The Acute and Chronic Effects of Flavonoids on The Absorption and Tissue Distribution of Selenium in Rats

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Abstract

The present study was designed to evaluate the effects of acute- and chronic use of the flavonoids silibinin, epigallocatechin gallate (EGCG), quercetin and rutin on the absorption and tissues distribution of selenium (Se) after single oral dose of Na-selenite. In the first part, thirty rats were allocated into 5 groups: 1st group treated with olive oil and served as control; the other 4 groups were treated with either silibinin (100mg/kg), EGCG (25mg/kg), quercetin (50mg/kg) or rutin (500mg/kg), administered orally as oily solutions for 30 days. Then, all groups received orally single doses of Na-selenite (0.5mg/kg) 2 hrs after administration of the last doses of the flavonoids and the vehicle. In the second part, similar protocol was followed as in the first part, except for the duration of flavonoids treatment, where only single doses were administered. The animals were sacrificed 3 hrs after Se administration. Blood samples, brains, kidneys and livers were obtained for evaluation of Se content using atomic absorption spectrometry. Chronic use of flavonoids increased serum and tissues Se significantly compared to control. While acute use did not change tissues Se levels, but significantly decreasing serum Se in all flavonoids treated groups, except for EGCG-treated group. In conclusion, chronic use of flavonoids increases serum and tissues levels of Se, while single doses approach reveal a significant decrease in serum levels without affecting tissues distribution; highly significant positive correlation between serum and kidney Se were also reported.

الخلاصة

صممت هذه الدراسة لمعرفة تأثير الاستخدام الحاد والمزمن لمركبات الفلافينويدsilibinin, EGCG, quercetin, rutin على امتصاص وتوزيع عنصر السيلينيوم بعد استخدام جرعة واحدة عن طريق الفم من هذا العنصر. في الجزء الأول من الدراسة، تم تخصيص ثلاثين الفئران وتوزيعهم إلى 5 مجموعات:تم اعطاء المجموعة الاولى زيت الزيتون وشغل منصب مجموعة السيطرة،في حين تم معالجة المجموعات الاربعة المتبقية كالاتي silibinin (١٠٠مغ/كغ)، EGCG(٢٥مغ/كغ)، quercetin (٥٠مغ/كغ) اوrutin (٥٠٠مغ/كغ) تم اعطاءها كمحاليل زيتية عن طريق الفم. ثم بعد ساعتين تلقت كل المجاميع جرعة واحدة من Na–selenite(٠.٥مغ/كغ) . في الجزء الثاني من الدراسة،استخدم بروتوكول مماثل كما هو الحال في الجزء الأول، باستثناء مدة العلاج الفلافونويد، حيث كانت تعطى جرعة واحدة فقط. تم التضحية بالحيوانات بعد ٣ ساعات من تناول عنصر السيلينيوم. تم الحصول على عينات من الدم،الدماغ ،الكلى والكبد وذلك لتقييم محتوى السيلينيوم باستخدام مطياف الامتصاص الذري.ان الاستخدام الطويل الأمد لمركبات الفلافونويد يؤدي الى زيادة ملحوظة بتراكيز عنصر السيلينيم في مصل الدم والأنسجة بشكل كبير مقارنة مع السيطرة. في حين استخدامها على المدى القصير لن يغير مستويات السيلينيم في الأنسجة ، بينما تتناقص بشكل ملحوظ تراكيز السيانيوم في جميع المجموعات المعالجة بمركبات الفلافونويد ، باستثناء المجموعة التي عولجت EGCG. في الختام،ان الاستخدام على المدى الطويل من مركبات الفلافونويد يزيد من تراكيز السيلينيوم في الدم والأنسجة ، في حين استخدام الجرعة واحدة ادت الى وجود انخفاض كبير في مستويات مصل الدم من السيلينيوم دون التأثير على توزيع الأنسجة؛ وذكرت أيضا الدراسة ان هناك علاقة إيجابية هامة للغاية بين مصل الدم والكلى .

Introduction

Flavonoids are a large group of polyphenolic compounds that are found in many fruits, vegetables, and are common substances in the daily diet [1]. There were large numbers of studies, as well as few cross-sectional observations and interventions linked this class of compounds to have beneficial effects towards health. From this, many experimental data in the previous reports have tried to investigate the mechanisms behind effects of flavonoids and health benefits. These molecules have wide variety of biological effects, as tested in both in vitro and in vivo studies. They can inhibit proliferation of cancer cells, reduce vascularization, protect neurons against oxidative stress, and stimulate vasodilation and improve insulin secretion [2,3]. A large body of evidence has concluded that consumption of dietary flavonoids, can have beneficial outcomes [4]. Nowadays there is an extensive range of flavonoid preparations on the market [5]. Suppliers of such flavonoid preparations accepted daily intakes in amounts greater than the amounts that can normally taken in normal diet. Actually such supra-physiological amount of flavonoid may exhibit adverse effects. Moreover, flavonoids-rich dietary supplements also contain essential trace elementsas add-on additives [6].Such combinations may lead to exaggerated pharmacological activity and/or toxicity of the adjunctly administered essential elements, or underexposure and loss of efficacy [7].Selenium(Se) is an essential micronutrient; its biological activity is concentration dependent, and trace concentrations are required for normal growth and development. Moderate concentrations can be stored and homeostatic functions maintained, while elevated concentrations can result in toxic effects [8].Selenium salts and organic seleno-compounds are easily absorbed from the gastrointestinal tract. More than 90% of seleno-methionine is absorbed in the small intestine, mainly through the Na^+-dependent neutral amino acid transport system [9]. Selenium delivered from vegetables sources achieves 85-100% bioavailability, while that from animal sources is only 50% bioavailable after oral intake [10].Selenium is required for normal animal growth and reproduction, and plays a potential role as an antioxidant via its involvement in the active site of the enzyme glutathione peroxidase (GSH-Px) in blood, liver, kidney and brain, which might be correlated with enhancing the immune response in mammals[11,12].Additionally,Schweizer et al reported that Se prevents brain ischemia and improve mood, cognitive function, and clarity[13]. The risk of flavonoids-selenium pharmacokinetic interaction poses two major challenges, pharmacotoxicity and treatment failure. Inhibition of the homeostatic mechanisms responsible for the absorption, tissue distribution and clearance of selenium can result in toxicity, while decrease absorption or faster clearance can lead to treatment failure. Accordingly, the present study was designed to investigate the effect of both acute and chronic use of supraphysiological doses of flavonoids (silibinin, epigallocatechingallate, quercetin and rutin) on serum concentration and tissues availability of Se in rats and to find correlation between these parameters.

Materials and Methods

Silibinin dihemisuccinate(SDH) (98% purity) was obtained from Tolbiac SRL, Argentina; Quercetin dehydrate (98% pure standardized extract) was purchased from Xian Co,China; Epigallocatechingallate(EGCG) was a gift from Al-Razi Pharm Ind, Syria; Rutin was obtained from Merck Laboratories, Germany; Sodium selenite was obtained

from Sigma Chemical Co., St Louis,MO, USA. The experiments were carried out on male Sprague-Dawley rats (200-250g). The animals were kept under controlled conditions (22-25°C) on a 12 h light/12 h dark cycle, and received the standard pellet diet and water *ad libitum*.All procedures were conducted according to National Institutes of Health Animal Care and Use Committee guidelines, and were approved by the Ethical Committee of the Institute of Pharmacology, Basra, Iraq. The present work includes two approaches, short- and long-term supply of the studied flavonoids; both of them involve the same schedule and the same number of animals. They differ only in the duration of flavonoids treatment. After acclimatization of rats for a period of one week, ٣٠ rats were allocated into five groups (6 rats each); first group was treated with vehicle (olive oil) as control group; the other four groups were treated with one of the flavonoids: SDH (100 mg/kg), EGCG (25 mg/kg); Quercetin (50 mg/kg) and Rutin (500 mg/kg). All flavonoids were prepared as oily solutions dissolved in olive oil and introduced orally as single daily doses using gavage tube for 30 consecutive days in the long-term study,and only single dose in short-term approach.The control group received 0.2 mL/day of olive oil in the same way. Then, all rats received single oral doses of Na-selenite (0.5mg/kg) 2 hrs after administration of the last doses of the flavonoids and the vehicle.After 3.0 hrs of Se administration, all animals were sacrificed with anesthetic ether; blood samples were drawn and collected in polyethylene tube, centrifuged at 10000 rpm for 20 min and the resulted serum was kept frozen at –20°C until Se analysis. The liver and both kidneys were quickly removed, and perfused with ice-cooled saline.the brain was carefully excised, rinsed with ice-cooled saline and the arachnoid membrane was carefully removed. One gram tissue of the obtained organs and 1.0 ml of serum were digested utilizing the acid wet digestion method [14,15].The digested samples were stored in refrigerator and used later for analysis of tissue and serum levels of Se [16]. The contents of Se in serum and tissue samples were first released from the protein matrix by wet digestion method as mentioned previously, and their concentrations were determined using atomic absorption spectrophotometer (Buck Scientific, Model 211-VGI, USA) with a combination Hydride/Cold Vapor Generatorsystem (Model 1018). Standard solutions of Se were used to prepare calibration carve for quantitative analysis.Selenium concentrations were presentedas µg/dlof serum or µg/g of tissues on wet weight basis.

Statistical analysis

Values were expressed as mean±S.D; the values were statistically evaluated using unpaired Student's ***t***-test and one way analysis of variance (ANOVA), supported by Bonferroni's*post hoc* analysis. Values with $P<0.05$ were considered significantly different. Analysis was performed using GraphPad Prism software for Windows (version 5.0, GraphPad Software, Inc., San Diego, CA).

Results

Figure 1 reveals the acute effect of silibinin, EGCG, quercetin and rutin on serum selenium concentration, after oral administration of sodium selenite in rats. Silibinin, quercetin and rutin decrease serum concentration of selenium significantly compared with control ($P<0.05$), on the other hand a cute use of EGCG does not affect serum selenium levels compared with control group rats ($P>0.05$). In kidney, selenium levels were not significantly affected by the single doses of the administered flavonoids ($P>0.05$), compared with control group

(Figure 2). Regarding brain and liver were not affected by acute effect of studied flavonoids, and no significant differences (P>0.05) observed with respect to non-treated control rats (Figures 3 and 4). Regarding chronic effects of flavonoids, figure 5 indicates that all tested flavonoids, increase serum levels of selenium significantly compared with acute effect of sodium selenite (P<0.05). However, EGCG produces the higher effect in this regard compared to other (P<0.05), and the effect of the later compounds appears to be comparable (P>0.05).In kidney tissue, chronic use of flavonoids produced significant increase in selenium levels, compared with control group (P<0.05); and when the effects of each flavonoid compared with others, they do not show significant differences among each other in this regard (P>0.05). In figure 7, all tested flavonoids increase Se levels significantly in brain tissue compared with control (P<0.05); while they showed comparable effects when compared with each (P>0.05).Similarly, figure 8 shows that chronic use of the targeted flavonoids produce significant increase (P<0.05) in liver levels of selenium as compared with a cute use of sodium selenite. Meanwhile, silibinin, EGCG and rutin produced comparable effect (P>0.05) in this respect, and found to be significantly higher than that produced by quercetin, which produces the least effect (figure 8). Table1 demonstrate correlation analysis among flavonoids treated groups in both acute and chronic study, the present study showed positive correlation between serum Se concentration and their concentrations in kidney, liver and brain actually it is significant with kidney in all flavonoids treated groups, this finding in acute study while in chronic study there were negative correlation observed in all flavonoids treated groups except in organs in silibinin treated groups and liver and kidney of quercetin treated groups showed positive correlation in this respect.

Discussion

Increase daily consumption of flavonoids-rich diet may potentiate the harmful effects of trace elements because of their different pharmacological properties. Moreover, it may change the activities of enzymes and environmental toxins. Thus, although there is evidence that flavonoids in fruits and vegetables provide protection against many diseases[18], the amount of flavonoid intake that may pose a potential hazard remains to be determined. Information on the bioavailability and organ distribution of selenium after acute and chronic administration of flavonoids is important for understanding whether flavonoids inhibit or enhance the absorption and organ distribution of this important element. Assessment of selenium availability in serum and tissues is an indirect measure of its kinetic inside the body [19]. According to our knowledge, this is the first project that studies the effect of silibinin, EGCG, quercetin or rutin (acute and chronic use) on the absorption and tissues distribution of selenium in rats. In the present study, chronic administration of all tested flavonoids increase significantly both serum and tissues concentrations of selenium compared to control animals. The explanation of such finding seems to be a little bit difficult, since there were conflicting reports in this regard. However, this may be explained by different mechanisms. One of the suggested mechanisms is through their action as vasodilators, with good hemodynamic effects improving localized blood flow in intestine, liver, kidney and cerebral blood flow. Hence, availability of selenium in serum and organs may be increased consequently. There is now a significant body of evidence supporting the idea that

many natural products produced vasodilatory activity. Several studies indicated that quercetin and EGCG can improve endothelial function via increasing NO bioavailability and/or NO production leading to vasodilatation[20,21].Silibinin also markedly improves endothelial function and have vasodilator effect by reducing circulating and vascular asymmetric dimethylargininelevels, an endogenous inhibitor of nitric oxide synthase that plays a pivotal role in endothelial dysfunction [22].In other hand, vasorelaxation induced by wines was correlated with the concentrations of certain polyphenols. Interestingly, those wines that contain higher concentrations of rutin and kaempferol showed a greater vasorelaxation[22,23].Fayed and Gad (2011) also supported the idea that a vasodilator enhances distribution of trace elements; they concluded that sildenafil citrate uses increased serum Se and Cu, as well as increased brain Se, Cu, and Cr concentrations in rats. This increment could be attributed probably to the increased absorption of trace elements from the intestinal tract and the increased uptake by brain tissue through the BBB[17].Accordingly, the reported increase in absorption and tissue distribution of Se after chronic use of polyphenols can be attributed to the increase in NO production, this idea needs need to be further investigated. However, overproduction of NO can be destructive and may cause irreversible cell damage [24].Other mechanism can explain the increase in serum and tissue selenium concentrations. This was related to enhancement of absorption and membrane transport of some metals ions when they form complexs and chelates with organic ligands. Trials in mammalian species have demonestrated that complexes of organic compounds with minerals increase relative bioavailability compared with inorganic ones, and provide another pathways for enhancing absorption of ingested minerals[25].In the second part of the present study, the serum and tissue levels of Se after concomitant administration of a pharmacological dose of sodium selenite with supra-physiological doses of flavonoids were evaluated. The results clearly showed that serum availability of Se decreases significantly compared with control group, with exception of EGCG, where no significant difference observed. Many studies showed such results, and consider chelation or complexation as a possible mechanism. Saha et al reported that absorption of selenium decreases with increase phytate level fed to rat [26].Furthermore, increasing flavonoids content in diet could influence the stability of Se species affecting absorption [27]. Other reports showed that interaction with other minerals like Zn or direct interactions in the grain content with flavonoids or phytate or other dietary ligands, this could explain the finding of the present study [28].In this part of the present work, orally administered doses of the four flavonoids (acute use) had no effects on tissues level of Se compared to the vehicle treated group. This is predictable since multiple doses of flavonoids required to give metal chelators property or to produce vasodilator effects [29].Control of plasma selenium levels, tissues and other biological matrices are necessary for assessment of absorption, distribution, metabolism and excretion of this element. The highest selenium content in the present study (µg/g tissue) was achieved in the liver, kidney and brain came in tune with Sohn et al report, which demonstrates high selenium concentration in the liver and plasma compared with negligible level in the kidney [30]. Furthermore, the high Se concentrations in these tissues are most likely the result of non-specific incorporation in to tissue proteins [19]. Meanwhile, the flavonoids

increase hepatic glutathione peroxidase activity that associated with the protection against oxidative stress in the presence of selenium in the liver; this may give another explanation for high liver selenium concentration [31,32].In conclusion, the chronic use of supraphysiological doses of silibinin, EGCG, quercetin or rutin increases the absorption and tissues(brain, kidney and liver) distribution of Se, while acute administration of these flavonoids did not significantly effecting the tissues availability of selenium.

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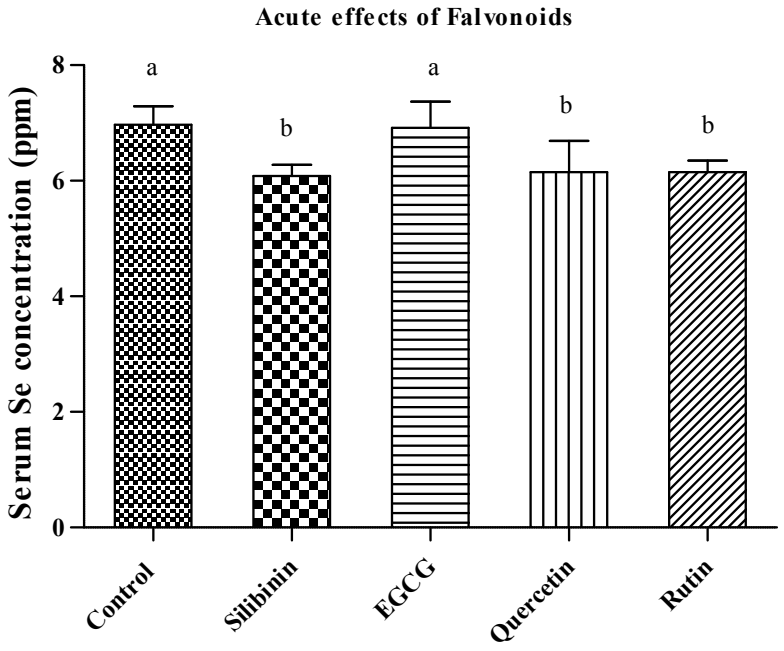

Figure 1. Acute effects of using silibinin, EGCG, quercetin and rutin on serum levels of Se after single oral dose of sodium selenite in rats; ANOVA: values with different letters (a,b) represent significant differences (P<0.05)

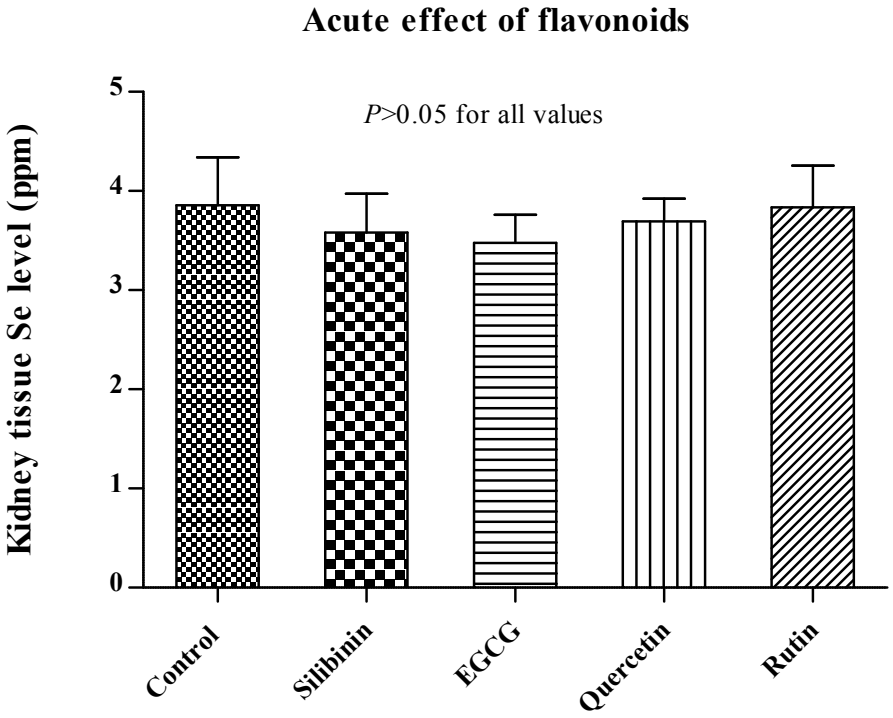

Figure 2. Acute effects of using silibinin, EGCG, quercetin and rutin on kidney tissue levels of Se after single oral dose of sodium selenite in rats; ANOVA: No significant differences among different groups (P>0.05)

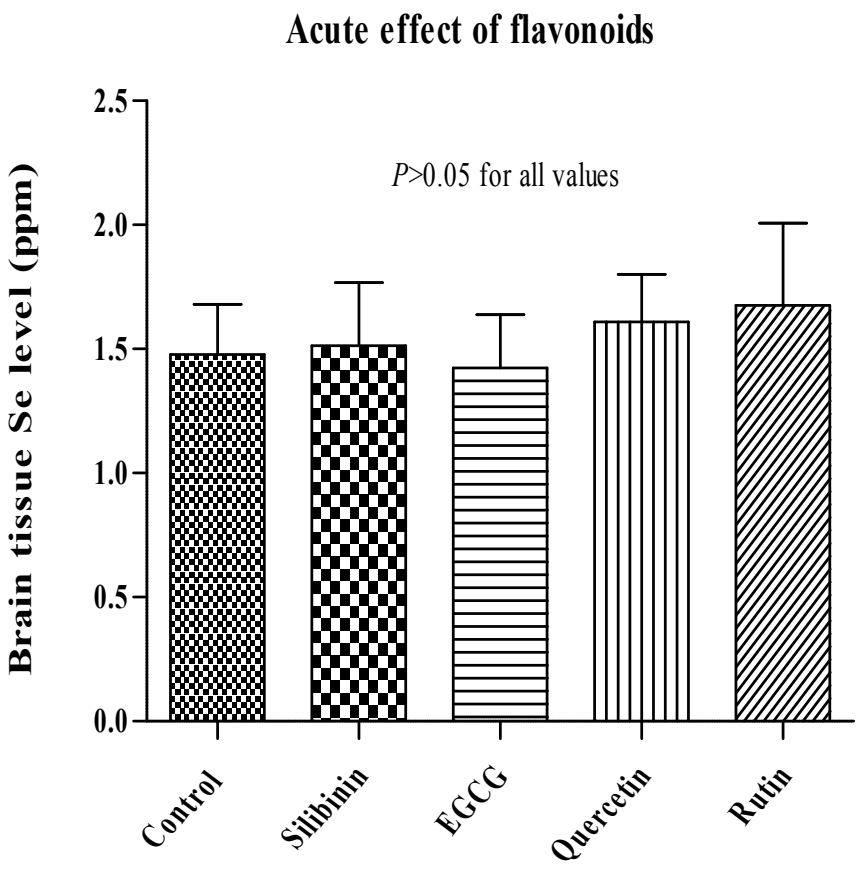

Figure 3. Acute effects of using silibinin, EGCG, quercetin and rutin on brain tissue levels of Se after single oral dose of sodium selenite in rats; ANOVA: No significant differences among different groups (P>0.05)

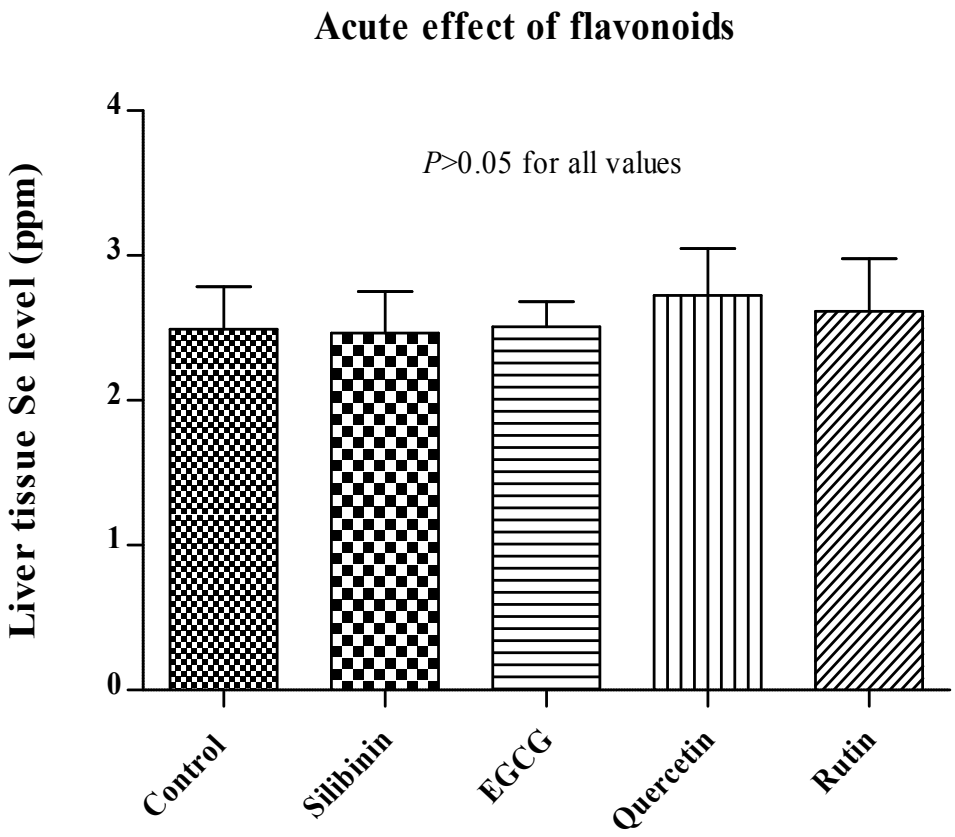

Figure 4. Acute effects of using silibinin, EGCG, quercetin and rutin on liver tissue levels of Se after single oral dose of sodium selenite in rats; ANOVA: No significant differences among different groups (P>0.05)

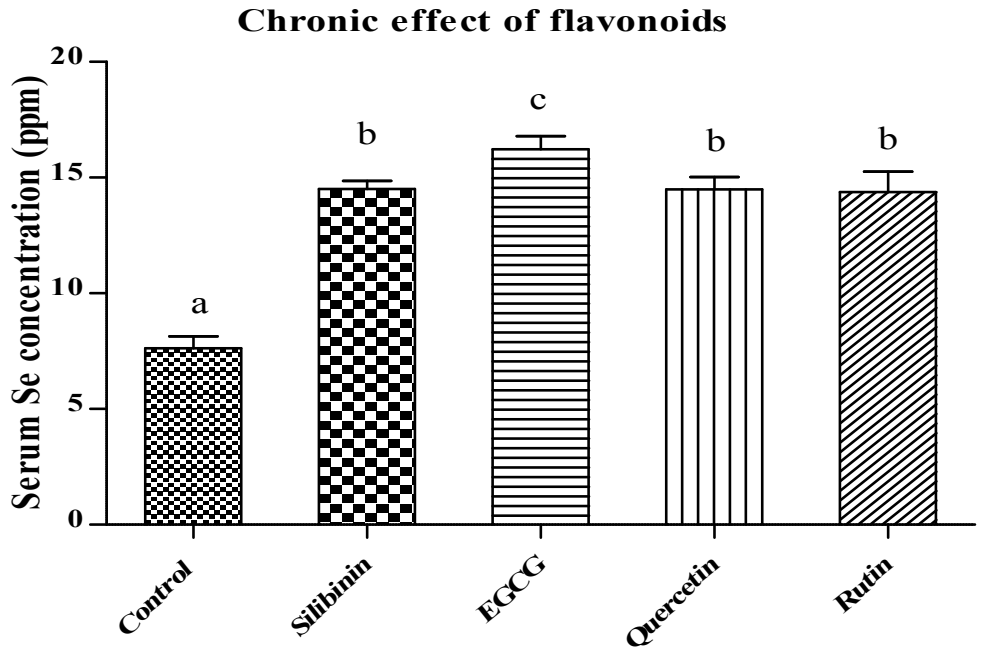

Figure 5. Chronic effects of using silibinin, EGCG, quercetin and rutin (xx days) on serum levels of Se after single oral dose of sodium selenite in rats; ANOVA: values with different letters (a,b,c) represent significant differences ($P<0.05$)

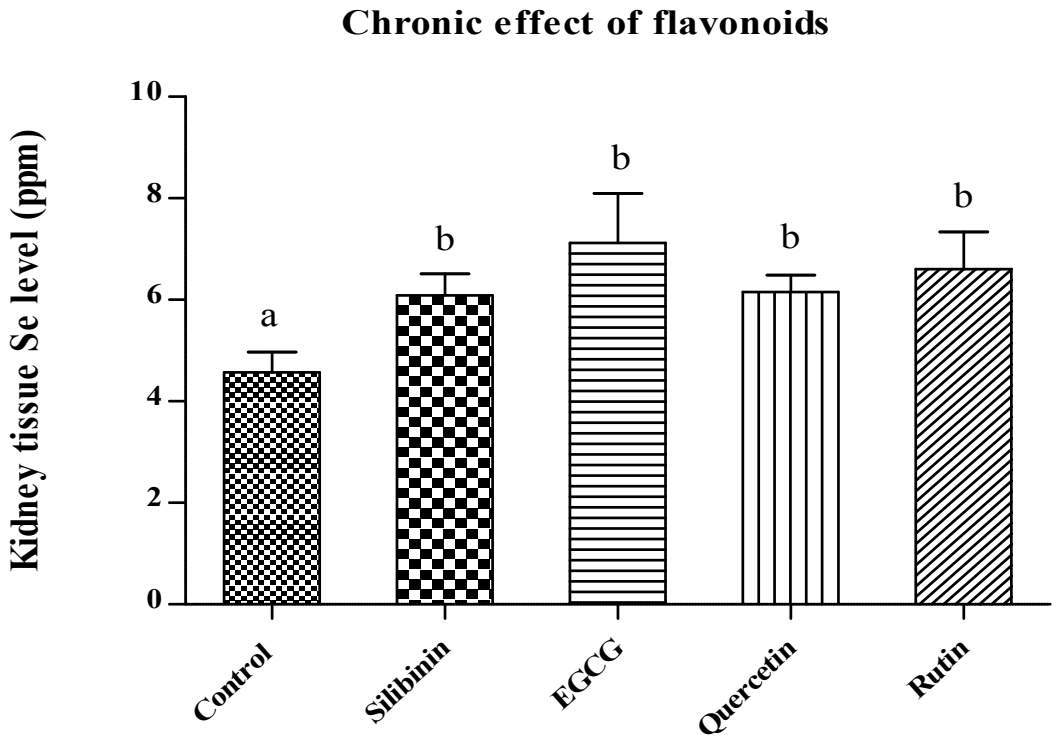

Figure 6. Chronic effects of using silibinin, EGCG, quercetin and rutin (xx days) on Kidney tissue levels of Se after single oral dose of sodium selenite in rats; ANOVA: values with different letters (a,b) represent significant differences (P<0.05)

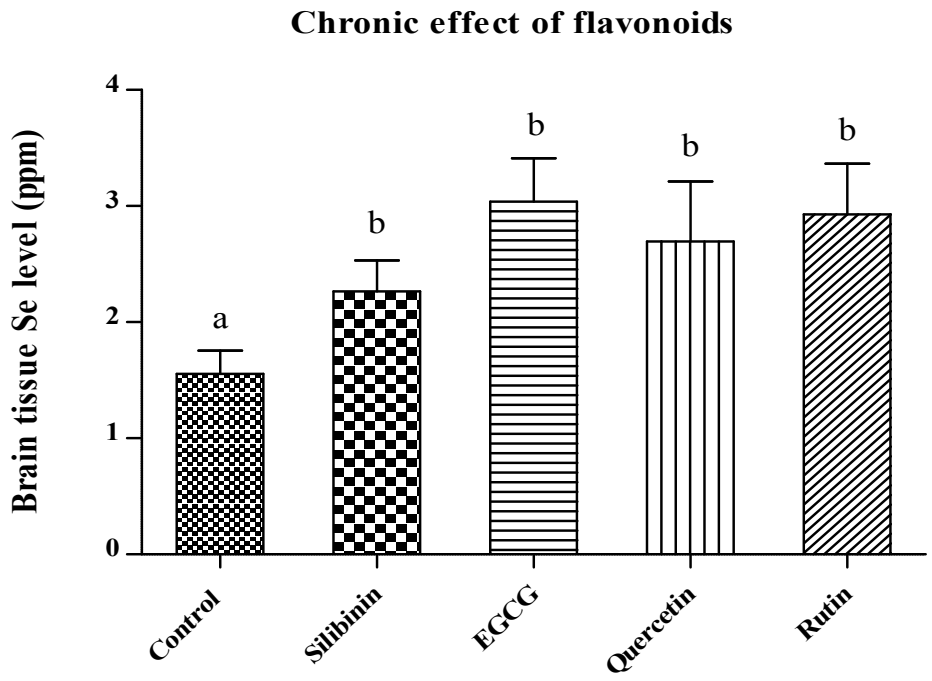

Figure 7. Chronic effects of using silibinin, EGCG, quercetin and rutin (xx days) on Brain tissue levels of Se after single oral dose of sodium selenite in rats; ANOVA: values with different letters (a,b) represent significant differences (P<0.05)

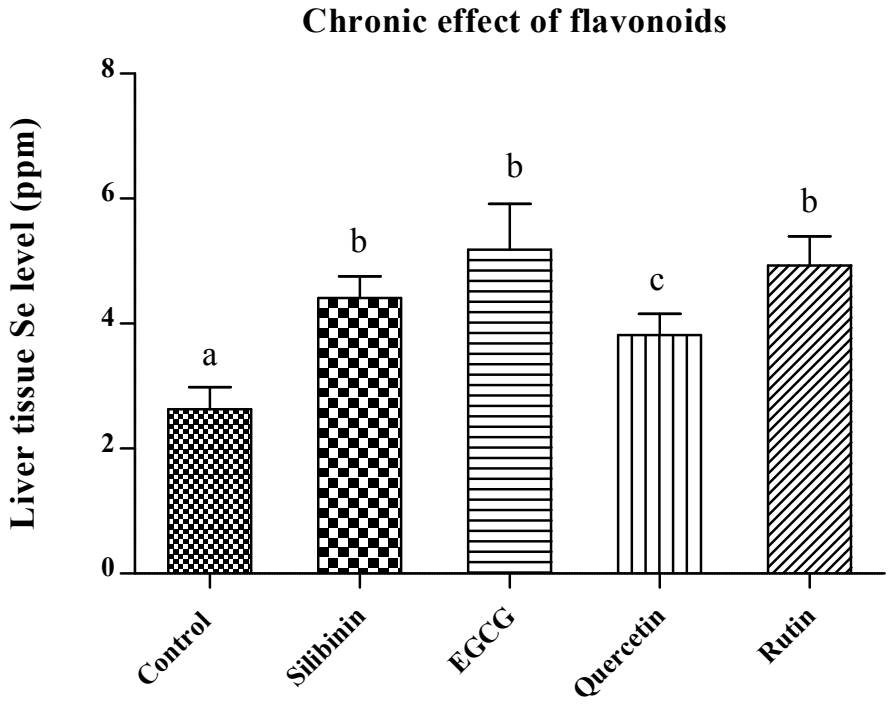

Figure 8. Chronic effects of using silibinin, EGCG, quercetin and rutin (xx days) on liver tissue levels of Se after single oral dose of sodium selenite in rats; ANOVA: values with different letters (a,b,c) represent significant differences ($P<0.05$)

Table 1. Correlation analysis of tissues selenium concentrations in acutely and chronically flavonoids treated groups in respect to serum selenium concentrations.

r = Correlation coefficient Significance: * = P<0.05

Parameter	Groups	Kidney selenium		Liver selenium		Brain selenium	
		r	P	r	P	r	P
Acute study	silibinin	0.8354	0.0384*	0.3766	0.4618	0.4095	0.4201
	EGCG	0.9703	0.0013*	0.6613	0.1527	0.3363	0.5146
	Quercetin	0.8551	0.0300*	0.3798	0.4577	0.3538	0.4915
	Rutin	0.8642	0.0264*	0.6020	0.2061	0.4209	0.4059
Chronic study	silibinin	0.1262	0.8117	0.1801	0.7327	0.04173	0.9374
	EGCG	-0.1958	0.7101	-0.9209	0.0091*	0.1267	0.8110
	Quercetin	0.2249	0.6683	0.5240	0.2859	-0.3030	0.5595
	Rutin	-0.1736	0.7422	-0.2742	0.5989	-0.4586	0.3603