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Effect of silymarin and pomegranate pretreatment on intestinal transport of buspirone across rat intestine

Shravan Kumar Yamsani, Ramesh Gannu, Adukondalu Devandla, Vamshi Vishnu Yamsani, Chinna Reddy Palem, Shiva Kumar Ravula, Madhusudan Rao Yamsani* and Sarangapani Manda

Centre for Biopharmaceutics and Pharmacokinetics, University College of Pharmaceutical Sciences, Kakatiya University, Warangal–506 009, A.P. India.

Abstract

To investigate the effect of silymarin and pomegranate juice pretreatment on the transport of buspirone, a CYP3A4 substrate across the rat intestine

The transport of buspirone across rat intestine (duodenum, jejunum and ileum) was studied by using non-everted sac method. The rats were pretreated with silymarin and pomegranate juice for 7 days. The rats were sacrificed by using anesthetic ether. The intestinal segments were isolated and used for the studies. The probe drug (buspirone) solution was placed in the isolated intestinal sac. Samples were collected at preset time points and replaced with fresh buffer. The drug content in the samples was estimated using high performance liquid chromatography method. Control experiments were also performed.

The results reveal that there was a significant ($p<0.05$) difference compared to control, in the transport of buspirone from the intestinal sacs which were pretreated with silymarin and pomegranate juice. It suggests that both silymarin and pomegranate juice might be acting by inhibiting the transporters and enzymes which are responsible for transport/metabolism of buspirone.

From the results it can be concluded that silymarin and pomegranate juice might be acting by inhibiting CYP3A4 enzymes as buspirone is extensively metabolized by CYP3A4. Further studies are recommended to prove their effects in human beings.

Introduction

Natural products, as used by the general population, are usually complex mixtures of many compounds. Both the putative active ingredient(s) and other constituents present in that mixture have the potential to cause interactions with various classes of drugs. Such interactions include induction or inhibition of metabolizing enzymes and drug efflux proteins. Prokaryotes and eukaryotes cannot distinguish between a natural chemical originating from plants and a chemical synthesized in laboratory. Consequently, physiological, pharmacological and toxicological effects of these chemicals irrespective of their origins remain the same. Ever-increasing use of herbs with western medicines raises the potential for drug–herbal interactions, which may alter drug bioavailability through altered absorption, distribution and metabolism.

Primary mechanisms of drug/herb interaction involve either induction or inhibition of intestinal

***Corresponding author:** ymrao123@yahoo.com

drug efflux pumps [efflux proteins such as P-glycoprotein (P-gp) and multiple resistance proteins (MRPs)] and intestinal and hepatic metabolism by cytochrome P450s (CYPs) (Evans 2000, Ioannides 2002). A concerted action by both drug efflux pumps and CYPs lower oral bioavailability of many drugs, i.e., protease inhibitors, macrolides and azoles. The most versatile enzyme system involved in the metabolism of xenobiotics is cytochrome P450. The CYP3A family of enzymes constitutes the most predominant phase-I drug metabolizing enzymes and accounts for approximately 30 % of hepatic CYP and more than 70 % of intestinal CYP activity. Moreover, CYP3A is estimated to metabolize between 50 % and 70 % of currently administered drugs (Watkins 1997). A congener of CYP family is CYP3A4, the most abundant form (Kolars et al. 1992). This CYP3A4 enzyme is present primarily in the hepatocytes and enterocytes (Parkinson 1996). It is now fairly established that naturally occurring dietary supplements can modulate hepatic and entrocytic CYP activity. Perhaps the best documented clinically relevant drug interaction is observed with grapefruit juice. Simultaneous consumption of grapefruit juice with a number of therapeutic agents that are subject to first pass intestinal/hepatic metabolism, resulted in higher plasma levels with subsequent adverse effects (Bailey et al. 1998). Grapefruit juice acts through inhibition of intestinal CYP3A4, which regulate pre-systemic metabolism (Guo et al. 2000). Although hepatic biotransformation can make a major contribution to systemic drug elimination, a combination of hepatic and intestinal drug metabolism may cause significant pre-systemic or first-pass drug loss.

Use of herbal drugs increased enormously because of their efficacy coupled with decreased risk of side effects. Silymarin is a flavanoid obtained from the plant *silybum marianum* belonging to family Asteraceae (USP-NF 2000). It is used in the supportive treatment of acute or chronic hepatitis and cirrhosis induced by drugs or toxins (Peepping 1999). It is a herbal drug that has the potential to influence drug metabolism by decreasing the metabolic activity of CYP3A4, a ubiquitous enzyme responsible for the hepatic and intestinal metabolism. It may also alter absorption, distribution and elimination through inhibition of p-glycoprotein (Zang and Morris 2003). Clinical data are sparse with regard to sylimarins effect on drugs that are metabolized by CYP450 system and effluxed by P-gp (Beakman et al. 2000). Because of the synergistic role of CYP3A4 and P-gp, the rate of elimination of drugs is very fast.

Pomegranate (*Punica granatum*) fruits are globally consumed fresh, in such processed forms as juice, jam, wine and oil and in extract supplements (Gil et al. 2000). They contain high levels of a diverse range of phytochemicals of which polyphenols are part of, including punicalagin (PA), ellagic acid (EA), gallotannins, anthocyanins (cyanidin, delphinidin and pelargonidin glycosides) and other flavonoids (quercetin, kaempferol and luteolin glycosides) (Kaplan et al. 2001, Kim et al. 2002, Cerda et al. 2003, Cerda et al. 2003). PA is the most abundant of these polyphenols, and EA has been previously shown to exhibit anti-carcinogenic properties, such as induction of cell cycle arrest as well as apoptosis and inhibition of tumor formation and growth in animals (Adams et al. 2006). Pomegranate juice (PJ) consumption has also shown potent anti-carcinogenic properties in various cancers (Albrecht et al. 2004, Kohno et al. 2004). *Punica granatum* L. (Punicaceae), also referred to as pomegranate, is commonly eaten around the world, and it has been used in folk medicine for a wide variety of therapeutic purposes (Langley 2000). Pomegranate is a rich source of crude fibers, pectin, sugars, and several

tannins (Gil et al. 2000). In addition, it has been reported that pomegranate contains certain species of flavonoids and anthocyanins in its seed oil and juice, and it shows potent antioxidant activity, resulting in beneficial health effects such as inhibition of low-density lipoprotein oxidation and decrease in cardiovascular diseases (Aviram et al. 2004). Furthermore, adjuvant therapeutic properties of the fruit have been suggested for use in cases of breast cancer (Kim et al. 2002). Based on these findings, pomegranate has been increasingly popularized in Japan. Higher pomegranate consumption allows for an increased possibility of pomegranate-drug interaction. Therefore, it is important to assess the interaction between pomegranate and CYP3A-mediated drugs because there are few reports on the inhibition of CYP3A activities. In the present study, using non everted sac method, we first investigated whether the components of pomegranate could inhibit the CYP3A-mediated drug metabolism of buspirone.

Buspirone is the first marketed anxiolytic drug from the azapirone class of compounds (Fulton and Brogden 1997). It is as effective as the benzodiazepines for the treatment of anxiety, but buspirone produces fewer adverse side-effects such as sedation, motor impairment, and dependence liability (O'Hanlon 1991). Unlike benzodiazepine anxiolytics, buspirone has little affinity for the aminobutyricacid benzodiazepine complex. Its primary pharmacological action is believed to be associated with the binding to 5-hydroxytryptamine subtype 1A (5-HT1A) receptor, resulting in the inhibition of the activity of serotonergic neurons through down-regulation (Goa and Ward 1986). Buspirone, originally approved by the Food and Drug Administration (FDA) for the treatment of generalized anxiety disorder in 1986, has been shown to be efficacious for the treatment of a variety of mental disorders, including panic disorder, major depression, obsessive-compulsive disorder, and social phobia (Sramek et al. 2002). Buspirone undergoes extensive first-pass metabolism in humans, resulting in a bioavailability of less than 5%, although it is almost completely absorbed after a single oral administration (Gammans et al. 1986).

Materials

Buspirone hydrochloride was gifted by Sun Pharmaceuticals, Simvasa, India. Silymarin was gifted by Ranbaxy Laboratories India Ltd), Dulbeccos Phosphate Buffer pH 7.4 (Hi Media Mumbai, India Ltd), Methanol HPLC (E. Merck Ltd., India), Acetonitrile HPLC (E. Merck Ltd) were used. All other chemicals used were of AR grade.

Preparation of pomegranate juice

Pomegrenate (*Punica granatum*) were cut into pieces, the rind was removed and the seeds were separated. The seeds were ground in mixer (Remi, Mumbai, India). The freshly prepared juice was adminstered to rats.

Noneverted intestinal sac study (Ruan et al. 2006)

The animal study was conducted according to the protocol approved by animal ethics committee, Kakatiya University, India. Male wistar rats weighing 200 ± 25 gm were selected for experiments. Pomegranate juice was administered to rats (n=3) at a dose of 10 mL Kg^{-1} for seven days. Silymarin suspension was prepared by suspending 1 gm of silymarin in 0.25 % w/v of sodium carbaoxy methyl cellulose and was administered at a dose of 150 mg Kg^{-1} to rats for seven days. Untretaed rats (n=3) were used as control.

The rats were fasted overnight with free access to water before the experiments. Control rats and pretreated rats on seventh day were sacrificed using anesthetic ether, the intestine was surgically removed and flushed with 50 mL of ice cold saline. The small intestine was cut into 3 segments,

duodenum, jejunum and ileum of equal length (10 cm). The probe drug (Buspirone 10 mg mL^{-1}) was dissolved in pH 7.4 isotonic Dulbecco's PBS (D-PBS) containing 25 mM glucose. The probe drug solution (1 mL) was filled in the normal sac (mucosal side), and both ends of the sac were ligated tightly. The sac containing probe drug solution was immersed in 40 mL of D-PBS, containing 25 mM glucose in the mucosal side. The medium was pre-warmed at 37° C and pre-oxygenated with 5 % CO_2/ 95 % O_2 for 15 minutes, under bubbling with a CO_2/O_2 mixture gas, the transport of the buspirone from mucosal to serosal surfaces across the intestine was measured by sampling the serosal medium periodically for 120 minutes. The samples of 1 mL were collected at predetermined time intervals from the serosal medium and replenished with fresh buffer. The drug transported was measured using high performance liquid chromatography (HPLC) method.

HPLC analysis

Shimadzu HPLC system equipped with a LC-10AT pump and SPD 10 AT UV visible detector and C18 Inertsil ODS–3V column (250 mm x 4.6 mm ID, particle size 5 µ) was used for the analysis of samples. The mobile phase used was a mixture of (30:70) of acetonitrile, buffer (0.02M of NaH_2PO_4, pH 2.5). The flow rate was 1 mL per minute and the detection was carried at 240 nm.

A calibration curve was plotted for buspirone in the range of 1-40 µg ml^{-1}. A good linear relationship was observed between the concentration of buspirone and the peak area of buspirone with a correlation coefficient (r^2 = 0.999). The required studies were carried out to estimate the precision and accuracy of the HPLC method of analysis of buspirone.

Sample preparation

To 200 µL of intestinal sac samples, 100 µL of methanol was added and vortexed on a cyclo-mixer for two minutes and centrifuged at 5000 rpm for 15 min using Biofuge Fresco Centrifuge (Heraeus, Germany). The supernatant (20 µL) was injected into HPLC.

Statistical analysis

The efflux results were tested for statistical significance using t-test. The difference in the sample means were considered significant at $p< 0.05$.

Results

In the present study, the mean transport of buspirone from mucosal to serosal (normal sac) was determined in duodenum, jejunum and ileum regions of rat intestine in the absence and presence of silymarin and pomegranate juice pretreatment. The time course of buspirone transport at different concentrations across rat small intestine of duodenum, jejunum and ileum was shown in Table 1.

Table 1. Mean ± S.D (n=3) cumulative transport of buspirone (10 mg mL^{-1}) in intestinal sacs in Wistar rats.

Region	Control	Buspirone +Pomegranate	Buspirone +Silymarin
Duodenum	39.19 ± 7.61	332.15 ± 25.18	590.61 ± 21.47
Jejunum	93.95 ± 15.44	779.31 ± 41.48	490.79 ± 40.60
Ileum	117.30 ± 6.79	672.16 ± 26.30	600.43 ± 25.92

The pomegranate juice pretreatment for 7 days increased the mean cumulative concentration of buspirone from 39.19 ± 7.61 to 332.15 ± 25.18 in duodenum (Figure 1), 93.95 ± 15.44 to 779.31 ± 41.48 in jejunum (Figure 2) and in ileum (Figure 3) the mean cumulative concentrations were increased from 117.30 ± 6.79 to 672.16 ± 26.30 respectively. The silymarin pretreatment for 7 days increased the mean cumulative concentration of buspirone from 39.19 ± 7.61 to 590.61 ± 21.47 in duodenum, 93.95 ± 15.44 to 490.79 ± 40.60 in jejunum and in ileum the mean cumulative concentrations were increased from 117.30 ± 6.79 to 600.43 ± 25.92 respectively. The transport of buspirone was increased 8.5, 8.3 and 5.7 times after pretreatment with pomegranate and 15.0, 5.2 and 5.2 times after pretreatment with silymarin

compared to respective controls. There was a statistically significant ($P<0.05$) difference. The transport was increased from duodenum to ileum. The results suggest that both pomegranate and silymarin pretreatment influences the transport of buspirone. As buspirone is the substrate for CYP3A, pomegranate and silymarin pretreatment might have inhibitory effect on the CYP3A, therefore the transport of buspirone was increased.

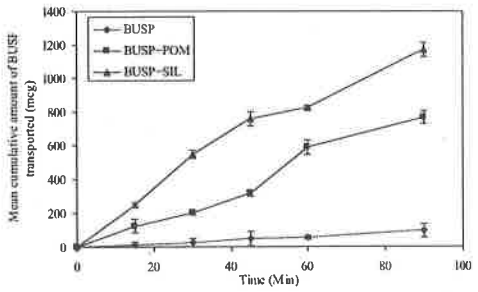

Figure 1. Effect of pomegranate and silymarin on transport of buspirone across duodenum.

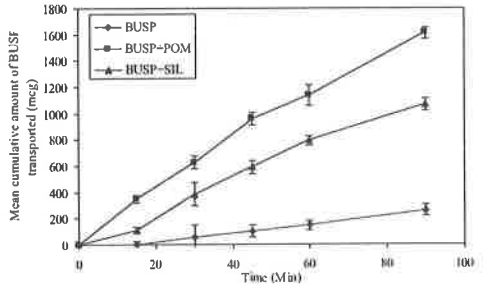

Figure 2. Effect of pomegranate and silymarin on transport of buspirone across jejunum.

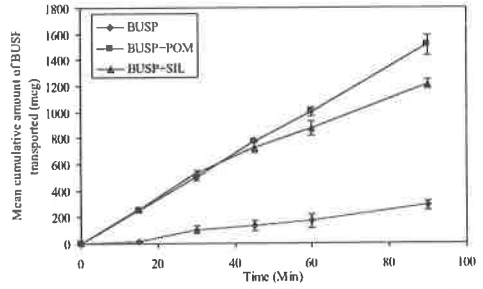

Figure 3. Effect of pomegranate and silymarin on transport of buspirone across ileum.

Discussion

The non-everted sac model was originally used to evaluate drug transport mechanisms (Kaul and Ritschel 1981). Genty et al. (2001) compared the permeability values of some actively transported molecules and passively absorbed compounds through everted and non-everted sacs and found that the permeability was higher for actively transported molecules when the sacs were everted. The permeability of passively absorbed drug diazepam remained the same whether the sacs were everted or not. These results suggested that the passive permeability of actively transported molecules can be determined through non-everted rat gut sacs (Kivisto et al. 1997).

The results from previous studies (Lamberg et al. 1998a, Lilja et al. 1998, Lamberg et al. 1998b) demonstrate that CYP3A inhibitors, verapamil, diltiazem, erythromycin, itraconazole and grapefruit juice, substantially increase the area under the curve (AUC) and the maximum concentration (C_{Max}) of buspirone in human plasma, presumably by inhibiting CYP3A

mediated metabolic clearance. In addition a CYP3A inducer, rifampicin decreases the AUC and C_{Max} of buspirone in human plasma by 90 and 84 %, respectively (Kivisto et al. 1999, Venkataramanan et al. 2000). These observations strongly suggest that CYP3A isoforms play an important role in the metabolism of buspirone in humans. From the present study, it appears that pretreatment with silymarin and pomegranate juice had effect on the intestinal transport of buspirone. Preliminary data suggested that silymarin might influence the metabolic activity of CYP3A4, a CYP450 iso-enzyme responsible for hepatic and intestinal metabolism of many important classes of drugs (Wacher and Benet 1995). Silymarin also may alter drug absorption, distribution and elimination through inhibition of P-gp. Synergistic role of CYP3A4 and P-gp in limiting the oral bioavailability of many drugs was proved (Scheuetz and Beck 1996, Garvan et al. 2000, Rajanarayana et al. 2004). Silymarin pretreatment appear to have a significant influence on CYP3A4 mediated intestinal metabolism of buspirone.

In the earlier studies pomegranate juice influenced the pharmacokinetics of carbamazepine in rats, particular in comparison with the effect of water, the AUC of carbamazepine increased approximately 1.5-fold in rats upon exposure to pomegranate juice 1 h before the administration of the drug. The component(s) of pomegranate inhibits the CYP3A-mediated metabolism of carbamazepine. Furthermore, pomegranate juice has an influence on the pharmacokinetics in rats (Hidaka et al. 2005). The manner in which inhibition is caused by the component(s) of pomegranate is similar to that caused by grapefruit.

Buspirone is an azapirone anxiolytic agent that produces less sedation and impairment of psychomotor performance than do benzodiazepines. It has poor bioavailability due to extensive first-pass metabolism. "High-dose grapefruit juice" has been shown to raise the AUC of buspirone between 3 and 20 fold and the maximum concentration between 2 and 16 fold (Muneaki Hidaka et al. 2005). Pomegranate juice pretreatment appear to have a significant influence on CYP3A4 mediated intestinal metabolism of buspirone. However, it is difficult to extrapolate our results which were obtained in rats to humans. Evaluation of pomegranate buspirone interaction in humans needs to be verified. Further investigations in humans are in progress.

Conclusions

From the results it can be concluded that silymarin and pomegranate juice might be acting by inhbiting the enzymes as buspirone is extensively metabolized by CYP3A4. Further studies are recommended to prove their influence in human volunteers or animals *in vivo*.

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References

Adams, L.S., Seeram, N.P., Aggarwal, B.B., Takada, Y.S. and Heber, D. (2006). Pomegranate juice, total pomegranate ellagitannins, and punicalagin suppress inflammatory cell signaling in colon cancer cells. *J. Agric. Food Chem.* 54: 980–985.

Albrecht, M., Jiang, W., Kumi-Diaka, J., Lansky, E.P., Gommersall, L.M. and Mansel, R.E. (2004). Pomegranate extracts potently suppress proliferation, xenograft growth, and invasion of human prostate cancer cells. *J. Med. Food.* 7: 274–283.

Apter, J.T. and Allen, L.A. (1999). Buspirone: future directions. *J. Clin. Psychopharmacol.* 19: 86–93.

Aviram, M., Rosenblat, M., Gaitini, D., Nitecki, S., Hoffman, A., Dornfeld, L., Volkova, N., Presser, D., Attias, J. and Liker, H. (2004). Pomegranate juice consumption for 3 years by patients with carotid artery stenosis reduces common carotid intima-media thickness, blood pressure and LDL oxidation. *Clin. Nutr.* 23: 423–433.

Bailey, D.G, Malcolm, J, Arnold, O and Spence, J.D. (1998) Grapefruit juice– drug interactions. *Br. J. Clin. Pharmacol* 46: 101–110.

Beckmann-knopp, S., Rietbrock, S., Weyhenmeyer, R., Bocker, R.H., Beckurts, T., Lang, W., Hunz, M. and Fuhr, U. (2000). Inhibitory effects of silybin on cytochrome P450enzymes in human liver. *Pharmacol. Toxicol.* 86: 250-256.

Cerda, B, Ceron, JJ, Tomas-Barberan, FA and Espin, JC. (2003). Repeated oral administration of high doses of pomegranate ellagitannin punicalagin to rats for 37 days is not toxic. *J Agric Food Chem.*51:3493–501.

Cerda, B., Llorach, R., Ceron, J.J., Espin, J.C. and Tomas-Barberan, F.A. (2003). Evaluation of the bioavailability and metabolism in the rat of punicalagin, an antioxidant polyphenol from pomegranate juice. *Eur. J. Nutr.* 42: 18–28.

Evans, A.M. (2000). Influence of dietary components on the gastrointestinal metabolism and transport of drugs. *Ther. Drug Monit.* 22: 131–136.

Fulton, B. and Brogden, R.N. (1997). Buspirone: an updated review of its clinical pharmacology and therapeutic applications. *CNS Drugs.* 7: 68–88.

Gammans, R.E., Mayol, R.F. and Labudder, J.A. (1986). Metabolism and disposition of buspirone. *Am. J. Med.* 80: 41–51.

Garvan, C., Kane, Md. and James, J. (2000). Lipsky, Md., Drug–Grapefruit Juice Interactions. *Mayo Clin. Proc.* 75: 933-942

Genty, M., Gonzalez, G., Clere, C.V., Desangle-Gouty and Legendre, J.Y. (2001). Determination of the passive absorption through the rat intestine using chromatographics indices and molar volume. *Eur. J. Pharm. Sci.* 12: 223–229.

Gil, M.I., Tomas-Barberan, F.A., Hess-Pierce, B., Holcroft, D.M. and Kader, A.A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J. Agric. Food Chem.* 48: 4581–4589.

Goa, K.L. and Ward, A. (1986). Buspirone: a preliminary review of its pharmacological properties and therapeutic efficacy as an anxiolytic. *Drugs* 32: 114–129.

Guo, L.Q., Taniguchi, M., Xiao, Y.Q., Baba, K., Ohta, T. and Yamazoe, Y. (2000). Inhibitory effect of natural furanocoumarins on human microsomal cytochrome P450 3A activity. *Jpn. J. Pharmacol.* 82: 122–129.

Hidaka, M., Okumura, M., Fujita, K., Ogikubo, T., Yamasaki, K., Iwakiri, T., Setoguchi, N. and Arimori, K. (2005). Effects of pomegranate juice on human cytochrome p450 3a (cyp3a) and carbamazepine pharmacokinetics in rats. *Drug Metab. Dispos.* 33: 644-648.

Ioannides, C. (2002). Pharmacokinetic interactions between herbal remedies and medicinal drugs. *Xenobiotica* 32: 451–478.

Kaplan, M., Hayek, T., Raz, A., Coleman, R., Dornfield, L. and Vaya, J. (2001). Pomegranate juice supplementation to atherosclerotic mice reduces macrophage lipid peroxidation, cellular cholesterol accumulation and development of atherosclerosis. *J Nutr.*131: 2082–2089.

Kaul, S. and Ritschel, W.A. (1981). Studies of the intestinal transfer of coumarin and 7-hydroxycoumarin across guinea pig and rat small intestine. *Arzneim. Forsch.* 31: 790–795.

Kim, N.D., Mehta, R., Yu, W., Neeman, I., Livney, T. and Amichay, A. (2002). Chemopreventive and adjuvant therapeutic potential of pomegranate (Punica granatum) for human breast cancer. *Breast Cancer Res. Treat.* 71: 203–217.

Kivisto, K.T., Lamberg, T.S., and Neuvonen, P.J. (1999). Interactions of buspirone with traconazole and rifampicin: effects on the pharmacokinetics of the active 1-(2-pyrimidinyl)-piperazine metabolite of buspirone. *Pharmacol Toxicol.* 84: 94–97.

Kivisto, K.T., Lamberg, T.S., Kantola, T. and Neuvonen, P.J. (1997). Plasma buspirone concentrations are greatly increased by erythromycin and itraconazole. *Clin Pharmacol Ther.* 62:348–354.

Kohno, H., Suzuki, R., Yasui, Y., Hosokawa, M., Miyashita, K. and Tanaka, T. (2004). Pomegranate seed oil rich in conjugated linolenic acid suppresses chemically induced colon carcinogenesis in rats. *Cancer Sci.* 95: 481–486.

Kolars, J.C, Schmiedlin-Ren, P., Schuetz, J.D., Fang, C. and Watkins, P.B. (1992). Identification of rifampin-inducible P450IIIA4 (CYP3A4) in human small bowel enterocytes. *J. Clin. Invest.* 90: 1871–1878.

Lamberg, TS, Kivisto, KT and Neuvonen, PJ. (1998a). Effects of verapamil and diltiazem on the pharmacokinetics and pharmacodynamics of buspirone. *Clin Pharmacol Ther.* 63:640–645.

Lamberg, T.S., Kivisto, K.T. and Neuvonen, P.J. (1998b). Concentrations and effects of buspirone are considerably reduced by rifampicin. *Br. J. Clin. Pharmacol.* 45: 381–405.

Langley P. (2000) Why a pomegranate? *Br. Med. J.* 321: 1153–1154.

Lilja, J.J., Kivisto, K.T., Backman, J.T., Lamberg, T.S. and Neuvonen, P.J. (1998). Grapefruit juice substantially increases plasma concentrations of buspirone. *Clin. Pharmacol. Ther.* 64: 655–660.

O'Hanlon, J.F. (1991). Review of buspirone's effect on human performance and related variables. *Eur. Neuropsychopharmacol.* 1: 489–501.

Parkinson, A. (1996). An overview of current cytochrome P450 technology for assessing the safety and efficacy of new materials. *Toxicol. Pathol.* 24: 48–57.

Peepping, J. (1999). Milk thistle: Silybuam marianum. *Am. J. Health Systems Pharm.* 56: 1195-1197.

Rajanarayana, K., Reddy, M.S., Vidyasagar, J. and Krishna, D.R. (2004). Study on the influence of silymarin pretreatment on metabolism and disposition of metronidazole. *Arzneimittelforschung.* 54: 109-113.

Ruan, L.P., Chen, S., Yu, B.Y., Zhu, D.N., Cordell, G.A. and Qiu, S.X. (2006). Prediction of human absorption of natural compounds by the non-everted rat intestinal sac model. *Eur. J. Med. Chem.* 41: 605–610.

Scheuetz, E.G. and Beck, W.T. (1996). Modulaters and substrates of p-gp and cyp3a coordinately up regulate these proteins in human colon carcinoma cells. *Mol. Pharmacol.* 49: 311-318.

Sramek, J.J., Zarotsky, V. and Cutler, N.R. (2002). Generalised anxiety disorder treatment options. *Drugs* 62: 1635–1648.

United States Pharmacopeia and National Formulary (2000). Anonymous, Silymarin. 24/19. RockvilleMD 2481.

Venkataramanan, R., Ramachandran, V., Komoroski, B.J., Zhang, S., Schiff, P.L. and Schiff, S.C. (2000). Milkthistle, a herbal supplement decreases the activity of CYP3A4 and uridine diphosphoglucuronosyl transferase in human hepatocyte cultures . *Drug Metab. Dispos.* 28: 1270-1273.

Wacher, V.J. and Benet, L.Z. (1995). Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and p-glycoprotien implication for drug delivery and activity in cancer therapy. *Mol. Carrinog.* 13: 129-134.

Watkins, P.B. (1997). The barrier function of CYP3A4 and P-glycoprotein in the small bowel. *Adv. Drug Deliv. Rev.* 27: 161–170.

Zang, S. and Morris, M.E. (2003). Effects of the flavonoids: Biochanin A, Morin, Phloretin and silymarin on the P-glycoprotien transport. *J. Pharamcol. Exp. Ther.* 304: 1258-1267.

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