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Effects of long-term use of polyphenols on the absorption and tissue distribution of orally administered metformin and atenolol in rats

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Abstract

Aim: To evaluate the effect of long-term use of silibinin, epigallocatechin (ECGC), quercetin and rutin on the absorption and tissue distribution of metformin and atenolol. Materials and

Methods: Thirty male rats were used, allocated into 5 groups and treated as follow: 1st group treated with olive oil and served as control; the other 4 groups were treated with either silibinin, EPGC, quercetin or rutin, administered orally as oily solutions for 30 days. At day 30, a 300mg/kg metformin and 50mg/kg atenolol were administered orally; 3.0 hrs later, the animals were sacrificed and blood samples, tissues of brain, kidney and liver were obtained for evaluation of the drugs level.

Results: The polyphenols increased both serum and tissue levels of metformin compared with controls. This effect was relatively varied according to the structural differences among flavonoids.

Conclusion: Long-term administration of silibinin, EGCG, quercetin or rutin increase oral absorption and tissue distribution of metformin, while atenolol was not affected; the effects of the studied polyphenols varied in accordance with the variations in their structural formulas.

INTRODUCTION

With the increasing interest in complementary therapy, plant-derived products are consumed by at least 10% of the general population and 30-70% of individuals with specific pathological conditions [1,2]. However, dietary supplements are not classified as drugs and do not require Food and Drug Administration (FDA) approval to be marketed. Co-administration of flavonoids with certain clinically used drugs may cause flavonoid-drug interactions by modulating the pharmacokinetics of certain drugs, which results in an increase in their toxicity or a decline in their therapeutic effect, depending on the structure of the ingested polyphenols [3]. This subject is of particular importance in assessing the safety of concentrated flavonoid food supplements or plant-derived products, particularly if their plasma concentrations stay high after ingestion [4,2]. Concerning the safety threatening aspect, the risks of adverse effects due to pharmacological interactions between herbal medicinal products and conventional therapies are highly considered [5]. These are often underestimated for two main reasons: consumers generally consider herbal medicinal products “safe” because of their natural origin, and as self-care products they are often taken without consulting a physician [6]. The efficacy of drug therapy depends on many factors related to a drug's pharmacokinetic and pharmacodynamic properties, which can be modified by differences in genetic polymorphisms, age, gender, circadian rhythms, intestinal bacteria,

pathophysiological conditions, pharmaceutical dosage form and xenobiotics [7-11]. One particular case is the co-administration of traditional drugs and herbal medicinal products (*i.e.* dietary supplements containing medicinal herbs or the herbal medicines traditionally used in phytotherapy for treating or preventing diseases), which may cause unexpected interactions [12]. Most interactions affecting absorption usually result in a reduction of the absorption of the drug, although increases in absorption can occur. Herbal drugs are more likely to inhibit absorption by forming complexes, for example with metal cations such as calcium, tannins and polyphenols in water extracts. Moreover, drug displacement from protein-bound forms, by concurrent drug administration, causes an increase in serum drug levels and therefore an increase in the therapeutic effect. It has been realized that this mechanism of interaction was grossly over-emphasized, because *in vitro* studies are not necessarily reflected by what happens *in vivo* [13]. The present study was designed to evaluate effect of long-term use of the flavonoids, silibinin, EGCG, quercetin and rutin, on the intestinal absorption and distribution of orally administered single doses of metformin and atenolol in rats.

MATERIALS AND METHODS

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; atenolol and metformin were obtained as a standardized powder from SDI, Iraq.

Animals and study design

Thirty male adult Sprague Dawly rats of 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 the Department of Pharmacology, College of Pharmacy, Al-Basra 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 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 (25mg/kg); Quercetin (50mg/kg) and Rutin (500mg/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 metformin (300mg/kg) and atenolol (50 mg/kg) by gavage needle administered 2.0 hrs after the last doses of the flavonoids and the vehicle.

Sample preparation

After 3.0 hrs of drugs administration, all animals were 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 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. Ten milligrams tissue from each organ was homogenized in ice-cooled phosphate buffer saline, and utilized for analysis of atenolol and metformin tissue levels.

Analysis of atenolol and metformin in serum and tissues

A 50 µL aliquot of serum or tissue homogenate sample was deproteinized with a 100 µL aliquot of acetonitrile. After vortex-mixing and centrifugation (16,000 g, 10 min), a 30 µL aliquot of the supernatant was injected directly onto a reversed-phase (C18) HPLC (Knauer, Germany). The mobile phase (25% acetonitrile and 75% pH 7.0, 0.03 M $(NH_4)_2$ HPO_4) was pumped at a flow-rate of 1.0 ml/min. The prepared mobile phase was filtered through a 0.45-mm Millipore filter and degassed ultrasonically before used. The UV-detector wavelength was set at 240 nm [14,15].

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

After administration of the four polyphenols (Silibinin, EGCG, Quercetin and Rutin) for 30 days, serum levels of metformin and atenolol were measured to explain drugs-polyphenols interactions. Figure 1 showed that all polyphenols produced significant increase (P<0.05) in serum levels of metformin compared with control group; the serum levels of metformin in all groups were found comparable. In other hand, serum atenolol levels revealed no significant differences compared with controls, except for silibinin (Figure 2), where significant increase was reported. No significant differences in serum atenolol levels were reported among all groups. In table 1, long-term administration of silibinin significantly increases tissue levels of metformin in brain and kidney (brain>kidney) compared to control group, while no such effect reported in liver tissue. Concerning the effect of EGCG in this respect, long-term use produced significant increase in metformin contents in the three tested organs (bran, kidney and liver) compared with control group; inter-groups analysis showed that highest level was reported in the kidney, which was significantly different with respect to that reported in brain and liver, where the latter two found comparable. In the quercetin-treated group, long-term use increases tissue levels of metformin in all the targeted organs (Table 1) compared to control group, with highest level reported in kidney tissues. Concerning the effect of rutin, long-term use significantly increases metformin levels in kidney tissue only compared with that reported in control group (Table 1). As shown in table 2, atenolol was not detected (within the limits of analysis method) in all targeted brain tissues and in all animal groups including the controls. Moreover, the detected levels of atenolol in kidney and liver tissues are not found significantly different after administration of polyphenols after long-term use, these levels are also found comparable to those reported in control group (Table 2). In the present study, the relative tissue availability in brain, kidney, and liver with respect to serum levels of metformin and atenolol were measured as an indicator of for the ability of polyphenols to modulate drug distribution in those organs, and give an idea about the ability of drugs to cross tissue specific barriers like blood brain barrier. Figure 3 demonstrates that in brain tissue, only silibinin, EGCG and quercetin increased the relative tissue availability of metformin in this organ; while rutin fails to show similar effect. The maximum organ delivery was attributed to the effect of quercetin compared with others. Meanwhile, in kidney tissue, all the four polyphenols significantly increased relative tissue availability of metformin in the kidney compared with control group (Figure 3), and their effects in this respect are found comparable and not significantly different. In the liver, only quercetin produced significant increase in the relative tissue availability of metformin compared with control; however, both silibinin and rutin significantly decreased such tissue availability compared with control. Meanwhile, EGCG do not show significant changes in this respect. In figure 4, long-term administration of the four polyphenols did not produce significant changes in relative tissue availability of atenolol in all targeted organs (brain, kidney and liver); as mentioned previously, atenolol was not detected in brain tissues in all circumstances.

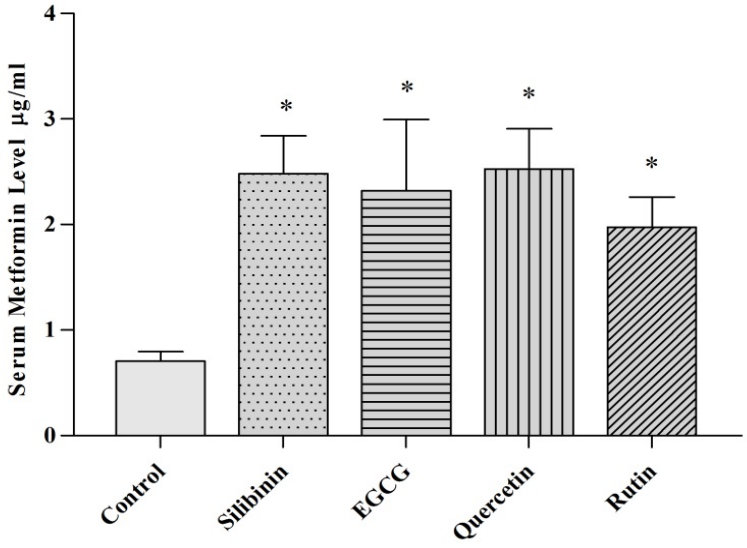

Figure 1. Effects of long-term use of Silibinin, EGCG, Quercetin and Rutin on serum levels of orally administered single dose of Metformin; values are presented as mean±S.D; * significantly different compared to control (P<0.05); no significant differences among treated groups (P>0.05).

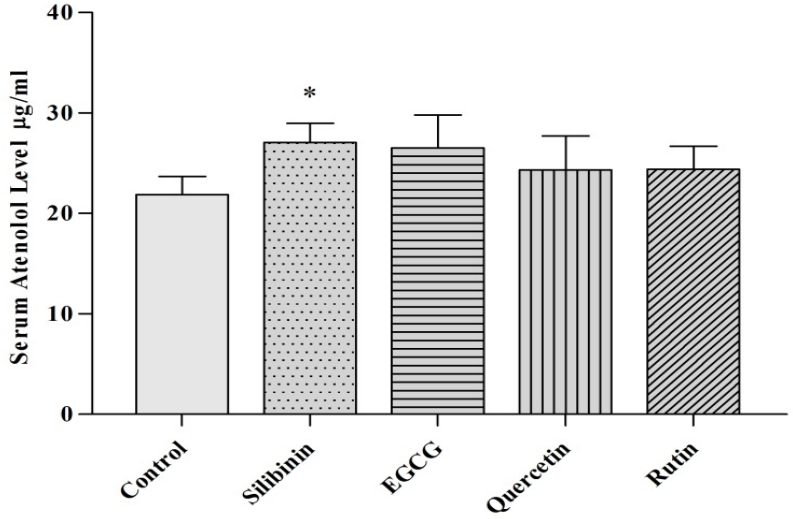

Figure 2. Effects of long-term use of Silibinin, EGCG, Quercetin and Rutin on serum levels of orally administered single dose of Atenolol; values are presented as mean±S.D; * significantly different compared to control (P<0.05); no significant differences among treated groups (P>0.05).

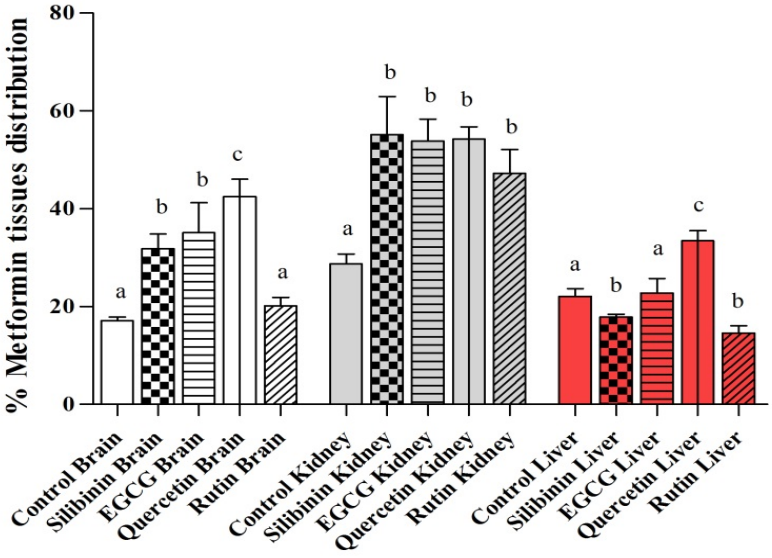

Figure 3. Effects of long-term use of silibinin, EGCG, quercetin and rutin on % metformin distribution in brain, kidney and liver after single oral dose; values are presented as mean±S.D; values with non-identical letters (a,b,c) represent significant differences among groups within the same organ.

Table 1. Effects of long-term administration of silibinin, EGCG, quercetin or rutin on the distribution of orally administered single dose (300) metformin in brain, kidney and liver tissues of rats.

Type of flavonoid	Metformin level µg/g tissue			*P* value
	Brain	Kidney	Liver	
Control	0.16±0.02	0.2±0.03	0.17±0.02	n.s
Silibinin	0.78±0.08*[a]	1.37±0.23*[a]	0.45±0.07[a]	0.01
EGCG	0.83±0.28*[a]	1.24±0.31*[a]	0.52±0.11*[a]	0.01
Quercetin	1.08±0.23*[a]	1.38±0.24*[a]	0.84±0.11*[b]	0.01 Kidney *vs*. Liver
Rutin	0.4±0.09[b]	0.93±0.18*[b]	0.3±0.05[a]	n.s Brain *vs.* Liver

Values were expressed as mean±SD; number of animals=6 in each group; * significantly different compared to the control within the same tissue (P<0.05); values with non-identical superscripts (a,b) within the same tissue were considered significantly different (P<0.05); P=0.01: significant differences between tissues within the same group.

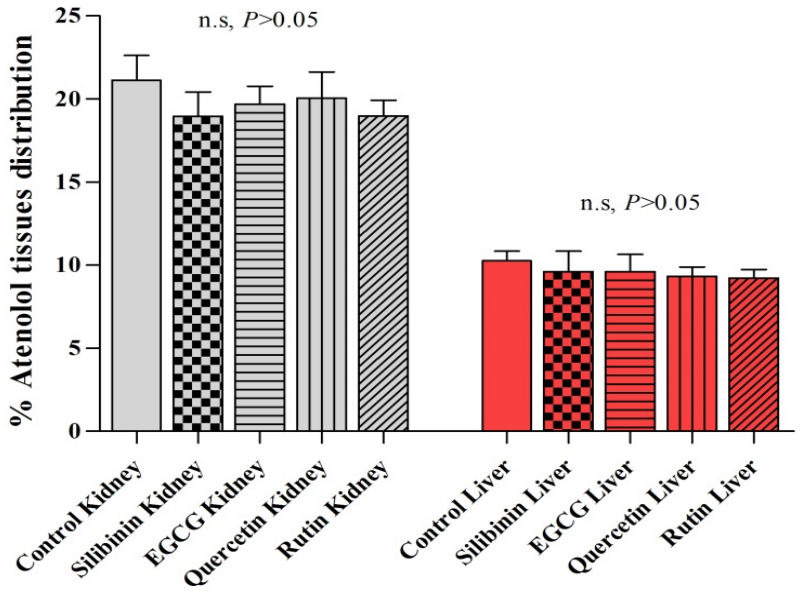

Figure 4. Effects of long-term use of silibinin, EGCG, quercetin and rutin on % atenolol distribution in kidney and liver after single oral dose; values are presented as mean±S.D; n.s=non-significantly different among groups within the same organ.

Table 2. Effects of long-term administration of silibinin, EGCG, quercetin or rutin on the distribution of orally administered single dose (50mg/kg) atenolol in brain, kidney and liver tissues of rats.

Type of flavonoid	Atenolol level µg/g tissue			*P* value
	Brain	Kidney	Liver	
Control	N. D	4.8±0.25	2.49±0.24	0.01
Silibinin	N. D	5.12±0.39	2.61±0.4	0.01
EGCG	N. D	5.2±0.7	2.56±0.5	0.01
Quercetin	N. D	4.86±0.64	2.28±0.39	0.01
Rutin	N. D	4.64±0.62	2.26±0.26	0.01

Values were expressed as mean±SD; number of animals=6 in each group; *P*=0.01: significant differences between tissues within the same group; N.D: not detected; no significant differences compared to control within the same tissue for each flavonoid (*P*>0.05).

DISCUSSION

Researches on polyphenols-drug interactions have illustrated the ability of some polyphenols to affect the bioavailability and activity of drugs, usually resulting in adverse effects. However, it seems that these interactions can be utilized to design specific synergies between polyphenols to potently increase their bioactivity, and hence, their beneficial health-promoting effects [16]. Information on the bioavailability and organ distribution of drugs after long term administration of polyphenols is important for understanding whether flavonoids inhibit or enhance absorption and organ distribution of several compounds. To our knowledge, this is the first project that studies the effect of silibinin, EGCG, quercetin or rutin (long-term use) on absorption and distribution of metformin and atenolol in rats. Interactions of nutritional components in food with these drug transporters and metabolizing enzymes give insight into several important issues. Thus, polyphenols may dramatically affect the blood concentrations of clinically used drugs, resulting in overdose or loss of therapeutic effect. Some polyphenols are high affinity inhibitors, whereas others may be substrates themselves [17]. In the present study, orally administered polyphenols significantly increased both serum levels and tissue availability of metformin; specific efflux transporters, which thought to be inhibited by

polyphenols, controlled metformin absorption and membrane transport. This inhibition largely depends on the polyphenolic structure and hydrophobicity, especially for their interaction with the hydrophobic regions of such transporters [18]. These transporters are widely expressed in the blood-brain barrier, intestine, kidney and liver, and have remarkable influence on the absorption and distribution of many drugs [19,20]. Accordingly, polyphenols can be considered as good candidate molecules to modulate the effects of these regulatory proteins, resulting in either enhancement or inhibition of their activities. This condition was highly expected in the finding of the present study, especially metformin. Metformin is a well-known substrate for influx transporters, including plasma monoamine transporter in the intestine, as well as efflux transporters including MDR and P-gp; since metformin is also a substrate for the efflux pump P-gp [21], the oral bioavailability of metformin might be affected by transporter inhibitors like polyphenols. Polyphenols are abundantly found in our daily foods; in addition to their antioxidant properties, they are capable to inhibit drug efflux by MDR [22]. The present study provides *in vivo* evidence that all studied polyphenols might increase absorption of metformin through the inhibition of P-gp-mediated efflux during the absorption phase in the intestine; this effect varies with variations in structural features of the flavonoids. In the present study, the increase in serum and tissue levels of metformin after long-term oral administration of polyphenols came in tune with many previously reported data. Shin and Choi (2009) reported that oral administration of quercetin, morin and EGCG significantly increased the C-max and the AUC of tamoxifen in rats [23]; while Rajnarayana *et al* (2004) demonstrated that pretreatment with silymarin led to significant increase in the disposition of metronidazole and its active metabolite, hydroxyl-metronidazole [24]. Moreover, Tamaki *et al* (2010) demonstrated that some herbal and dietary supplements and isoflavonoids increase the systemic availability of many BCRP substrates when concomitantly given orally [25]. The clinical outcome of such types of interactions may be of serious consequences during treatment with drugs that have low margin of safety and narrow therapeutic index. In this respect, quercetin increases oral bioavailability of digoxin in pigs and resulted in serious toxicity [22]. After long-term administration, polyphenols significantly increase tissue availability of metformin compared with control; the order of increase can be ranked as follow: kidney>brain>liver; this may be attributed to the highest concentration of polyphenols achieved in intestine and kidney compared to liver, which makes modulation of transporters in those organs more prominent. This result was in agreement with previous observation by Chen *et al* (1997), who reported that highest EGCG, epigallocatechin and epicatechin AUC levels were detected in the intestine and kidney and excreted through both the urine and bile [26]. The reported differences between the effects of the studied polyphenols can be related to the variation 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. Kitagawa (2006) reported that EGCG, as inhibitor of P-gp, was more effective than verapamil and quercetin; so, there is a possibility many polyphenols have such inhibitory activities on P-gp function, and the large hydrophobic region in addition to the phenolic hydroxyl groups seems to be commonly necessary for this activity [18]. The present study demonstrates also that metformin can pass the BBB, and the polyphenols can affect the transport function of this barrier, with consequent increase in relative availability of metformin in brain tissue; this finding was in tune with previous data in this respect [27]. In the present study, we also evaluate the effects of long-term administration of polyphenols on GIT absorption and tissue availability of atenolol, not P-gp substrate, to enable precise estimation of polyphenols effects on membrane transporters. Orally administered doses of the four polyphenols (multiple doses) had no effects on either serum levels or tissue availability of atenolol compared to control group. This finding can be explained according to that atenolol is not a well-known substrate for P-gp; so, its absorption and distribution are not influenced by the polyphenols as in case of metformin. This finding was in tune with the report of Yang et al (2000), who indicate that atenolol, metoprolol and alprenolol are not substrates for P-gp (in contrast to propranolol) and not affected by modulating its activity *in vitro* [28]. Such finding was also supported by the idea that atenolol was absorbed and distributed mainly through paracellular absorption pathway [29,30]. Atenolol follows the passive diffusion pathway with minimal first pass metabolism effect, implying no effect of P-gp on its transport or movement in apical basolateral direction [31]. In tune with this finding, the present study showed that there is no effect for the evaluated polyphenols, as p-gp modulators, on absorption or tissue distribution of atenolol. In the present study, atenolol was not detected (within the method limitations) in brain tissues of all animal groups including the controls. This result was consistent with many other studies; Wang *et al* (2005) considered atenolol as CNS negative compound, and a P-gp substrate [32], while others considered passage of atenolol across BBB into the brain as very limited [33].

CONCLUSION

Long-term administration of silibinin, EGCG, quercetin or rutin increase oral absorption and tissue distribution of metformin, while atenolol was not affected; the effects of the studied polyphenols varied in accordance with the variations in their structural formulas.

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