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Effects of Single Oral Doses of Flavonoids on Absorption and Tissue Distribution of Orally Administered Doses of Trace Elements in Rats

Saad Abdulrahman Hussain[1,*], Ausama Ayoob Jaccob[2]

[1]Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad, Baghdad, Iraq
[2]Department of Pharmacology and Toxicology, College of Pharmacy, Al-Basra University, Basra, Iraq
*Corresponding author: saad_alzaidi@yahoo.com

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Abstract Many flavonoids have the capacity to modulate the activity of drug metabolizing enzymes and transporters, thus raising the potential for alterations in the pharmacokinetics of drugs and other essential elements. The present study evaluates the effect of single supraphysiological orally administered doses of silibinin, epigallocatechin gallate (EGCG), quercetin and rutin on the absorption and tissue distribution of orally administered doses of the trace elements zinc copper and iron in rats. Thirty rats were allocated into 5 groups treated as follow: 1st group treated with olive oil, served as control; the other 4 groups were administered orally single doses of either silibinin, EPGC, quercetin or rutin, after 2 hr a solution contains sulphate salts of zinc, copper and iron was administered orally. The animals were sacrificed, blood samples, tissues of brain, kidney and liver were obtained for evaluation of the plasma and tissues concentrations of Zn, Cu and Fe using atomic absorption spectrometry. All four flavonoids decreased serum and tissues concentrations of the trace elements compared with control. In conclusion, concomitant administration of single doses of Zn, Cu and Fe with supra-physiological doses of the flavonoids silibinin, EGCG, quercetin and rutin significantly decreases serum and tissue levels of these trace elements.

Keywords: *flavonoids, trace elements, absorption, tissue distribution*

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1. Introduction

Flavonoids are a large group of naturally occurring polyphenolic compounds that are found abundantly in fruits, vegetables, nuts and plant-derived beverages [1]. The increased use of flavonoids has attracted attention, as they are associated with a range of health benefits, including the prevention of diabetes, cancer, cardiovascular and neurodegenerative diseases [2,3,4]. Most of researches regarding pharmacodynamic and pharmacokinetics of flavonoids are often carried out in animals and their effects in humans remain uncertain [1]. However, a large body of evidence, mainly derived from preclinical studies in animals, has concluded that dietary polyphenols, when given in large quantities, can have desirable outcomes [5]. Additionally, the increasing popularity of herbal medicines, many of which contain flavonoids as the active ingredient, may also expose the individual to larger than expected amounts of flavonoids. This is currently of great concern, as there is accumulating evidence that many flavonoids have the capacity to modulate the activity of drug metabolizing enzymes and transporters, thus raising the potential for alterations in the pharmacokinetics of drugs and other essential elements [6,7,8]. This may lead to exaggerating pharmacological activity and/or toxicity of the adjunctly administered drugs or essential elements on the one hand, or underexposure and loss of efficacy on the other [9,10]. This lack of information, combined with the fact that natural products are usually a mixture of many active ingredients, increases the likelihood of harm. Moreover, this additionally raises concerns about the safe use of dietary flavonoids. The risk of polyphenols-trace elements pharmacokinetic interaction poses two major extremity challenges, pharmacotoxicity and treatment failure. The former can result from the inhibition of the homeostatic mechanisms responsible for the absorption, tissue distribution and clearance of the trace elements, while the latter may be the consequence of inducing processes that lead to faster clearance. The present study was designed to evaluate the effect of single supraphysiological orally administered doses of silibinin, epigallocatechin gallate, quercetin and rutin on the absorption and tissue distribution of orally administered doses of the trace elements zinc copper and iron in rats.

2. Materials and Methods

Silibinin dihemisuccinate (98% purity) was obtained from Tolbiac SRL, Argentina; Quercetin dehydrate (98% pure standardized extract) was purchased from Xian Co,

China; Epigallocatechin gallate was a gift from Al-Razi Pharm Ind, Syria; Rutin was obtained from Merck Laboratories, Germany; Ferrous sulphate, Copper sulphate and Zinc sulphate were obtained from SD Fine Chemicals, India. Thirty male adult Sprague Dawly rats, with body weight 200-250g, were obtained from the Animal House, Department of Pharmacology and Toxicology, College of Pharmacy, Baghdad University, and the experiments were carried out in Department of Pharmacology, College of Pharmacy, Al-Basrah University, Iraq. The rats were housed under controlled conditions (22-25°C) on a 12 h light/12 h dark cycle, and received the standard pellet diet (National Center for Drug Research and Quality Control, Baghdad) and water *ad libitum*. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC), College of Pharmacy, University of Baghdad. After acclimatization for one week, the animals were allocated into five groups consisting of 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: silibinin dihemisuccinate (SDH) (100 mg/kg), Epigallocatechin gallate (EGCG) (25mg/kg); Quercetin dehydrate (Quer) (50mg/kg) and Rutin (500mg/kg). All flavonoids are prepared as oily solutions dissolved in olive oil and introduced as single doses administered orally using gavage tube; the control group receives 0.2 ml/day of olive oil in the same way. After 2 hrs, all groups of rats received orally single doses of zinc sulphate (60mg/kg), copper sulphate (60mg/kg) and ferrous sulphate (60mg/kg), all these elements were administered 2 hrs after administration of the last doses of the flavonoids and the vehicle. Three hours administration of the trace metals, all animals are sacrificed after short duration anesthesia 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 trace elements 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 the serum were digested utilizing the wet digestion method [11,12]; the digested samples were stored in refrigerator and used later for analysis of tissue and serum levels of zinc, copper and iron [13]. The contents of Zn, Cu and Fe 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) at wavelength of 214 nm for zinc, 247 nm for Fe and 324 for Cu [14]. Standard solutions of these elements were used to prepare calibration carve for quantitative analysis.

3. 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).

4. Results

Figure 1, Figure 2, Figure 3 showed the effect of single doses of silibinin, EGCG, quercetin and rutin on the absorption of orally and concomitantly administered essential elements (Zn, Cu and Fe); serum zinc level was significantly decreased ($P < 0.05$) in all groups of rats treated with silibinin, EGCG, quercetin or rutin compared to that found in vehicle-treated group (Figure 1). The effect of all polyphenols was found comparable in this respect. In Figure 2, the four polyphenols also significantly decreased serum copper level compared with control group, but their effects were not comparable; where EGCG and quercetin produced greater effect in this respect, which was significantly differ from that reported in both silibinin and rutin treated groups. Regarding the effects on serum iron levels, Figure 3 clearly indicates that single doses of the four polyphenols significantly decreased serum iron levels, compared with that reported in control group; these effects were comparable for silibinin, EGCG and rutin, and significantly different with that reported for quercetin; the later produced lower decrease in serum iron levels, compared to the formers. Administration of single doses of the polyphenols silibinin, EGCG, quercetin or rutin, produced significant decrease in tissue Zn levels in brain, kidney and liver of rats received these polyphenols, compared with that reported in control group (Table 1). In brain tissue, all polyphenols showed comparable effects (not significantly differ), while in the kidney silibinin produced the highest decrease in tissue Zn levels, which was significantly different compared to other polyphenols. In liver tissues, similar profile for the effects of polyphenols in kidney tissues was clearly demonstrated in Table 1. Table 1 also shows that tissue levels of copper in the brain, kidney and liver were significantly decreased after concomitant administration of silibinin, EGCG, quercetin or rutin, with single dose of copper sulphate, compared to that reported in placebo group. Silibinin produced the highest effect in this respect compared with the other three polyphenols, which were found comparable in their effect. Table 1 clearly shows that tissue levels of iron in the three organs were significantly decreased after concomitant administration single doses of silibinin, EGCG, quercetin or rutin with single dose of the ferrous sulphate, compared to that reported in placebo group. In brain and kidney tissues, silibinin produced the lowest effect compared to other three polyphenols ($P < 0.05$), and found comparable in their effect. Meanwhile, all four polyphenols produced comparable effects in decreasing tissue iron levels in the three targeted organs. Figure 4 indicates that although all the four polyphenols (when administered as single doses) not significantly affecting Zn relative availability in brain tissue compared with control group; only rutin decreases significantly this value compared with that produced by EGCG ($P < 0.05$). Concerning kidney tissue, although the single doses of polyphenols did not produce significant changes in Zn relative availability compared with control group, silibinin decreases significantly this value compared with those produced by EGCG and rutin. However, only EGCG and rutin produced significant increase in Zn relative availability in liver tissue compared with control group, while the four polyphenols showed comparable effects when compared with each others

(Figure 4). In Figure 5, all polyphenols decreased Cu relative availability significantly compared with control in brain tissue; when compared with each others, silibinin demonstrates the lowest effect ($P < 0.05$) in this respect. Regarding kidney tissue, Figure 5 indicates that silibinin only significantly decreases Cu relative availability compared with control, and it demonstrates significantly lower effect in this respect compared with that produced by rutin. In liver tissue, there were no significantly different changes in Cu relative availability regarding the four polyphenols, both when compared with control group or with each others. In Figure 6, only silibinin, when administered as a single dose, significantly increases relative availability of Fe in brain tissue compared with control and the other three polyphenols. In kidney tissue, silibinin and EGCG significantly increased Fe relative availability compared with control and the other two polyphenols, while quercetin produces significant decrease in this parameter. Regarding liver tissue, EGCG increases significantly Fe relative availability compared with control and the other three polyphenols, while quercetin significantly decreases this value; silibinin and rutin showed no significant changes in this parameter compared with control group (Figure 6).

Table 1. Effects single orally administered doses of silibinin (100mg/kg), EGCG (25mg/kg), quercetin (50mg/kg) and rutin (500mg/kg) on tissue availability of Zn, Cu and Fe in Brain, Kidneys and Liver of rats after single oral doses of these metals

Treatment groups	Tissue Levels of Trace Elements µg/g tissue								
	Zn			Cu			Fe		
	Brain	Kidney	Liver	Brain	Kidney	Liver	Brain	Kidney	Liver
Control	45.1±3.4	61.7±4.8	72.2±4.2	45.8±3.0	53.1±3.8	59.2±5.0	41.7±6.8	297.5±24.2	428.2±71.0
Silibinin	33.9±2.7*	42.9±2.5*^{a}	54.7±3.0*^{a}	22.9±1.6*^{a}	33.8±2.6*^{a}	41.9±1.4*^{a}	25.7±6.0*a	171.3±14.1*a	201.2±13.2*
EGCG	37.5±2.7*	49.8±2.1*^{b}	59.6±2.2*^{a}	33.3±2.7*^{b}	45.8±2.9*^{b}	51.7±3.0*^{b}	14.9±2.0*b	130.8±10.1*b	171.8±19.9*
Quercetin	36.9±2.8*	50.7±2.6*^{b}	63.1±2.0*^{b}	30.8±3.0*^{b}	42.2±1.8*^{b}	52.4±2.5*^{b}	20.5±2.8*b	136.0±22.0*b	188.0±16.8*
Rutin	32.1±3.0*	52.2±3.0*^{b}	61.0±2.4*^{b}	32.4±2.6*^{b}	42.9±4.0*^{b}	49.6±4.3*^{b}	19.9±3.4*b	115.8±9.4*b	178.5±9.7*

Values are presented as mean±S.D; n=6 rats in each group; * significantly different compared to control group ($P < 0.05$); values with non-identical superscripts (a, b) for the same metal in the same organ are considered significantly different ($P < 0.05$).

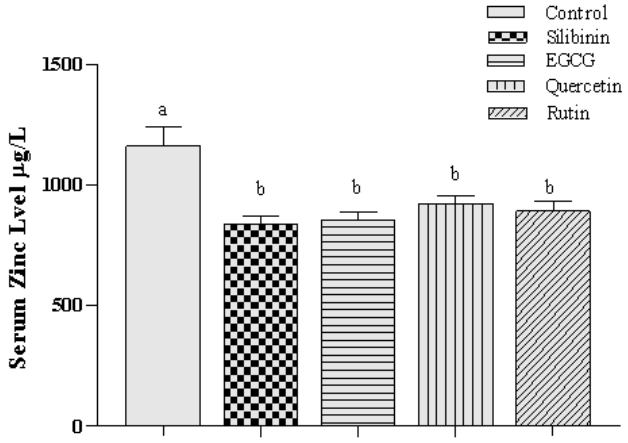

Figure 1. Effects of orally administered single doses of silibinin (100mg/kg), EGCG (25mg/kg), quercetin (50mg/kg) and rutin (500mg/kg) on serum levels of Zn in rats after single oral dose of this element

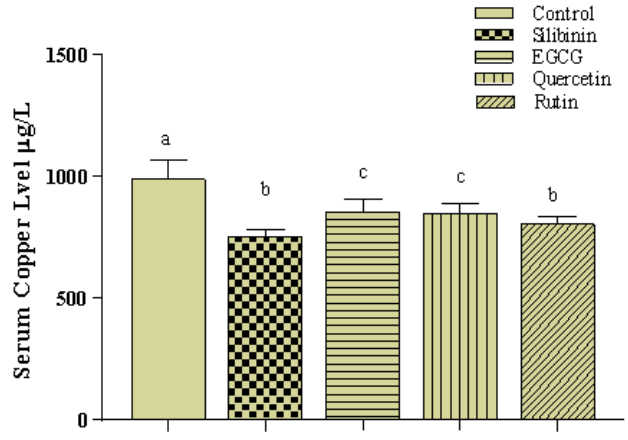

Figure 2. Effects of orally administered single doses of silibinin (100mg/kg), EGCG (25mg/kg), quercetin (50mg/kg) and rutin (500mg/kg) on serum levels of Cu in rats after single oral dose of this element

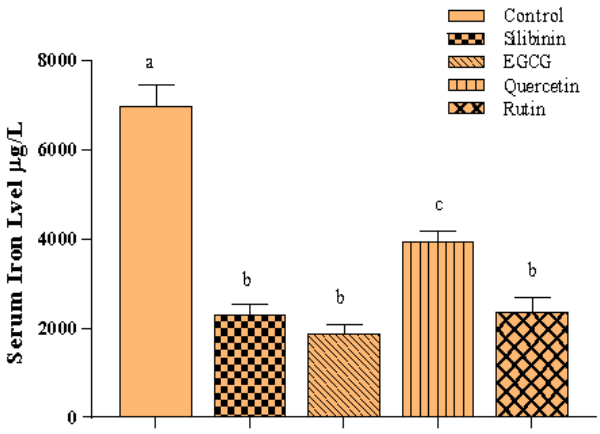

Figure 3. Effects of orally administered single doses of silibinin (100mg/kg), EGCG (25mg/kg), quercetin (50mg/kg) and rutin (500mg/kg) on serum levels of Fe in rats after single oral dose of this element

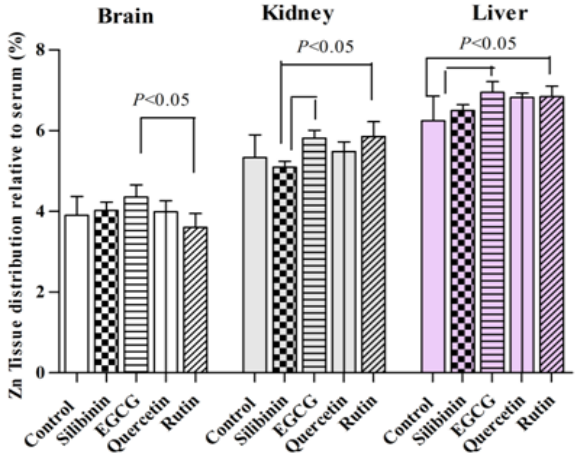

Figure 4. Effects of single doses silibinin, EGCG, quercetin or rutin on %Zn distribution in brain, kidney and liver tissues of rats after oral administration of single dose (60mg/kg) $ZnSO_4$. Values were expressed as mean ± SD; number of animals=6 in each group; $P<0.05$ consider significantly different among groups in the same tissues

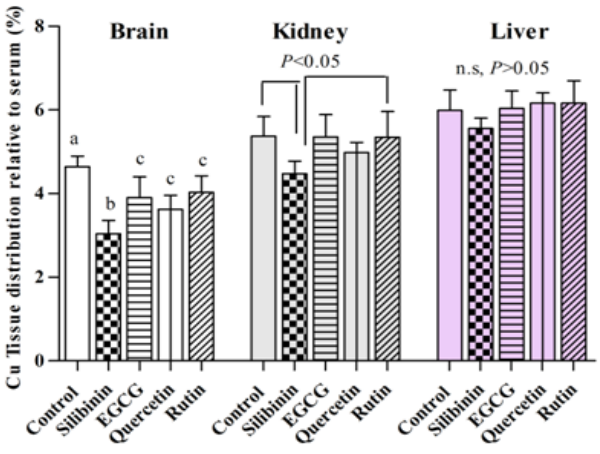

Figure 5. Effects of single doses silibinin, EGCG, quercetin or rutin on %Cu distribution in brain, kidney and liver tissues of rats after oral administration of single dose (60mg/kg) $CuSO_4$. Values were expressed as mean±SD; number of animals=6 in each group; $P<0.05$ consider significantly different among groups in the same tissues

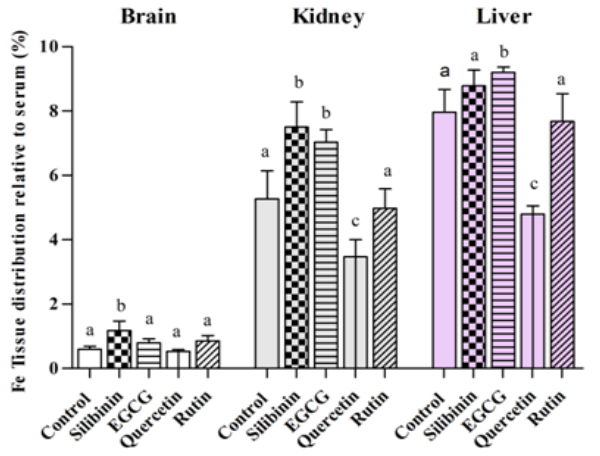

Figure 6. Effects of single doses silibinin, EGCG, quercetin or rutin on %Fe distribution in brain, kidney and liver tissues of rats after oral administration of single dose (60mg/kg) $FeSO_4$. Values were expressed as mean±SD; number of animals=6 in each group; values with non-identical superscripts (a,b,c) within the same tissue were considered significantly different($P<0.05$)

5. Discussion

High consumption of flavonoids rich diet may influence the pharmacokinetics of drugs or trace elements because of their diverse pharmacological properties. Moreover, it may modulate trace elements levels and the activities of environmental toxins and metalloenzymes. Thus, although there is evidence that a flavonoid-rich diet or supplements may promote good health and provide protection from many diseases, the conditions and the levels of flavonoid intake that may pose a potential hazard remains to be determined. In the present study, serum and tissue levels of Zn, Cu and Fe after concomitant administration of pharmacological doses of those elements with supra-physiological doses of polyphenols were evaluated. Our finding clearly showed that serum and tissues availability of trace elements decreased significantly compared with control group. Many studies showed such results, and consider chelation or polyphenols-trace element complexation as possible mechanism for such finding. Purified flavonoids as well as flavonoid-rich extracts have been reported to chelate iron *in vitro* [15]. Ren *et al* studied the complexation mechanisms of several flavonoids (quercetin, luteolin, galangin, kaempferol, and chrysin) with iron. The most important chelation site was the 3-hydroxyl-4-carbonyl group followed by the 4-carbonyl-5-hydroxyl group. In this respect, quercetin and iron form orthogonal Fe-O bonds, where three quercetin molecules are required to saturate the bonds of a single Fe ion *in vitro* [16]. As far as green tea catechins are concerned, the galloyl group seems to be mainly responsible for iron binding, with the green tea catechin EGCG containing 2 galloyl groups. In this context, in a randomized, double-blind, placebo controlled trial, Ullmann *et al* studied nonheme iron absorption in response to pure crystalline EGCG; non-heme iron absorption was dose-dependently decreased by 14 and 27% due to 150 and 300 mg/d EGCG relative to the placebo group [17]. Ma *et al* have recently shown that polyphenols also inhibit heme iron absorption mainly by reducing basolateral iron exit rather than decreasing apical heme iron uptake in intestinal cells [18]. Furthermore, rutin taken in proper amount can effectively improve antioxidant status, whereas at an increased dosage, it may cause trace elements (such as iron, zinc, and copper) deficiencies and a decrease in the activities of related metal-containing enzymes [13]. During our work in the same respect, we reported in our lab a statistically significant decrease in zinc, copper and iron levels in experimental animals compared with both control group and the long-term administration of polyphenols [19]. Likewise, Frejnagel *et al* (2010) reported a statistically significant decrease in utilization of zinc and copper in experimental animals compared to control rats; particularly in animals fed the honeysuckle diet [20]; this was opposite to the outcome of a long-term experiment performed by Coudray *et al*, where polyphenols had neither positive nor negative influence on zinc or copper availability [21]. The phenolic compounds are released from food or beverages during digestion, and can combine with iron in the intestinal lumen, making it unavailable for absorption [22]. The polyphenols are such powerful inhibitors of non-heme iron that substantial changes for iron absorbed are more likely to occur if the timing of consumption is altered, rather than the quantity. For example, a serving of yod kratin (leaves of the lead tree, *Leucaena glauca*, a vegetable with a high content of polyphenols and widely consumed in Thailand) reduced iron absorption from a composite meal of rice, fish and vegetables by almost 90% [23]. Interestingly, all major types of food polyphenols can strongly inhibit dietary iron absorption, and a dose-dependent inhibitory effect of polyphenols compounds on iron absorption has been demonstrated. In particular, it has been reported that any beverage providing 20-50 mg total polyphenols reduce iron absorption from a bread meal by 50-70%, whereas beverages containing 100-400 mg total polyphenols reduce iron absorption by 60-90% [24]. The inhibitory effect of polyphenols on iron absorption has largely been demonstrated, but the capability of complex formation with iron in the intestine and thereby reduction of iron uptake into the body depends on their structure [25]. This gives an idea for the reported variation in the effect of polyphenols on the absorption of trace elements reported in the present study. Zinc absorption process has been

shown to consist of a specific, saturable carrier-mediated component and a nonspecific, unsaturable diffusion-mediated component [26]; when dietary zinc was increased from 5 to 40 mg/kg, the amount of zinc absorbed increased linearly at higher dietary levels, which would be consistent with a diffusion process. In the presence of dietary ligands, like polyphenols, the concentration of "free" zinc is likely to be considerably lower. Therefore, zinc is likely to predominantly be transported via the saturable, specific transport mechanism leading to lower serum zinc concentration [27]. The results of the present study are found relatively comparable with this finding, where concomitant or co-ingestion of polyphenols with trace elements reduce the availability of free trace elements that absorbed by passive diffusion, and only saturable carrier-mediated transport will be available for trace elements absorption and hence decrease serum concentration. In the present study, the results showed that tissues concentration of trace elements were decreased significantly compared with control group. These results give more accurate and predictable indication rather than serum level only; since serum trace elements concentration was decreased compared to control, but when measured as relative tissue availability related to serum levels (or organ distribution relative to serum), elements levels were not decreased in all organs. The relative tissue availability obtained in liver, kidney and brain showed wide variations depending on the element itself and the type of polyphenols administered. Furthermore, it can be inferred that with single-dose polyphenol approach, the concentration of polyphenols in human and animal tissues would not be enough to displace physiological metal chelators. This situation would limit the role of polyphenols in transition metal sequestration to those conditions characterized by excessive amounts of redox active metals, and/or to compartments with high polyphenol concentration, e.g. the GIT [28]. The highest percent of tissue extraction reported in the present study was found in liver tissue for all trace elements. Linder *et al* reported that after intestinal absorption, in the first phase, copper goes from the intestine to the liver and kidney; in the second phase, copper usually goes from the liver (and perhaps the kidney) to other organs including the brain, with highest concentration observed in liver [29]. In addition, copper was incorporated into ceruloplasmin where under normal dietary conditions, much of the copper entering the liver and kidney from the diet reemerges in the plasma in bound form with ceruloplasmin. It is of interest to know that not only liver but also the kidney may be a source of the ceruloplasmin in blood plasma. Indeed, these two tissues have the highest copper concentrations in mammalian organs; this may reflect the fact they are not only the first tissues in which dietary copper is deposited, but both tissues synthesize and secrete ceruloplasmin [30]. In conclusion, concomitant oral administration of single doses of Zn, Cu and Fe with supra-physiological doses of the flavonoids silibinin, EGCG, quercetin and rutin significantly decreases serum and tissue levels of these trace elements.

Conflict of Interest

None declared.

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