American Journal of Pharmacological Sciences, 2015, Vol. 3, No. 1, 7-12
Available online at http://pubs.sciepub.com/ajps/3/1/2

DOI:10.12691/ajps-3-1-2

Effects of *Ginkgo biloba* Extract on the Oral Bioavailability of Fluoxetine and Venlafaxine in Rats

Fatima Adnan Alzubaidi[1], Saad Abdulrahman Hussain[2,*]

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

Received January 01, 2015; Revised January 15, 2015; Accepted January 18, 2015

Abstract Since herbal supplements are not evaluated for interactions with other drugs, there are concerns about their interactions with conventional drugs, which are mostly used as multiple drug treatment approach. The present study aims to evaluate the effects of long-term use of Ginkgo biloba (GK) extract on the intestinal absorption of orally administered, single doses of fluoxetine and venlafaxine in rats. AUC calculations and cumulative diffused calculations are utilized for evaluation of physicochemical interactions in vitro. Rats administered 100 and 200 mg/kg of GK extract, and oral bioavailability of single doses of fluoxetine and venlafaxine were evaluated using HPLC method. The results showed that GK extract has no significant effects on the bioavailability of fluoxetine and venlafaxine. Fluoxetine and venlafaxine serum levels are not affected by 100 mg/kg GK extract, while 200 mg/kg significantly increases venlafaxine level, with no effect on that of fluoxetine. In conclusion, GK extract shows no physicochemical interactions with fluoxetine or venlafaxine. Long-term administration of a low dose of GK extract (100 mg/kg) had no significant effect on serum levels of fluoxetine and venlafaxine, while lager dose (200 g/kg) increases significantly only the serum level of venlafaxine, while that of fluoxetine was not affected.

Keywords: *Ginkgo biloba, fluoxetine, venlafaxine, oral availability, drug interaction*

Cite This Article: Fatima Adnan Alzubaidi, and Saad Abdulrahman Hussain, "Effects of Ginkgo biloba Extract on the Oral Bioavailability of Fluoxetine and Venlafaxine in Rats." *American Journal of Pharmacological Sciences*, vol. 3, no. 1 (2015): 7-12. doi: 10.12691/ajps-3-1-2.

1. Introduction

The use of supplements from natural sources is increasingly adopted as anapproach to promote health and well-being, even in developed countries that mostly depend on synthetic drugs in this regard [1]. Since natural products are not evaluated for the possibility of interactions with other drugs, there are many concerns about the expected interactions of these supplements with conventional drugs, which are mostly used as multiple drug treatment approach. However, many animal and clinical studies provide reliable data regarding the interactions of natural products with conventional drugs, which may predispose to serious adverse effects [2,3]. *Ginkgo biloba*(GK) extract is a complex mixture of different compounds. It has been used, for thousands of years, in traditional Chinese medicine for the treatment of many health disorders [4]. GK contains three groups of compounds: ginkgo flavonol glycosides (quercetin, kaempferol, and isorhamnetin), terpenes with lactone rings (ginkgolides A, B, C, J and M and bilobalides) and specific organic acids (ginkgoin and ginkgolin acids) [5]. The extract of GK contains the largest percentage of ginkgo flavonol glycosides (22-27%) from the flavonoid group [6]. These compounds exhibit therapeutic properties such as antioxidant, anti-allergic, anti-inflammatory, antiviral, anti-proliferative, anti-tumorigenic, anti-anxiety, and anti-carcinogenic actions [7]. As with conventional drugs, herbal medicines interact with drugs pharmacokinetics. Pharmacokinetic interactions result in alterations in the absorption, distribution, metabolism or elimination of many drugs or natural products [8]. These interactions affect drug action by quantitative alterations, either increasing or decreasing the amount of drug availability, which may have impact on the pharmacological activity. Pharmacokinetics interactions often occur because of changing the activity of drug metabolizing enzymes and/or transporting proteins, especially cytochrome P450 enzymes and P-glycoprotein (P-gp) [9]. GK extract inhibits P-glycoprotein in humans. Adjunct use of this extract with drugs, such as talinol, which are primarily transported by P-glycoprotein, may require a necessary dose adjustment [10,11]. In other study, GK decreases the oral availability of cyclosporine in rats [12], indicating induction of P-gp in the small intestine after long-term exposure. This finding apparently contradicts other studies, which show that GK extract was the strongest inhibiting herb for P glycoprotein [13]. However, GK may inhibit metabolizing enzymes and drug transporters; while induction of these systems was the outcome after long-term, repeated exposure [14]. The present study aims to evaluate the effects of long-term use (30 days) of GK extract on the intestinal absorption of

orally administered, single doses of fluoxetine and venlafaxine in rats, and verapamil as standard comparator.

2. Materials and Methods

2.1. In vitro Study

Utilizing a modified *in vitro* model of drug diffusion through an artificial membrane [15], this part of the study is performed to determine if there is a direct physicochemical interaction between GK extract constituents with fluoxetine or venlafaxine at the site of administration. Two buffer solutions are used, the first is phosphate buffer solution (pH 6.8), in which the drugs are dissolved (reservoir); it represents the intestinal media, i.e., the site of drug absorption. The second is phosphate buffer saline (pH 7.4); it represents the blood compartment to which drugs are absorbed (recipient). A dialysis membrane (pore size 12000 Dalton, Sigma Company) separates the two buffers. The *Ginkgo biloba* extract, fluoxetine and venlafaxine are dissolved in phosphate buffer solution (pH 6.8) to prepare a concentration of 5 µg/ml of fluoxetine and venlafaxine, and 25 µg/ml of GK extract. The drugs are mixed, using a magnetic stirrer, and the temperature of the solution is adjusted to 37°C. Different samples aspirated from the receiver compartment (pH 7.4), every 30 minute (for four hours), centrifuged, and filtered through a 0.45-mm Millipore filter. Then a 30 µl of filtrate is injected directly into a reversed-phase C18 high performance liquid chromatography (HPLC) column, for determination of fluoxetine and venlafaxine concentrations that diffused through the dialysis membrane. This procedure is repeated three times, where the drugs (fluoxetine and venlafaxine) are dissolved in the phosphate buffer solution (pH 6.8) without GK, and their concentrations are measured and served as control.

2.2. Analysis of Drugs Concentrations

High performance liquid chromatography (HPLC) method is utilized, and validated to determine the concentrations of fluoxetine and venlafaxine, using Knauer HPLC system, and C18 column 250*4.6 mm is used. The mobile phase includes Acetonitrile: potassium dihydrogen phosphate buffer: Triethylamine (40:60:0.2 v/v/v), and the pH is adjusted to 3.1 with o-phosphoric acid [16]. The samples are pumped at flow rate of 1.0 ml/min. The mobile phase is passed through nylon 0.45 µm membrane filters. The detector wavelength is set at 233 nm.

2.3. In vivo Study

In this part of the study, we evaluate the effects of long-term administration of low and high doses of GK extract, on the intestinal absorption of orally administered, single doses of fluoxetine and venlafaxine. The obtained results are compared with the effects produced by verapamil, as a standard modulator of membrane transporters, in adult male rats.

2.4. Laboratory Animals

Forty-six adult male of Wistar albino rats (150-200 gm) are used in this part of the study. The animals obtained from the animal house of the College of Pharmacy, University of Baghdad and the animal house of the College of Veterinary Medicine, University of Kufa. The rats housed in the animal house of the College of Medicine, University of Babylon, and kept in a polypropylene cages at controlled temperature (25±2°C), 12hr light/dark cycle, and allowed to acclimatize for at least 1 week prior to the experiment. The animals had free access to commercial pellet diet and water *ad libitum*. The rats are randomly allocated into eight groups. First group: six rats are given carboxymethylcellulose 5% (the vehicle), orally by gastric tube for 30 days; then a single dose of fluoxetine (20 mg/kg) orally, and served as control [16]. The second group: six rats are given carboxymethylcellulose 5% (the vehicle), orally by gastric tube for 30 days; then a single dose of venlafaxine (20 mg/kg) orally, and served as control [18]. In third and fourth groups, twelve rats administered GK extract (100 mg/kg/day), suspended in carboxymethylcellulose 5%, orally by gastric tube for 30 days; then given a single dose of either fluoxetine or venlafaxine (20 mg/kg) orally [19]. In fifth and sixth groups, twelve rats administered GK extract (200 mg/kg/day), suspended in carboxymethylcellulose 5% orally, by gastric tube for 30 days; then given a single dose of either fluoxetine or venlafaxine (20 mg/kg) orally. In seventh and eighth groups (5 rats each), the rats are given verapamil (40 mg/kg/day) orally, by gastric tube for 10 days; then a single dose of either fluoxetine or venlafaxine (20 mg/kg) orally [20]. All animals are sacrificed after 5.5 hours after administration of the fluoxetine dose [21], and 2.0 hours after the administration of venlafaxine dose [22]. They underwent laparotomy under light anesthesia by inhalation of diethyl ether, and blood is aspirated directly from the heart into polyethylene tube. The blood samples are left to clot, and then centrifuged at 10000 rpm for 20 minutes. The resulted serum is kept frozen (- 40°C) until the time of drugs analysis.

2.5. Measurement of Fluoxetine and Venlafaxine Levels

A 50 µl aliquot of serum sample is deproteinized with a 100 µl aliquot of acetonitrile. After vortex mixing and centrifugation (16,000 rpm, 10 min), a 30 µl aliquot of the supernatant is injected directly into a reversed-phase (C18) HPLC column [23].

3. Statistical Analysis

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

4. Results

4.1. In vitro Study Results

Based on the AUC calculations, and cumulative diffused calculations, Figure 1, Figure 2, Figure 3 and Figure 4 showed that GK extract has no significant effects on the diffused amounts of fluoxetine and venlafaxine through the artificial cellophane membrane, when compared with control.

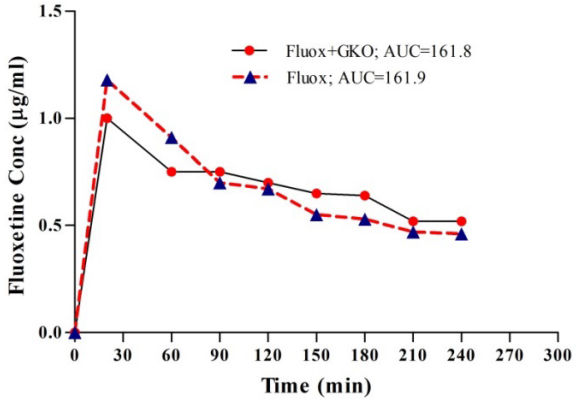

Figure 1. Effects of *Ginkgo biloba* extract on the diffusion of fluoxetine through artificial cellophane membrane. Evaluation was based on the AUC calculations of the diffused fractions

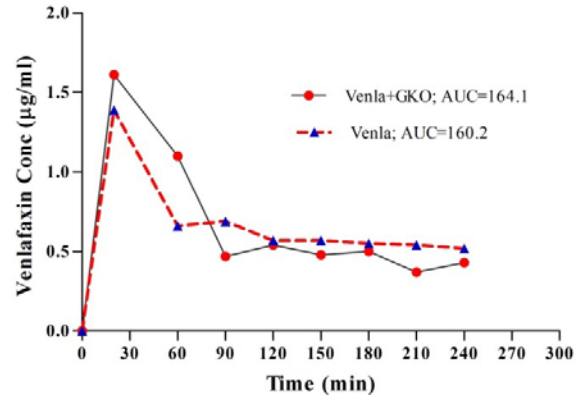

Figure 2. Effects of *Ginkgo biloba* (GK) extract on the diffusion of Venlafaxine through artificial cellophane membrane. Evaluation was based on AUC calculations of the diffused fractions

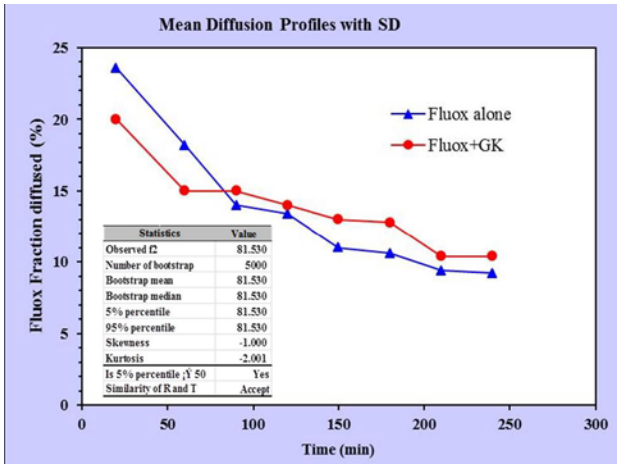

Figure 3. Effect of GK extract on the fractions diffused of through artificial cellophane membrane, using in-vitro Dissolution Profile Comparison method

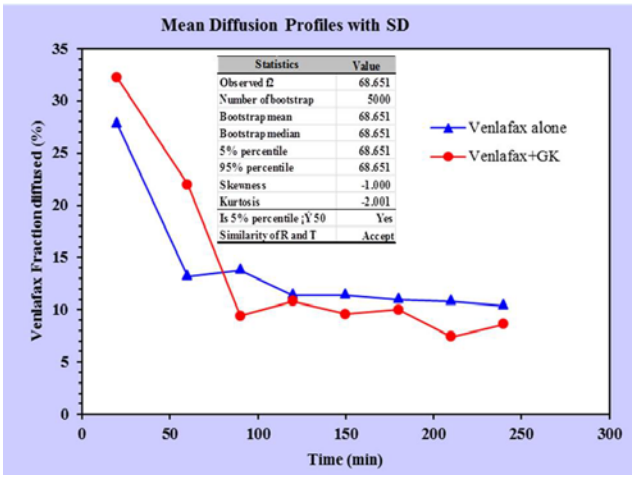

Figure 4. Effect of GK extract on the fractions diffused of Venlafaxine through artificial cellophane membrane, using in-vitro Dissolution Profile Comparison method.

4.2. In vivo Study Results

Figure 5 shows that oral administration of verapamil (40 mg/kg) to the rats for 10 days significantly decreases (P=0.001) the serum concentration of fluoxetine, after single oral dose (20 mg/kg), compared to control group (vehicle-treated animals). Figure 5 also shows that treatment of rats with verapamil (40 mg/kg for 10 days) significantly decreases (P=0.001) serum fluoxetine, administered as single oral dose (20 mg/kg), compared with GK extract treated animals (100 and 200 mg/kg/day, for 30 days). As shown in Figure 5, long-term administration of GK extract (100 and 200 mg/kg) does not significantly affect serum fluoxetine concentration (P=0.58 and 0.118, respectively) after single oral dose (20 mg/kg), compared to control group (vehicle-treated animals). Meanwhile, the later approaches show comparable effect, when compared with each other using ANOVA (P>0.05). Although increasing the dose of GK extract decreases serum fluoxetine concentration, but it does not reach significant level compared with the lower dose. Figure 6 shows that oral administration of verapamil (40 mg/kg) to the rats for 10 days does not significantly affect serum concentration of venlafaxine (P= 0.24), after single oral dose (20 mg/kg), compared to control group (vehicle-treated animals). As shown in Figure 6, long-term administration of GK extract (100 mg/kg) does not significantly affect serum venlafaxine concentration (P= 0.06) after single oral dose (20 mg/kg), compared with control group (vehicle-treated animals).Meanwhile, increasing the dose GK extract (200 mg/kg) significantly increases serum venlafaxine concentration (P=0.001) after single oral dose (20 mg/kg), compared to control group (vehicle-treated animals). One-way ANOVA and Bonferroni's *post hoc* analysis are used to compare the effects of the vehicle, verapamil (40 mg/kg, for 10 days), and GK extract (100 and 200 mg/kg, for 30 days) on the serum concentrations of venlafaxine, after single oral dose (20 mg/kg). Figure 6 demonstrates that GK extract (200 mg/kg/day) significantly (P<0.05) increases serum concentration of venlafaxine, compared with verapamil and GK extract (100 mg/kg/day). There is no significant difference between the effect of verapamil and GK extract (100 mg/kg/day) on serum concentration of venlafaxine.

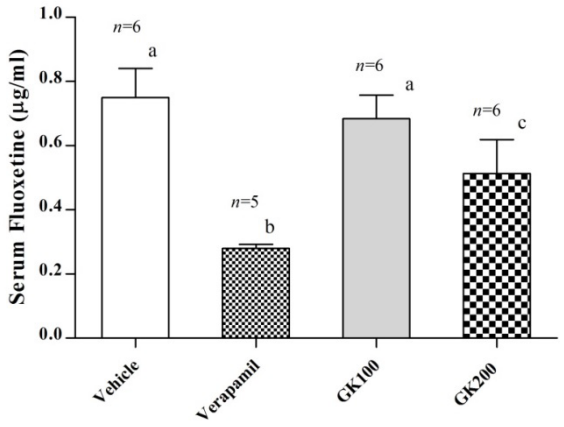

Figure 5. Effect long-term administration of GK extract (100 and 200 mg/kg) and verapamil (40 mg/kg) on the fluoxetine serum levels of rats administered single oral dose of fluoxetine (20 mg/kg); n: number of rats; values with different letters (a,b,c) are significantly different (P<0.05) using ANOVA and *post hoc* test

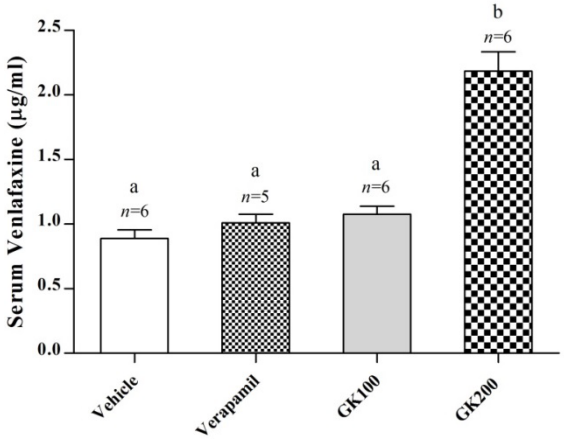

Figure 6. Effect long-term administration of GK extract (100 and 200 mg/kg) and verapamil (40 mg/kg) on the venlafaxine serum levels of rats administered single oral dose of venlafaxine (20 mg/kg); *n*: number of rats; values with different letters (a,b) are significantly different (P<0.05) using ANOVA and *post hoc* test

5. Discussion

As the use of herbal drugs increases, the public health consequences of drug-herb interactions are becoming more significant. Herb-drug interactions, resulting from concurrent use of herbal drugs may cause adverse reactions such as toxicity and treatment failure [24]. Additionally, there is a well-defined epidemiological evidence for the health benefits of many herbal products [25], and there have been proposals to make use of them as purified extracts or as complexes of their constituents with metals or other compounds, in the treatment of many diseases and health disorders [26,27]. Accordingly, it is important to understand, as fully as possible, the reaction between constituents of herbal extracts and the other prescribed drugs or trace elements. Especially if the chemical features, the extracts mirrors that of the active constituents, enable herb-drugs or herb-metals interactions that occur at the site of administration and at physiological pH values [28]. Although the mechanistic studies underlying herb-drug interactions do not report physicochemical interactions between the herb constituents and many administered therapeutic agents, the mostly studied mechanisms involve the inhibition or induction of metabolizing enzymes, and membrane drug transporters [3,29]. Interactions of several commonly used herbal drugs, such as GK and St. John's wort, with many drugs, many of them have very narrow therapeutic indices, may predispose to fatal consequences. A good understanding of the mechanisms of herb-drug interactions is also essential for assessing and minimizing clinical risks [30]. Regarding the possibility of physicochemical interactions at the site of administration, the absorption of co administered drugs can be modulated when natural products, which contain colloidal fibers, gums, and mucilage, are taken together. Such products include aloe gel, flaxseed, marshmallow, psyllium, and rhubarb [31]. The constituents of natural compounds can form complex with drugs and prevent absorption and, subsequently, decrease plasma and tissue availability. In this regard, psyllium inhibits the absorption of lithium, and there are many case reports of decreased plasma lithium concentrations, when administered concomitantly with psyllium [32]. In the present study, *in vitro* diffusion method shows that GK extract has no significant effect on the amount of fluoxetine and venlafaxine that diffused through the artificial cellophane membrane, when compared with control. This means that there is no physicochemical interaction between the constituents of GK extract with fluoxetine and venlafaxine, when administered concomitantly. P-glycoprotein (P-gp) is an ATP-binding cassette transmembrane protein, responsible for the active efflux of a broad spectrum of structurally unrelated compounds [33]. This transport system limits xenobiotic tissue penetration, and prevents substances from accumulating at cytotoxic levels. In particular, the expression of P-gp in organs that have an excretion (liver and kidney), absorption (intestine) or barrier function, such as the blood-brain barrier, impacts the oral bioavailability and tissue distribution of a drugs [34]. Importantly, alterations in the expression and/or activity of drug transporters can dramatically influence the pharmacokinetic profile of drugs that interact with these proteins, often resulting in clinical drug-drug interactions. In the present study, the orally administered dose (40 mg/kg) of verapamil to the rats for 10 days decreases significantly the serum level of orally administered single dose fluoxetine. This result can be explained according to the fact that fluoxetine is a P-gp substrate in rodents, and this efflux transporter [35] controls its membrane transport processes. This efflux transporter is expressed on the apical membrane of the intestinal epithelium, and may limit intestinal absorption and determines oral bioavailability. It is also expressed constitutively on the blood-brain barrier, liver and kidney, affecting drug distributionat these sites [36]. Verapamil is a P-gp modulator [37,38], so administration of verapamil may modulate intestinal absorption and uptake of fluoxetine by other tissues. The significant decrease in serum level of fluoxetine may also be attributed to its large uptake of by other tissues, i.e. high transport of fluoxetine from the serum to these tissues. In general, when investigating P-gp induction or inhibition by a test compound in wild-type animals, its influence on the pharmacokinetic profile of the investigated drug should be carefully investigated. In this regard, qualitative and quantitative evidences frequently supported these phenomena, based on

immunofluorescence tests, polymerase chain reaction, western blot analysis and/or *in vitro* bidirectional transport investigations [39]. According to a case study, verapamil (240 mg) improves the antidepressant activity of lofepramine (140 mg twice daily), in in 46-year-old man, who did not show any improvement in symptoms with Lofepramine alone [40]; although the mechanism of action is not well defined, verapamil itself does not have antidepressant properties. The possible explanation for the apparent efficacy of verapamil in treatment resistant depression may be the blockade of the efflux transporter (P-gp) [41]. Based on the present study result, it seems logic to suggest that using synthetic or natural compounds, which can modulate drug transporters in the intestine, will increase the possibility of lowering the dose of adjunctly administered drugs to limit their peripheral side effect profile. While at the same time, maintaining effective tissue drug levels would be of great interest to both drug development and clinical practice. In the present study, orally administered GK extract (in both doses) does not change serum fluoxetine level significantly. Other researchers study the effect of acute and chronic use of GK extract on the activity of drug transporters, without specific emphasis on the dose-response relationship in this respect; i.e., they utilize specific single dose effect. Yang *et al*. (2006) reported that GK decreases oral availability of cyclosporine in rats, indicating an induction of P-gp in the small intestine after long-term exposure [42]. This finding apparently contradicts the results obtained in our study. However, the effect of GK extract might be the same as that reported for St. John's wort. It reflects inhibition at short-term (acute exposure) *in vitro* and *in vivo*, and induction *in vivo*, only after a long-term administration (repeated exposure) [14]. The orally administered GK extract (100 mg/kg) has no significant effect on serum venlafaxine level, while 200 mg/kg dose significantly increases venlafaxine levels in the serum. This elevation may be attributed to the potent inhibitory effect of GK extract on the activity of P-gp in the intestine. On the other hand, venlafaxine is a substrate to cytochrome CYP3A4, and high dose of GK extract inhibits its activity [43]; this effect, together with the inhibition of P-gp, may lead to high serum levels of venlafaxine. Although the present study demonstrates significant effect of GK extract on the absorption of fluoxetine and venlafaxine in rats, such activity may not be necessarily observed in human. This idea comes in accordance with many reports in this respect [24,44], and human studies in this regards are highly suggested. In the intestine, most of the interactions that involve drug transporters responsible for efflux of drugs often result in poor absorption and low oral bioavailability, as the administered drugs are readily effluxed back into the intestinal lumen, and excreted out of the body. Since majority of drugs are developed as oral dosage forms, the use of animal models to evaluate the interactions at that site have become critical tools for assessing a drug's potential *in vivo* absorption properties in the presence of expected modulators of membrane transporters. In conclusion, GK extract does not show physicochemical interactions with concomitantly administered fluoxetine or venlafaxine. Long-term administration of a low dose of GK extract (100 mg/kg) has no significant effect on serum levels of fluoxetine and venlafaxine, while lager dose (200 g/kg) increases significantly only the serum level of venlafaxine, and that of fluoxetine is not significantly affected.

Acknowledgement

The present data was abstracted from a PhD thesis, submitted by Fatima A. Alzubaidi to the Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad. The authors thank University of Baghdad for supporting the project, and Moafaq A. Ghareeb and Abather A. Sadiq for technical assistance.

References

[1] Bardia A, Nisly NL, Zimmerman MB, Gryzlak BM, Wallace RB. Use of herbs among adults based on evidence-based indications: findings from the National Health Interview Survey. *Mayo Clin. Proc*., 82: 561-566. 2007.

[2] Gardiner P, Phillips R, and Shaughnessy AF. Herbal and dietary supplement-drug interactions in patients with chronic illnesses. *Am. Fam. Physician*, 77: 73-78. 2008.

[3] Mohamed MF, Frye RF.Inhibition of intestinal and hepatic glucuronidation of mycophenolic acid by *Ginkgo biloba* extract and flavonoids. *Drug Metab. Dispos*., 38 (2): 270-275. 2012.

[4] Zhao XD, Dong N, Man HT, Fu ZL, et al. Antiproliferative effect of the *Ginkgo biloba* extract is associated with the enhancement of cytochrome P450 1B1 expression in estrogen receptor‐negative breast cancer cells. *Biomed. Rep*., 1 (5): 797-801. 2013.

[5] American Herbal Pharmacopoeia. Gingko Leaf & Ginkgo Leaf Dry Extract Monograph. Santa Cruz: American Herbal Pharmacopoeia, 2003.

[6] Pilija V, Mirjana R, Brenesel MD, Popovic M, et al. Inhibitory effect of *Ginkgo Biloba* extract on the tonus of the small intestine and the colon of rabbits. *Molecules*, 15: 2079-2086. 2010.

[7] He L, Guoying Z, Xu J, Liu, J, et al. Research progress on polysaccharides from *Ginkgo biloba.J. Med. Plants Res*., 6: 171-176. 2012.

[8] Robertson SM, Davey RT, Voell J, Formentini E, et al. Effect of *Ginkgo biloba* extract on lopinavir, midazolam and fexofenadine pharmacokinetics in healthy subjects. *Curr. Med. Res. Opin*., 24 (2): 591-599. 2008.

[9] Abad MJ, Bedoya LM, Bermejo P.An update on drug interactions with the herbal medicine *Ginkgo biloba*. *Curr. Drug Metab*., 11: 171-181. 2010.

[10] Fan L, Tao GY, Wang G, Chen Y, et al. Effects of *Ginkgo biloba* extract ingestion on the pharmacokinetics of talinolol in healthy Chinese volunteers. *Ann. Pharmacother*., 43: 944-949. 2009.

[11] Fan L, Mao XQ, Tao GY, Wang G, et al. Effect of *Schisandrachinensis* extract and *Ginkgo biloba* extract on the pharmacokinetics of talinolol in healthy volunteers. *Xenobiotica*, 39: 249-254. 2009.

[12] Hellum BH, Nilsen OG. In vitro inhibition of CYP3A4 metabolism and P-glycoprotein-mediated transport by trade herbal products. *Basic Clin. Pharmacol. Toxicol*., 102:466-475. 2008.

[13] Nuhu AA. *Ginkgo biloba*: A 'living fossil' with modern day phytomedicinal applications. *J. Appl. Pharm. Sci*., 4 (3): 96-103. 2014.

[14] Pal D, Mitra AK. MDR- and CYP3A4-mediated drug-herbal interactions. *Life Sci*., 78: 2131-2145. 2006.

[15] Rao V, Raj MK, Ravinder S, Sowmya K, Kumar KP, Sudhakar M.Effect of Permeation enhancers on diffusion of lamotrigine drug through cellophane membrane. *Am. J. Adv. Drug Deliv*., 1 (4): 606-610. 2013.

[16] Eswarudu MM, Anitha M, Gayathri N, chaithanya T. A validated RP-HPLC method for the simultaneous estimation of fluoxetine hydrochloride and olanzapine in pharmaceutical dosage form.*Int. Res. J. Pharmacy*., 3 (4): 310-313. 2012.

[17] Caccia S, Cappi MC, Garattini S. Influence of dose and route of administration on the kinetics of fluoxetine and its metabolite norfluoxetine in the rat. *Psychopharmacology*, 100 (4): 509-514. 1990.

[18] Eren I, Naziroglu M, Demirdas A, Celik O, et al. Venlafaxine modulates depression-induced oxidative stress in brain and medulla of rat. *Neurochem. Res*., 32 (3): 497-505. 2007.

[19] Abeer MA, Maha AA. Histological study of prolonged exposure to mobile phone radiations on young male albino rat's cerebellar cortex and the role of *Ginkgo biloba* supplementation. *J. Am. Sci*., 9 (11): 156-166. 2013.

[20] Kirchengast M. Preclinical considerations and results with the combination of verapamil and trandolapril: blood pressure reduction and beyond. *J. Hypertens*., 15 (2): S27-S33. 1997.

[21] Keller T, Cambon N, Genevray M, Crivelli F, et al. Bioequivalence study of fluoxetine hydrochloride in healthy volunteers. *Arzneimittelforschung*, 2005; 55 (9): 491-497. 2005.

[22] Goodnick PJ. Pharmacokinetic optimization of therapy with newer anti-depressants. *Clin. Pharmacokinet.*, 27 (4): 307-330. 1994.

[23] Cheng CL, Chou CH. Determination of metformin in human plasma by high-performance liquid chromatography with spectrophotometric detection. *J. Chromatography B*, 762: 51-58. 2001.

[24] Ji HY, Liu KH, Kong TY, Jeong HU, et al. Evaluation of DA-9801, a new herbal drug for diabetic neuropathy, on metabolism-mediated interaction. *Arch. Pharm. Res*., 36: 1-5. 2013.

[25] McKay DL, Chen CO, Yeum KJ, Matthan NR, et al. Chronic and acute effects of walnuts on antioxidant capacity and nutritional status in humans: a randomized, cross-over pilot study. *Nutr. J*., 9: 21. 2010.

[26] Mandel SA, Amit T, Kalfon L, Reznichenko L, Youdim MB. Targeting multiple neurodegenerative diseases etiologies with multimodal-acting green tea catechins. *J. Nutr*., 138: 1578S-1583S. 2008.

[27] Hou RR, Chen JZ, Chen H, Kang XG, et al. Neuroprotective effects of (-)-epigallocatechin-3-gallate (EGCG) on paraquat-induced apoptosis in PC12 cells. *Cell. Biol. Int*., 32 (1): 22-30. 2008.

[28] Jaccob AA, Hussain SA. Effects of long-term use of flavonoids on the absorption and tissue distribution of orally administered doses of trace elements in rats. *Pharmacol. Pharmacy*, 3 (4): 474-480. 2012.

[29] Choi YH, Chin YW, Kim YG. Herb-drug interactions: focus on metabolic enzymes and transporters. *Arch. Pharm. Res*., 34: 1843-1863. 2011.

[30] Na DH, Ji HY, Park EJ, Kim MS, et al. Evaluation of metabolism-mediated herb-drug interactions. *Arch. Pharmacol. Res*., 34 (11): 1829-1842. 2011.

[31] Samson RR. Effects of dietary garlic and temporal drift on platelet aggregation. *Atherosclerosis*, 44 (1): 119-120. 1982.

[32] Perlman BB. Interaction between lithium salts and ispaghula husk. *Lancet*, 335 (8686): 416. 1990.

[33] Bachmeier C, Levin GM, Abdelahad DB, et al. Effect of Venlafaxine and Desvenlafaxine on drug efflux protein expression and biodistribution in vivo. *J. Pharm. Sci*., 102: 3838-3843. 2013.

[34] Najafzadeh H, Fatemi SR, Ashna A. Effect of verapamil on serum level of salinomycin in diabetic rats. *Am. J. Appl. Sci*., 8 (9): 860-863. 2011.

[35] Doran A, Obach RS, Smith BJ, Hosea NA, et al. The impact of P-glycoprotein on the disposition of drugs targeted for indications of the central nervous system: evaluation using the MDR1A/1B knockout mouse model. *Drug Metab. Dispos*., 33: 165-174. 2005.

[36] Shugarts S, Benet LZ. The role of transporters in the pharmacokinetics of orally administered drugs. *Pharm. Res*., 26 (9): 2039-2054. 2009.

[37] Bansal T, Mishra G, Jaggi M, Khar RK, Talegaonkar S. Effect of P-glycoprotein inhibitor, verapamil, on oral bioavailability and pharmacokinetics of irinotecan in rats. *Eur. J. Pharm. Sci*., 36 (4-5): 580-590. 2009.

[38] De Klerk OL, Willemsen AT, Bosker FJ, Bartels AL, et al. Regional increase in P-glycoprotein function in the blood brain barrier of patients with chronic schizophrenia: A PET study with [^{11}C]-verapamil as a probe for P-glycoprotein function. *Psychiatry Res*., 183: 151-156. 2010.

[39] Milane A, Fernandez C, Vautier S, Bensimon G, et al. Minocycline and riluzole brain disposition: interactions with p-glycoprotein at the blood-brain barrier. *J. Neurochem*., 103: 164-173. 2007.

[40] Adlersberg S, Toren P, Mester R, Rehavi M, et al. Verapamil is not an antidepressant in patients resistant to tricyclic antidepressants. *Clin. Neuropharmacol*., 17: 294-297. 1994.

[41] Aszalos A, Thompson K, Yin JJ, Ross DD. Combinations of P-glycoprotein blockers, verapamil, PSC833, and cremophor act differently on the multidrug resistance associated protein (MRP) and on P-glycoprotein (Pgp). *Anticancer Res*., 19: 1053-1064. 1999.

[42] Yang CY, Chao PD, Hou YC, Tsai SY, et al. Marked decrease of cyclosporin bioavailability caused by coadministration of ginkgo and onion in rats. *Food Chem. Toxicol*., 44: 1572-1578. 2006.

[43] Uchida S, Yamada H, Li XD, Maruyama S, et al. Effects of *Ginkgo biloba* extract on pharmacokinetics and pharmacodynamics of tolbutamide and midazolam in healthy volunteers. *J. Clin. Pharmacol*., 46: 1290-1298. 2006.

[44] Song IS, Kong TY, Jeong HU, Kim EN, et al. Evaluation of the transporter-mediated herb-drug interaction potential of DA-9801, a standardized dioscorea extract for diabetic neuropathy, in human in vitro and rat in vivo. *BMC Complment. Altern. Med*., 14: 251-259. 2014.