Eur J Clin Pharmacol (2003) 58: 803–807
DOI 10.1007/s00228-002-0543-5

PHARMACOKINETICS AND DISPOSITION

K. Rajnarayana · Mada S. Reddy
Devarakonda R. Krishna

Diosmin pretreatment affects bioavailability of metronidazole

Received: 15 April 2002 / Accepted: 6 November 2002 / Published online: 1 March 2003

Abstract *Objective*: To screen for inhibitory effects of diosmin on cytochrome P_{450}-mediated metabolism of metronidazole in healthy volunteers. *Design*: Before/after non-blinded investigation conducted in healthy male volunteers.
Methods: After an overnight fast, metronidazole (two 400-mg tablets) was administered to 12 volunteers, either alone or after a 9-day pretreatment period with a once-daily dose of diosmin 500-mg tablets under direct observation. Serum concentrations of metronidazole up to 48 h postdose and urinary concentrations of metronidazole and its two major metabolites up to 24 h postdose were measured using reversed-phase high-performance liquid chromatography.
Results: Metronicazole plasma $AUC_{(0-\infty)}$ and C_{max} were significantly higher after diosmin pretreatment by (mean) 27% and 24%, respectively. However, time to reach peak concentration (t_{max}) was not affected significantly. Urinary excretion of acid and hydroxy metabolites in urine was decreased significantly, while excretion of unchanged metronidazole was increased.
Conclusion: Diosmin pretreatment significantly altered the metabolism of metronidazole, as demonstrated by changes in plasma pharmacokinetics as well as by urinary recovery of both parent drug and its major metabolites. This may be caused by the inhibition of cytochrome P_{450} enzymes.

Keywords Diosmin · Metronidazole · CYP3A4

K. Rajnarayana · M.S. Reddy · D.R. Krishna (✉)
Drug Metabolism and Clinical Pharmacokinetics Division,
University College of Pharmaceutical Sciences,
Kakatiya University, AP-506009,
Warangal, India
E-mail: dr_krishna@hotmail.com
Tel.: +91–8712–572567
Fax: +91–8712–438844

Introduction

Diosmin (3', 5, 7-trihydroxy-4'-methoxyflavone 7-rutinoside) is a flavone (Fig. 1) [1] used for the treatment of haemorrhoids and of chronic venous insufficiency of the lower limbs [2]. A pure synthetic diosmin (Venex) was used in the study. Diosmin and diosmetin are natural dietary agonists of the aryl hydrocarbon receptor (AhR), causing a potent increase in CYP1A1 transcription and its activity; however, only diosmetin is capable of inhibiting CYP1A1 enzyme activity [3]. In pharmacokinetic studies on diosmin and diometin, both flavonoids were rapidly metabolised, diosmetin was partly excreted in bile as the glucuronide and sulphate, and diosmin was partly excreted in bile as such and as the glucuronide conjugate. Both the parent compounds were completely absent in urine. Diosmetin had a long plasma elimination half-life ranging from 26 h to 43 h [4, 5].

Flavones and flavonols are in general more potent enzyme inhibitors specifically for CYP1A1 and CYP1A2 than flavanones, isoflavones and chalcones [6]. The inhibitory effects of tangeretin, green tea flavonoids and other flavonoids on CYP1A, CYP2B, CYP2E1 and CYP3A enzymes were examined in rat and human liver microsomes [7]. Specifically, flavones or flavonoids that contain C5, C7 and C4' hydroxyl groups were potent inhibitors of CYP1A1 and 1A2 activity [8]. Furthermore, naturally occurring plant flavonoids may acutely upregulate the apparent activity of P-glycoprotein (P-gp), while flavonoids like quercetin, rutin, galangin, kaempferol, genistein and daidzein inhibited the P-gp pump efflux activity [9]. Thus, in general, flavones have the potential to alter the pharmacokinetics of drugs by at least two mechanisms.

Metronidazole, a nitroimidazole derivative (1-hydroxy-ethyl-2-methy-5-nitroimidazole), is widely used for the treatment of protozoan and anaerobic bacterial infections. Metronidazole is metabolised primarily in the liver by CYP 3A4 and CYP 2C9, the two major

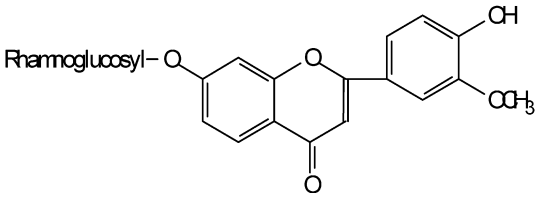

Fig. 1 Chemical structure of diosmin

metabolites being 1-(2-hydroxyethy)-2-hydroxymethy-5-nitroimidazole (hydroxymetronidazole) and 2-methyl-5-nitroimidazole-1-acetic acid (acid metabolite). The acetic acid metabolite is only found in urine and does not possess any pharmacological activity [10, 11].

As metronidazol is an important antibacterial with widespread use, especially in developing countries, administration in patients receiving long-term therapy with diosmin may occur. It is thus important to consider the effect of diosmin-mediated enzyme inhibition on the pharmacokinetics of metronidazole. Metronidazole is metabolised by the CYP3A4 and CYP2C9 in liver microsomal enzymes. Flavonoids and flavones are well-known inhibitors of both enzymes, but there is no report available on the effect of diosmin-mediated enzyme inhibition on the pharmacokinetics of metronidazole. In the present study, we examined the hypothesis that the flavone diosmin might interact with the metabolism of metronidazole in vivo.

Materials and methods

Drugs and chemicals

Diosmin (800 mg, Venex) was obtained from Elder pharmaceuticals Ltd. (Mumbai, India), metronidazole tablets (400 mg, Flagyl) from RhonePoulene (Mumbai, India) and metronidazole pure substance from Aristo Phamaceutical Ltd. (Mumbai, India). All other chemicals and solvents were of highest analytical grade available and purchased from Sd. Fine chemicals Ltd.(Mumbai, India). They were used without further purification.

Study population

Twelve healthy male volunteers with a mean ±SD age of 24.3 ± 3.3 years (range 20–30 years), a mean height of 172.4 ± 5.0 cm (range 165–180 cm) and mean body weight of 61.8 ± 6.6 kg (range 54–70 kg) participated in the study after undergoing a thorough physical examination. All volunteers were briefed about the study and written informed consent was obtained. The investigational protocol was approved by the institutional ethics committee (University College of Pharmceutical Sciences, Kakatiya University). All subjects fasted for 12 h before the administration of metronidazole. They had to avoid nicotine, alcohol, caffeine and citrus fruit products for 1 week before and throughout the study period. Study drugs were taken in the morning with 100 ml tap water just after voiding.

Study protocol

The first part of the study consisted of oral administration of two 400-mg tablets of metronidazole alone, and blood was sampled from an antecubital vein. Blood samples were drawn predose and 0.5, 1, 2, 4, 6, 8, 12, 24, 36 and 48 h postdose, and urine was collected during 0 to 4, 4 to 8, 8 to 12 and 12 to 24-h intervals, respectively, after the administration of metronidazole. The second part of the study was conducted after a wash-out period of 4 days (about ten metronidazole half-lives). Diosmin (500 mg) was administered daily for 9 days. On the 10th day, metronidazole (2×400 mg) tablets were administered. Blood and urine samples were collected as described above. Blood samples were centrifuged at about 1200 *g* for 15 min. Serum samples and 5-ml aliquots of urine were stored at 80°C until analysis.

Analytical method

Metronidazole in serum samples was estimated using a modified reversed-phase, high-performance liquid chromatography (HPLC) method [12]. The system (Shimadzu, Japan) consisted of LC-8A solvent delivery module and a SPD-10A*VP* UV-visible spectrophotometric detector. The mobile phase consisted of acetonitrile: 5 μM potassium dihydrogen orthophosphate buffer, pH 3.0 (15:85) with a flow rate of 1 ml/min. The column used was Hichrom C-18 (stainless-steel column of 25 cm length and 4.6 mm internal diameter packed with porous silica spheres of 5 μm diameter, 100 A pore diameter), and UV absorption of the eluent was monitored at 313 nm. Sensitivity was set at 0.001 a.u.f.s. To 250 μl of serum 5 μl tinidazole (1.0 mg/ml) was added as internal standard and vortexed for 2 min. An equal volume (250 μl) of 5% trichloroacetic acid was added only to serum samples for protein precipitation and mixed on a cyclo-mixer for 1 min and centrifuged at about 1200 *g* for 8 min using a Biofuge Fresco (Heraeus, Germany). Urinary metabolite levels were estimated by taking 100 μl urine and diluting the samples five times with deionised water. The supernatant (20 μl; both serum and urine) was injected onto the column. A linear calibration curve in the range of 2–36 μg/ml was established ($r^2 = 0.999$) in serum matrix. The lower limit of quantification was 50 ng/ml. Interassay variability at 2, 10 and 30 μg/ml gave with coefficients of variation of 7.2, 5.0 and 3.0%, respectively.

Calculation of pharmacokinetic parameters

The pharmacokinetic parameters peak serum concentration (C_{max}) and time to reach peak concentration (t_{max}) were directly obtained from concentration–time data. In the present study, AUC_{0-t} refers to the AUC from 0 h to 8 h which was determined using linear trapezoidal rule and $AUC_{0-\infty}$ refers to the AUC from 0 to infinity. The AUC_{0-t} value is more than 90% of the $AUC_{0-\infty}$ in the present study and hence the extrapolation to ∞ is valid. $AUC_{0-\infty}$ was calculated using the formula $AUC_{0-t} + (C_{last}/K_{el})$, where C_{last} is the concentration in milligrams per litre at the last time point and K_{el} is the elimination rate constant.

Statistical analysis

Analysis of variance on log-transformed AUC_{0-t}, $AUC0_{-\infty}$ and C_{max} data was performed, and 90% confidence intervals were calculated for the ratios after/before diosmin pretreatment for these parameters. Student's *t*-test (paired data) was applied to $t_{1/2}$, t_{max} and urinary excretion data. A value of $p < 0.05$ was considered to be statistically significant.

Results

Diosmin pretreatment showed a significant effect on all pharmacokinetic parameters of metronidazol examined except t_{max}. Metronidazole plasma concentrations were increased by approximately 25%, while urinary

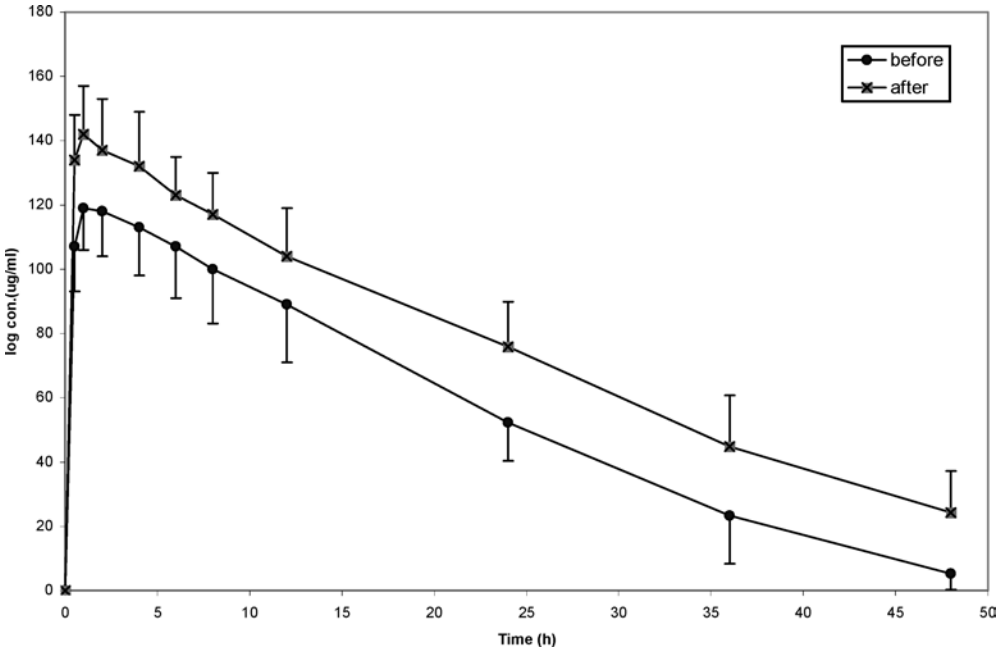

Fig. 2 Serum metronidazole log concentration–time profiles before and after pretreated with diosmin. Before treatment (*circles*), after treatment (*squares*)

excretion of metronidazole was decreased and that of the metabolites was increased to a similar extent. The mean ± SD serum concentrations of metronidazole at different time points before and after diosmin pretreatment are shown in Fig. 2, and the pharmacokinetic parameters of metronidazole are presented in Table 1. The mean ± SD of urinary recovery of metabolites (acid- and hydroxy-metronidazole) and unchanged metronidazole during 0–24 h are given in Table 2. Metabolic ratios of acid metabolite and of hydroxy metabolite over metronidazole are shown in Fig. 3 and Fig. 4, respectively. The 90% confidence intervals calculated for the ratios of metronidazole AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} values after pretreatment with diosmin over the values obtained without diosmin pretreatment were 1.18–1.37, 1.19–1.38 and 1.13–1.35, respectively. All three lie beyond the 0.80–1.25 range, indicating that differences between periods are of practical relevance.

Discussion

Our results suggest that diosmin pretreatment caused a decreased metabolism and/or transport of metronidazole

Table 2 Urinary excretion of metronidazole and its metabolites (mean ± SD, $n = 12$) collected over 24 h

Parameters (moles)	Before	After	*P* value
Acid metabolite	0.49 ± 0.19	0.37 ± 0.15	< 0.005
Hydroxy metabolite	0.59 ± 0.30	0.46 ± 0.24	< 0.001
Metronidazole	0.67 ± 0.20	0.83 ± 0.27	< 0.002

Table 1 Pharmacokinetic parameter values of metronidazole in human volunteers before (B) and after (A)pretreatment with diosmin

Vol. code	C_{max} (mg/l)			t_{max} (h)			AUC_{0-t} (mg h/l)			$AUC_{0-\infty}$ (mg h/l)			$t_{1/2}$ (h)		
	B	A	A/B	B	A	A/B	B	A	A/B	B	A	A/B	B	A	A/B
1	12.5	20.1	1.60	1	1	1	185	342	1.84	188	350	1.86	9.0	8.2	0.91
2	19.0	21.0	1.10	1	1	1	305	352	1.15	326	376	1.15	12.8	11.4	0.89
3	20.3	21.0	1.03	1	1	1	325	365	1.12	338	380	1.12	10.7	8.7	0.81
4	22.3	21.3	0.95	0.5	1	2	199	224	1.12	199	224	1.12	1.1	5.06	4.60
5	19.3	22.1	1.14	1	0.5	0.5	301	343	1.14	306	357	1.16	6.8	9.1	1.33
6	23.4	29.3	1.25	1	1	1	391	478	1.22	409	513	1.25	8.4	10.8	1.28
7	15.2	19.4	1.27	2	2	1	209	282	1.34	212	285	1.34	6.2	4.8	0.77
8	20.4	21.1	1.03	1	1	1	270	316	1.17	272	324	1.19	4.9	7.9	1.61
9	16.2	19.7	1.21	2	1	0.5	209	257	1.22	209	260	1.24	1.5	5.3	3.53
10	15.9	24.3	1.52	2	1	0.5	214	266	1.24	215	267	1.24	5.1	5.5	1.07
11	17.4	21.4	1.22	1	1	1	170	260	1.52	170	265	1.55	2.1	7.4	3.52
12	8.7	13.9	1.59	1	0.5	0.5	145	182	1.25	145	183	1.26	2.1	7.5	3.57
Mean	17.5	21.2	1.24	1.2	1	0.9	244	306	1.27	249	315	1.29	5.7	7.6	1.99
S.D.	4.1	3.5	0.22	0.4	0.3	0.4	71	75	0.28	80	88	0.20	4.1	2.2	1.38

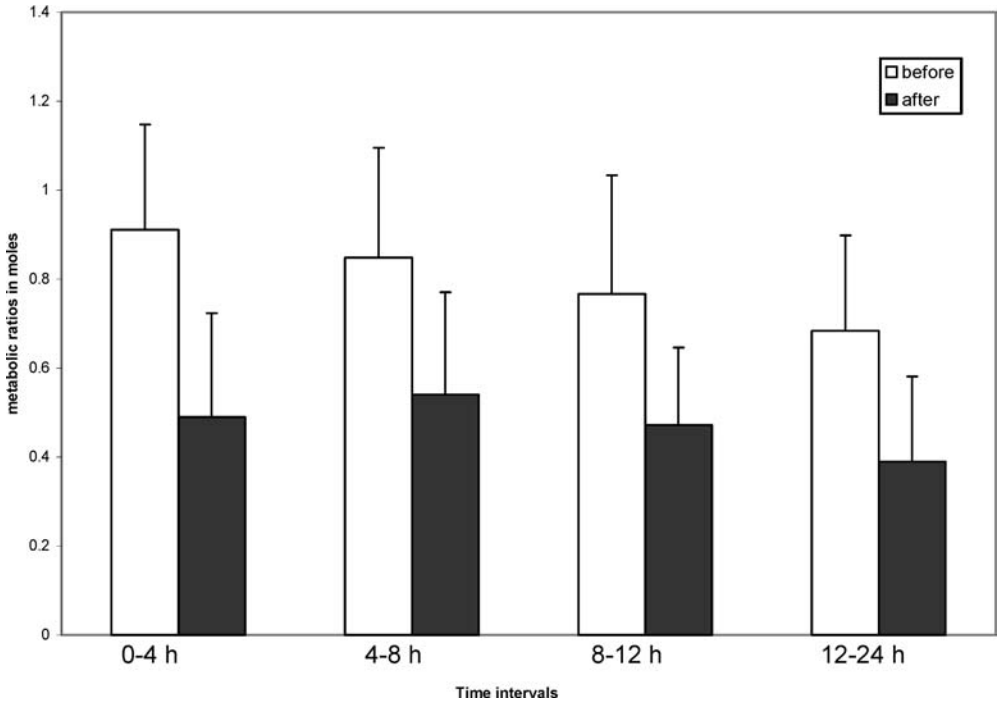

Fig. 3 Metabolic ratios of acid metronidazole in moles. Before treatment (*open bars*), after treatment (*closed bars*)

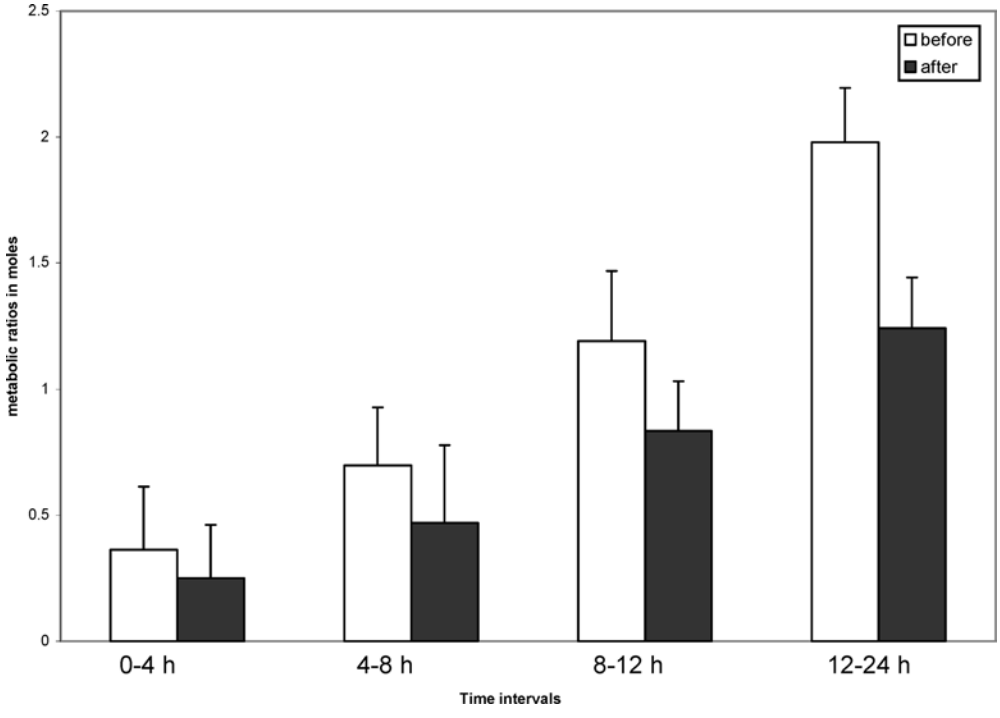

Fig. 4 Metabolic ratios of hydroxy-metronidazole in moles. Before treatment (*open bars*), after treatment (*closed bars*)

as both AUC and C_{max} showed a significant increase probably owing to decreased clearance, and urinary excretion changed towards a decreased ratio of metabolites over parent compounds. These changes may be due to a decreased activity of CYP2C9 and/or CYP3A4 in liver/ intestine, and/or decreased P-gp-mediated exsorption into the intestines.

Metronidazole is extensively metabolised by the liver into five metabolites, and oxidative metabolic enzymes (CYP2C9/3A4) are responsible for formation of the major hydroxy and acetic acid metabolites [10]. Pharmacokinetic interactions of a broad spectrum of drugs often occur as a result of a change in drug metabolism mediated by CYP3A4. The location of CYP3A4 in small bowel and liver permits an effect on both presystemic and systemic drug disposition. It was reported that citrus phytochemicals such as diosmin, naringin, naringenin, quercetin or rutin inhibited the metabolic activation of

tobacco-specific nitrosamine by inhibiting cytochrome P_{450} enzymes including 1A1, 1A2, 2B1, 2D6 and 2E1 [13]. Diosmin exhibited inhibition of alkoxyresorufin-O-dealkylase reactions selective for various cytochrome P_{450} enzymes [14]. Diosmetin, the metabolite of diosmin, had a protective effect on lipid peroxidation and increased glutathione content [15]. This is evidence that diosmin and diosmetin indeed are substances with the capability of affecting drug metabolism.

The assumption about a possible role of P-gp in the higher concentrations of metronidazole after diosmin pretreatment was based on a previous report [16] where genistein, quercetin and daidzein were found to increase the accumulation of daunorubicin, whereas the substances decreased accumulation of rhodamine-123 (a p-gp substrate) in cell lines with high expression of multi-drug resistance (MDR) protein. If diosmin inhibits P-gp activity within the intestinal epithelium, the absorption and oral bioavailability of P-gp substrates might be increased. This, however, requires further experimental evidence.

From the literature, it is evident that metronidazole, the prototype drug of the nitroimidazoles, is able to inhibit carbamazepine metabolism and elevate plasma carbamazepine concentrations to potentially toxic levels by inhibiting CYP3A4 [17]. Metronidazole is also known to inhibit CYP2C9 and elevate phenytoin concentrations [18]. These reports suggest that under steady-state conditions inhibition of metronidazole metabolism by diosmin may be amplified, to some extent, by the self-inhibition of these enzymes by metronidazole.

In summary, diosmin pretreatment increases metronidazole concentrations in vivo, most probably by inhibition of CYP3A4 and/or CYP2C9. Based on bioequivalence criteria, the extent of effect may be of clinical relevance. Taking the metronidazole single dose setting and the fixed sequence design of this study into account, further studies are required for final judgement.

Acknowledgements The authors are thankful to University Grants Commission (UGC), New Delhi, India and Council of Scientific and Industrial Research (CSIR), New Delhi, India, for financial support.

References

1. Rajnarayana K, Reddy MS, Chaluvadi MR, Krishna DR (2001) Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. Ind J Pharmacol 33:2–16
2. Bouskela E, Donyo KA, Verbeuren TJ (1995) Effects of Daflon 500 mg on increased microvascular permeability in normal hamsters. Int J Microcirc Clin Exp15:22–26
3. Ciolino HP, Wang TT, Yeh GC (1998) Diosmin and diosmetin are agonists of the arylhydrocarbon receptor that differentially affect cytochromeP4501A1 activity. Cancer Res 58:2754–2760
4. Cova D, Angelis DL, Giavarini F, Palladini G, Perego R (1992) Pharmacokinetics and metabolism of oral diosmin in healthy volunteers. Int J Clin Pharmacol Ther Toxicol 30:29–33
5. Perego R, Beccaglia P, Angelini M, Villa P, Cova D (1993) Pharmacokinetic studies of diosmin and diosmetin in perfused rat liver. Xenobiotica 23:1345–1352
6. Edenharder R, Rauscher R, Platt KL (1997) The inhibition by flavonoids of 2-amino-3-methyl imidazo-[4,5-f]-quinoline metabolic activation to a mutagen; a structure activity relationship study. Mutat Res 379:21–32
7. Obermeier MT, White RE, Yong CS (1995) Effect of bioflavonoids on hepaticP450 activities. Xenobiotica 25:575–584
8. Suoping Z, Renke D,Fred KF, Robert EV (1998) Comparative inhibition of human cytochromeP450 1A1 and 1A2 by flavonoids. Drug Metab Dispos 26:989–993
9. Phang JM, Poore CM, Lopaczynska J, Yeh GC (1993) Flavonol stimulated efflux of 7,12-dimethyl benz-(a)-anthracene in multi drug-resistant breast cancer cells. Cancer Res 53:5977–5981
10. Sidelmann UG, Cornett C, Tjournelund J, Hansens H (1996) A comparative study of precision cut liver slices, hepatocytes, and liver microsomes from the Wistar rats using metronidazole as a model substrate. Xenobiotica 26:709–722
11. Pendland Sl, Piscitelli SC, Schrecken berger PC, Danzier LH (1994) In vitro activities of metronidazole and its hydroxy metabolite against Bacteroides Spp Antimicrob Agents Chemother 38:2106–2110
12. Galmier MJ, Frasey AM, Bastide M (1998) Simple and sensitive method for determination of metronidazole in human serum by high-performance liquid chromatography. J Chromatogr B 720:239–243
13. Bear WL, Teel RW (2000) Effect of citrus phytochemicals on liver and lung cytochrome P450 activity and on the in vitro metabolism of the tobacco specific nitrosamine NNK. Anticancer Res 20:3323–3329
14. Teel RW, Huynh H (1998) Modulation by phytochemicals of cytochrome P450-linked enzyme activity. Cancer Lett 133:135–141
15. Villa P, Cova D, De FL (1992) Protective effect of diosmin on in vitro cell membrane damage and oxidative stress in cultured rat hepatocytes. Toxicology 73:179–189
16. Reddy MS, Rajnarayana K, Chaluvadi MR, Krishna DR (2001) Bioflavonoids—their pharmacokinetics and interaction with cytochrome P450 isozymes and P-glycoprotein. Ind J Pharm Sci 63:187–195
17. Spina E, Fisani F, Perucca E (1996) Clinically significant pharmacokinetic drug interactions with carbamazepine. An update. Clin Pharmacokinet 31:198–214
18. Levy RH (1995) CytochromeP450 isozymes and antiepileptic drug interactions. Epilepsia 720:8–13