Effects of Long-Term Use of Flavonoids on the Absorption and Tissue Distribution of Orally Administered Doses of Trace Elements in Rats

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ABSTRACT

The risk of pharmacokinetic polyphenols-trace elements interaction may undesirable therapeutic outcomes. We evaluate the long-term use of silibinin, epigallocatechin (EGCG), quercetin and rutin on the absorption and tissue distribution of zinc, copper and iron after single oral doses in rats. Five groups of rats received either with olive oil as control or one of the polyphenols silibinin, EPGC, quercetin or rutin, administered orally as oily solutions for 30 days. At day 30, a solution contains sulphate salt of zinc, copper and iron was administered orally; 3 hrs later blood samples, tissues of brain, kidney and liver were obtained for evaluation of the elements levels. The results showed that the polyphenols increased both serum and tissue levels of these elements compared with controls. This effect was relatively varied according to the structural differences among flavonoids. In conclusion, long-term use of supraphysiological doses of flavonoids increase absorption of Zn, Cu and Fe and their tissue availability in brain, kidney and liver; this effect seems to be different with variations in structural features.

Keywords: Flavonoids; Trace Elements; Absorption; Tissue Distribution

1. Introduction

Flavonoids (polyphenolic compounds) are one of the bioactive compounds widely available in fruits and vegetables [1]. Flavonoids have long been associated with a variety of biochemical and pharmacological properties, including antioxidant, antiviral, anticarcinogenic, and anti-inflammatory activities [2], and believed to be beneficial to human health. Many peoples are motivated by scientific research that is widely carried in the news media, which indicated these flavonoids and polyphenols could prevent cancer, ageing, and cardiovascular diseases [3,4]. However, these researches are often carried out in animals and their effects in humans remain uncertain [5]. 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 [6]. There is currently an extensive range of flavonoid supplements on the market [7]. Suppliers of such supplements recommend daily flavonoid intakes in amounts that are many times higher than those doses which can normally be achieved from a flavonoid-rich diet. The question arises whether supplements containing such supra-physiological flavonoid levels may exhibit adverse effects. In addition, it is likely that a large proportion of individuals taking dietary flavonoid supplements are also taking conventional drugs or trace elements [8]. The concomitant intake of “supra-nutritional” flavonoid doses together with conventional drugs may lead to flavonoid-drug interactions [8]. Approximately 38 million adults in the US (18.9% of the population) use herbal products that contain flavonoids or other natural supplements, but only one third tell their physician about this use [9]. 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 pharmacokinetic polyphenols-trace elements 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 the lead to faster clearance. This is in addition to the intrinsic pharmacodynamic actions of the polyphenols themselves which may include potentiating, additive, antagonism, or neu-
tralization effects. The present study was designed to evaluate the effect of long-term use of supraphysiological 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

2.1. Chemicals and Reagents
Silibinin dihemisuccinate (SDH) (98% purity) was obtained from Tolbiac SRL, Argentina; Quercetin dihydrate (98% pure standardized extract) was purchased from Xian Co, China; Epigallocatechin gallate (EGCG) 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.

2.2. Animals and Study Design
Thirty male adult Sprague Dawly rats of body weight 200 - 250 g 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-Basra University, Iraq. The rats were housed under controlled conditions (22°C - 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 a period of 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 are 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 are prepared as oily solutions dissolved in olive oil and introduced as single daily doses administered orally using gavage tube for 30 consecutive days; the control group receives 0.2 mL/day of olive oil in the same way. At day 30, all groups of rats received orally single doses of Zinc sulphate (60 mg/kg), Copper sulphate (60 mg/kg) and Fe sulphate (60 mg/kg), all these elements were administered 2 hrs after administration of the last doses of the flavonoids and the vehicle.

2.3. Samples Preparation
After 3.0 hrs of administration of 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 serum were digested utilizing the wet digestion method [10,11]; the digested samples were stored in refrigerator and used later for analysis of tissue and serum levels of zinc, copper and iron [12].

2.4. Analysis of Trace Elements
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 [13]. Standard solutions of these elements were used to prepare calibration curve for quantitative analysis.

3. Results

Figure 1 showed that all the flavonoids used in the present study significantly increased (P < 0.05) the GI absorption of Zn, when administered as single oral dose compared to control group; meanwhile, no significant differences in serum Zn levels were reported among the effect of the four flavonoids (P > 0.05) in this respect. Concerning long-term effects of the tested flavonoids on the absorption of Cu, all of them produced significant increase (P < 0.05) in serum Cu levels compared to the values reported in controls; only SDH and quercetin demonstrated significantly different effects in this respect (lower effect for quercetin), when the effects of the four flavonoids compared with each others (Figure 2). In Figure 3, treatment of rats with one of the four flavonoids used in the study for 30 days resulted in significant increase (P < 0.05) in the oral absorption of Fe when administered as single dose of ferrous sulphate compared to control group. When the effects of the studied flavon-
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Flavonoids were compared, non-significant differences were reported between the effects of SDH and quercetin, while the others showed significant differences in the order: EGCG > SDH = quercetin > rutin (Figure 3).

The effects of flavonoids on the organ availability and percent amounts of Zn, Cu and Fe distributed to the brain, kidneys and liver relative to serum levels were evaluated after administration of single oral doses of those elements. In Table 1, the results showed that all administered flavonoids significantly increased the tissue availability of the essential metals (Zn, Cu and Fe) compared to the value reported in control group. The most prominent effect for the studied flavonoids in this respect was reported on iron tissue availability, where EGCG produced consistent increase in the three targeted organs while rutin showed the lowest effect in this respect compared to others. Concerning the effects on the tissue availability of Zn and Cu, the influence is relatively comparable for all flavonoids in all organs (especially for Cu) and EGCG demonstrates the lowest effect on Zn availability in the three organs. In Figure 4, the influence of the flavonoids on percent Zn availability in the brain tissue relative to serum levels indicated comparable effects,

![Figure 1. Effects of long-term administration of silibinin (100 mg/kg), EGCG (25 mg/kg), quercetin (50 mg/kg) and rutin (500 mg/kg) on serum levels of Zn in rats after single oral dose of this element.](image1)

![Figure 2. Effects of long-term administration of silibinin (100 mg/kg), EGCG (25 mg/kg), quercetin (50 mg/kg) and rutin (500 mg/kg) on serum levels of Cu in rats after single oral dose of this element.](image2)

![Figure 3. Effects of long-term administration of silibinin (100 mg/kg), EGCG (25 mg/kg), quercetin (50 mg/kg) and rutin (500 mg/kg) on serum levels of Fe in rats after single oral dose of this element.](image3)

![Figure 4. Effects of long-term administration of silibinin (100 mg/kg), EGCG (25 mg/kg), quercetin (50 mg/kg) and rutin (500 mg/kg) on tissue availability of Zn in Brain, Kidneys and Liver relative to serum levels in rats after single oral dose of this metal.](image4)
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Table 1. Effects of long-term administration of silibinin (100 mg/kg), EGCG (25 mg/kg), quercetin (50 mg/kg) and rutin (500 mg/kg) on tissue availability of Zn, Cu and Fe in Brain, Kidneys and Liver of rats after single oral doses of these metals.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Zn μg/g Tissue</th>
<th>Cu μg/g Tissue</th>
<th>Fe μg/g Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Brain</td>
<td>Kidney</td>
<td>Liver</td>
</tr>
<tr>
<td>Control</td>
<td>32.9 ± 2.1</td>
<td>35.6 ± 3.1</td>
<td>44.8 ± 5.7</td>
</tr>
<tr>
<td>Silibinin</td>
<td>70.8 ± 4.9*a</td>
<td>94.0 ± 12.2*a</td>
<td>63.5 ± 4.6*a</td>
</tr>
<tr>
<td>EGCG</td>
<td>58.4 ± 6.3*b</td>
<td>60.3 ± 4.3*b</td>
<td>75.6 ± 7.6*b</td>
</tr>
<tr>
<td>Quercetin</td>
<td>61.0 ± 7.5*b</td>
<td>95.7 ± 9.0*b</td>
<td>114.6 ± 10.7*b</td>
</tr>
<tr>
<td>Rutin</td>
<td>63.7 ± 6.4*b</td>
<td>81.7 ± 2.8*b</td>
<td>68.3 ± 9.1*b</td>
</tr>
</tbody>
</table>

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

4. Discussion

High consumption of flavonoids rich diet may potentiate other deleterious effects of drugs or trace elements because of their diverse pharmacological properties; moreover, it may modulate drugs activity 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. Globally, dietary intake of mixed flavonoids is estimated to be in the range of 500 - 1000 mg, but it can be as high as several grams in those persons supplementing their diets with flavonoids or flavonoid-containing herbal preparations such as ginkgo biloba or grape seed extract [14]. These high doses may lead to pharmacological concentrations in body fluids and tissues. In the present study, orally administered doses of SDH, EGCG, quercetin and rutin to rats for 30 days, significantly improved both serum levels and tissue availability of orally administered doses of the essential elements, Zn, Cu and Fe compared control animals. The explanation of such finding seems to be a little bit difficult, since conflicting reports are available.
These phenomena, combined with lower complex for-
resorption from the gut and transfer to the enterocyte.
competition between minerals of similar charge in their
negatively charged mucin layer and results in lower
binding activity of polyphenols, which is related to the presence
of ortho-dihydroxy polyphenol, i.e., molecules bearing
catechol or galloyl groups and condensed tannins; the
possibility of occurrence of chelation in physiological pH
also supports the physiological significance of this phe-
nomenon [23]. In tune with our finding, in an in vitro
study, polyphenol-rich beverages such as red wine, red
grape juice, and green tea or certain specific polyphenols
(tannic acid and quercetin) have the ability to enhance the
uptake of zinc in Caco-2 cells [24]. Luminal interactions
with ligands have drastic consequences for the bioavaila-
ibility of metals. Some metal complexes are very stable.
Depending on the lipophilicity, such a complex may be
absorbed, distributed and possibly excreted without re-
leasing its metal moiety. Thus, in spite of sufficient ab-
sorption, the metal may not be metabolically available.
Although the available information suggests that poly-
phenolic compounds can chelate many essential elements
(Zn, Cu and Fe) and may affect their availability for
absorption [25], Coudray et al. (2000) reported that short-
or long-term consumption of polyphenols present in wine
did not have a negative effect on intestinal absorption or
tissue levels of zinc and Cu in rats [26]; the results of the
present study are found relatively comparable with this
finding, even when supraphysiological doses of poly-
phenols were introduced in pure form, and for the first
time showed some differences between certain flavo-
noids in this respect. The differences between flavonoids
reported in the present study could be related to the
differences in certain structural properties, including the
number and distribution of hydroxyl groups at specific
parts of the structural formula; this will consequently
affect the physicochemical properties of these flavonoids,
especially lipid solubility and interactions with biological
targets. Meanwhile, the interaction between dietary fla-
vonoids and trace minerals may affect metal homeostasis
in a structure-specific fashion. Since fluid properties of
biological membranes were essential for numerous cell
functions including solute transport and membrane-
associated enzymatic activities [27], it is possible that
even mild alterations produced by the lipophilic struc-
tures of flavonoids on membrane fluidity could cause abherent function and changes in membrane permea-
bility [28]. The D-ring hydroxyl groups of the flavonoid
structure occupy the first coordination sphere around
metal ion to form a diolate combination ring, while B-ring

Figure 6. Effects of long-term administration of silibinin
(100 mg/kg), EGCG (25 mg/kg), quercetin (50 mg/kg) and
rutin (500 mg/kg) on tissue availability of Fe in Brain, Kid-
neys and Liver relative to serum levels in rats after single
oral dose of this metal.

Regarding the influence of polyphenols intake on trace
elements homeostasis. Flavonoids can act as transition
metal ion chelators [15,16]. This feature plays an
important role in their antioxidant activity because the
free radical generation is mainly catalyzed by transition
metals in vivo and in vitro. However, excessive intake of
flavonoids may cause a decrease in essential trace
elements (Cu and Zn) and their related enzyme activities.
According to many previously reported data, flavonoids
as transition metal chelators, when used in excessive
amounts may cause a decrease of trace minerals, such as
iron, copper, and zinc [17]. In contrast to this idea, we
have shown in this study that with oral administration of
supraphysiological doses of SDH, EGCG, quercetin and
rutin, the availability of iron, copper, and zinc levels in
the serum and tissues after single oral doses of these
elements was increased compared to controls. In the
present study, the higher serum and tissue availability of
the essential elements can be explained according to the
fact that absorption and membrane transport of some
metals ions were enhanced when they form complexes
and chelates with organic ligands. In tune with this
finding, many data were reported on metal binding to
proteins in the cells [18,19] and the higher availability of
chelated elements may be linked to the shielding of the
minerals positive charge during chelation. This allows
the mineral to withstand the binding activity of the
negatively charged mucin layer and results in lower
competition between minerals of similar charge in their
resorption from the gut and transfer to the enterocyte.
These phenomena, combined with lower complex for-
formation in the intestinal lumen with compounds such as
phytate, may contribute to the higher absorption of
minerals from the gut. Moreover, feeding trials in mam-
malian species have shown that complexes of organic
compounds with trace minerals have higher relative bio-
availability than inorganic ones and provide alternative
pathways for absorption, thus leading to a reduction in
the excretion of minerals [20-22]. Another possible ex-
planation for this behavior is based on the metal chelat-
ing ability of polyphenols, which is related to the presence
of the metal ion to form a diolate combination ring, while B-ring

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5. Acknowledgements

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