronic dietary selenite or methy-selenosysteine toxicity initiates at 3-5 ppm, and there was almost no survival of rats fed 16 ppm Se (Harr *et al*., 1967; Martin and Hurlbut, 1976; Koller and Exon, 1986). This mirrors previous reports which revealed that Nano-Se (LD_{50} 113 mgSe kg^{-1}b.w.) has a 7-fold lower acute toxicity than sodium selenite (LD_{50} 15 mgSe kg^{-1}b.w.) in mice (Zhang *et al*., 2004), Nano-

Se exposed a lower short-term toxicity than selenite in mice (Zhang *et al*., 2004), selenium-enriched probiotics (LD_{50} of was 18.49 g kg^{-1}b.w.) in mice (Shun-yi and Ke-he , 2007), selenium- enriched yeast (LD_{50} of was 37.3 mgSe kg^{-1}b.w.) in weanling Sprague-Dawley rats (Aguilar *et al*., 2008). In a previous report it was (Maneetong *et al*., 2013) revealed that where high content (386.18 mgSe kg^{-1}) of selenium were in Se-KS; almost 80% of selenium in Se-KS was found in SeMet and MeSeCys forms. They are involved in many anti-disease functions. For the *in vivo* experiment of Se-KS, it was reported that the toxicity was less than the toxicity of Se from SS (Chantiratikul *et al*., 2011; 2015; 2016).

Using our understanding and knowledge, we are expected to find an alternative useful product for patients and health promoters who wish to use an enriched selenium plant as a new food supplement that can reduce the risk of disease. Therefore, this research concentrates on the development and application of the physical, biological and toxicological properties of selenium enriched kale. Specifically, the research investigated the chemical composition of kale seedlings. Se bio-accessibility was performed by using a gastro-intestine enzyme to ascertain the capacity of available selenium content in kale seedlings that would be useful for animals and humans. The antioxidant activities were monitored after simulation of gastrointestinal digestion in order to follow the potential for free radical scavenging activity. The activities were then compared with the different kinds of kale seedling. The correlation significant value of total selenium content, chemical composition and antioxidant activity in kale seedlings was evaluated. Additionally, to confirm a safety evaluation, the oral acute toxicity of kale seedlings in Wistar albino rats was investigated for the effects of varied doses and fixed doses.

1.2 Purposes of the research

1. To investigate the proximate analysis of the kale seedlings by AOAC methods
2. To determine total selenium and Se bioaccessibility of the kale seedlings by HG-AAS techniques

3. To determine the total polyphenol and flavonoid contents of the kale seedlings using the spectrophotometric method and to determine antioxidant activity by using DPPH, $ABTS^{\bullet+}$ and FRAP assay

4. To evaluate the relation between total protein content and antioxidant activities (DPPH, $ABTS^{\bullet+}$ and FRAP) of selenium enriched kale seedling

5. To investigate the toxicity of selenium enriched kale seedling.

6. To investigate the accumulation of total selenium content in different Wistar albino rat organs after administering kale seedlings

1.3 Scope of Research

1. Cultivation and preparation of the kale seedlings according to Maneetong *et al.* (2013).

2. Determination of the proximate analysis in the kale seedlings could be evaluated according to an adapted previously method (AOAC, 1980)

3. Determination of total selenium and Se bio-accessibility in the kale seedlings

4. Determination of total polyphenol and flavonoid content in the kale seedlings - The methods were estimated according to a modified procedure (Singleton *et al*., 1999; Chang *et al*., 2002).

5. Investigation and evaluation of antioxidant activities in kale seedlings were estimated according to a modified procedure (Pulido *et al*., 2006; Ursini *et al*., 1994; Long and Halliwell, 2001; Benzie and Strain, 1996; Pulido *et al*., 2000).

6. Investigation of the toxicity of the kale seedlings was conducted by modifying standard procedure (OECD, 420 and 423).

7. Determination of total selenium content in different organs of Wistar albino rats after being administered kale seedlings.

1.4 Benefit of Research

1. Provide chemical composition data of kale seedlings, such as the contents of protein, carbohydrate, fat, fiber, ash and moisture by AOAC methods.

2. Provide data of the total selenium and Se-bioaccessibility in kale seedlings.

3. Provide data of total polyphenol and flavonoid contents in the kale seedlings.

4. Provide data of the correlation efficiency values between total protein contents and bioactivities (antioxidant; ABTS and DPPH free radical scavenging assay) of the kale seedlings.

5. Provide data of the toxicity of the kale seedlings.

6. Provide data of selenium accumulation in different Wistar albino rat's organs after administering kale seedlings.

CHAPTER 2

LITERATURE REVIEW

2.1 Selenium

In 1817 the selenium was discovered by Swedish chemist and Jons Jacob Berzelius. Selene is Greek's name of selenium, it mean goddess of the moon. Schwarz and Foltz was detected selenium as necessary to animal and human health about 140 years later, the micronutrient was protected against liver necrosis in vitamin E deficient rats (Schwartz and Foltz, 1957; Kim and Mahan, 2003; Kieliszek *et al*., 2012). Additionally, in 1960 the roles of selenium in human health were interested gather momentum and scrutiny looked for human diseases related to those of Se-responsive animal disarrays. Although selenium was identified as essential to human nutrition 42 years ago, a universal marker of daily requirement remains elusive. Research has extended our knowledge of the vital functional roles attributed to selenium, which have both short-term, and long-term public health implications (Navarro-Alarcon and Cabrera-Vique, 2008). The evidence of selenium toxicity occurred after observance a lethal disease in the middle of horses grazing in certain areas of China and USA (Moxon *et al*., 1943; Dumont *et al*., 2006). The lower concentration of selenium are essential, whereas higher concentration it revealed toxicity. Wada *et al*. (1993) also reported that the narrow concentration of selenium between deficiency, essential and toxicity. For the consumption of food comprising less than 40 µg day $^{-1}$effects in deficiency, while regular consumption of food comprising more than 400 µg day $^{1-}$effects in toxicity (WHO, 1996).

2.2 Selenoprotein

Selenium is importance micro element that is specifically incorporated into selenoprotein are enzyme. The potential of selenoprotein and seleno-enzyme are use in prevention and treatment of cancer, cardiovascular and neurodegenerative disease, diabetes, immune system disorder, antioxidant oxidoreductase function (Alexander, 2015). The 25 selenoprotein are generally occurred in human organism as well as 12 and

24 selenoprotein in yeast cell and rodent, repectively. Selenoprotein can be classified into housekeeping and stress-related protein that is an essential part at a cellular level in several metabolic process. The greatest biological importance of selenium in the organism is related with its occurrence in active sites of many enzyme and proteins. In 1973, the first selenoprotein were discovered. There were the glycine reductase show in *Clostridium stricklandii* bacterium cell and for mate dedydrogenase show in Clostridium thermoaceticum bacterium cell (Rayman, 2000). The GPx activity are depended on the incorporation of selencystein reduce into each of its four polypeptide chains. Selenium in the form of selenocystein in the peptide chain 45, is the fixed fragment of the GPx active site (Pyrzynska, 1996). Under physiological conditions. Selenoprotein is completely dissociated (pK~5) and breaks down hydrogenperoxide into alcohol. The five difference human GPx enzymes constitute a family of enzyme that have the ability to reduce inorganic (H_2O_2) and organic (ROOH) peroxide, with the formation of seleninic acid as an intermediate product. GPx family are enzymes that contain selenium in the form of selenocystein in theirs active site. It is role in these processes in estimate that of tocopherals (vitamin E) (Wasowicz *et al*., 2003; Berry *et al*., 1997; Combs *et al*., 2001; Zeng and Combs, 2008; Encinar *et al*., 2003; Gharieb *et al*., 2004). GPx enzymes are protected membrane from proxidantive damage (Ursini *et al*., 1982), it can decrease phospholipid, cholesterol and cholesterol ester hydroperoxide to be harmless toxic derivatives (Maiorino *et al*., 1990). Enzyme thioredoxin reductase are related in the redox regulation of cellular process; activation and inactivation of thyroid hormones (Fairwether-Tait *et al*., 2010; Berry *et al*., 1991; Davey *et al*., 1995; Arthur *et al*., 1990a; 1990b; 1991; 1999). Tamura and Stadtman are reported and identified thioredoxin reductase, which reduces oxidized thioredoxin. Thioredoxin are strong donor of electron for enzyme catalyzing reductions, including the ribonucleotide reductase and thioredoxin peroxidase. (Kieliszek and Blazejek, 2013). Additionally, thioredoxin redutase certain low-molecular-weight compound for example oxidized glutathione, dehydroascorbic acid vitamin K, lipid peroxidase and hydrogen peroxide (Kieliszek and Blanzejak, 2013). Moreover, selenoproteins and other include selenoprotein-P are decrease of lipid peroxidanse (Saito *et al*., 1990) and other. Twenty-five selenoprotein known in human and their function are summarized and described in Table 2.1.

Table 2.1 Summary of human selenoproteins and biological functions

Abbreviate	MW(kDa)	Subcellular	Tissue distribution	Functions
GPx-1	87	Cytoplasm	Ubiquitous, highly expressed in erythrocytes, liver, kidney and lung	-Cystolic enzyme -Reduce retroviral virulence by preventing viral mutations deficiency causes cardiomyopathy -Antioxidant defence in cell cytosol -The reduction of H_2O_2 and a limited number of organic hydroperoxide for example cumen hydroperoxide and tert-butyl hydroperoxide
GPx-2	87	Cytoplasm	Liver, epithelium of the gastrointestinal tract	-Reduce lipid peroxide during food digestion in alimentary tract -Protection of gastrointestinal tract from oxidative damage -Antiapoptic function colon crypts; helps to maintain intestinal
GPx-3	93	Plasma	Plasma10-30% selenium in plasma, extracellular fluid, express in liver, kidney, heart, lung, thyroid, gastrointestinal tract and breast	-Plasma antioxidant can decrease lipid hydroperoxides -Antioxidant in extracellular fluid, kidney is source of GPx-3 in plasma thyroid protection from hydrogen peroxide in thyrocytes and follicular lumen
GPx-4	22	Cytoplasm mitochondria nucleus	Testes; widely expressed high expression in the testes, cytosol and membrane bound form	-Antioxidant in membranes; structured protein in sperm -Reduction of phospholipid hydroperoxide -Can decrease phospholipid, cholesterol and a cholesterol ester hydroperoxides to less toxic derivatives

Table 2.1 Summary of human selenoproteins and biological functions (Cont.)

Abbreviate	MW(kDa)	Subcellular	Tissue distribution	Function
DOI-2	30-34	ER and membrane	Thyroid, CNS, pituitary brown adipose tissue, skeletal muscle, heart, kidney, pancreas	-Activation of thyroid hormones -Thyroid hormone control
DOI-3	31	Cell and endosome membrane	Placenta, fetal tissue, skin membrane	-Convert thyroxine (T4) to bio-inactive 3',5,',3; reverse –tri-iodothyronine
Sel-P	45-57	Plasma	Plasma 30-50% of selenium in plasma, Ubiquitously expressed in most tissue; high expression in brain, liver and testes, heart and also found in the kidney	-Selenium home ostasis and transport of selenium to tissues/antioxidant activity and decrease of lipid hydroperoxide -Protect against oxidizers -Prevent liver necrosis and lipid peroxidation -Protect against free radical Transports selenium to other tissue (brain, kidney, testes, erythrocytes) -Play main part in selenium organification and metabolism -Protect endothelial cell against damage for peroxynitrite
Sel-W	9	Cytoplasm	Various tissue, abundant in muscle, brain, colon, heart, skeletal muscle and prostate	-Involved in skeletal and cardiac muscle metabolism/function, antioxidant function and an intracellular transporting medium for selenium metabolizes muscle -Can reduce peroxide using glutathione as electron donor

Table 2.1 Summary of human selenoproteins and biological functions (Cont.)

Abbreviate	MW(kDa)	Subcellular	Tissue distribution	Function
Sel-M	14	Perinuclear region, ER lumen, Gogi	Mainly brain, kidney, lung and other tissue	-Involved in protein folding in the ER -Protein folding in the endoplasmic reticulum antioxidant activity
Sel-S	21	ER membrane	Plasma, various tissue membrane protein, endoplasmic reticulum	-Inflammatory response, regulation of inflammatory cytokines (interleukin1β and 6 and tumor necrosis factor alpha) -Remove of misfolded protein from the endoplasmic reticulum -Transmembrane protein, putative role in endoplasmic reticulum stress
Sel-H	13	Nucleus	Embryonic and tumor cell	-DNA binding protein, regulation of glutathione synthesis genes and phase II detoxification
Sel-T	20	ER, Golgi	Ubiquitous	-Calcium mobilization
Sel-V	17	Testes	Unknown	-Testes specific expression
Sel-R	5-14	Cytoplasm	Herat, liver, muscle, kidney	-Contains antioxidant properties -Participates in methionine metabolism -Participates in protein repair

Table 2.1 Summary of human selenoproteins and biological functions (Cont.)

Abbreviate	MW(kDa)	Subcellular	Tissue distribution	Function
Sel-K	61-62	ER-membrane	Most tissue, ubiquitous expression transmembrane glucoprotein associated with endoplasmic reticulum, brain, lung and placenta	-May be important in muscle and development -Degradation of H_2O_2 -May affect glycoprotein folding -May regulate calcium mobilization required for early muscle devilment; mutation cause myophathies including multiminicore disease
SPS2	47	Cytoplasm	Liver	-Synthesis of selenophosphate for selenoprotein synthesis
Sel-I	45	ER membrane	Various tissue, abundant in brain	-Unknown
Sel-O	73	-	Various tissue	-Unknown

Source: Fairweather-Tait *et al*. (2010); Rayman (2012); Zwolak and Zaporwska (2012); Kieliszek and Blazejak (2013); Duntas and Benvenga (2015)

2.3 Source of selenium

The naturally occurring metalloid element and present inorganic and organic forms. Selenium is chalcogens in group 16 (VIA) of the periodic table. The physical properties are showed in Table 2.2 and revealed that chemical symbol, atomic number, atomic mass and other properties.

Table 2.2 Physical properties of selenium

Property	Detail
Element name	Selenium
Chemical symbol	Se
Atomic number	34
Periodic table group	VIA
Atomic mass	78.96
Density	4,808 kg m^{-3}
Melting point	220 °C
Boiling point	685 °C
Vapor pressure	1 mmHg at 356 °C
Natural isotope	Abundance:
^{74}Se	0.87%
^{76}Se	9.02%
^{77}Se	7.58%
^{78}Se	23.52%
^{80}Se	49.82%
^{82}Se	9.19%

Source: ATSDR (2003); Fordyce (2013)

Six stable isotope of selenium are occur in natural. The chemical behavior of selenium resembles that of sulphur. Oxidation state of selenium described in Table 2.3 selenium can exist in the 2^-, 0, 4^+ and 6^+.

Table 2.3 Chemical forms of selenium in the environment

Oxidative state	Chemical forms
Se^{2-}	Selenide (Se^{2-}, HSe-, H_2Se_{aq})
Se^{0}	Elemental selenium (Se^{0})
Se^{4+}	Selenite (SeO_3^{2-}, $HSeO_3^{-}$, H_2SeO_{3aq})
Se^{6+}	Selenate (SeO_4^{2-}, $HSeO_4^{2-}$, H_2SeO_{4aq})
Organic Se	SeMet, SeCys, MeSeCys, γ-glutamyl-Se-methylselenocysteine

Source: Rayman (2012); Fordyce (2013)

2.3.1 Selenium in the environment

At the global scale, selenium is constantly recycled in the environment via the atmospheric, marine and terrestrial systems (Fordyce, 2013). Selenium cycling through the atmosphere is significant because of the rapidity of transport, but the terrestrial system is most important in terms of animal and human health because of the direct links with agricultural activities and the food chain (Fordyce, 2013).

2.3.2 Selenium in food

Food is the major source of selenium contents for the general population (Dumont *et al*., 2006). The concentration of selenium in food and beverages is influenced by geographical location, seasonal change, protein content and food processing (Navarro-Alarcon and Cavrera-Vique, 2008). Selenium content of food is highly dependent on the amount of selenium in the soil, which related on the capacity of plants to take up, accumulate, transform and assimilate the element. (Ellis and Salt, 2003). Food protein content is another important factor influencing selenium presence in food since selenium and replace sulphur in amino acid as SeMet, SeCys and selenocystathionine due to their physicochemical similarity (Navarro-Alarcon and Cavrera-Vique, 2008). Selenoproteins, which selenium is available under the SeCys form. The Se-containing proteins are synthesized by plants and animals (Dumont *et al*., 2006). SeMet is the principal form of

selenium in plants. It seems that animals do not divide between SeMet and its S-analogue methionine. SeMet and SeCys are combined in proteins via the same enzymatic pathway. The occurrence of these compounds in the protein sequence depends on the respective availability of both compounds (Esaki *et al*., 1981). Beside, selenite, MeSeCys and CysSeSeCys are the most intensively analyzed selenium compounds in laboratory and epidemiological investigates into several diseases prevent and treatment (Ganther, 1999; Suhajda *et al*., 2000; Fernandes and Gandin, 2015). They are found selenium compound reference this review that showed in Figure 2.1.

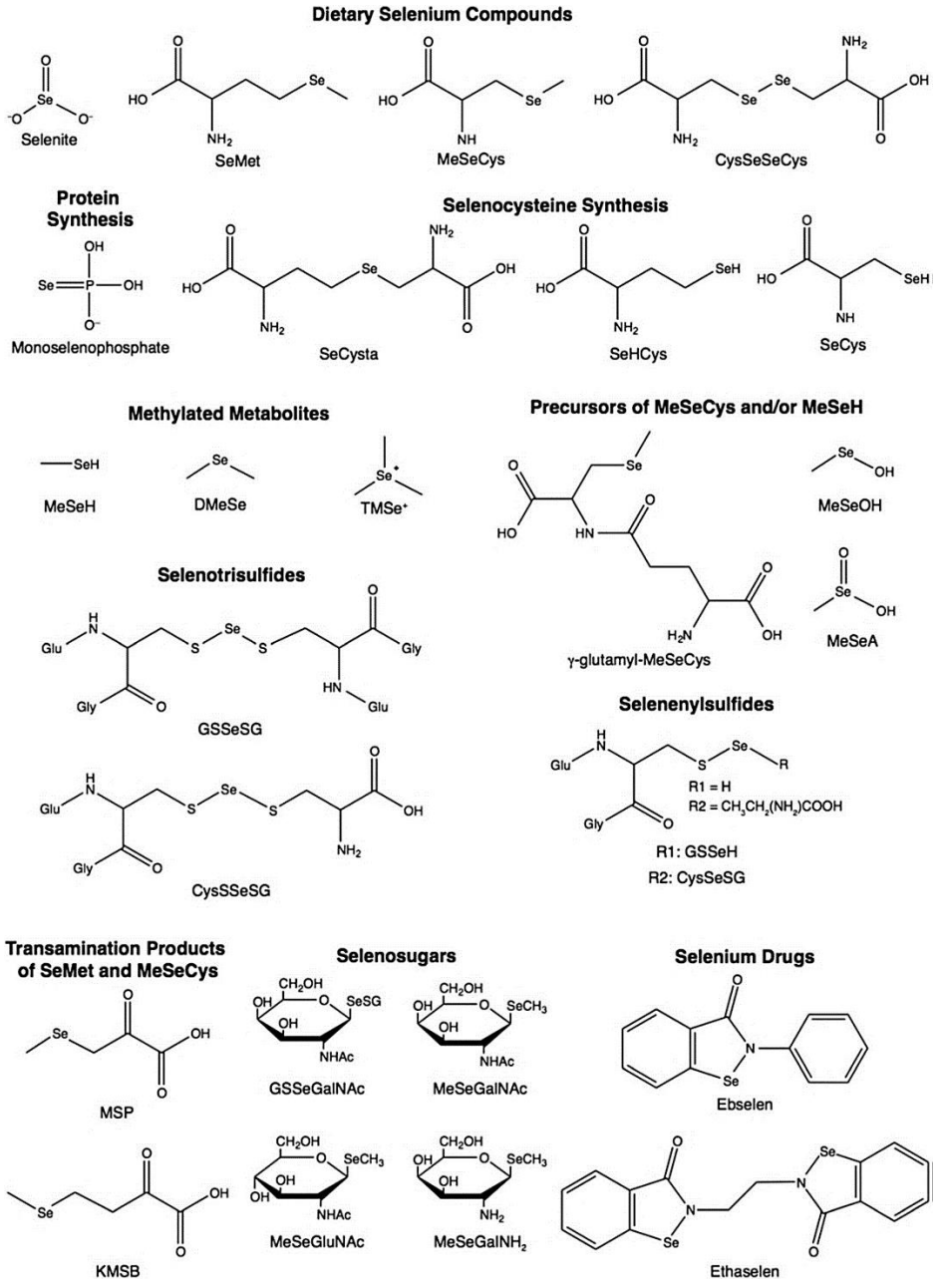

Figure 2.1 Structures of the selenium compound (Weekley and Harris, 2013)

Selenium accumulator is present in SeMet as free SeMet (Ogra *et al*., 2004). The methylated form of SeCys, SeMeCys are exhibit in selenium accomulators in the form of free and γ-glutamyl-Se-methylselenocystenine. Because, free mercapto (-SH) groups are

lower less inactive than free selenol (-SeH) and SeCys is reactive to be present in the free form (Suzuki, 2005). Similarly, Selenium element is accumulates in accumulators on form of non-active amino acid and peptides (-$SeCH_3$ group more than -SeH group) for example MeSeCys, SeMet and γ-glutamyl-Se-methylselenocystenine (Ogra *et al*., 2004; Suzuki, 2005). Selenite are accumulators as broccoli and cucumber, SeMet-accumulators as grains, wheat and mushroom and MeSeCys-accumulators as garlic, kale and onion. Selenium source in normal food id thus selenium bound to protein such as SeCys in seleno enzyme and SeMet in general protein in food, selenite and selenate in drinking water and foods. Data on selenium contents in several foods are summarized in Table 2.4. Tuna, salmon, sardines, pork, ham and oyster are protein-rich foods containing high levels of selenium (Marro, 1996; Akl *et al*., 2006).

Table 2.4 Selenium content in food according to several researchers

Food type	Origin	Selenium contents ($\mu g\ kg^{-1}$)	Reference
Salmon	Australia	270-368	Marro (1996)
Tuna	Egypt	810	Akl *et al*. (2006)
Sardine	Australia	570	Fardy *et al*. (1994)
Rice	Greece	19.1	Pappa *et al*. (2006)
Rice	Italy	20.1	Panigati *et al*. (2007)
Rice jasmine	Thailand	37-59	Sirichakwal *et al*. (2005)
Pork	USA	144-450	USDA (1999)
Pork	Thailand	142-201	Sirichakwal *et al*. (2005)
Ham	Australia	200	Tinggi (1999)
Oster	Australia	770	Fardy *et al*. (1994)
Beef	Thailand	75-226	Sirichakwal *et al*. (2005)
Beef	Australia	80-200	Fardy *et al*. (1994)
Lamb	Spain	27-30	Diaz-Alarocon *et al*. (1996)
Pork kidney	Spain	849-1543	Diaz-Alarocon *et al*. (1996)
Pork liver	Spain	256-800	Diaz-Alarocon *et al*. (1996)
Chicken	Thailand	156-271	Sirichakwal *et al*. (2005)
Chicken	Brazil	330	Lemire *et al*. (2010)

Table 2.4 Selenium content in food according to several researcher (Cont.)

Food type	Origin	Selenium contents ($\mu g\ kg^{-1}$)	Reference
Lamb	Spain	27-30	Diaz-Alarcon *et al*. (1996)
Rabbit	Spain	74-106	Diaz-Alarcon et al. (1996)
Egg hen	Thailand	327	Sirichakwal *et al*. (2005)
Eggs	Greece	172.8	Pappa *et al*. (2006)
Eggs	Australia	0.72-14.2	Marro (1996)
Egg duck	Thailand	485	Sirichakwal *et al*. (2005)
Cow's milk	Greece	13.1-21.9	Pappa *et al*. (2006)
Cow's milk	Ireland	14-18	Murphy and Cashman (2001)
Cow's milk	Thailand	19-36	Sirichakwal *et al*. (2005)
Gouda cheese	Greece	85.4	Pappa *et al*. (2006)
Yoghurt	Greece	21.9-26.9	Pappa *et al.* (2006)
Yoghurt	Croatia	29.9	Klapec *et al*. (2004)
Yoghurt	Spain	50.0	Cabrera *et al*. (1996)
Acacia pennata (Cha-om)	Thailand	127	Sirichakwal *et al*. (2005)
Broccoli	Thailand	60	Sirichakwal *et al*. (2005)
Garlic	Brazil	80	Lemire *et al*. (2010)
Onion	Brazil	70	Lemire *et al*. (2010)
Onion	India	127	Singh and Garg (2006)
Green peas	India	180	Singh and Garg (2006)
Peper	India	150	Singh and Garg (2006)
Peper, sweet	Thailand	1	Sirichakwal *et al*. (2005)
Chili peper	Thailand	1	Sirichakwal *et al.* (2005)
Black peper	India	116	Singh and Garg (2006)
Celery	Australia	9.3-14.2	Marro (1996)
Lettuce	Australia	3.-22.8	Marro (1996)
Green pea	India	180	Singh and Garg (2006)
Bean	Brazil	50	Lemire *et al*. (2010)
Lentils	USA	28	USDA (1999)
Kale, Chainese	Thailand	3	Sirichakwal *et al.* (2005)
Radish	Thailand	2	Sirichakwal *et al.* (2005)
Spinach	Thailand	6	Sirichakwal *et al.* (2005)

Source: Navarro-Alarcon and Cabrera-Vique (2008)

2.3.3 Selenium in supplement

Various researcher considered that the selenium supplementation can be essential for individuals in regions with very low levels of selenium (Simonoff *et al*., 1992; Chan et al., 1998; Navarro-Alarcon and Cabrera-Vique, 2008). The several forms of selenium suggest the role and efficacy of the functions it carries out (Fairweather-Tait *et al*., 2010). The commonly form of selenium supplements are including selenite (Se(IV)) or selenate (Se(VI)), SeMet, SeCys and MeSeCys. Specifics vary among studies, but in general, inorganic forms of selenium are absorbed less effectively than the organic forms, with the latter being slightly less toxic (DRI, 2000; Tiwary *et al.,* 2006). SeCys and SeMet have been shown to be incorporated into proteins in humans and plants **(**Ogra, 2004; Cheajesadagul *et al*., 2014; Kubachka *et al*., 2017). Selenium enriched plants have been widely studied for alfalfa (*Medicago sativa*: Hajiboland *et al*., 2015), lentil (*Lens culinaris*: Funes-Collado *et al*., 2013; Ekanayake *et al*., 2015;), cabbage (*Brassica oleracea*), lettuce (*Lactuca sativa*), chard (*Beta vulgaris*) and parsley (*Petroselinum crispum*: Funes-Collado *et al*., 2013), chives (*Allium schoenoprasum*: Kapolna *et al*., 2007), garlic (*Allium sativum*: Tsuneyoshi *et al*., 2006), green tea (*Camellia sinensis*: Xu *et al*., 2007; Li *et al*., 2008; Taheri *et al.,* 2011), green onions (*Allium fistulosum*: Wrobel *et al*., 2004; Kápolna *et al*., 2006), carrot (*Daucus catota*), spinach (*Spinaia oleracea*), barley (*Hordeum vulgare*), buck wheat (*Fagopyrumesculentum*: Sugihara *et al*., 2004), radish sprouts (*Kaiware*: Yoshida *et al*., 2004), soybean (*Glycine max*: Chan *et al*., 2009; Funes-Collado *et al*., 2013), pakchoi (*Brassica chinensis*: Thosaikham *et al*., 2014), kale (*Brassica oleracea*: Meneetong *et al*., 2013), mushroom (Zhao *et al*., 2004; Cremades, *et al*., 2012), tobacco (*Nicotiana tabacum* L. Fan *et al*., 2015; Jiang *et al*., 2015), pear (*Pyrus bretschneideri*), grape (*Vitis vinifera*), peach (*Prunus persica*) (Feng *et al*., 2015), cucumber (*Cucumis sativus* L.: Hawrylak-Nowak *et al*., 2015), coleus (*Coleus blumei* Benth: Hu and Yuan, 2015), Danshen (*Salvia miltiorrhiza*: Hu *et al*., 2015), sugar cane (*Saccharum* spp. hybrids Var. CoLk 94184), wheat (*Triticum aestivum* L.: Liu *et al*., 2015), sweet basil seedlings (*Ocimum basilicum*: Oraghi-Abdebili *et al*., 2015) and rape seedlings (*Brassica napus* L. cv. Xiangnongyou 571: Liu *et a*l., 2015) Table 2.5 was summarized the total selenium contents and selenium species in several selenium enriched plants.

Table 2.5 Total selenium and selenium species concentration in dried

Plant	Selenium addition	Selenium species	Total selenium ($\mu g\ g^{-1}$)	Reference
Broccoli	Sodium selenate	SeMet, MeSeCys	467.1	Thosaikham *et al.* (2014)
Broccoli	Sodium selenite	Selenite, SeMet, MeSeCys	27.0	Pedrero *et al*. (2007)
Chickpea	Sodium selenate	-	214.0	Zhang *et al*. (2012)
Chicory	Sodium selenate	selenite, selenate, SeCys, SeMet, MeSeCys,	480.0	Mazej *et al*. (2006)
Dill	Sodium selenite	selenite, SeCys, SeMet, MeSeCys	10.3	Cankur *et al*. (2006)
Kale	Sodium selenite	SeMet, MeSeCys	386.1	Maneetong *et al*. (2013)
Garlic	Sodium selenate	selenite, selenate, SeCys, SeMet, MeSeCys, γ-Glu-MeSeCys	969.0	Larsen *et al*. (2006)
Garlic	Sodium selenate	selenite, selenate, SeCys, SeMet, MeSeCys, γ-Glu-MeSeCys	296.0	Ip *et al*. (2000)
Garlic	Sodium selenate	selenite, selenate, SeCys, SeMet, MeSeCys, γ-Glu-MeSeCys	96.0	Dumont *et al*. (2006)
Garlic	Sodium selenite	selenate, SeCys, SeMet, MeSeCys, γ-Glu-MeSeCys	68.0	Kotrebai *et al*. (2000)
Garlic	Sodium selenite	selenate, SeCys, SeMet, MeSeCys, γ-Glu-MeSeCys	235.0	Kotrebai *et al*. (2000)
Garlic	Sodium selenite	selenate, SeMet, MeSeCys, γ-Glu-MeSeCys	1355.0	Kotrebai *et al*. (2000)
Green onion	Sodium selenite	SeCys, SeMet, MeSeCys	30.3	Shah *et al*. (2004)
Onion	Sodium selenite	SeCys, SeMet, MeSeCys	154.0	Wrobel *et al*. (2004)
Onion	Sodium selenate	SeCys, SeMet, MeSeCys	601.0	Wrobel *et al*. (2004)
Onion	Sodium selenite	selenate, SeMet, MeSeCys, γ-Glu-MeSeCys	96.0	Kotrebai *et al*. (2001)
Onion	Sodium selenite	selenate, SeMet, MeSeCys, γ-Glu-MeSeCys	140.0	Kotrebai *et al*. (2001)
Pakchoi	Sodium selenate	SeMet, MeSeCys	311.7	Thosaikham *et al*. (2014)
Pumpkin	Sodium selenite	Se-Met	1.1	Smrekolj *et al*. (2005)

Table 2.5 Total selenium and selenium species concentration in dried (Cont.)

Plant	Selenium addition	Selenium species	Total selenium ($\mu g\ g^{-1}$)	Reference
Ramp	Sodium selenate	selenate, MeSeCys, SeMet, γ-Glu-MeSeCys	252.0	Whanger *et al*. (2000)
Ramp	Sodium selenite	selenate, MeSeCys, SeMet, γ-Glu-MeSeCys	48.0	Kotrebai *et al.* (2001)
Ramp	Sodium selenite	selenate, MeSeCys, SeMet, γ-Glu-MeSeCys	524.0	Kotrebai *et al*. (2001)
Radish	Sodium selenite	selenate, SeCys, SeMe, MeSeCys	112.0	Ogra *et al*. (2005)
Radish	Sodium selenate	selenate, SeCys, SeMe, MeSeCys	120	Ogra *et al*. (2005)
Radish	Sodium selenite	selenate, SeCys, SeMe, MeSeCys	47.8	Pedrero *et al*.(2006)
Radish	Sodium selenate	selenate, SeCys, SeMe, MeSeCys	110.4	Pedrero *et al*.(2006)
Rice	Sodium selenite	SeMet	1.1	Xu *et al*. (2004)
Shallot	Sodium selenite and Sodium selenate	selenate, MeSeCys, γ-Glu-MeSeCys	226.8	Ogra *et al*. (2005)

2.4 Recommendation dietary intake of selenium

The current US recommendations for dietary selenium intake are summarized in Table 2.6 These recommendations differ from those issued in some other countries in having a greater number of life-stage groups.

Table 2.6 US RDA (Recommended Dietary Allowances) dietary reference intake ($\mu g\ kg^{-1}$) for selenium

Age	Male	Female	Pregnancy	Lactation	UL
0-6 months	15	15	-	-	45
7-12 months	20	20	-	-	60
1-3 years	20	20	-	-	90
4-8 years	30	30	-	-	150
9-13 years	40	40	-	-	280
14-18 years	55	55	-	-	400
≤18 years	-	-	60	70	400
19-30 years	55	55	60	70	400
31-50 years	55	55	60	70	400
50-70 years	55	55	-	-	400
≥70 years	55	55	-	-	400

Source: Food and Nutrition Board National Academy of Sciences (2002)

2.5 Metabolism of selenium in mammal

The chemical property of selenium are similarity with sulfur. Therefore, various plants and other organisms are taken up and metabolize selenium via sulfur transporters pathways (Pilon-Smits and Quinn, 2010). The substitution of sulfur by selenium in proteins and other compound interferes the function of their molecules. Ip (1998) was studied the assimilation of selenium intake was first proposed consists of the reduction of the different species to hydrogen selenide (HSe). This selenium species shows the role of a central gateway for both utilization and excretion. The waste of selenium was occured

through a mechanism of methylation in DMSe, excreted by the breath and feces. Selenosugars and trimethylselenide (TMSe) forms are excreted into the urine. The absorption, transport, distribution, excretion, retention and metabolic transformation of selenium is dependent on the chemical form and amount of element ingested and on the presence or absence of numerous interacting dietary factors (Whanger, 1996). The metabolism of selenium in mammals is schematically exhibited in Figure 2.2.

2.5.1 Absorption

The absorption of selenium species are arises mainly in duodenum part of the small intestine by the different routes and mechanisms, in various cases shared with their sulphur analogues. Practically all forms of selenium, inorganic as well as organic, are readily absorbed with total efficacy close to be complete (70–90%) under normal physiological and intake conditions (Fairweather-Tait *et al*., 2012; Roman *et al*., 2014). Whanger (1996) was confirmed the complete absorption of selenium species in rats (91% and 81% for SeMet and SeCys). SeMet is better absorbed than selenite (Bopp *et al*., 1982; Swanson *et al*., 1991; Daniels, 1996; Whanger, 1996) and actively absorbed in the similarly mechanism as methaionine (Wolffram *et al*., 1989; Combs *et al*., 1984; Daniels, 1996). Selenite is an exception because its direct absorption does not exceed 60%. Christensen (1983) confirmed the relatively poor absorbability by human of selenium as selenite as compared to organic form. Levander (1986) conducted with human have demonstrated that the absorption of dietary selenium intake ranges from 55-70%. In the current of reduced glutathione (GSH), as occurs in the gastrointestinal fluid, selenite was increased absorption up to quantitative proportions (Grammelgaard *et al*., 2010). In this conditions, selenite responds non-enzymatically with thiol groups of GSH to form selenodiglutathione (GS-Se-SG, Figure 2.2 path a), as follows: (Weekly *et al*., 2012; Roman *et al*., 2014)

$$2H^+ + 4GSH + SeO_3^{2-} \text{ --------> } GSSG + Gs\text{-}Se\text{-}SG + 3H_2O$$

GS-Se-SG is consequently decomposed by glutathione reductaseinto selenide following the steps (Figure 2.2 path b):

$$GS\text{-}Se\text{-}SG + NADPH \text{ ---------> } GS\text{-}Se^- + GSH + NADP^*$$

$$GS\text{-}Se^- + H_2O \text{ ---------> } Se^0 + GSH + OH^-$$

$$GS\text{-}Se^- + NADPH + H_2O \text{ ---------> } HSe^- + GSH + NADP+ + OH^-$$

$$GS\text{-}Se^- + GSH \text{ ---------> } HSe^- + GSSG$$

$$HSe^{-}(O) \text{ ---------> } Se^{0} + OH^{-}$$

$$HSe^{-} + (O) + 2GSH \text{ ---------> } HSe^{-} + GSSG + H_2O$$

The GS-Se-SG are remained stable in the stomach due to at low pH conditions, that estimated to become unstable and reactive in the intestine (Gabel-Jensen *et al*., 2006; Roman *et al*., 2014). The transport proteins process related in the direct or indirect absorption of selenite are unclear. The fraction of selenite which is directly absorbed undergoes the same reduction in red blood cells (RBCs), so that the overall pool of the species is converted into selenide (Suzuki, 2005; Orga *et al*., 2013; Roman *et al*., 2014). Selenide anions are the key of metabolic intermediates, which can be converted to selenoprotein synthesis or selenium excretion (Ogra *et al*., 2013; Chantiratikul *et al*., 2016). Otherwise, selenite can be a substrate for the thioredoxin system (thioredoxin, NADH and thioredoxin reductase, itself a Se-protein) and directly reduced to selenide (Figure 2.2 path c) following a reaction path similar to that reported above for glutathione reductase (Weekley *et al*., 2011; Roman *et al*., 2014). The diglutathione (GSSG) is not a substrate for thioredoxin reductase and is a poor disulfide substrate for reduced thioredoxin. However, the addition of a selenium atom makes this compound a highly reactive substrate for the thioredoxin system, able of redox cycling in the presence of oxygen. Selenate is absorbed paracellularly, with elevated efficiency, via a passive diffusional process (Gammelgaard *et al*., 2012; Roman *et al*., 2014). After absorption, it is reduced to selenite (Figure 2.2 path d), as in sulfate reduction, by ATP sulfurylase via the still unidentified Se-isologue of 3-phosphoadenosine 5-phosphosulfate (Se-PAPS) (Roman *et al*., 2014). Moreover, selenate may share the absorption pathway as sulphur (Shennan, 1988) and higher absorbed than selenite (95%). The Se-amino acids SeMet and SeCys are absorbed through transcellular pathways mediated by transporters which are basically shared with their sulphur-containing analogues (Nickel *et al*., 2009; Roman *et al*., 2014). SeMet is absorbed through a Na^{+}-dependent process, but the identity and affinity of the transport proteins is still to be established. SeMet can also be incorporated non-specifically into proteins such as serum albumin and haemoglobin, by randomly replacing the (sulphur) methionine (Fig. 1 path e) (Suzuki and Ogra, 2002). Instead, it can be transformed into SeCys (Figure 2.2 path f) (Schrauzer, 2002) and then into selenide (Figure 2.2 path g) via the trans-selenation pathway, analogous to the trans-sulfuration pathway. The SeMet released through protein catabolic processes enters the trans-

selenation pathway in the same way. Excess of SeMet has been also proposed to undergo direct methylation by γ-lyase (Figure 2.2 path h) (Suzuki *et al*., 2006; Roman *et al*., 2014). The absorption of MeSeCys may share with SeMet part of the transport mechanism, but some distinctions are still not clearly understood.42 The Se-dipeptide GGSeMCys is assumed to play the role of a carrier of MeSeCys. After ingestion as a dietary constituent, the bulk (not necessarily the entire amount) of GGSeMCys is hydrolyzed by γ-glutamyl transpeptidase in the gastrointestinal tract (Figure 2.2 path i), releasing MeSeCys for absorption and systemic delivery to the other tissues (Dong and Lisk, 2001; Roman *et al*., 2014). GGMeSeCys is quantitatively absorbed from the gastrointestinal tract like MeSeCys. MeSeCys and GG MeSeCys are directly methylated by β-lyase to MSe (Figure 2.2 path j) so that urinary excretion is the major route for eliminating the excess of Se from these species (Suzuki *et al*., 2006; Roman *et al*., 2014)

2.5.2 Transport, distribution and utilization

The Se-species absorbed into the gastro-intestinal tract are firstly transported into the liver: SeMet is usually transported in the form of Se-albumin (SeAlb) (Schrauzer, 2002; Roman *et al*., 2014) while selenate and the other organic species may be transported intact or through mechanisms which are still not elucidated. The liver is the foremost organ in Se metabolism, since it synthesizes most of the Se-proteins and regulates the excretion of Se metabolites (Suzuki *et al*., 2010; Roman *et al*., 2014). The SelP produced into the liver is released into the bloodstream and is responsible for the distribution of Se to the other organs, where other Se-proteins can be synthesized. The local uptake of Se from plasma has been shown to occur by endocytosis mediated by receptors of the apolipoprotein family such as apoER2 in testis and brain (Olson *et al*., 2007) and megalin (Lrp2) in kidney (Olson *et al*., 2007; Roman *et al*., 2014). Thus, the liver regulates the whole-body Se distribution by sorting the metabolically available Se between the two pathways of selenoproteins synthesis and the excretory metabolite synthesis. (Burk and Hill, 2009). Such regulation might be passive, so that the fraction of Se that cannot be utilized for Se-proteins synthesis enters the excretory pathway. Active regulation of the excretory metabolites has been also hypothesized (Burk and Hill, 2009; Roman *et al*., 2014) but not yet investigated.

The utilization of selenium requires the generation of Se-donor selenophosphate (SePhp) from selenide and ATP (Figure 2.2 path k) that is mediated by seleno-

phosphate synthetase 2 (SPS2). Different from all the other amino acids that are synthesized before being aminoacylated onto their tRNAs, SeCys is directly synthesized on its tRNA, designated $tRNA^{[Ser]}Sec$, by the mechanism represented in Figure 2.2 (Turanov *et al*., 2011; Roman *et al*., 2014). The $tRNA^{[Ser]}Sec$ is initially aminoacylated with serine by seryl-tRNA synthetase (SerRS). The hydroxyl moiety of Ser is then replaced by a phosphate group to form O-phospho-seryl-$tRNA^{[Ser]}Sec$ by a specific kinase (PSTK). Finally, SeCys synthase (SeCysS) exchanges the phosphate group with activated SePhp to form selenocysteyl-$tRNA^{[Ser]}Sec$. The $tRNA^{[Ser]}Sec$ reads the UGA codon and is used for the integration of SeCys into the amino acidic sequence to form selenoproteins (Figure 2.2 path l) (Turanov *et al*., 2011; Roman *et al*., 2014). Thus, SeCys is recognised as the 21st amino acid because its synthesis is genetically encoded in the ribosome-mediated system. Interestingly, in mice Cys can replace SeCys in Se-proteins such as thioredoxin reductases in proportions that depend on the Se status (Turanov *et al*., 2011; Roman *et al*., 2014). The catabolism of selenoproteins releases SeCys (Figure 2.2 path m) which is cyclically reconverted to selenide.

2.5.3 Excretion

The excretion of Se in humans follows two possible routes, leading in both cases to methylated products. The balance among the metabolites depends on the source species and the selenium status. Under supplemented or toxic selenium status, TMSe is well recognized as the core metabolite. The production starts from selenium sources which are already mono-methylated species, such as SeMCys and selenobetaine (SeBet), and is subsequently transformed following a stepwise methylation pathway mediated by methyltransferases (Figure 2.2 path n) (Ohta and Suzuki, 2008; Roman *et al*., 2014). The intermediate species, DMSe, excreted trough the breath route, seems to be kinetically favoured with respect to TMSe. Under low-toxic Se status the metabolism of Se follows another route, where selenide is converted into an intermediate selenosugar-GS conjugated (GS-Se-N-acetyl-galactosamine, GS-SeGal) and then into SeMethyl-*N*-acetyl-galactosamine (MSeGalNAc), excreted into the urine (Figure 2.2 path o) (Suzuki, 2005; Roman *et al*., 2014). Minor selenosugars have been also detected in urine, including SeMethyl-N-acetyl-glucosamine (MSeGluNAc) (Gammelgaaard and Bendahl, 2004; Roman *et al*., 2014) and the deacylated analogue of SeGalNAc, SeMethyl-N-amino-galactosamine ($MSeGalNH_2$) (Letseou *et al*., 2007; Roman *et al*., 2014). It has been also

hypothesized that in case of Se excess, hydrogen selenide can be metabolized entering into the stepwise methylation pathway (Figure 2.2 path p) (Ohta and Suzuki, 2008; Roman *et al*., 2014).

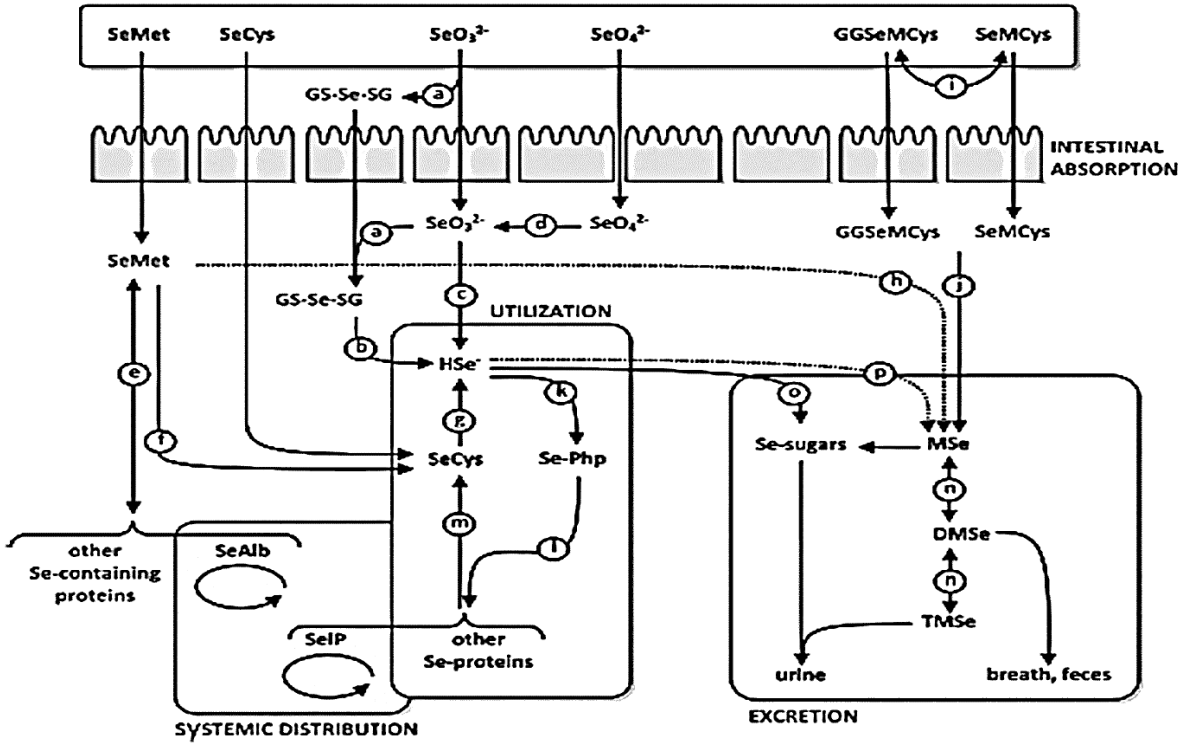

Figure 2.2 Metabolism pathway of nutritionally available selenium in mammals.

Source: Roman *et al*. (2014)

2.6 Selenium bioacessibility and bioavailability

The bioacessibility and bioavailability are important when assessing chemical risk to human, it purports to simulate the release of chemical from sample. The fraction of a substance that is available for absorption through the gastrointestine (GI) tract into the blood stream (Ruby *et al*., 1993; Ruby *et al*., 1996). Pedrero *et al*. (2006) focused the potential bioavailability of selenium and its species form radish. The examination of the GI showed that almost 100% of selenium present in fresh plant. Bioaccesible percentage for total selenium were 55-88% in Barker's yeast and yeast tablets (Dumont *et al*., 2004; Dumont *et al*., 2006). The estimation of bioavailability and bioaccessibility is special concern because some of them are essential for human. The efficiency of bioavailability are different depends on the chemical form of the element. The organic forms can be absorbed higher bioavailability than inorganic forms of selenium about two-fold and has been investigated more than 90 percentage in humans (Moreda-Pineiro *et al*., 2011). Selenium bioavailability can also be studied using in vitro GI digestion and antioxidant assays (Vendeland, 1992; Moon and Shibamoto, 2009; Wang *et al*., 2013). These approaches are faster and simpler, and do not use laboratory animals, and thus provide a good alternative to in vivo studies.

2.7 Antioxidant role of selenium

The antioxidant mechanism of selenium for example ROS scavenging, glutathione peroxidase, and metal-binding were concerned and described. Selenium compound are well known for their ability to scavenging ROS (Battin and Brumaghim, 2009). Kunwar *et al*. (2007) investigated the power potential of 3,3-diselenobispropionic acid to scavenge peroxyl radical using UV-vis spectrophotometer to confirm the reaction. The scavenging superoxide capacity of six selenocarbamates and selenourea compound by using chemiluminescence to monitor was examined. (Takahashi *et al*., 2005). Sies and coworker was determined the effectiveness of selenomethionine and ebselen to scavenge peroxynitrete. In addition, Lin *et al*. (2005) was studied the combining polyphenol and selenium functionality in polyphenolic ester. These compound were investigated by using

DPPH radical scavenging activity and prevent peroxynitrite oxidation. It was found that addition of a selenium atom in these molecules improve their antioxidant ability more than the regular polyphenolic ester. For the mechanism of glutathione peroxidase. The mechanism of the hydrogen peroxide reduction by two molecules of glutathione catalyzed by the selenoprotein glutatione peroxidase (GPx) has been computationally studied. It has been shown that the first elementary reaction of this process, (E-SeH) + H_2O_2 --> (E-SeOH) + H_2O (1). The second elementary reaction, (E-SeOH) + GSH --> (E-Se-SG) + HOH (2) and the last elementary reaction, (E-Se-SG) + GSH --> (E-SeH) + GS-SG (3), is initiated with the coordination of the second glutathione molecule (Prabhakar *et al*., 2005). Finally, metal-binding mechanism of selenium. Zimmerman *et al*. (2015) concerned about selenium compounds more efficiently inhibited Cu (I)-mediated DNA damage compared to Fe (II)-mediated DNA damage. It revealed that simply the existence of selenium atom is not sufficient for the inhibition of DNA damage, and that functional groups with the capacity to interact with metal ions considerably affect antioxidant behavior. Mass spectrometry confirmed copper and iron coordination for selenium compounds, regardless of antioxidant ability, indicating that metal binding is necessary but not sufficient for sulfur and selenium prevention of DNA damage. Because sulfur and selenium antioxidants can prevent DNA damage through multiple pathways. Methylselenocysteine and selenomethionine with CuI established that coordination occurs through the selenium atoms in addition to the nitrogen and/or oxygen atoms by using NMR and X-ray absorption spectroscopy. Zainal *et al*. (1995) was reported structural of metal-selenium complexes for biological relevant metal ion $(SeMet)_2Cu$ and $(SeMet)_2Zn$ there structure characterized by using IR and Raman spectroscopy. Similarly study, the antioxidant activity of several selenium compounds with metal-mediated DNA damage caused by iron, copper and hydrogenperoxide. It was found that SeMet, selenolcysteine, methyl-selenocysteine prevent copper-mediated DNA damage**.**

CHAPTER 3

METHODOLOGY

3.1 Research designs

In this research, the experiments were designed as shown in Figure 3.1

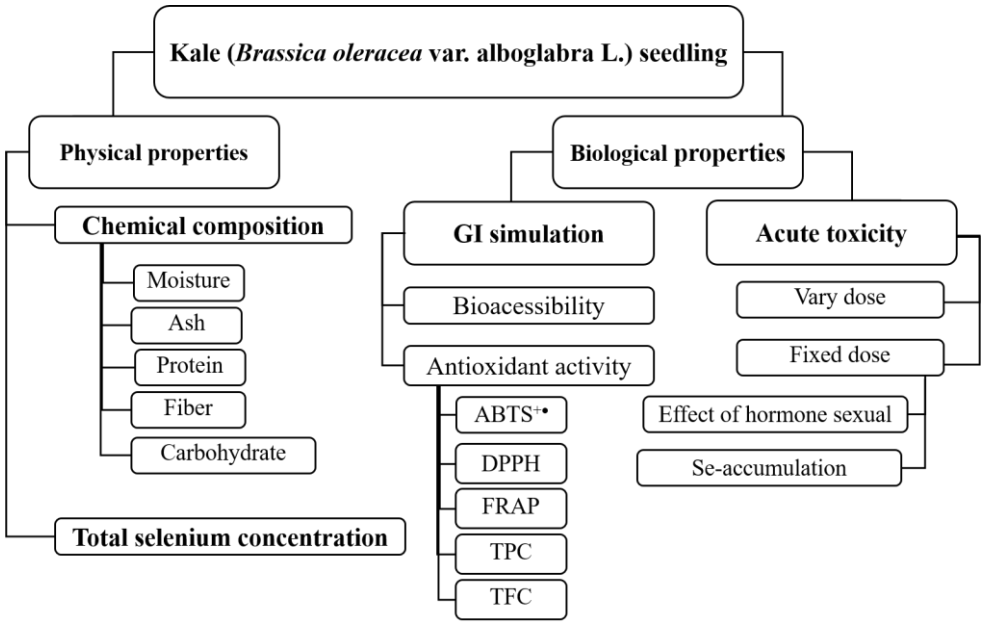

Figure 3.1 The schematic scope of this research

3.2 Chemicals and reagents

Sodium selenite pentahydrate, α-amylase from porcine, pancreatin from porcine, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (trolox), ascorbic acid, Folin–Ciocalteu reagent, 2,2-azino-bis-(3-ethylenebenzo-thiozoline-6-sulphonic acid) (ABTS), gallic acid and quercetin were purchased from Sigma-Aldrich (USA). Pepsin and 2,4,6-tripiridyl-S-triazine (TPTZ) were obtained from Acros Organics (Belgium). Alluminium trichloride, ethanol, hydrochloric acid, iron (III) chloride hexahydrate, potassium acetate, potassium iodide, magnesium sulphate heptahydrate, propylene glycone and sodium nitrile were purchased from BHD (UK). Ammonium thiocyanate, methyl red and sodium carbonate were

purchased from Univar (USA). Bromocresol green, boric acid, boric indicator solution, nitric acid, potassium nitrate, potassium sulphate, sodium hydroxide, sodium molybdate, sulfuric acid and zinc chloride were purchased from Carlo Erba (Italy). Sodium borohydride was purchased from HIMDIA (India). All chemicals and reagents used in this study were of analytical grade.

3.3 Experimental instruments materials, instruments and apparatus

The experimental equipment used in this study is listed in Table 3.2

Table 3.1 List of experimental materials, instruments and apparatus used in this work

Equipment	Supplier
Analytical balance	Denver Instrument Company
Air pump	Thailand
Chinese kale seeds	Chia Tai Company Limited, Thailand
Filter paper No.1	Whatman, England
Fluorescent lamp	Thailand
HG-AAS	Agilent Technologies, Inc., USA
Micropipette	Gilson, France
Sponge	Thailand
Shelf	Handmade
Spectrophotometer	GENESYS^{TM} 10S, Thermo Scientific, USA
Plastic pot	Thailand

3.4 The cultivation and preparation of the kale seedlings

The plantings of the selenium enriched kale seedlings were evaluated according to a modified previous procedure (Meneetong *et al.*, 2013). The kale seeds were soaked in tap water for 15 h before planting. After that, kale seeds were planted into wet sponges in plastic pots (35×40×30 cm) and closed until the seeds began to germinate. After almost all of the kale seeds were germinated, the plastic pots were opened to light from a fluorescent lamp (12/12 h day/night) and watered daily with tap water for 4 days. After

that, the plant was supplemented with a selenium element form of Na_2SeO_3 and could be treated within 10 L of adapted half formula of Hoagland's solution at a concentration of 30 μgSe mL^{-1} by using the hydroponic system. After 15 days, the plants were harvested and washed thoroughly with deionized water to exclude contamination from their surfaces. They were dried at 60 °C in a hot air oven to a constant weight, ground and stored at -20 °C until analysis.

3.5 Determination of total selenium

The total selenium content of the dried samples was analyzed by 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 then 1.5 mL deionized water was added and mixed in a glass vessel. The vessel was heated at 100 °C in a metal bath for 45 min. After cooling, 5 mL of 37%w/w HCl was added to reduce Se (VI) to Se (IV) for 30 min. The digest solution was transferred and stored in a freezer at 4 °C until analysis. The digestion process was accurately validated with a percentage recovery test by spiking the selenium standard solution. The total selenium compounds; selenite ion (SeO_3^{2-}), Se-methyl selenocysteine (SeMCys) and selenomethionine (SeMet) were determined by HG-AAS. The standard solution of each Se compound was prepared by diluting stock solutions to a final concentration of 20 μg Se L^{-1} with DI water. Afterward, the standard solutions of each selenium compound were individually injected into the hydride generation system. The method of selenium hydride generation was recommended in the user's guide of VGA (Agilent technology, 2014). The conditions of HG-AAS for Se determination were summarized. The parameters Setting VGA 77, reducing solution (0.6% w/v $NaBH_4$ and 0.5% w/v NaOH), acid solution (5 M HCl), carrier gas (Ar gas) and ETC 60 (atomization temperature 900 ^{o}C and hollow cathode lamp UltrAA lamp for selenium detection at λ_{max} 196 nm. The method of validation was carried out by spiking 20 μL of Se standard solution, containing 10 mg Se/L, into the mixture of the sample and nitric acid. The Se from the Se standard solution recovered at 83.03-133.46% and the linear range from 1-20 μg Se L^{-1}.

3.6 Chemical compositions of the kale seedlings

3.6.1 Determination of moisture content

The determination of moisture content was evaluated according to an adapted method from a previous procedure (AOAC, 1980). One gram of the kale seedlings was placed in the crucible and heated at 105° C until a constant weight was obtained. The moisture content of each variety was calculated as loss in weight of the original sample and expressed as percentage of moisture content (n=3).

3.6.2 Determination of crude protein

The crude protein was determined by the Kjeldahl method, which was modified from a previous method (AOAC, 1980). The 0.5 g of the powdery form of the kale seedlings was digested with 5 ml of 98% w/w sulphuric acid in the presence of a Kjeldahl catalyst by using Kjeldatherm block digestion unit (Gerhardt, Germany). The nitrogen from the protein in the sample was converted to ammonium sulphate that was distilled with 40%w/v sodium hydroxide and back titrated with 0.1 N of hydrochloic acid. The percentage nitrogen was calculated and multiplied by 6.25 to obtain the value of crude protein

3.6.3 Estimation of crude lipid

Crude lipid was performed using the soxhlet extraction method, which was evaluated according to a modified previous method (AOAC, 1980). Two grams of the powdery form of each of the kale seedlings were weighed and wrapped with a filter paper and placed in a thimble. The thimble was covered with cotton wool and placed in the extraction column that was connected to a condenser by using auto extractor (BUCHI, Japan). Then, two hundred milliliters of hexane was used to extract the lipid. It was cooled in a desiccator and weighed at room temperature to obtain the weight of the crude lipid.

3.6.4 Determination of crude fibre

Crude fiber was analyzed using the AOAC method (1980). Two grams of the powdery form of the selenium enriched kale seedling and 200 ml of 1.25 % H_2SO_4 were heated for 30 min and filtered with a buchner funnel. The residue was washed with distilled water until it was acid free. 200 ml of 1.25% NaOH were used to boil the residue 30 min, it was filtered and washed several times with distilled water until it was alkaline free. Finally, it was rinsed with ethanol three times. The residue was put in a crucible and

dried at 105^{o} C in an oven overnight. After cooling in a desiccator, it was ignited in a muffle furnace at 550 ^{o}C for 90 minutes to obtain the weight of the crude fiber.

3.6.5 Determination of ash content

The method used was adapted from previous procedure (AOAC, 1980). The total ash content of a substance was the percentage of inorganic residue remaining after the organic matter was ignited. One gram of all samples of kale seedlings was placed in a crucible and ignited in a muffle furnace at 550 ^{o}C for 6 h. It was cooled in a desiccator and weighed at room temperature to get the weight of the ash.

3.6.6 Determination of carbohydrate

The carbohydrate content was evaluated according to a previously method (AOAC, 1980). The carbohydrate was calculated by the difference of the summed up percentage compositions including protein, lipid, fibre, and ash contents from 100 percent.

3.7 The evaluation of Se bio-acessibility and antioxidant activity

3.7.1 Simulation of gastrointestinal digestion

The gastrointestinal digestion was simulated by using a previously described method (Pedrero *et al*., 2006). 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 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 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 volumetric flask and filtered through filter paper and stored at -20 $^{\circ}$C until used for the antioxidant analysis. The blank of gastrointestinal digestion, 2.5 mL of deionization water and above described was used. Total selenium of the samples after GI digestion was determined by previously described conditions. Total selenium GI digestion and blank (without sample) were measured after the GI process. % Bioaccessiblity was calculated by equation below.

$$\%\text{Bioacessibility} = \frac{\text{Total selenium GI digestion} - \text{total selenium in blank}}{\text{Total selenium in sample}}$$

3.7.2 Estimation of total phenolic content

Total phenolic content was determined according to a modified procedure (Singleton *et al*., 1999). Briefly, 100 µL of previously prepared sample was oxidized with 500 µL of 0.2 N Folin-Ciocalteu's reagent and neutralized by adding 400 µL of 7.5%w/v sodium carbonate. The absorbance was measured at 765 nm after mixed and incubated in room temperature for 60 min. The results were calculated as milligram per gram gallic equivalent (mgGE gDW^{-1}).

3.7.3 Estimation total flavonoid

Total flavonoid content was estimated using the aluminum chloride colorimetric method (Chang *et al*., 2002). The previously prepared sample was mixed with 500 µL of 2.5%w/v, sodium nitrite 400 µL of 5% w/v aluminum chloride. The mixture was allowed to incubate at room temperature for 30 min. The absorbance of the reaction mixture was read at 415 nm. The results were expressed as milligram per gram quercetin equivalent (mgQE gDW^{-1}).

3.7.4 DPPH free radical scavenging activity

The radical scavenging capacities of samples against DPPH radical were evaluated by Ursini *et al*. (1994). Appropriate dilutions, 100 µL of the digestion of samples from previous experimental (range from 0.025-10 mg mL^{-1}) in 1000 µL of 0.1 mM ethanolic solution of DPPH radical Absorbance was measured at 515 nm after 30 min of reaction in the dark. Trolox and ascorbic acid were used as standard substances. The percentage inhibition was calculated by the following equation as**:**

$$\%\text{Inhibition DPPH radical scavenging} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

3.7.5 $ABTS^{\bullet+}$ radical scavenging assay

In ABTS assay, 2,2-azinobis (3-ethylbenzothiazolin-6-sulphonic acid) (ABTS) cation radical was generated with potassium persulphate (Long and Halliwell, 2001). The ABTS radical was generated by mixing two stock solutions of 7 mM ABTS and 2.4 mM potassium persulphate in the same ratio and allowed to react in the dark for

12 h at room temperature. The $ABTS^{+\bullet}$ radical solution was diluted to an absorbance at 734 nm in deionized water. The 100 µL of different concentrations of the previously prepared sample (0.1-1.0 mg mL^{-1}) were mixed with 900 µL of $ABTS^{+\bullet}$ radical 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 solution. The percentage inhibition was calculated by the following equation as**:**

$$\%\text{Inhibition ABTS radical scavenging} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

3.7.6 Ferric reducing antioxidant power (FRAP)

The ferric reducing ability of the extract was measured at low pH (Benzie and Strain, 1996; Pulido *et al*., 2000). FRAP reagent (900 µl) was mixed with an aliquot of 100 µl of different concentrations of the selenium enriched kale seedling extract (1-40 µg/ml). This could be incubated for 15 min at 37 °C. An intense blue colored complex was formed when Fe^{3+}-TPTZ complex was reduced to the ferrous (Fe^{2+}-TPTZ) form. The absorbance at 595 nm was recorded. The reducing power of the samples increased with the absorbance values.

3.8 Acute toxicity of selenium enriched kale seedling

The experimental procedures were modified from the Organization for Economic Corporation and Development (OECD) guideline and approved by the Animal Ethic Committee of Mahasarakham University (Approval No. 005/2557). For the preparation of animal and feed condition for all acute toxicity experimental were described below.

Principle

Acute toxicity category method is a method for assessing acute oral toxicity. It involves the identification of a dose level that causes mortality. The test involves the administration of a single dose of test chemical or substance to fasten healthy young adult rodents by oral gavage, observation behavior for up to 14 days after dosing and

recording of body weight, food intake and the necropsy of all the animals. In this method a pre-specified fixed dose of the test substances was used.

Animals and treatment

Healthy young adult male and female Wistar albino rats**,** 8- 12 weeks old, weighing 200- 230 g for males and 180- 220 g for females were used. The animals were purchased from National Laboratory Animal Center, Mahidol University. Moreover, female rats were non-nulliparous and non-pregnant.

Housing and feeding conditions

All rats were housed and maintained in aluminum cages (2 rats per cage) at the temperature of the experimental animal room of 22 ±3 ^{o}C and 50-60% relative humidity, with 12:12 light/dark cycle. For feeding, conventional laboratory diets were used with an unlimited supply of drinking water.

Preparation of animals

All animals are randomly selected and marked to permit individual identification and kept in their cages for at least 5 days prior to the start of administrating to allow for acclimatization to laboratory conditions.

Preparation of doses

The preparation of kale seedlings was dissolved by using propylene glycol as vehicle. The animals were given the maximum volume not normally exceeding 1 mL/ 100g of body weight: however in the case of aqueous solutions 2 mL/ 100 g body weight can be considered. The doses were prepared shortly prior to administration unless the stability of the preparation over the period during which it were used is known and shown to be acceptable (OECD, 2000).

Administration of doses

The simultaneous solution of kale seedlings were administered by gavage needle. In the unusual circumstance that a single dose was not possible, doses were given in smaller fractions over a period not exceeding 24 h (OECD, 2000).The animals were fasted prior to dosing (e.g. with the rat, food but not water should be withheld over-night). The doses were provided over two periods (withheld 3 h after administration), because the single dose over stuffed in animals. (OECD, 2000).

Post-procedural monitoring

The animals were monitored at 30 min, 60 min and every 3 h until 24 h after the administration. The observation of the animal for the following signs; labored breathing, discharge from the nose or mouse, ruffled fur, hunched posture, lethargy, minimal responsiveness, diarrhea, immobility. Lethargy, labored breathing, minimal responsiveness and uncontrolled bleeding or discharges are criteria for immediate euthanasia. Recordation was observed on the experimental health monitoring sheet. The Attending Veterinarian was alerted about any ill or injured rodents (OECD, 2000).

Data Collection

Body weight and food consumption were collected before and after the administered test every day until the 14th day. Weight gain was collected and calculated. At the end of the test, surviving animals were weighed and then humanely killed. After 14 days, all animals were sacrificed with nambutol; 45 mg/kg b.w. (i.p.) anesthesia and collected blood samples were put into heparinized tubes by cardiac puncture. The blood samples were collected in EDTA for studying haematological and biochemical blood parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), dehydrogenase (LDH), total protein (TP), glucose (GLU), uric acid (UA) and creatinine (CREA). All parameters of blood were analyzed by the Animal hospital faculty of Veterinary Medicine Khon Kaen University. The organs including liver, kidney, heart, spleen and lung were collected, weighted and the relative organ weight was determined (based on terminal body weight). The relative organ weight and body weight gain were calculated as follows:

$$\%\text{Relative organ weight} = \frac{\text{Absolute organ weight(g)}}{\text{Body weight (g)}} \times 100$$

$$\text{Body weight gained} = \frac{\text{Final weight(g)} - \text{Initial weight}}{\text{Initial weight (g)}} \times 100$$

Additionally, data were collected from some organs such as heart, liver, and kidney for pathology especially for varying dosages in the acute toxicity test.

Waste or residual material from this study

Non- infectious materials were kept in waste plastic bags tightly sealed and landfilled. The infected animals were kept in black bags. Infectious waste was deeply landfilled to avoid environment pollution.

3.8.1 Vary dose acute toxicity

For this experiment, female rats were used. Literature reviews of LD_{50} methods (OECD guideline 423) report that females are generally slightly more sensitive (Lipnick *et al.*, 1995; Ateba *et al.*, 2014) and OECD recommends the use of female animals for acute toxicity study. Twenty eight Wistar albino rats were randomly divided in three groups including control and test groups. The control group was administrated propylene glycol as a vehicle while the test groups varied and performed both kale seedlings (R-KS and Se-KS) at 1250, 2500 and 5000 mg kg^{-1}b.w. (n=4 for each group)(OECD, 2000). After administration, behavior signs were monitored and data collected as described above (post–procedural monitoring and data collection).

3.8.2 The effect of fixed doses acute toxicity in male and female rats modified based on OECD guideline 420

The Wistar albino rats of either gender catalog (n=4) were randomly divided into three groups including control and test groups. The control group was administrated propylene glycol as vehicle while, the test groups were administrated both kale seedlings (R-KS and Se-KS) at 5000 mg kg^{-1}b.w. The acute toxicity test was modified based on OECD 420 (fixed dose) method. After administration, behavior signs were monitored and data collected as described above (post–procedural monitoring and data collection).

3.8.3 The effect of fixed dose acute toxicity on the selenium accumulation in several tissues

This experimental research project used male rats to avoid the hormone sexual effect (n=4). The OECD guideline 420 (fixed dose) was used and handled the acute toxicity test. Forty eight Wistar albilno rats were randomly divided into three groups including control and test groups. The control group was administrated propylene glycol as a vehicle while, the test groups were administrated both kale seedlings at 5000 mg kg^{-1}b.w. After administration, behavior signs were monitored and data collected as described above (post–procedural monitoring and data collection). Some of the blood samples were especially collected in EDTA to prevent blood clotting. This allowed for a thorough study of studying biochemical blood parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine (CREA) and blood urea nitrogen (BUN). Blood chemicals were used to monitor liver and kidney

functions. Additionally, this part focused on the accumulation of selenium in tissues. The time periods were studied from a range of 6 h, 12 h, 24 h and 14 days for either group for any clear changes in the selenium content in tissues both before and/ or after administration of kale seedlings. For the selenium accumulated experiment, plasma and several tissues such as brain, heart, liver, kidney, gastric, small and large intestine were collected and washed with normal saline, dried with hot air oven and digested for determination of total selenium using HG-AAS. Tissues sample preparation and detection of selenium used the same method as in kale seedlings. Moreover, the body weight, food and feces were collected before and after administering the test substance every day until the 14^{th} day. The monitoring of selenium accumulation in feces was performed.

3.9 Data analysis

The results were performed in triplicate and expressed as mean±SD. The significant difference was analyzed by one way analysis (ANOVA) followed by Scheffe's post hoc test for multiple comparison. Independent t-test was used to comparison test at p <0.01 and 0.05 were considered significant between R-KS and Se-KS. The correlations among variables were performed with Pearson's correlation test. In the test acute toxicity was used and presented as mean±SEM and analyzed by one way analysis (ANOVA) followed by Scheffe's post hoc test for multiple comparison.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Chemical composition, total selenium and Se-bioaccessibility of kale seedlings

The AOAC (1980) method was used to determine the chemical composition of R-KS and Se-KS. Table 4.1 showed the chemical composition, total selenium content and Se-bioaccessibility in vitro simultaneous gastrointestine model of R-KS and Se-KS. The moisture and crude fat content of all kale seedlings was not significantly different *(p* > 0.05). The results for crude proteins, total selenium and Se-bioacessibility of the Se-KS were 36.42±0.21%, 432.54±22.28 mg kg^{-1} D.M. and 50.43±0.27%, respectively. These were significantly ($p < 0.05$) higher than those of the R-KS, 32.68±2.24%, 16.63±3.07 mg kg^{-1} D.M. and 20.47±5.53%. It can be seen that the total crude protein increased with association with biosynthesis and bioaccumulation of selenium in their plants (Sors *et al*., 2005). Various data revealed that the protein content of enriched selenium plants is higher than selenium accumulated in regular plants (Thavarajah *et al.*, 2008; Broadley *et al.*, 2010; Premarathna *et al.*, 2012; Bhatia *et al*, 2013; Poblaciones *et al.*, 2013; Poblaciones *et al.*, 2014; Banuelos *et al.*, 2015; Lavu *et al.*, 2016; Bachiega *et al.*, 2016). The supplementation of a suitable content of selenium increases synthesis of seleno-amino acid and protein content (Zhao *et al.*, 2004; Sors *et al.*, 2005; Zhu *et al.*, 2009). In this study, Se-KS exhibited 26 fold higher selenium accumulations than the R-KS. This finding was in good agreement with LeDuc *et al*. (2004), who reported the accumulation of enriched selenium in *B. juncea*, 2-4 fold more than regular plant. Pilon-Smits *et al*. (1999) revealed that the total foliar Se accumulation and higher Se tolerance increased 2-3 fold. Additionally, the accumulation of selenium in *A. thaliana* had an 8 fold increase (Ellis *et al.*, 2004). Meanwhile, Se-bioaccessibility gained after *in vitro* simulated gastrointestinal ingestion. Fifty percent of bioavailable capacity in the Se-KS that showed high selenium contents could be taken up in human system simulation. These results are in accordance with a previous investigation that exhibited high Se-bioavailability of Se-

KS in laying hens (69.72% and 64.54% for selenium food supplemention at 5 mgSe kg^{-1} and 10 mgSe kg^{-1}) (Chantiratikul *et al.*, 2016). Conversely, ash mainly consists of inorganic metal, salt and trace elements in kale seedlings. R-KS showed a significantly higher ($p < 0.05$) ash content than the Se-KS. This is because Se-KS, more than R-KS (Maneetong *et al*., 2013) was transformed from an inorganic to organic form.

Table 4.1 Proximate analysis and total selenium of R-KS and Se-KS

Proximate Composition	**R-KS**	**Se-KS**
Moisture (%)	94.89±0.12	94.35±0.09
Crude protein (%)	32.68±2.24[a]	36.89±1.03[b]
Crude fat (%)	3.55±0.44	3.42±0.21
Crude fibre (%)	5.52±1.28[a]	7.09±1.46[b]
Ash (%)	22.74±0.43[a]	15.45±0.04[b]
Carbohydrate (%)	31.59±2.10	31.61±2.10
Se-Bioaccessibility (%)	20.47±5.53[a]	50.43±0.27[b]
Total selenium (mgSe kg^{-1})	16.63±3.07[a]	432.54±22.28[b]

The data are expressed as mean ±SD (n=3)

The different lower case letter indicates significant difference between rows at the $p < 0.05$.

The correlation between each chemical composition of our data was confirmed by Pearson correlation coefficient (r) and is presented in Table 4.2. Carbohydrates had no relationship with Se-bioaccessibility ($r = 0.033$, $p > 0.05$), crude protein ($r = 0.283$, $p > 0.05$) and crude fat ($r= 0.187$, $p > 0.05$). Se-bioaccesibility had a strong relationship with crude protein ($r = 0.889$, $p < 0.05$), crude fibre ($r = 0.865$, $p < 0.05$) and ash ($r = 0.977$, $p < 0.01$). Similarly, crude protein had a strongly negative relationship between ash ($r = 0.948$, $p < 0.01$). The amount of crude protein was the key determinant for Se-bioacessibility in kale seedlings. The finding was in good agreement with Lavu *et al*. (2016) who revealed that Se-bioavailability capacity had a good relationship with the selenium content in selenium enriched rice. Meanwhile, total selenium content (TSeC) had a strong relationship between bioaccesibility ($r = 0.978$, $p < 0.01$) and crude protein ($r = 0.948$, $p < 0.01$). This study was in accordance with previous reviews which reported that the concentration of selenium in food was influenced by geographical location,

seasonal change, protein content and food processing (Navarro-Alarcon and Cavrera-Vique, 2008). The Selenium content of food is highly dependent on the amount of selenium in the soil, which relates to the capacity of plants to take up, accumulate, transform, assimilate and eliminate the element (Ellis and Salt, 2003). TSec has a strong negative relationship with ash ($r = 0.999$, $p < 0.01$), because the selenium element replaces the sulphur in amino acid as SeMet, SeCys and selenocystathionine due to their physicochemical similarity (Navarro-Alarcon and Cavrera-Vique, 2008). Several plants can transform seleno-inorganic to seleno-orgnic form (Shen *et al*., 2010; Liu *et al*., 2012; Maneetong *et al*., 2013; Thosaikham *et al*., 2014; Bachiega *et al*., 2016) which indicates that the inorganic content in plants decreases. Therefore, the ash content in the enriched selenium plants decreased.

Table 4.2 The correlation coefficients (r*)* between chemical compositions

	Se-bioacessibility	Crude protein	Crude fat	Crude fibre	Ash	Carbohydrate
TSeC	0.978**	0.948**	-0.751	0.826*	-0.999**	0.016
Se-bioaceesibility	1	0.889*	-0.809	0.865*	-0.977**	0.035
Crude protein		1	-0.723	0.794	-0.948**	-0.201
Crude fat		-	1	-0.795	0.794	0.178
Crude fibre		-	-	1	-0.821*	-0.406
Ash		-	-	-	1	-0.026
Carbohydrate		-	-	-	-	-

*Significant model difference at $p < 0.05$.

**Significant model difference at $p < 0.01$.

4.2 The antioxidant capacities of kale seedlings

4.2.1 Total polyphenol and flavonoid and antioxidant capacities

After the processes of *in vitro* simulated gastrointestinal digestion, R-KS and Se-KS supernatants were evaluated for total polyphenol and total flavonoid content and antioxidant activities (DPPH, ABTS and FRAP) by the visible spectrophotometric method. The total polyphenol and flavonoid contents were determined as gallic acid and quercetin equivalents. The results are presented in Table 4.3. The contents of phenolic (30.06 mgGE gDW^{-1}) and flavonoid (10.99 mgQE gDW^{-1}) in Se-KS were higher than that of R-KS (18.49 mgGE gDW^{-1} and 10.16 mgQE gDW^{-1}, respectively). These results are in agreement with Lu *et al*. (2007) in that the accumulation and transformation of phenolic compounds are related to selenium contents. Total polyphenol content (TPC) of the Se-KS was significantly higher than R-KS ($p < 0.05$). However, there was no significant difference of total flavonoid content (TFC) between R-KS and Se-KS. Antioxidant activities trials 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 mg mL^{-1}) and ABTS (0.1-1.0 mg mL^{-1}) which were expressed as IC_{50} values (showed in Table 4.3). In addition, free radical scavenging of the R-KS was significantly less than Se-KS ($p < 0.05$). The high potential antioxidant of Se-KS was found in both assays, DPPH and ABTS (80.72% and 75.52%). Selenium content significantly increases in total polyphenol and antioxidant capacities. Bachiega *et al*. (2016), reported on the samples with contents of total polyphenol that also had higher antioxidant capacity and the biofortification of plants with selenium significantly increased the amount of phenolic compounds. Several studies on antioxidant activity on different sources of selenium biofortified plants have been published for at least a decade and are listed on Table 4.3.

4.2.2 Correlation analysis between TSeC, PC and antioxidant activities

The correlation coefficient analysis between protein content (PC), total selenium content (TSeC) and Se-bioaccessibility was reported. The relationship between TSeC, PC and Se-bioaccessibility with antioxidant activity measured by DPPH, ABTS and FRAP assay as exhibited Table 4.4 is interesting. All data were analyzed by SPSS

(version16). The correlation among variables was performed with Pearson's correlation test. The results demonstrated that Se-bioaccessibily and antioxidant assay were strongly correlated, DPPH (r = 0.961); ABTS (r = 0.923) and FRAP assay (r =0.952). The PC of all kale seedlings showed a high correlation coefficient with antioxidant capacities of DPPH and ABTS of 0.914 and 0.935, respectively. Moreover, the highest correlation was found for TSeC/TPC (r = 0.997). Moreover, we also found the relationship between antioxidant methods of the kale seedlings by DPPH, ABTS and FRAP assay exhibited strong correlation by FRAP/DPPH (r = 0.967), FRAP/ABTS (r = 0.941) and DPPH/ABTS (r = 0.897). Additionally, there was a significantly positive correlation coefficient between TSeC, Se-bioaccessibility, TPC and antioxidant activity. In concordance with previous studies, it indicated that there is a positive correlation among TSeC and antioxidant activity in several selenium enriched plants such as green tea (Xu *et al*., 2003; Li *et al*., 2008), *Spirulina platenis* (Chen *et al*.,2008), brown rice (Liu *et al*., 2012), mushroom (Zhao *et al*., 2008). The Selenium element can form complexes with polyphenol, in which the phenolic is complexed by metal. This can increase antioxidant capacity, because hydrogen-donating affects the scavenging of free radicals (Yo *et al*., 2007). Further evidence revealed the structures of metal-selenium complexes for biologically relevant metalion $(SeMet)_2Cu$ and $(SeMet)_2Zn$, This shows the importance of selenium-containing compounds as an antioxidant defense system. (Zainal *et al*., 1995). Additionally, when combining polyphenol and selenium functionalities in polyphenolic acid aster; this compound showed a strong ability to free radical scavenging (Lin *et al*., 2005). This suggests that a combining or complexing between polyphenol and selenium in Se-KS may occur. It causes Se-KS to exhibit enriched antioxidant activity. In conclusion, the absorption, accumulation and transformation of a selenium inorganic form to a bioactive organic compound in Se-KS are responsible for higher Se-bioaccessibility and stronger antioxidant activities more than an untreated source.

Table 4.3 Antioxidant capacities and total polyphenol and flavonoid in R-KS and Se-KS

Method	Sample	
	R-KS	Se-KS
TPC (mgGE gDW^{-1})	18.49 ± 0.67^{a}	30.06 ± 0.09^{b}
TFC (mgQE gDW^{-1})	10.16 ± 0.10^{a}	10.99 ± 0.67^{a}
IC_{50} DPPH (mg mL^{-1})	0.79 ± 0.00^{a}	0.74 ± 0.01^{b}
IC_{50} ABTS (mg mL^{-1})	0.58 ± 0.03^{a}	0.45 ± 0.03^{b}
FRAP (mgTE gDW^{-1})	18.83 ± 0.21^{a}	15.32 ± 0.40^{b}

The data are expressed as mean ±SD (n=3).

The different lower case letter indicates significant difference between rows at the $p < 0.05$.

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Table 4.4 Comparison between antioxidant activity (DPPH, ABTS and FRAP) with our result from the several sources of Se-enriched plant and standard

	IC_{50} (DPPH) (mg mL^{-1})	IC_{50} (ABTS) mg mL^{-1})	TPC (mgGE g^{-1}D.W.)	FRAP (mgTE g^{-1}D.W.)	Ref.
*Se-KS	0.74	0.45	30.06	15.32	These study
Se enriched rice grains	-	1.1	-	-	Wang *et al*. (2013)
Se enriched rice seedling	5.01	1.03	-	-	Wang *et al*. (2013)
Se enriched mycelium of *L. edodes* (Berk.)	> 0.10	-	13.18	-	Turło *et al*. (2010)
Pro-Se of B. animalis 01.	> 0.10	-	-	-	Shen *et al*. (2010)
Se-containing green tea 1% extract	71.5% (scavenging)	-	60.5	9947.6 µM	Molan *et al*. (2009)
Se-containing green tea 1% extract	74.2% (scavenging)	-	94.2	11132.7 µM	Molan *et al*. (2009)
Se-enriched green tea (ethanolic extract)	92.5.2%(scavenging)	-	-	-	Li *et al*. (2008)
Se-enriched green tea (aqueous extract)	72.8% (scavenging)	-	-	-	Li *et al*. (2008)
Se-enriched peanut (water soluble)	1.14				Zhao *et al*. (2015)
Se-enriched peanut (alkaline soluble)	1.28				Zhao *et al*. (2015)
Se-enriched peanut (prolamin)	2.11				Zhao *et al*. (2015)
Se-enriched peanut (salt soluble)	1.66				Zhao *et al*. (2015)
Se-*Zn* containging in *P. ostreatus*	3.84	-	13.38	-	Gasecka *et al*. (2016)
Se-*Zn* containging in *P. eryngii*	3.35	-	10.86	-	Gasecka *et al*. (2016)
Se-enriched broccoli sprout	5.48 µM Trolox g^{-1}	5.59 µM Trolox g^{-1}	1.20	28.04 µM $FeSO_4$ g^{-1}	Bachiega *et al*. (2016)
Se-enriched broccoli seedling	7.30 µM Trolox g^{-1}	8.12 µM Trolox g^{-1}	1.88	45.87 µM $FeSO_4$ g^{-1}	Bachiega *et al*. (2016)
Se-enriched broccoli inflorescence	5.12 µM Trolox g^{-1}	7.54 µM Trolox g^{-1}	1.05	31.94 µM $FeSO_4$ g^{-1}	Bachiega *et al*. (2016)
Trolox	1.02	0.99	-	-	Wang *et al*. (2013)
asorbic acid	4.20 µg mL^{-1}	0.0254	-	-	These study
Rutin	9.10 µg mL^{-1}	0.0654	-	-	These study

* Theses study.

Table 4.5 The correlation coefficients (r) between difference of Se-bioaccessibility, total selenium and antioxidant capacity methods

	PC	Se-bioaccessibility	TSeC	TPC	TFC	DPPH	ABTS	FRAP
PC	1	0.889*	0.946**	0.947**	0.789	-0.914**	-0.935**	0.983**
Se-bioaccessibility	-	1	0.978**	0.976**	0.731	-0.961**	-0.923**	0.952**
TSeC	-	-	1	0.997**	0.723	-0.990**	-0.927**	0.986**
TPC	-	-	-	1	0.730	-0.987**	-0.913*	0.989**
TFC	-	-	-	-	1	-0.716	-0.853*	0.784
DPPH	-	-	-	-	-	1	0.897*	-0.967**
ABTS	-	-	-	-	-	-	1	-0.941**
FRAP	-	-	-	-	-	-	-	1

*Significant model difference at $p < 0.05$.

** Significant model difference at $p < 0.01$.

4.3 Acute toxicity of kale seedling

Se-KS shows strong potential for free radical scavenging and high selenium bioacessibility. Hence, the next experiment was performed to investigate the toxicity of R-KS and Se-KS *in vivo* to confirm a safety evaluation for a new dietary supplement of Se for patients and health promoters. Vary dose (OECD guideline 423) and fixed dose (OECD guideline 420) were used to monitor the effects of acute oral toxicity of the R-KS and Se-KS (OECD, 2000). The investigation was based on a basic understanding of the overall selenium absorption, distribution, accumulation, elimination and excretion that was provided thorough monitoring of the tissue selenium content. However, this study was concerned with the specific process of selenium accumulation in tissues.

4.3.1 Vary doses of acute toxicity modifying method based on OECD guideline 423

This research evaluated the potential toxicity and approximate 50% lethal dose (LD_{50}) of both kale seedling including R-KS and Se-KS following a single oral administration to female rats. The R-KS and Se-KS were orally administered in single doses of 1250, 2500 and 5000 mg kg^{-1} b.w. The general behavior of all kale seedling groups was observed from 6 h, 12 h and 24 h after administration and continuously monitored until the 14th day. The observation of the animal behavior and physical appearance is shown in Table 4.6. There were no deaths. In addition, there was no evidence of any of the behavioral signs of toxicity including labored breathing, discharge from nose or mouse, ruffled fur, hunched posture, lethargy, minimal responsiveness and uncontrolled bleeding or discharges during the experimental period. We also measured the amount of selenium in each dose of R-KS and Se-KS (1250, 2500 and 5000 mg kg^{-1} b.w.). R-KS at doses of 1250, 2500 and 5000 mg kg^{-1} b.w. were composed of selenium 0.02, 0.04 and 0.08 mgSe kg^{-1} b.w. unit, respectively. Whereas, Se-KS at doses of 1250, 2500 and 5000 mg kg^{-1} b.w. were 0.54, 1.08 and 2.16 mgSe kg^{-1} b.w. unit, respectively. It has been reported that selenium compounds selenite and methyselenosystein occurred as a signal of toxicity at 3-5 mgSe kg^{-1} with almost no survival at 16 mgSe kg^{-1} (Harr *et al*., 1967; Martin and Hurbut, 1976; Koller and Exon, 1986). These

data confirmed that the amount of selenium in our kale seedling did not exceed the toxicity doses and as such, no signs of toxicity in animal behavior or physical appearance were seen in this experiment.

4.3.1.1 Effect of kale seedlings on relative organ body weight

The results showed that the body weight and food intake experienced no significant differences ($p > 0.05$) when compared with control group. These results are shown in Table 4.7 and Figure 4.1 and 4.2. There was no significant difference in weight of average organs and relative organs between control and both kale seedling treated groups at a dose 1250, 2500 and 5000 mg kg^{-1} b.w. The effects of kale seedling on principal organ weights relative to body weight are shown in Figure 4.4. When toxicity occurs in tissues, it prompts a decrease or increase in the body weight which is associated with toxic effect of the chemicals and substance (Tofovic and Jackson, 1999; OECD, 2000; Kifayatullah *et al.*, 2015). For the liver, the enlargement of the organ might be an exhibition of hepatic injury, which often varies in degree and localization depending on the mechanism involved. It may reflect as hepatic tenderness and enlargement (Orafidiya *et al*., 2004). There was no significant difference in the change of weight. The results indicated that the vital organs, such as liver, kidney, heart, spleen and lung may not be adversely affected throughout the treatment by kale seedlings. The average and relative organ weight of the tested plant and control treated group showed statistically no significant differences ($p > 0.05$).

4.3.1.2 Effect of kale seedlings on hematological parameters

Hematological parameters are one of the most sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animal (Mukinda and Syce, 2007). A low level of platelets is the first evidence of drug induced toxic effect on haemopoiesis (Mdhluli, 2003) and the level of platelets decreases that exhibit the increased risk of bleeding (Slichter, 2004). The results of hematological experiments are summarized in Table 4.8. All the tested hematological parameters including total blood count, hemoglobin, red blood cell, total white blood cell, neutrophil, monocyte, lymphocyte, packed cell volume and platelet count were within normal limits compared to the control group. No toxicologically significant differences ($p > 0.05$) between treated

animals and control were explored. There were generally no significant differences noted between control and treated groups (both kale seedlings; R-KS and Se-KS) for the hematological parameters measured.

4.3.1.3 Effect of kale seedlings on biochemical parameters

The results of the various biochemical tests on the experimentally tested animals with kale seedlings and the control group are summarized in Table 4.9. Oral administration of kale seedlings at doses of 1250, 2500 and 5000 mg kg^{-1} b.w. did not cause any significant changes in serum biochemical parameters such as CHO, TG, LDL, HDL, ALT, AST, ALP and others when compared with control group. For liver markers, the transaminases (AST and ALT) are well known enzymes used as good indicators of liver function (Hilaly *et al*., 2004) and biomarkers predicting possible toxicity (Rahman *et al*., 2001). Generally, any damage to the parenchymal liver cell results in elevations of both transaminases in the blood (Wolf *et al*., 1972). Moreover, AST is found in high concentrations in liver, heart, skeletal muscle and kidney. It is presented in both cytoplasm and mitochondria of cells. The increases of serum AST can be taken as a first sign of cell damage that leads to the outflow of the enzymes into the serum (Mdhluli, 2003). Both kale seedlings (R-KS and Se-KS) altered the hepatocytes and consequently the metabolism of the rats. For kidney function tests, creatinine is well known as a good indicator of renal function (Hilaly *et al.*, 2004) Any rise in creatinine levels is only observed if there is marked damage to functional nephrons (Lameire *et al*., 2005). Neither of the kale seedlings (R-KS and Se-KS) altered the renal function. Indeed, the animals were obtained with an over dose of selenium compound in both selenium forms. Selenium is capable of generating oxygen free radicals such as super oxide anion ($O_2^{-\bullet}$) by catalysis (Seko *et al*., 1989; Mezes and Balogh, 2009) in which selenite reacts with glutathione endogenously in the cell or extracellularly causing toxicity by formation of superoxide and selenium (Seko *et al*., 1989; Spallholz, 1994; Seko and Imura, 1997; Mezes and Balogh, 2009). Additionally, selenium dioxide, SeCys, diselenodipropinate and diphenylselenide can generate superoxide anion together. The oxygen free radicals cause oxidation damage to lipid, lipoprotein and other biochemical parameters such as enzyme

nucleic acid and protein. Oxidative damages play a significant pathological role in human diseases. Cancer emphysema, cirrhosis, arteriosclerosis, and arthritis have been correlated with oxidative damage (Halliwell and Gutteridge, 1985). Ganther (1999) and Spallholz (2001) revealed that selenium compound such as methyl selenide anion (selenol) had been exhibited to induce cellular apoptosis even in tumor cell and MeSeCys induced apoptosis in cancer cells through activation of caspase, a likely mechanism for other selenium compounds that also induces apoptosis (Mezes and Balogh, 2009). These results confirmed that both kale seedlings (R-KS and Se-KS) did not induce damage to the liver, kidney, heart and other organs in rats.

4.3.1.4 Effect of kale seedling on histopathological changes

The examination photomicrographs of several tissues are shown in Figure 4.5. From observation, there was no morphological abnormality of fresh tissues seen in any of the groups of rats (control, R-KS at 1250, 2500 and 5000 mg kg^{-1} b.w. and Se-KS at 1250, 2500 and 5000 mg kg^{-1} b.w.). Fresh tissues from a target organ did not show any changes in color, volume and texture when compared with control group.

The photomicrographs include the liver (Figure 4.5, 1a-1g), kidney (2a-2g) and heart (3a-3g). Histological feature of the liver, kidney and heart of control rats exhibited normal structures (1a, 2a and 3a for liver, kidney and heart, respectively). Rats administered with 1250, 2500 and 5000 mg kg^{-1} b.w. of R-KS and with 1250, 2500 and 5000 mg kg^{-1} b.w. of Se-KS showed no adverse affect to the morphology of organs as shown in Figure 4.4.

The liver is a main target organ of acute toxicity; chemical agents or natural substances can induce hepatotoxicity. The histological investigation of all doses of R-KS and Se-KS showed no lesion or abnormal pathology found in liver tissues. In addition, there was no abnormal pathology change in the kidney or heart tissue. Cellular arrangement was similar to the organs of the control group. Moreover, there was no potential toxicity or cellular lesions as well as no necrosis or inflammation reaction observed in all tissues. Likewise, there were no significant changes of serum biochemical

parameters in all groups of rats (Table 4.9). Therefore, it can be suggested that oral acute administration of R-KS and Se-KS did not alter toxicity to rats.

A summary of the results of this part reveals the administration of R-KS and Se-KS at 1250, 2500 and 5000 mg kg^{-1} b.w. did not cause any mortality or abnormal toxic behavior. Moreover, no difference was observed in body weight, organ weight, food intake, and there were no abnormalities in necropsy or histopathological observations. For the hematological and biochemical of blood values were in range of the control group. The finding recommend that acute experience with both kale seedling (R-KS and Se-KS) did not cause toxic effect, and its LD_{50} value was considered to be greater than 5000 mg kg^{-1} b.w. in rodents. Various researches exhibited the Se-KS revealed that good biological activity and low toxicity compared with selenite (Chantiratikul *et al*. 2011; 2013; 2014; 2016**)**.

Table 4.6 General appearance and behavioral observation of acute toxicity study for control and treated group (n=4)

Observation	Control	R-KS (mg kg^{-1} bw)			Se-KS (mgkg^{-1} bw)		
		1250	2500	5000	1250	2500	5000
Labored breathing	Normal	No effect	No effect	No effect	No effect	No effect	No effect
Discharge from nose or mouse	Normal	No occur	No occur	No occur	No occur	No occur	No occur
Ruffled fur	Normal	No occur	No occur	No occur	No occur	No occur	No occur
Lethargy	No occur	No occur	No occur	No occur	No occur	No occur	No occur
Minimal Responsiveness	No occur	No occur	No occur	No occur	No occur	No occur	No occur
Uncontrolled bleeding	No occur	No occur	No occur	No occur	No occur	No occur	No occur
Body weight	Normal	No effect	No effect	No effect	No effect	No effect	No effect
Urination	Normal	No effect	No effect	No effect	No effect	No effect	No effect
Excrete	Normal	No effect	No effect	No effect	No effect	No effect	No effect
Change in skin	No effect	No effect	No effect	No effect	No effect	No effect	No effect
Diarrhea	No occur	No occur	No occur	No occur	No occur	No occur	No occur
Coma	Not present	Not present	Not present	Not present	Not present	Not present	Not present
Death	Alive	Alive	Alive	Alive	Alive	Alive	Alive

Table 4.7 Body weight gain, food intake and water consumption of Wistar albino rat treated orally with kale seedlings

	Initial weight (g)	Final weight (g)	Body weight gained (%)	Food intake (g)
Control	178.75±13.56	208.00±8.89	14.44±3.17	12.79±3.51
R-KS-1250	175.00±9.95	213.00±10.08	17.82±2.89	13.58±3.41
R-KS-2500	170.25±6.98	208.25±6.41	18.33±0.93	12.67±3.54
R-KS-5000	167.75±11.63	207.50±7.22	19.40±3.15	13.68±3.03
Se-KS-1250	161.25±10.746	203.00±5.31	20.77±3.79	12.81±2.93
Se-KS-2500	168.50±2.50	207.25±3.84	18.62±1.90	13.98±3.87
Se-KS-5000	160.72±17.85	204.50±11.64	21.36±1.01	13.69±3.66

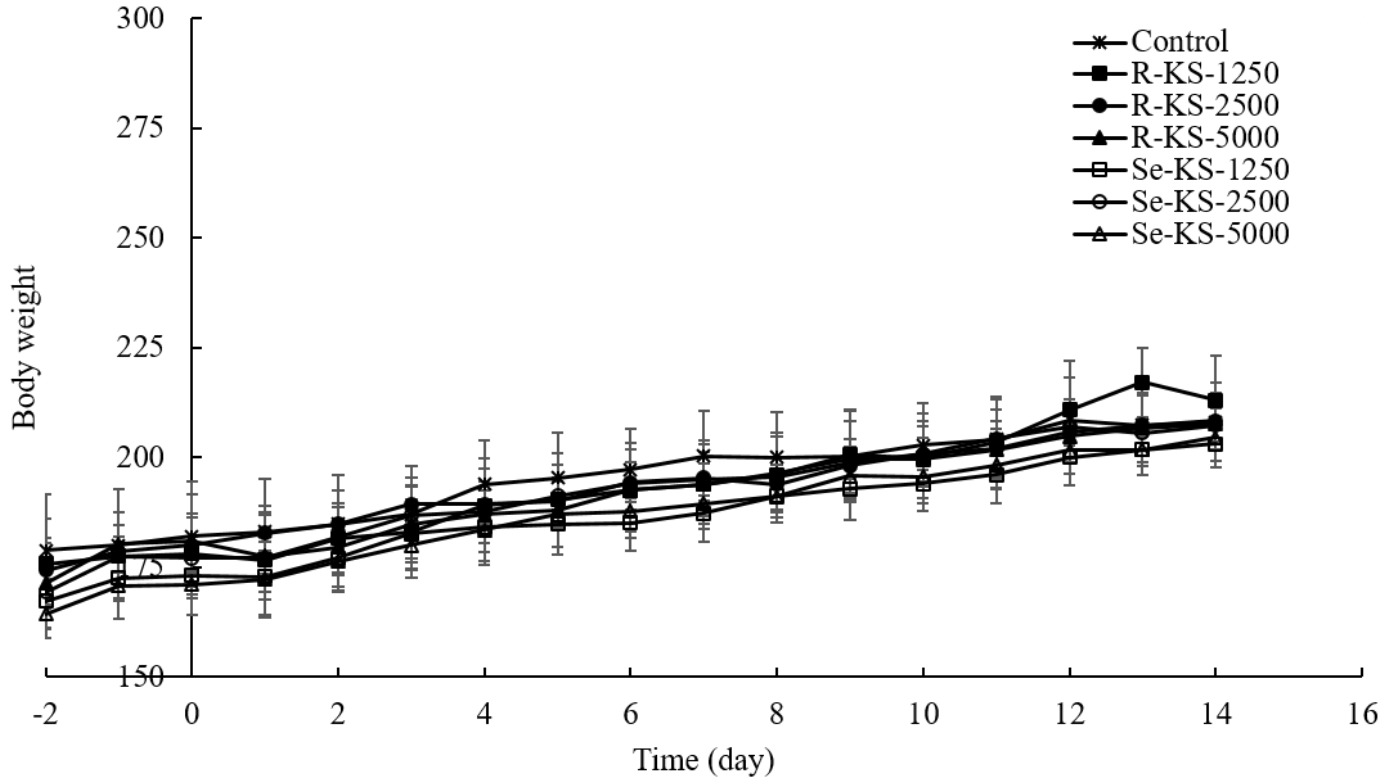

Figure 4.1 Body weight in both genders of rats treated with control, R-KS at 5000 mg kg^{-1} b.w. and Se-KS at 5000 mg kg^{-1} b.w. for 14 day. The values are expressed as mean±SEM (n=4)

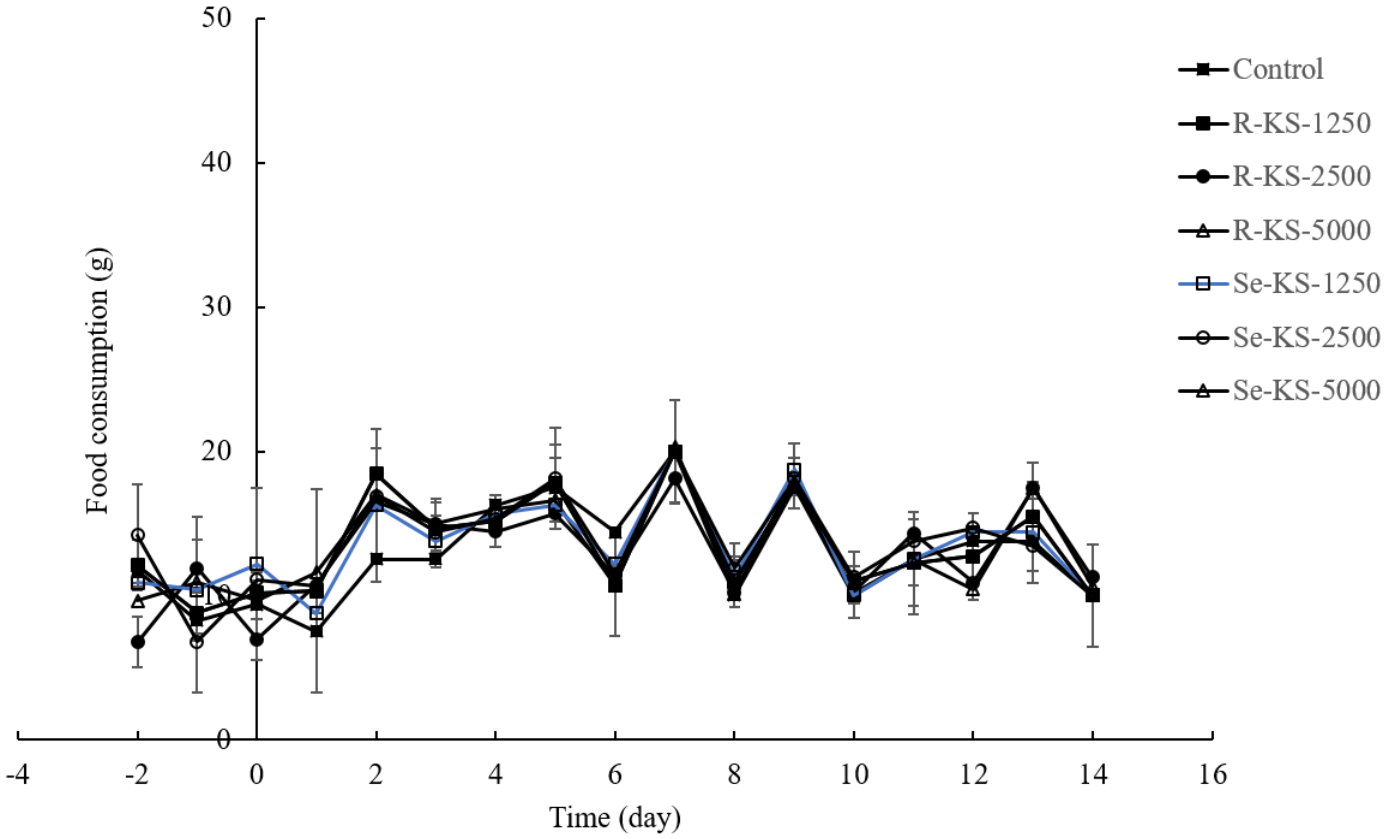

Figure 4.2 Food consumption in both genders of rats treated with control, R-KS at 5000 mg kg^{-1} b.w. and Se-KS at 5000 mg kg^{-1} b.w. for 14 day. The values are expressed as mean±SEM (n=4)

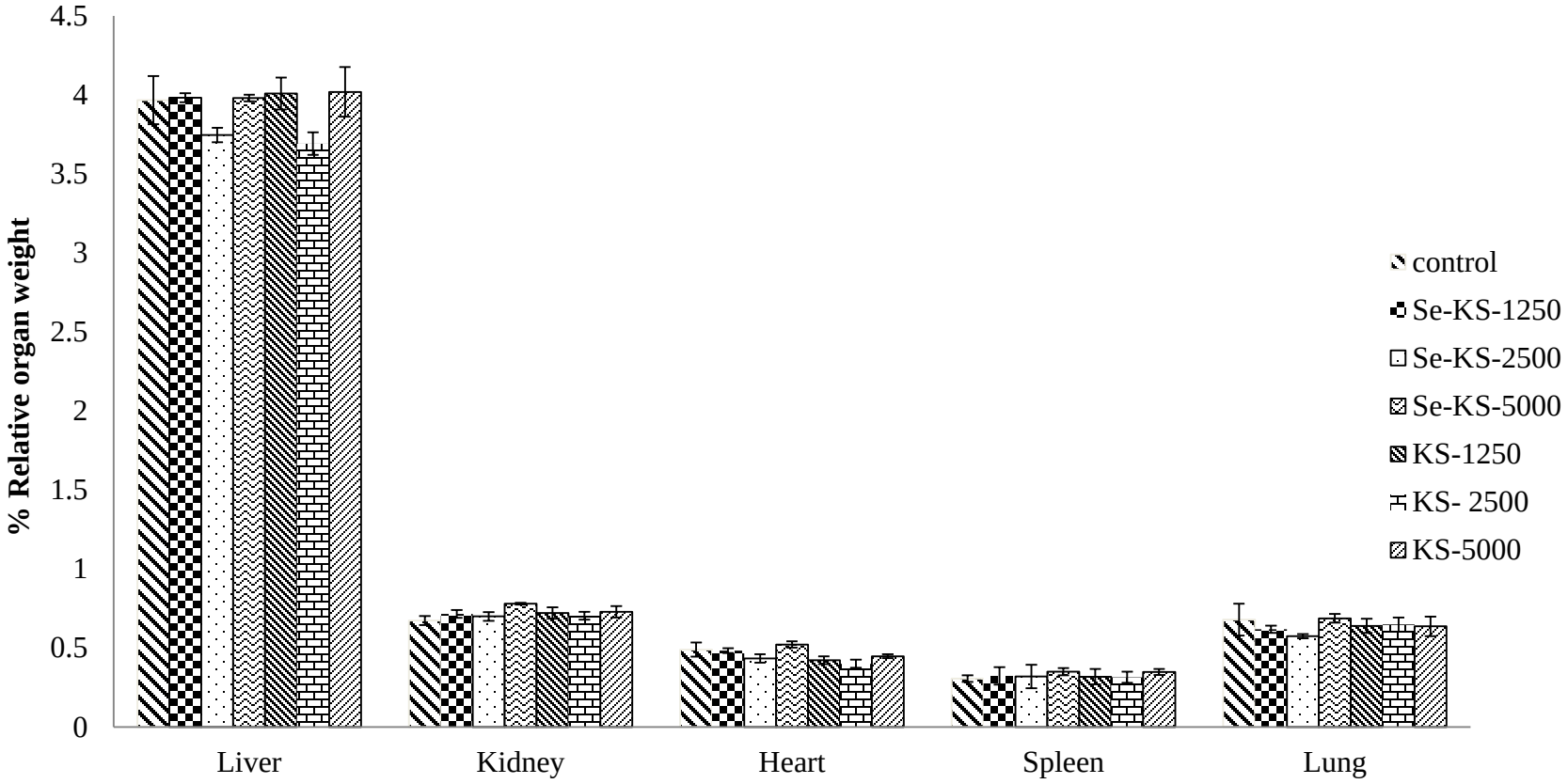

Figure 4.3 Relative organ weight of rats after 14 days of treatment with control and kale seedling (n=4)

Table 4.8 Hematological parameters of rats treated with kale seedlings for acute toxicity administration (n=4)

mg kg^{-1} b.w.	WBC	RBC	HB	Hct	MCV	MCH	MCHC	Neu	Lym
Control	2400.00±594.42	8.16±0.06	16.75±0.31	49.25±0.48	60.25±1.03	20.30±0.33	33.70±0.34	7.50±0.65	92.25±0.75
R-KS-1250	2475.00±460.75	8.60±0.13	16.68±0.30	50.50±0.29	58.50±1.19	19.43±0.64	33.13±0.40	6.75±1.49	91.25±2.43
R-KS-2500	1800.00±141.42	8.37±0.12	15.78±0.22	48.50±0.29	57.50±0.65	18.83±0.17	32.78±0.27	10.50±1.26	89.25±1.25
R-KS-5000	2025.00±322.43	8.23±0.25	16.78±0.10	50.25±0.48	57.00±2.94	20.48±0.73	33.95±0.58	9.00±4.45	89.50±5.92
Se-KS-1250	1650.00±206.16	8.45±0.24	16.75±0.28	50.50±0.65	59.50±1.76	19.88±0.62	33.43±0.52	7.25±1.93	90.50±1.71
Se-KS-2500	1600.00±339.12	8.44±0.08	16.53±0.46	49.25±1.25	58.00±0.82	19.60±0.36	33.73±0.22	7.00±1.05	95.75±1.65
Se-KS-5000	2500.00±635.09	8.61±0.25	16.48±0.33	49.50±1.04	57.50±0.65	19.43±0.57	33.42±0.31	4.00±1.15	94.00±1.47

The data are expressed as mean ±SEM (n=4)

Table 4.9 Biochemical values of rats treated with kale seedlings for acute toxicity administration (n=4)

Dose (mg kg^{-1} b.w.)	CHO (U/L)	TG (U/L)	HDL (U/L)	LDL (U/L)	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	67.50±2.63	101.50±11.81	53.50±1.55	30.25±0.63	73.75±10.73	25.75±0.75	109.00±5.12
R-KS-1250	102.75±23.89	106.75±23.45	58.50±7.44	35.25±3.52	93.50±24.47	30.25±2.17	142.00±16.42
R-KS-2500	89.00±7.63	89.00±7.63	54.00±0.91	28.75±1.03	100.50±18.83	29.00±1.87	116.50±7.77
R-KS-5000	108.00±6.89	108.00±6.89	55.75±1.89	23.75±1.03	104.00±12.80	25.00±3.37	104.00±13.71
Se-KS-1250	63.00±3.19	75.00±8.26	49.75±1.38	29.25±1.55	62.25±11.88	30.75±2.59	144.00±13.90
Se-KS-2500	68.00±9.46	102.50±30.66	50.00±5.05	29.00±1.94	102.00±8.61	24.25±0.85	126.50±10.81
Se-KS-5000	59.00±4.32	96.75±9.58	50.50±1.76	28.50±1.94	104.50±37.97	31.75±3.47	147.00±13.67

Dose (mg kg^{-1} b.w.)	BUN (mg/dL)	CREA (mg/dL)	UA (mg/dL)	TP (mg/dL)	Alb (mg/dL)	Glob (mg/dL)	TB (mg/dL)
Control	20.93±0.88	0.63±0.02	0.85±0.10	6.15±0.18	3.73±0.08	2.43±0.13	0.47±0.06
R-KS-1250	24.25±1.68	0.85±0.12	2.00±0.36	5.83±0.09	3.58±0.05	2.25±0.06	0.33±0.02
R-KS-2500	24.48±1.01	0.78±0.05	1.90±0.56	6.38±0.19	3.85±0.22	2.53±0.22	0.53±0.10
R-KS-5000	22.40±0.92	0.75±0.06	1.85±0.71	6.38±0.07	3.95±0.18	2.43±0.18	0.38±0.03
Se-KS-1250	21.29±1.35	0.80±0.06	1.58±0.25	7.05±0.25	4.23±0.10	2.83±0.10	0.60±0.00
Se-KS-2500	22.90±0.70	0.68±0.05	0.73±0.11	6.75±0.21	4.23±0.17	2.53±0.17	0.45±0.05
Se-KS-5000	23.10±4.29	0.58±0.11	1.28±0.48	6.50±0.17	4.28±0.05	2.23±0.05	0.45±0.03

The data are expressed as mean ±SEM (n=4)

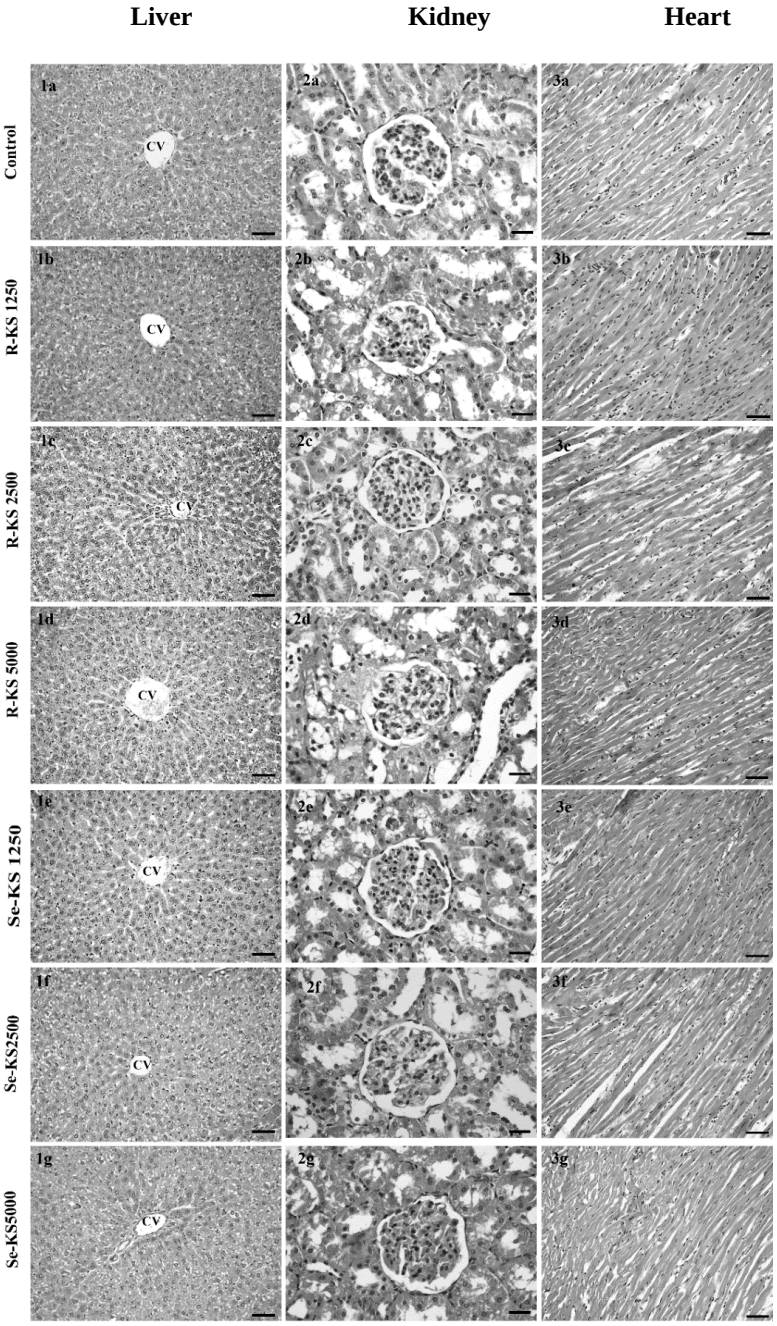

Figure 4.4 Histopathological analysis of organs treated with control (vehicle), R-KS and Se-KS at 1250, 2500 and 5000 mg kg^{-1} b.w. (column 1) liver; scale bar are 50 µm; (column 2) kidney glomerulus; scale bar are 20 µm and (column 3) heart muscle; scale bar are 50 µm (CV is central vein)

4.3.2 The effect of fixed doses of acute toxicity in male and female rats modifying based on OECD guideline 420

This experiment compared the acute toxicity of both kale seedlings between male and female rats at the highest exposure level (5000 mg kg^{-1} b.w.). The general behavior of the animals was observed between 6, 12 and 24 h after administration and continuously monitored for 14 days. No deaths in any animals were observed during the experimental period. No effect on toxic behavior caused by kale seedlings was noted. These results indicate that acute experience to both kale seedlings (R-KS and Se-KS) did not cause a toxic effect, and its LD_{50} value was confirmed to be greater than 5000 mg kg^{-1} b.w. in rats.

4.3.2.1 Effect of kale seedlings on relative organ body weight

The results found that the body weight and food intake in male groups was higher than in female groups. This was attributed to the high level of metabolism in males, more so than in females (Wang *et al*., 2010). These results are shown in Table 4.10 and Figure 4.5 and 4.6.There was no significant difference in average organs and relative organs weight between control and both kale seedlings treated groups at a dose 5000 mg kg^{-1} b.w. The effects of kale seedling on principal organ weights relative to body weight are shown in Figure 4.7. There was no significant difference in the change of each weight. The results indicated that the vital organs such as liver, kidney, heart, spleen and lung were not adversely affected by kale seedlings throughout the treatment. The average and relative organ weight of tested plants and the control treated group showed statistically non-significant differences ($p > 0.05$). These results are presented in Figure 4.7.

4.3.2.2 Effect of kale seedling on biochemical parameters

The results of the various biochemical tests on the experimentally tested animals with kale seedlings and control group are summarized in Table 4.11. Oral administration of kale seedlings at dose of 5000 mg kg^{-1} b.w. in 14 day did not cause significant changes in serum biochemical parameters such as BS, CREAT, ALT, AST, ALP and other parameters when compared with control group. The metabolism in male rats was at a higher level than in female rats (Wang *et al*., 2010). .Therefore, these results found that

the biochemical parameters including BS, ALT and ALP in male groups was higher than in female groups.

Table 4.10 Body weight gain, food intake and water consumption of male and female Wistar albino rat treated orally of kale seedlings

	Male			Female		
	Control	R-KS	Se-KS	Control	R-KS	Se-KS
Initial weight (g)	224.75±2.39[b]	217.50±4.09[b]	220.50±1.25[b]	199.00±2.74[a]	194.00±3.39[a]	197.00±2.86[a]
Final weight (g)	344.50±12.33[b]	342.75±12.51[b]	341.00±5.90[b]	213.00±5.44[a]	206.50±0.64[a]	208.50±2.21[a]
Body weight gained (%)	34.36±3.39[b]	36.23±6.06[b]	35.27±1.25[b]	13.93±3.04[a]	13.13±2.48[a]	11.85±2.17[a]
Food intake (g)	24.17±3.42	23.69±3.16	23.19±4.11	15.87±4.38	14.40±4.12	14.67±4.07

The data are expressed as mean ±SEM (n=4).

The different low case letter denote significance $p < 0.05$ between column**.**

Table 4.11 Biochemical values of male and female rats treated with kale seedlings for acute toxicity administration

Parameters	Male			Female		
	C-14 d	R-KS-14 d	Se-KS-14 d	C-14 d	R-KS-14 d	Se-KS-14 d
BS (mg/dL)	135.00±6.67^{bc}	173.00±11.49^{c}	154.00±9.65^{c}	96.00±4.96 ^{ab}	59.25±1.54^{a}	69.25±7.76^{a}
BUN (mg/dL)	19.43±0.85	20.30±1.45	21.70±0.85	23.25±1.66	22.27±1.45	25.20±2.23
CREA (mg/dL)	0.80±0.04^{bc}	0.60±0.07^{a}	0.77±0.04^{ab}	1.20±0.05^{c}	1.02±0.07^{bc}	1.05±0.08^{bc}
UA (mg/dL)	0.62±0.10	0.97±0.21	0.93±0.17	1.07±0.08	1.22±0.27	1.10±0.26
TP (mg/dL)	5.35±0.15	5.65±0.11	5.70±0.14	6.07±0.23	5.70±0.08	6.02±0.41
Alb (g/dL)	3.57±0.04	3.57±0.11	3.67±0.02	3.95±0.03	3.62±0.11	3.82±0.17
Glob (g/dL)	1.78±0.17	2.67±0.06	2.02±0.07	2.12±0.23	2.07±0.11	2.20±0.29
TB (mg/dL)	0.17±0.06	0.39±0.10	0.10±0.05	0.25±0.09	0.20±0.07	0.25±0.06
AST (U/L)	89.25±2.95	100.60±3.89	97.5±7.99	96.15±4.13	92.00±9.65	126.75±20.27
ALT (U/L)	110.50±7.54^{b}	105.33±18.92^{b}	105.25±9.13^{b}	50.25±7.05^{a}	65.25±9.18^{ab}	46.00±5.87^{a}
ALP (U/L)	154.00±7.51^{b}	135.50±6.78^{b}	161.5±12.98^{b}	60.5±3.61^{a}	65.75±7.95^{a}	60.75±3.35^{a}

The data are expressed as mean ±SEM (n=4) and the different low case letter denote significance $p < 0.05$ between column**.**

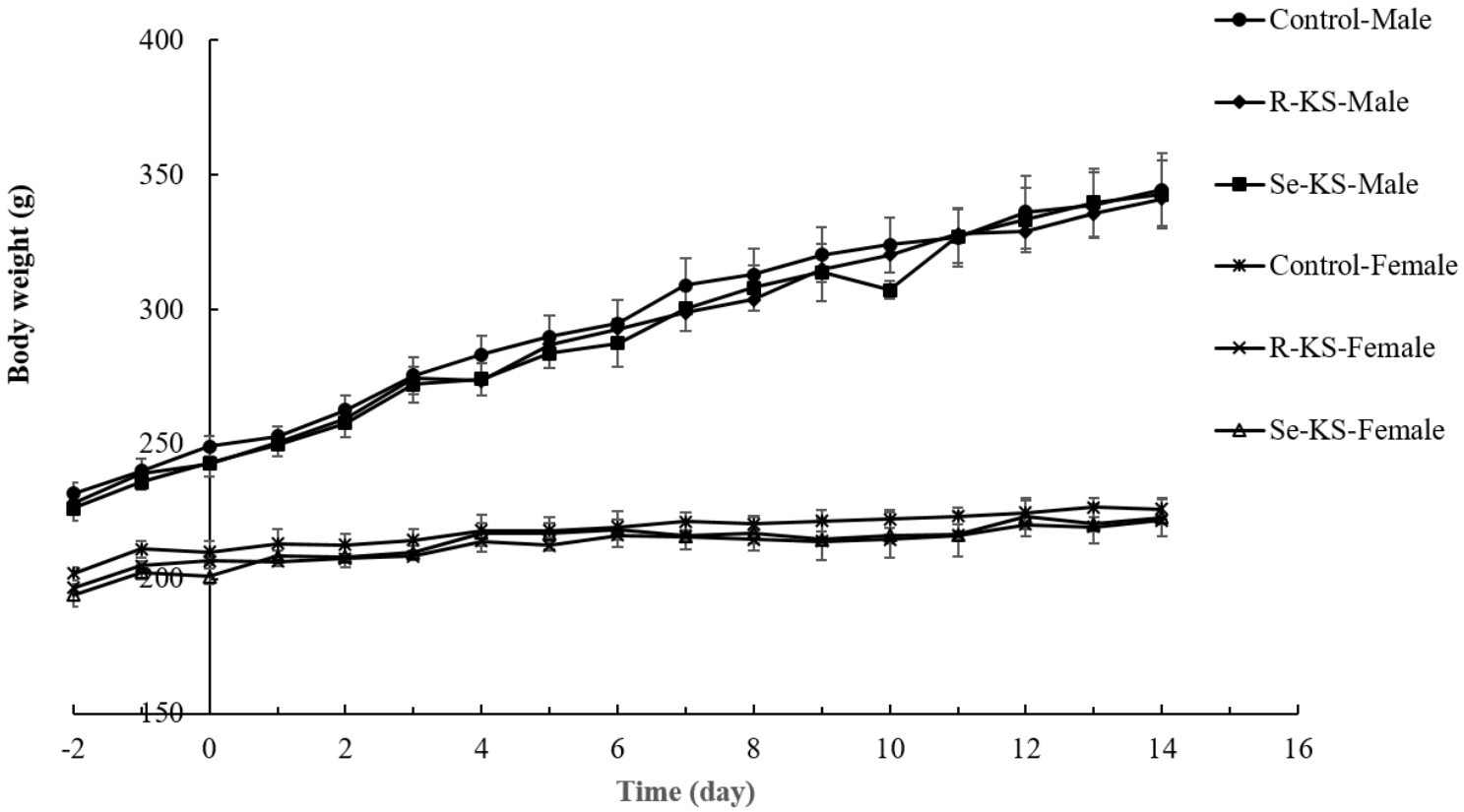

Figure 4.5 Body weight in rats of treated with control, R-KS at 1250, 2500 and 5000 mg kg^{-1} b.w. and Se-KS at 1250, 2500 and 5000 mg kg^{-1} b.w. for 14 days. The values are expressed as mean±SEM (n=4)

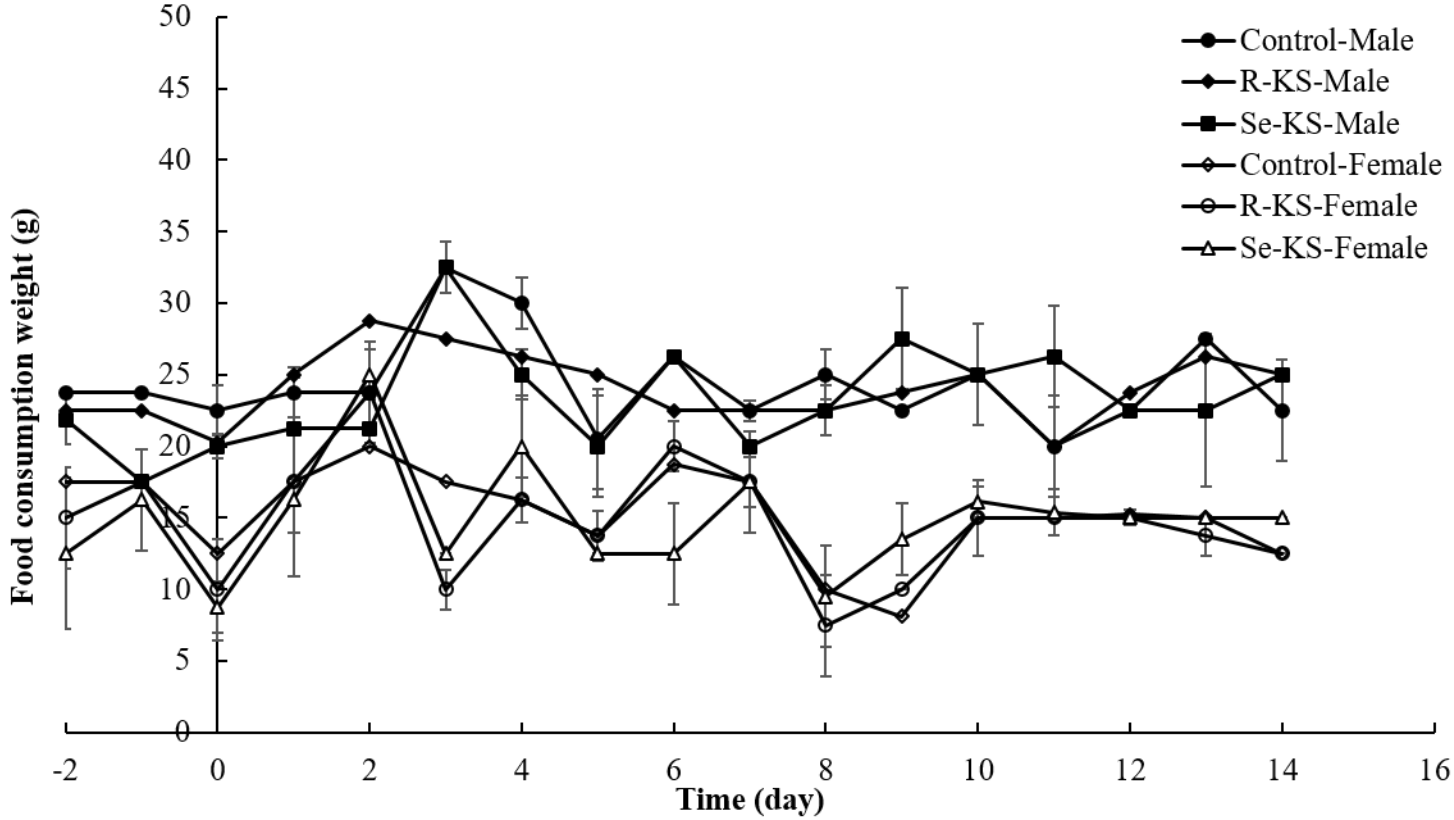

Figure 4.6 Food consumption in rats treated with control, R-KS at 1250, 2500 and 5000 mg kg^{-1} b.w. and Se-KS at 1250, 2500 and 5000 mg kg^{-1} b.w. for 14 day. The values are expressed as mean±SEM (n=4)

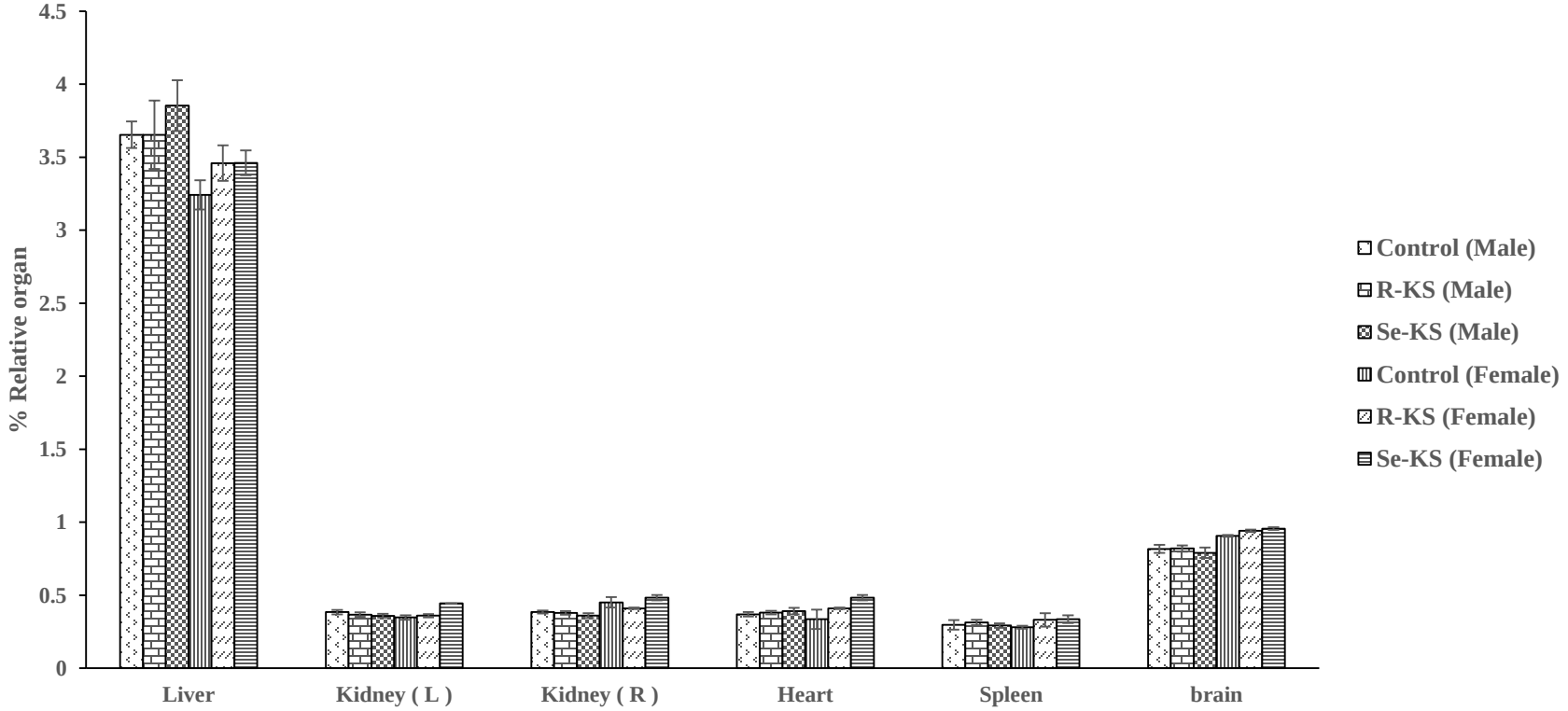

Figure 4.7 Relative organ weight of rats after 14 days treatment with control and kale seedling (n=4)

The data are expressed as mean ±SEM (n=4). No statistically significant difference $p > 0.05$

4.3.3 Monitoring of selenium accumulation in organs after administering fixed doses of acute toxicity test

This section evaluates the effect of the highest dose of both kale seedlings (R-KS and Se-KS) in selenium accumulation organs after a single oral administration. The kale seedlings were orally administered at doses of 5000 mg kg^{-1} b.w. The general behavior was observed at 6 h, 12 h and 24 h after administration and continuously monitored for 14 days. No effect on toxic behavior caused by kale seedlings was noted. These results recommend that experience with kale seedling showed no toxic effect and its LD_{50} value is repeated confirmation to be more than 5000 mg kg^{-1} b.w. in rats.

4.3.3.1 Effect of kale seedlings on relative organ body weight

The results found that the body weight and food intake experienced no significant differences ($p > 0.05$) when compared with control group. These results are shown in Figure 4.8 and 4.9. There was no significant difference in average organs and relative organs weight between control and both kale seedlings treated groups at a dose of 5000 mg kg^{-1} b.w. (vary times; 6, 12, 24 h and 14 day). The effect of kale seedlings on principal organ weights relative to body weight are shown in Figure 4.10. There were no significant differences in the change of each weight. The results indicated that, the vital organs such as liver, kidney, heart and lung were not adversely affected throughout the treatment by kale seedlings. The average and relative organ weight of tested plant and control treated group shown statistically non-significant differences ($p > 0.05$).

4.3.3.2 Effect of kale seedling on biochemical parameters

Biochemical parameters of both kale seedlings are shown in Table 4.11. A recent investigation concentrated on the enzyme in kidney function tests (BUN and CREAT) and liver makers (ALT, AST and ALP) which are main organs that relate to the signal of toxicity. The results of the various biochemical tests on the experimentally tested animals with kale seedlings and control group are summarized in Table 4.13. Oral administration of kale seedlings at a dose of 5000 mg kg^{-1} b.w. (vary times; 6, 12, 24 h and 14 day) did not

cause significant changes in serum biochemical parameters such as BUN, CREAT, ALT, AST and ALP when compared with control group.

Table 4.12 %Relative organ weight of rats after 14 day treatment with control and kale seedling

	Liver	Kidney	Heart	Lung
C-6 h	4.50±0.19	0.43±0.01	0.42±0.03	0.64±0.04
C-12 h	4.25±0.25	0.42±0.03	0.38±0.01	0.60±0.04
C-24 h	4.72±0.54	0.44±0.03	0.41±0.02	0.60±0.11
C-14 d	3.46±0.10	0.30±0.02	0.32±0.02	0.43±0.01
KS-6 h	5.27±0.22	0.48±0.01	0.44±0.01	0.72±0.03
KS-12 h	4.40±0.27	0.45±0.01	0.42±0.02	0.68±0.14
KS-24 h	3.91±0.21	0.44±0.01	0.42±0.02	0.63±0.02
KS-14 d	3.52±0.16	0.31±0.01	0.32±0.02	0.40±0.04
Se-KS-6 h	4.76±0.34	0.44±0.02	0.42±0.02	0.64±0.02
Se-KS-12 h	4.47±0.41	0.42±0.01	0.45±0.03	0.71±0.05
Se-KS-24 h	4.64±0.26	0.42±0.02	0.40±0.02	0.63±0.04
Se-KS-14 d	3.41±0.13	0.42±0.01	0.31±0.01	0.41±0.03

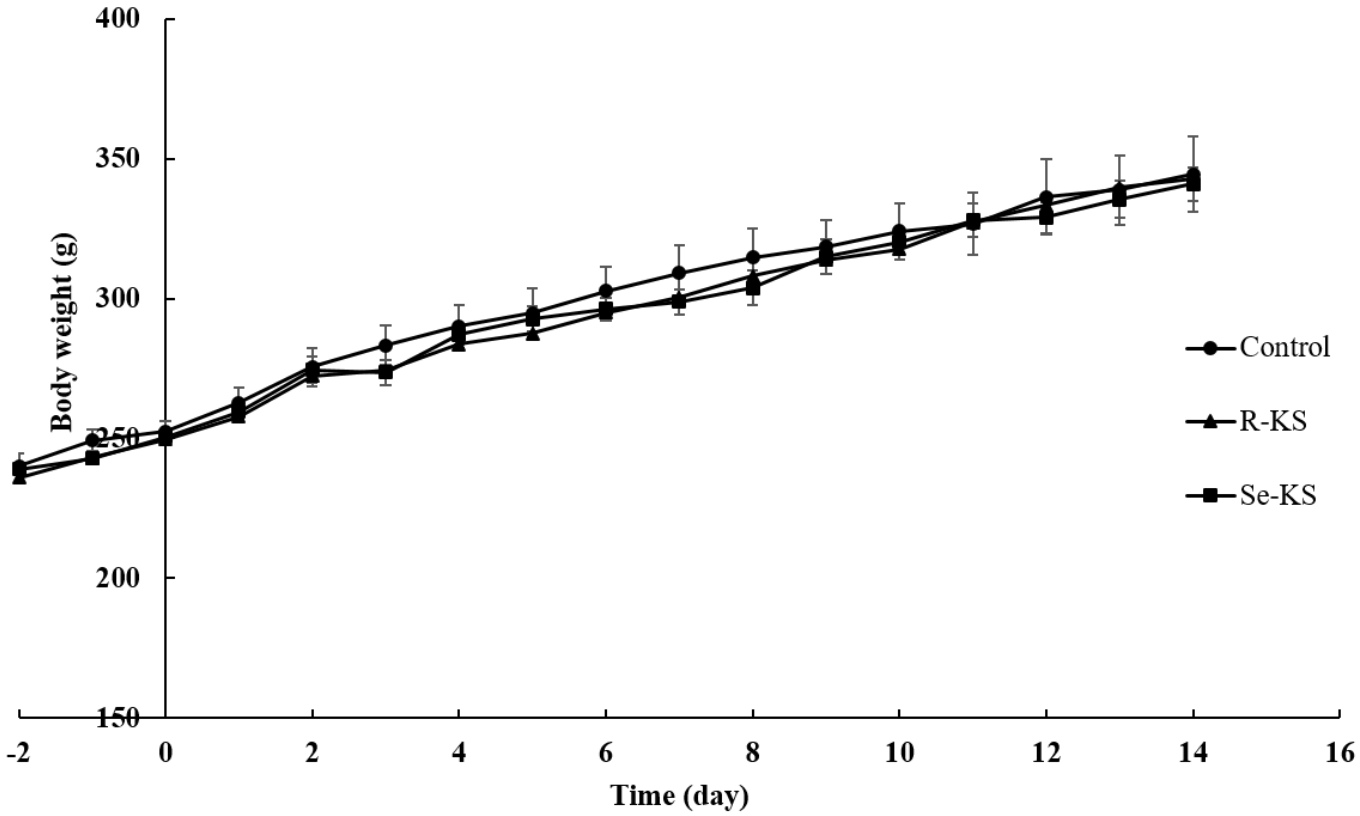

Figure 4.8 Body weight in both genders of rats treated with control, R-KS at 5000 mg kg^{-1} b.w. and Se-KS at 5000 mg kg^{-1} b.w. for 14 day. The values are expressed as mean±SEM (n=4)

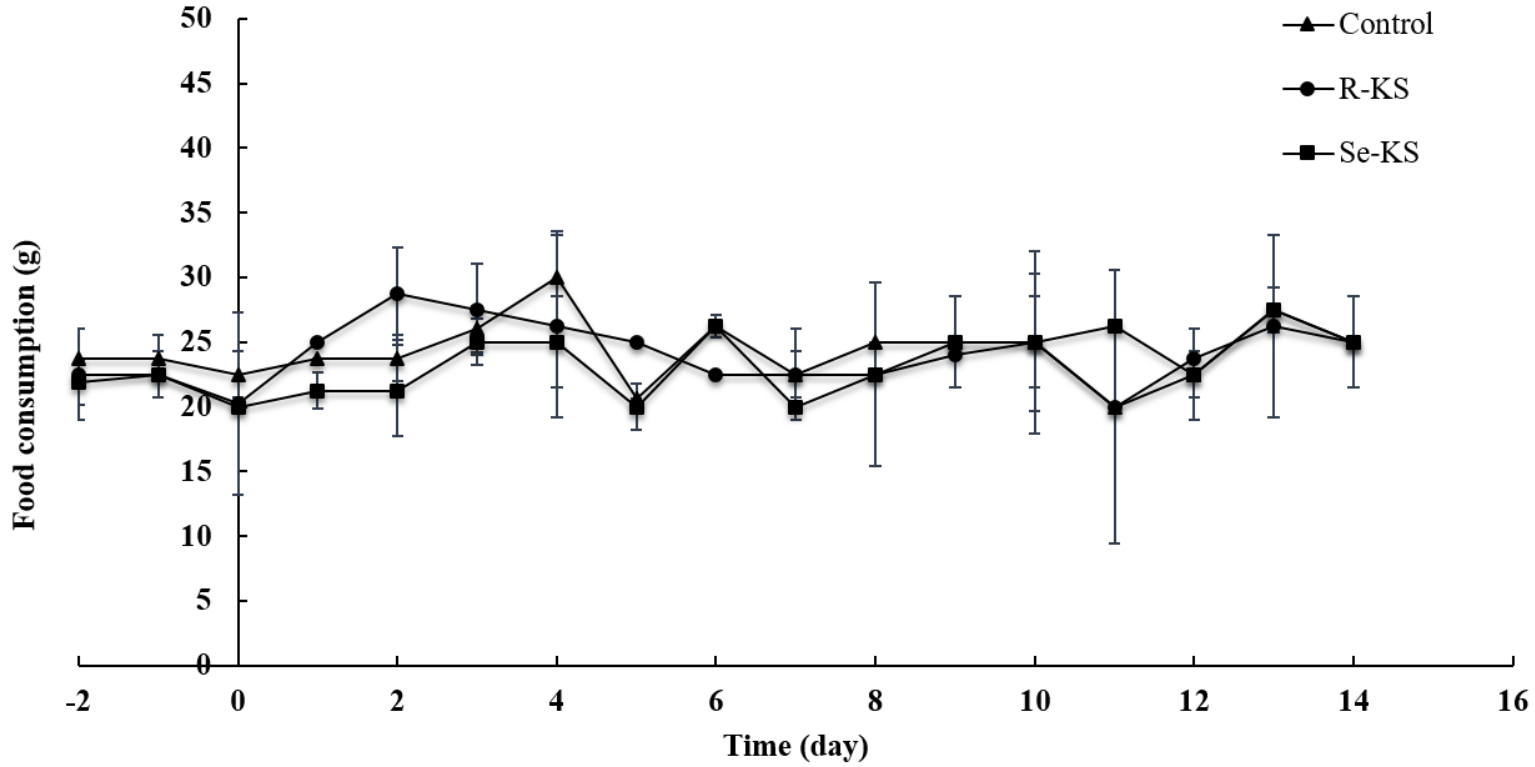

Figure 4.9 Food consumption in both genders of rats treated with control, R-KS at 5000 mg kg^{-1} b.w. and Se-KS at 5000 mg kg^{-1} b.w. for 14 day. The values are expressed as mean±SEM (n=4)

Figure 4.10 Relative organ weight of rats after 14 day treatment with control and kale seedling (n=4).

*The difference was significant $p < 0.05$

Table 4.13 Biochemical values of rats treated with kale seedlings for acute toxicity administration (n=4)

	BUN (mg/dL)	CREAT (mg/dL)	AST (U/L)	ALT (U/L)	ALP (U/L)
C-6 h	16.72±1.13[a]	0.29±0.06[a]	147.25±32.69[ab]	35.24±3.77[a]	177.50±43.37[a]
C-12 h	19.32±3.56[a]	0.29±0.06[a]	106.75±17.93[a]	30.25±1.50[a]	137.75±11.14[a]
C-24 h	20.27±2.26[a]	0.38±0.01[a]	120.50±20.17[a]	43.00±12.19[a]	140.75±15.98[a]
C-14 d	20.40±0.36[a]	0.36±0.05[a]	100.25±7.88[a]	38.75±2.06[a]	138.00±16.75[a]
R-KS-6 h	14.90±1.64[a]	0.33±0.08[a]	148.00±32.45[ab]	44.25±9.21[a]	177.75±29.88[a]
R-KS-12 h	17.82±1.95[a]	0.34±0.01[a]	114.50±13.37[a]	32.75±2.75[a]	157.00±4.16[a]
R-KS-24 h	18.60±4.21[a]	0.28±0.02[a]	99.75±13.57[a]	38.50±7.72[a]	153.50±13.82[a]
R-KS-14 d	19.12±1.55[a]	0.37±0.06[a]	115.25±40.63[a]	43.75±6.23[a]	141.75±20.9[a]
Se-KS-6 h	18.52±1.98[a]	0.31±0.03[a]	206.50±36.17[b]	48.75±4.85[a]	179.00±39.40[a]
Se-KS-12 h	19.07±4.19[a]	0.29±0.02[a]	110.75±18.94[a]	31.75±12.55[a]	152.00±16.43[a]
Se-KS-24 h	18.30±3.22[a]	0.33±0.03[a]	98.50±6.13[a]	34.00±5.16[a]	155.00±16.43[a]
Se-KS-14 d	19.52±0.92[a]	0.40±0.01[a]	106.00±16.04[a]	43.50±9.03[a]	150.75±20.33[a]

The different low case letters denote significance $p < 0.05$ between column**.**

4.3.3.3 Effect of kale seedling on accumulation of selenium in different organs

To investigate the selenium accumulation in the digestive system, circulatory and vital organs, stomach, small intestine, large intestine, liver, spleen, heart, kidney and brain were processed for the amount of selenium at different times (6, 12, 24 h and 14 day). The investigation was conducted after a single oral administration of kale seedlings at a high dose of 5000 mg kg^{-1} b.w. Furthermore, feces excretion was collected; 2 days before administration and collected every day after kale seedlings administration. The results of selenium accumulation tests in animal organs are summarized in Table 4.14 and Figure 4.11., 4.12 and 4.13. Selenium contents were evidently increased in several organs such as liver, kidney and plasma at 6 h to 24 h after administered of Se-KS. The selenium forms were changing; elimination, accumulation and biotransformation in organs (Raman *et al*., 2014). A basic understanding of the overall selenium absorption, distribution, accumulation, elimination and excretion was provided through monitoring tissue selenium content. It was found that the selenium content of Se-KS in several tissues was significant and increased over the period of time range from 6 h to 24 h. Selenium content decreased and reverted to a normal condition in 14 days. The selenium content from Se-KS was higher and remained in organs longer than selenium from R-KS, but the difference was insignificant when compared with control group because, the half-life of selenium was 162 days (Janghorbani *et al*., 1990).

Kale seedlings were ingested to gastric, which contained many enzymes, and the digestion and absorption process started. For the gastric organ, the selenium contents of Se-KS were slightly increased in the period of time ranging from 6 h to 24 h and selenium content decreased and reverted to normal condition in 14 days.

The selenium compound in R-KS and Se-KS was completely digested in the small intestine which contained pancreatine, lipase and other enzymes. In the small intestine, selenium contents were less accumulated, because the maximum absorption occurred in this organ, especially in the duodenum (Wrihght, 1966; Vendeland, 1992; Whanger, 1996). This result was in accordance with previous reports (Glenn *et al*., 1964; Maag *et al*., 1960). The lowest selenium concentration was in the small intestine. It has been reported that the absorption of inorganic and organic selenium compounds were absorbed more abundantly

than inorganic selenium compounds (Chantiratikul *et al*., 2014; 2016). Selenite, SeMet and SeCys achieved almost complete absorption (92%, 91% and 81%, respectively) in rats. Wright (1996) reported on the selenium absorption in swine and sheep (85% and 35%). Se-KS was digested through the GI process where it obtained selenium compounds such as selenite, SeMet and MeSeCys (Maneetong *et al*., 2013). These three compounds may have high absorption in the small intestine which confirms lowest selenium accumulation when compared with server organs. In fact, all nutrients absorbed by the intestines pass through the liver and are processed before traveling to the rest of the body. The selenium compounds were carried in the blood stream. These experiments investigated the selenium concentration in the plasma of Se-KS and found that it had significantly increased in the time range from 6 h to 24 h. The selenium content decreased and reverted to normal condition in 14 days. The main selenium species was found in plasma including SelP (68%), GPx (25%) and association to albumin (7%) which carried the selenium element throughout the body (Xu *et al*., 2008).

Continually, the selenium compounds were absorbed and distributed to the liver and spleen by villi and capillary blood, respectively. The selenium concentration of Se-KS in the liver significantly increased in the time ranging from 6 h to 24 h and selenium content decreased and reverted to normal condition in 14 days. The metabolism of selenium occurred in the liver. Selenium compounds can be converted to hydrogen selenide that is used for synthesis of selenoprotein, selenium containing protein and metallprotien (Suzuki, 2005; Weekley and Harris, 2013; Roman *et al*., 2014, Chantiratikul *et al*., 2016). Grnther and Hsieh (1974) found the final methylation process was completed in the soluble fraction of the renal cell and in the micromal fraction of hepatic cell. The details of seleno-metabolite depicted that the selenium amino acids SeMet and SeCys are absorbed through the transcellular pathway mediated by transports which are basically shared with their sulphur-containing analogues. SeMet is absorbed through a Na^{+}-dependent process and SeMet can also be incorporated non-specifically into protein such as serum albumin and haemoglobin by randomly replacing the methionine (sulphur). Alternatively, it can be transformed into SeCys and into selenide via the tran-selenation pathway. For elimination SeMet, SeCys, SeMCys and GGSeMCys are directly methylated by β-lyase in the liver and kidney to methylselenide

such as trimethylselenide (TMSe) and dimethylselenide (DMSe) so that urinary excretion is the major route for eliminating excess selenium from these species (Soda *et al*., 1987; Roman *et al*., 2014).

The selenium concentration of Se-Ks in the spleen significantly increased in period of time ranging from 6 h to 24 h and selenium content decreased and reverted to normal condition at 14 days. The spleen is well known as a main organ with a crucial role with respect to erythrocyte and protecting immune response. Seleno amino acid is related with the globin portion of hemoglobin as GPx and DOI **(**Burk *et al***.**, 2003; Fairweather**-**Tait *et al***.**, 2010; Rayman, 2012; Zwolak and Zaporwska, 2012; Kieliszek and Blazejak, 2013; Roman *et al***.**, 2013; Duntas and Benvenga, 2015**).** The Se**-**KS may be used for immunity synthesis**.**

In the heart organ, the selenium concentration of Se-KS in the heart slightly increased in the period of time ranging from 6 h to 24 h. Selenium content decreased and reverted to a normal condition at 14 days. The whole blood from GI tract was carried to the heart**.** The heart is a muscle organ which pumps blood through the blood vessels of the circulatory system **(**Clarence and Danald, 2009**).** The selenium compound was carried to all tissues in the animal and human body**.**

In the brain, the selenium concentration of Se-KS in the brain slightly increased in the period of time ranging from 6 h to 24 h. Selenium content decreased and reverted to normal condition in 14 days. The selenium content from Se-KS was higher and remained in the brain longer than selenium from R-KS, but the difference, when compared with control group, was insignificant. These results assumed the selenium compound can be carried in a blood brain barrier. Additionally, selenium can reduce lipid peroxidation in many brain regions (Whanger, 2000). Moreover, selenium will influence compounds with hormonal activity and neurotransmitters in the brain, It is possible that this is the reason selenium affects moods in humans and behavior in animals (Whanger, 2000).

Selenium excretion occurred through the urinary route (kidney), respiratory route (lung), fecal route (feces) and skin. The selenium element in the kidney was carried from the renal artery. The selenium concentration of Se-KS in the kidney significantly increased in the period of time ranging from 6 h to 24 h and selenium contents decreased and reverted to normal condition in 14 days. Renal selenium elimination is dependent on glomerular

filtration and degree of reabsorption. Increasing renal fluid reabsorption did not increase the selenium content in urine, indicating a tubular re-absorptive process where the selenium follows retention of water (Oster and Prellwitz, 1990; Davis and Hall, 2015). Thus dehydration or renal insufficiency would decrease rates of elimination. Excretion and renal clearance rates correlate with creatinine, indicating glomerular filtration is the mechanism of elimination (Davis and Hall, 2015). Byard (1969) and Palmer *et al*. (1970) found trimethylselenide (TMSe) in the main excretory product of selenium metabolism. It amounted to 30-50% from all urinary excretion and did not depend on form or dose of selenium intake (Roman *et al*., 2014). Minor selenium metabolite product, selenosugar along with other compounds including SeMethyl-*N*-acetyl-glucosamine and deacylated analogue of SeGalNac, and SeMethyl-*N*-amino-galactosamine were eliminated from the urine. Each of these metabolite products had low toxic selenium status (Roman *et al*., 2014).

The selenium concentration of Se-KS in the lung slightly increased in the period of time ranged from 6 h to 24 h and selenium content decreased and reverted to normal condition in 14 days. The respiratory route of excretion is essential when a toxic dose of selenium is administrated. Ewan (1978) investigated dimethylselenide (DMSe). It produced a garlic-like odor on the breath of animals and humans, and the elimination of DMSe increased by raising protein, methionine or selenium content in dietary food (Ganther *et al*., 1986). Burk *et al*. (1972) suggested the DMSe is an intermediate in the selenium detoxification pathway and the conversion of DMSe to TMSe is rate limited when there is excess selenium. The methlylated detoxification product such as DMSe and TMSe was excreted by the lungs (Weekley and Harris, 2013; Roman *et al*., 2014).

In the large intestine, which temporarily stores the undigested material, the selenium concentration of Se-KS in the large intestine increased significantly in the period of time ranging from 6 h to 24 h. The selenium content decreased and reverted to normal condition on day 14. After the digestive process, the fiber of both kale seedlings (R-KS and Se-KS) was carried to the large intestine. In this process the rest of selenium element can be absorbed. Consequently, the remaining undigested fibers were stored as feces before being removed by defecation.

The selenium concentration of Se-KS in feces significantly increased more than R-KS and the control group at a period of time 1-2 days after administration. Data are shown in Figure 4.14. These results occurred because selenium content decreased and reverted to a normal condition 3 days after administration. The elimination and detoxification of selenium occurred through the fecal route, but fecal excretion was a minor route of selenium elimination (Pedrosa *et al*., 2012). Burk (1972) reported the fecal excretion of ^{75}Se –selenite remained at 10% of the dose over a dosage range of 0.004-1 ppm. Selenite was reduced to an insoluble or unavailable form by rumen microorganism (Petersen and Spedding, 1993). DMSe was eliminated through feces and breathing (Patching and Gardiner, 1999; Roman *et al*., 2014).

Therefore the selenium element accumulation in several tissues describes the pathway of metabolism of Se-KS in Wistar albino rats. At periods 6 h-24 h and 14 day after administration of Se-KS, selenium was unequally digested, transported, distributed and accumulated between the tissues. The highest selenium level was found in the kidney follow by plasma, liver, spleen, gastric, heart, large intestine, lung, brain and lowest level in small intestine, respectively. Additionally, selenium from Se-KS may eventually be methylated and secreted in the breath and feces as DMSe and in urine as Se-sugar and TMSe.

Table 4.14 Selenium accumulation in organs of control, R-KS and Se-KS after administration 6 h, 12 h, 24 h and 14 day

Gastric/times	Control (mg kg^{-1})	R-KS (mg kg^{-1})	Se-KS (mg kg^{-1})
6 h	0.71 ± 0.07^{Aa}	0.63 ± 0.07^{Aa}	1.33 ± 0.33^{Ba}
12 h	0.67 ± 0.09^{Aa}	0.60 ± 0.06^{Aa}	1.39 ± 0.36^{Ba}
24 h	0.67 ± 0.43^{Aa}	0.68 ± 0.11^{ABa}	2.09 ± 0.22^{Cb}
14 day	0.84 ± 0.18^{Aa}	0.76 ± 0.05^{Ba}	0.71 ± 0.12^{Aa}

Small intestine/times	Control (mg kg^{-1})	R-KS (mg kg^{-1})	Se-KS (mg kg^{-1})
6 h	0.67 ± 0.07^{Ab}	0.33 ± 0.08^{Aa}	0.40 ± 0.04^{Ca}
12 h	0.50 ± 0.06^{Bb}	0.38 ± 0.02^{Aa}	0.52 ± 0.03^{Bab}
24 h	0.62 ± 0.01^{Ab}	0.32 ± 0.04^{Aa}	0.32 ± 0.05^{Aa}
14 day	0.49 ± 0.04^{Ba}	0.40 ± 0.08^{Aa}	0.62 ± 0.06^{Db}

Plasma/times	Control (mg kg^{-1})	R-KS (mg kg^{-1})	Se-KS (mg kg^{-1})
6 h	2.90 ± 0.21^{Aa}	3.17 ± 0.28^{Aa}	5.24 ± 0.48^{Bb}
12 h	3.00 ± 0.27^{Aa}	4.51 ± 1.53^{Ab}	5.15 ± 0.17^{Bb}
24 h	3.22 ± 0.42^{Aa}	3.69 ± 0.46^{Aa}	7.96 ± 0.45^{Cb}
14 day	3.32 ± 0.17^{Aa}	4.22 ± 0.10^{Ab}	4.31 ± 0.24^{Ab}

The different capital letter denote significance $p < 0.05$ between column (n=4)**.**

The different low case letter denote significance $p < 0.05$ between row (n=4)**.**

Table 4.14 Selenium accumulation in organs of control, R-KS and Se-KS after administration 6 h, 12 h, 24 h and 14 day (Cont.).

Brain/times	Control (mg kg^{-1})	R-KS (mg kg^{-1})	Se-KS (mg kg^{-1})
6 h	0.78 ± 0.10^{Ab}	0.66 ± 0.04^{ABa}	0.71 ± 0.05^{Aab}
12 h	0.85 ± 0.38^{Aa}	0.60 ± 0.11^{ABa}	0.82 ± 0.04^{Aa}
24 h	1.02 ± 0.57^{Aa}	0.53 ± 0.02^{Aa}	1.03 ± 0.05^{Ba}
14 day	0.84 ± 0.40^{Aa}	0.76 ± 0.21^{Ba}	1.01 ± 0.44^{Ba}

Liver/times	Control (mg kg^{-1})	R-KS (mg kg^{-1})	Se-KS (mg kg^{-1})
6 h	2.36 ± 0.57^{Aa}	1.96 ± 0.32^{Aa}	4.77 ± 0.49^{Bb}
12 h	2.45 ± 0.20^{Aa}	2.12 ± 0.13^{Aa}	6.38 ± 1.06^{Cb}
24 h	2.86 ± 0.23^{Ab}	1.82 ± 0.07^{Aa}	7.65 ± 0.53^{Dc}
14 day	2.58 ± 0.50^{Aa}	2.44 ± 0.17^{Ba}	2.61 ± 0.45^{Aa}

Heart/times	Control (mg kg^{-1})	R-KS (mg kg^{-1})	Se-KS (mg kg^{-1})
6 h	1.02 ± 0.18^{Aa}	1.13 ± 0.10^{Aa}	1.18 ± 0.16^{Aa}
12 h	0.96 ± 0.06^{Aa}	0.97 ± 0.24^{ABa}	1.39 ± 0.27^{Ab}
24 h	1.03 ± 0.17^{Aa}	0.89 ± 0.09^{Ba}	2.22 ± 0.63^{Ba}
14 day	1.17 ± 0.09^{Ab}	0.98 ± 0.08^{Ba}	0.90 ± 0.10^{Aa}

The different capital letter denote significance $p < 0.05$ between column (n=4)**.** The different low case letter denote significance $p < 0.05$ between row (n=4)**.**

Table 4.14 Selenium accumulation in organs of control, R-KS and Se-KS after administration 6 h, 12 h, 24 h and 14 day (Cont.).

Lung/times	Control (mg kg^{-1})	R-KS (mg kg^{-1})	Se-KS (mg kg^{-1})
6 h	1.41 ± 0.10^{Aa}	1.19 ± 0.43^{Aa}	1.16 ± 0.12^{Ba}
12 h	1.59 ± 0.05^{Ab}	1.07 ± 0.19^{Aa}	1.26 ± 0.30^{Ba}
24 h	1.52 ± 0.09^{Ac}	0.92 ± 0.10^{Aa}	1.17 ± 0.11^{Bb}
14 day	0.56 ± 0.30^{Ba}	0.83 ± 0.02^{Aa}	0.73 ± 0.05^{Aa}

Kidney/times	Control (mg kg^{-1})	R-KS (mg kg^{-1})	Se-KS (mg kg^{-1})
6 h	3.69 ± 0.63^{Aa}	4.46 ± 0.32^{Aa}	9.39 ± 0.97^{Bb}
12 h	4.45 ± 0.25^{ABa}	4.84 ± 0.13^{Aa}	9.15 ± 1.46^{Bb}
24 h	4.30 ± 0.59^{ABa}	4.54 ± 0.64^{Aa}	14.11 ± 0.75^{Cb}
14 d	4.88 ± 0.37^{Ba}	4.38 ± 1.02^{Aa}	6.04 ± 0.44^{Ab}

Large intestine/times	Control (mg kg^{-1})	R-KS (mg kg^{-1})	Se-KS (mg kg^{-1})
6 h	0.89 ± 0.07^{Aa}	0.71 ± 0.08^{Aa}	1.31 ± 0.09^{Bb}
12 h	0.29 ± 0.05^{Ba}	0.82 ± 0.06^{ABb}	1.83 ± 0.25^{Bc}
24 h	0.28 ± 0.05^{Ba}	0.85 ± 0.21^{ABb}	2.39 ± 0.27^{Bc}
14 day	0.44 ± 0.19^{Aa}	0.92 ± 0.10^{Bb}	2.07 ± 0.31^{Ac}

The different capital letter denote significance $p < 0.05$ between column (n=4)**.**
The different low case letters denote significance $p < 0.05$ between row (n=4)**.**

Table 4.14 Selenium accumulation in organs of control, R-KS and Se-KS after administration 6 h, 12 h, 24 h and 14 day (Cont.).

Spleen/times	Control (mg kg^{-1})	R-KS (mg kg^{-1})	Se-KS (mg kg^{-1})
6 h	0.89 ± 0.07^{ABa}	0.82 ± 0.09^{ABa}	1.31 ± 0.09^{Ab}
12 h	0.92 ± 0.18^{Ba}	0.67 ± 0.42^{Aa}	1.83 ± 0.25^{Bb}
24 h	0.68 ± 0.05^{Aa}	0.70 ± 0.14^{Aa}	2.39 ± 0.27^{Cb}
14 day	0.88 ± 0.19^{ABa}	1.29 ± 0.57^{Ba}	2.07 ± 0.31^{BCb}

The different capital letter denote significance $p < 0.05$ between column (n=4)**.**
The different low case letters denote significance $p < 0.05$ between row (n=4)**.**

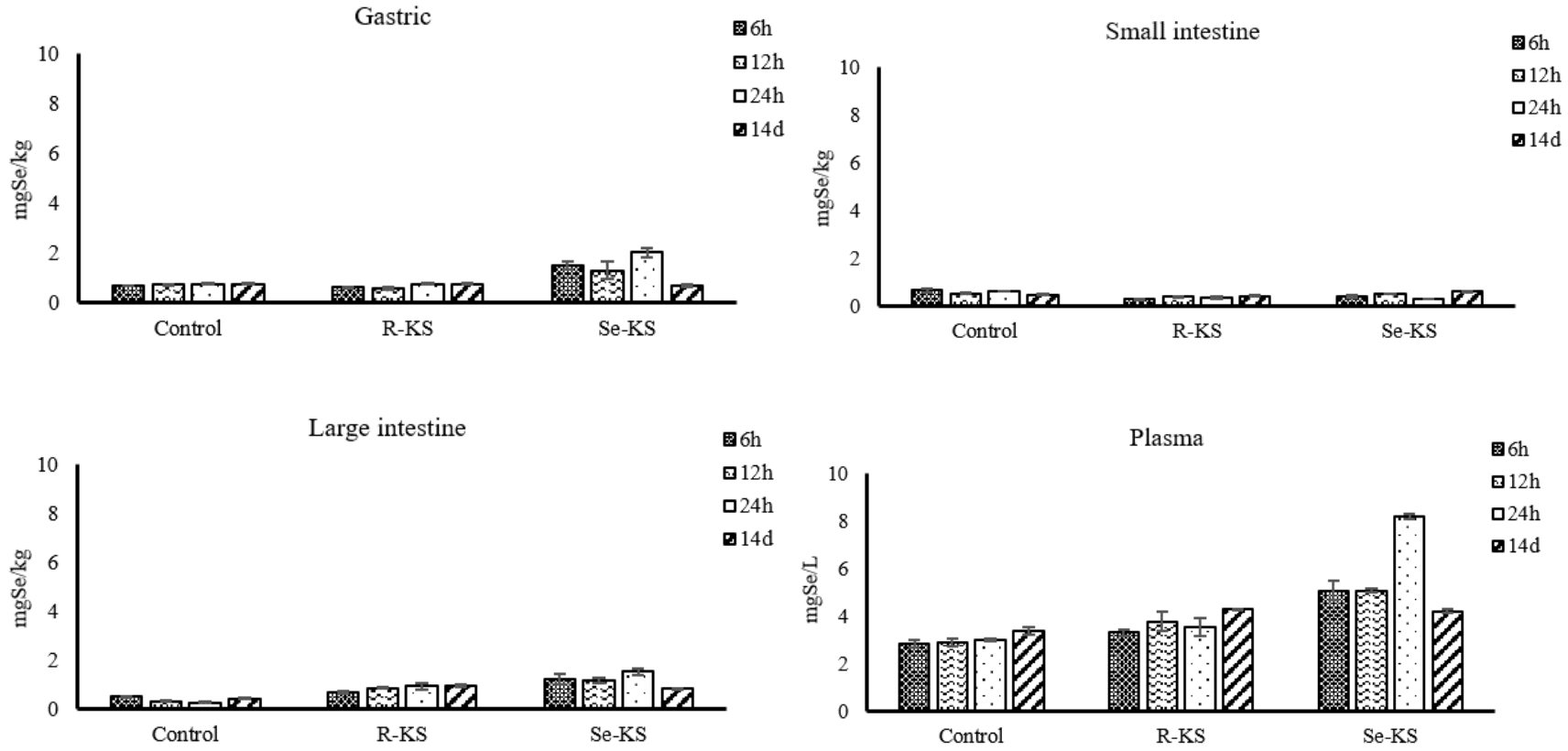

Figure 4.11 Selenium accumulation in gastric, small intestine, large intestine and plasma.

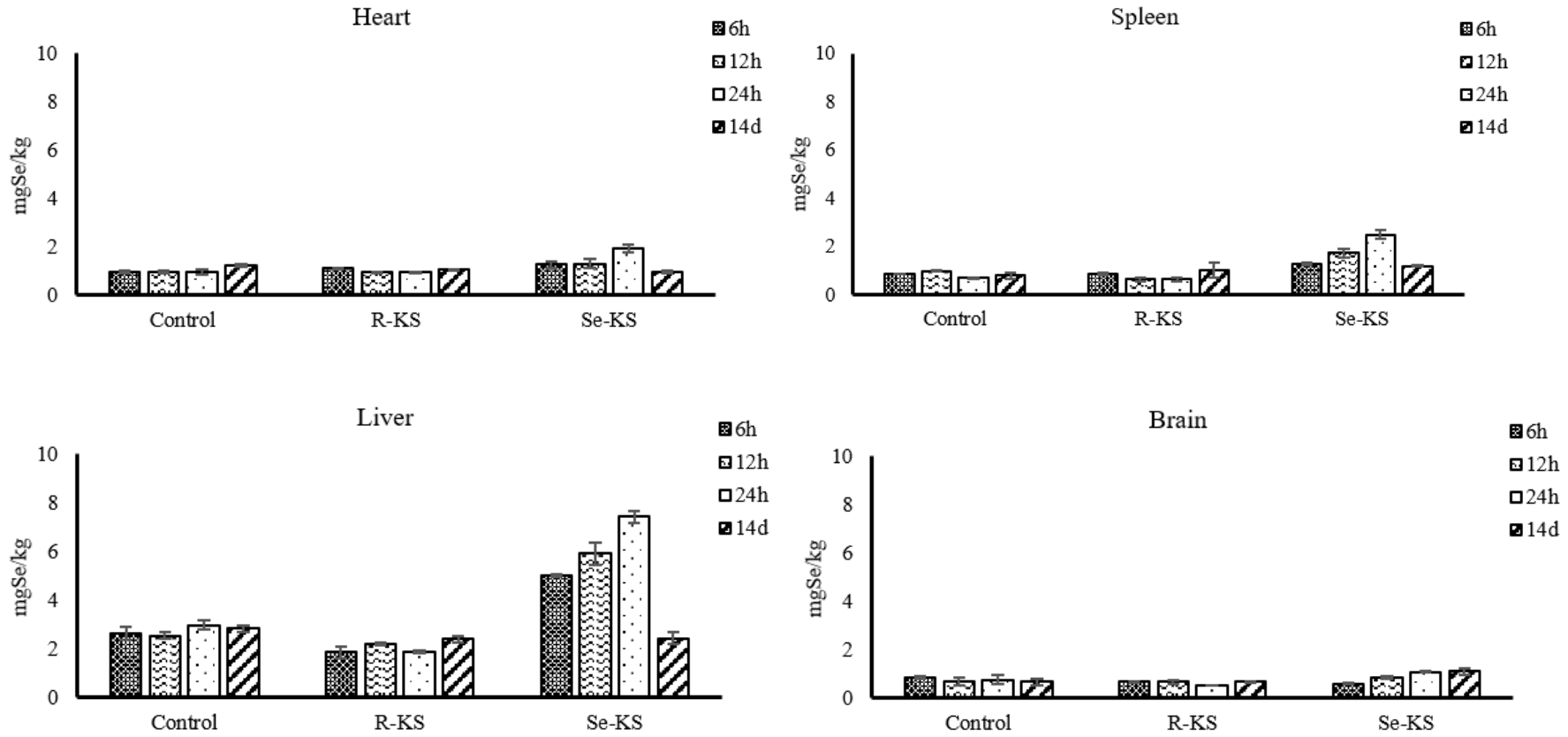

Figure 4.12 Selenium accumulations in heart, spleen, liver and brain.

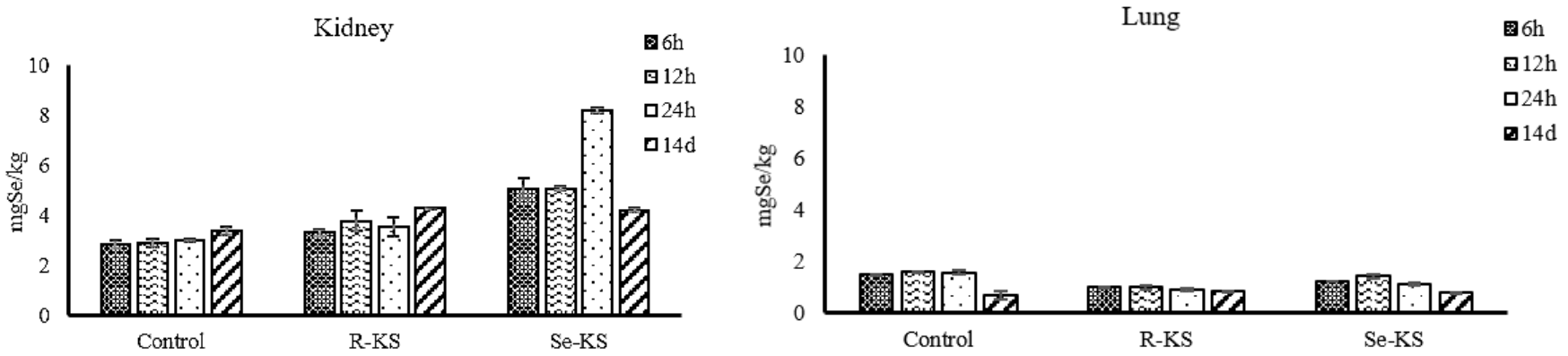

Figure 4.13 Selenium accumulations in kidney and lung

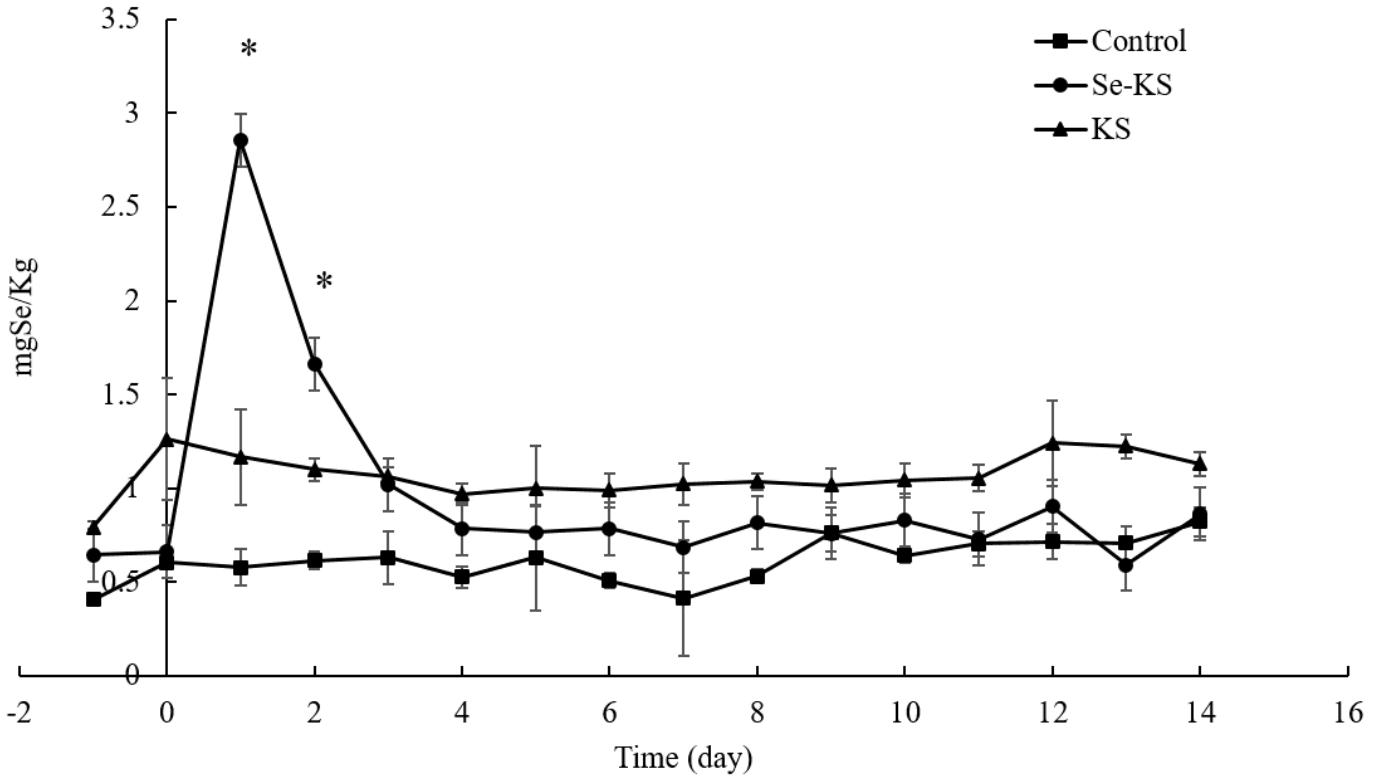

Figure 4.14 The selenium accumulation in feces of rats after administered kale seedlings and control. Means ± SD (n=4)
*The data are presented p <0.05 compared with control group

CHAPTER 5

CONCLUSION

5.1 Conclusions

The findings of this study can be summarized as follows:

The absorption, accumulation and transformation of the selenium inorganic form to bioactive organic compounds from Se-KS were responsible for greater Se-bioaccessibility and stronger antioxidant activities more so than the untreated form. The Se-KS was germinated under a selenite hydroponic condition that revealed the main selenium species including SeMet and MeSeCys, which the primary species had exposed. Additionally, kale readily accumulated selenium from the substrate, so the Se-KS could also be an excellent natural source of the above revealed selenoamino acid and selenoprotein content, which may improve health promotion.

The highest dose of Se-KS was 5000 mg kg^{-1} b.w., and did not cause any toxicity. As such, Se-KS has been supported by safety products, which are relatively nontoxic. In this study the acute toxicity researches were considered nontoxic if no mortality or no death occurred. No clinical signs of toxicity were observed at doses at or below 5,000 mg kg^{-1} (Brock *et al.*, 1995). Se-KS was evaluated at the median lethal dose (LD_{50}) rather than 5000 mg kg^{-1} b.w. which indicated Se-KS as being a safe substance.

Selenium content accumulation in several tissues was described as the pathway of metabolism of Se-KS in Wistar albino rats. At periods of 6 h to 24 h after administration of Se-KS, selenium was unequally digested, transported, distributed and accumulated between the tissues. The highest selenium level was found in the kidney followed by plasma, liver, spleen, gastric, heart, large intestine, lung, brain and lowest level in small intestine, respectively. The inorganic and organic form of Se-KS after thorough GI-digestion will be directly converted to hydrogen selenide (intermediate form)

which is used for selenprotein synthesis such as GPx, DOI, Sel-P, Sel-W, Sel-15 etc. Belenium will be eventually get methylated and secreted in the breath and feces as DMSe and in urine as Se-sugar and TMSe.

The selenium content could encourage the biosynthesis of phenolic content, which is postulated as a bioactive compound having a beneficial effect on human health, and positively affecting antioxidant activities. The Se-KS also had strong antioxidant activity, and showed superior bioaccessibility values and safety evaluation. Therefore, our understanding and knowledge of these matters will be expected to be a potential source of natural and alternative uses for patients and health promoters who want to use this enriched selenium plant as a new food supplement and reduce the risk of disease.

5.2 Suggestions

According to these results, Se-KS has exhibited a potential for being a good source of selenium species content, especially SeMet and SeMetSeCys, which rated excellent for antioxidant and bioacessibility. Therefore, further studies should be conducted on the evaluation of the biological activities (antioxidant and anti-cancer) *in vivo* experiments, and investigations of their toxicity effects (sub-acute, sub-chronic and chronic) in animal or human body tests. Furthermore, the effect of consuming a product produced from this plant on stress and immunity in humans needs to be studied further to fully realize the application of this plant for use as a drug for anti-cancer prevention and as a food supplement.

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