*Section: Research Paper*
*ISSN 2063-5346*

# EFFECT OF BLACK GRAPE JUICE ON TIZANIDINE PHARMACOKINETICS IN RATS

**Dipika S. Sherkar [1], Dr. Vaibhav G. Bhamre *, [2], Dr. Minal R. Narkhede, Jitesh H. Shet[3]**

1. Department of Pharmaceutics, MGV's Pharmacy College, Panchavati, Nashik, Maharashtra, India.
2. Department of Pharmaceutics, K. K. Wagh College of Pharmacy, Hirabai Haridas Vidyanagari, Panchavati, Nashik, Maharashtra, India.
3. Department of Pharmaceutics, SMBT College of Pharmacy, Dhamangaon, Nashik, Maharashtra, India.
4. Department of Pharmcology, MGV's Pharmacy College, Panchavati, Nashik, Maharashtra, India.

E-mail: vaibhav.bhamre@gmail.com

**Abstract:** Objective of this work was to study the effect of black grape juice (BGJ) on bioavailability and other pharmacokinetic parameters of tizanidine in rats. A single dose parallel design was used with 36 animals randomly divided in reference group and test group. All the rats received 7 mg tizanidine orally and in test group 10 mL-20 mL freshly prepared BGJ was co-administered with tizanidine. Nine blood samples were collected from each animal over a 24-hour period. Plasma tizanidine concentrations were determined by HPTLC using UV detection, and pharmacokinetic parameters were determined by non-compartmental method. The mean value of the peak plasma concentration (Cmax) of tizanidine increased significantly (31.51%, P value <0.001; 90% CI, 131.35% -131.71%) in animals who had given the drug with BGJ (Cmax , 45.32 ± 0.12 µg/mL) than those who had given the drug with water (Cmax, 34.46 ± 0.07 µg/mL). The area under the plasma concentration time curve from t=0 to time of the last measureable concentration (AUC0-t) was also increased significantly (104.65%, P value <0.001; 90% CI, 204.47% -204.78%). Similarly, the value of area under the concentration-time curve from t=0 to infinity (AUC0-∞) value was increased significantly (78.28%, P value <0.001; 90% CI, 177.13% -179.68%); these changes were not within the 90% CI range of 80.000 - 125.000 % which is the acceptable range of bioequivalence. Tmax, T1/2, terminal elimination rate constant (λz), CL/F value, Vd/F value, AUMC0-t and AUMC0-∞ values, MRT0-t and MRT0-∞ values and % relative bioavailability (Fr) value for test group were also determined and compared with

*Section: Research Paper*
*ISSN 2063-5346*

reference group. Form results the values of Cmax and AUC0-∞ were not within the bioequivalence acceptable range and from statistical analysis the reference and test samples were found to be bio-in-equivalent, suggesting the improved tizanidine oral bioavailability and therapeutic efficacy due to co-administration of BGJ.

**Key words:** Black grape juice, Tizanidine, Pharmacokinetics, Bioavailability.

## INTRODUCTION

Tizanidine HCl is an orally administered, centrally acting α-2 adrenergic agonist muscle relaxant. It is used to treat the spasms, cramping and tightness of muscles caused by medical problems such as multiple sclerosis, spastic diplegia. It is thought that tizanidine reduces spasticity by increasing presynaptic inhibition of motor neurons [1,2,3]. Tizanidine undergoes extensive first-pass metabolism in liver with less than 3% of unchanged parent drug excreted in urine, and the estimated absolute tizanidine bioavailability of 21% [4].The main enzyme involved in tizanidine metabolism *in vitro* is cytochrome P450 (CYP) lA2 [5]. On the basis of *in vivo* or *in vitro* evidence, inhibition of the CYP isoforms responsible for the metabolism of co-administered drugs has been proposed as the mechanism responsible for the pharmacokinetic interactions caused by flavonoids [6,7]. This inhibition may result in reduced first-pass clearance of many xenobiotics by the liver and hence result in greater delivery of drugs to extrahepatic tissues [8]. Flavonoids like quercetin and catechin are known inhibitors of CYP1A-dependent metabolism [9,10]. Crude polyphenolic extracts (seeds, pulp + skin, whole) of Indian grapes and catechin inhibited the microsome catalysed activity of cytochrome P450 isozymes (1A1, 1A2, 2B1) in a dose-dependent manner, by the decreased formation of resorufin. The inhibitory activity of grape polyphenolic extracts appears to correlate significantly with its total polyphenolic contents [11]. Black grapes contain polyphenols like Anthocyanidins, Quircetin, Kaempferol, Procyanidins, Proanthocyanidins, (+)-Catechin, Gallic acid, Caffeic acid, Rutin and Resveratrol [12,13]. Fresh grape skin contains about 50 to 100 micrograms of resveratrol per gram[14]. *In-vitro* Grape polyphenol, Quercetin is an inhibitor of CYP1A2 isoenzyme [15] while resveratrol inhibits the expression and activity of CYP 1A1/1A2 in microsomes and intact HepG2 cells [16]. Naturally occurring analogues of resveratrol were also shown to be inhibitors of CYP1A2 [16]. Moreover, resveratrol was demonstrated to inhibit human CYP1A1 and the other AhR gene battery

*Section: Research Paper*
*ISSN 2063-5346*

products, cytochromes 1B1 and 1A2 [17] by inhibiting a receptor on cells called AhR to which polycyclic aromatic hydrocarbons bind [18]. Therefore, our hypothesis is that coadministration of black grape juice with tizanidine could increase the bioavailability of tizanidine by inhibitory action of grape polyphenols on CYP1A2 mediated phase I metabolism of tizanidine.

## MATERIALS AND METHODS

### Materials:

Tizanidine HCl was obtained as a gift sample from Blue Cross Pvt. Ltd. Nashik. Sonaka Black grapes were obtained from local commercial sources and were stored at 40C until use. Fruit juice was obtained by crushing whole grapes, and the juice was filtered to remove the residues. AR grade toluene, methanol and acetone were obtained from Merck Chemicals, India and ammonia liquor was obtained from Qualigens Fine Chemicals, India.

### Subjects:

Wistar rats of either sex weighing between 200-250 g were purchased from Bharat Serums & Vaccines Ltd., Thane. The study protocol was approved by the institutional Animal Ethics Committee of MGV and animals were maintained under standard conditions in an animal house (M.G.V.'s Pharmacy College, Panchavati, Nashik-03.)

### Study design:

A total of 36 healthy wistar rats of either sex were selected for studies. For noncompartmental pharmacokinetic analysis single-dose parallel study design was selected. Study involves two groups reference group and test group. Both groups receive 7 mg tizanidine orally and in test group black grape juice (10 mL-20 mL) was co-administered with tizanidine. Animals were fasted from 12 hr before the commencement of study and through the complete study but allowed to drink water. Freshly prepared black grape juice had been used in this study.

### Blood sampling:

Animals were anesthetized and blood samples were drawn from retro-orbital plexus of rats using fine bored glass capillary. Blood samples were drawn (1.5 mL) ½ hr before the oral drug

*Section: Research Paper*
*ISSN 2063-5346*

administration and ½, 1, 2, 3, 4, 8, 12, 24 hr after the drug administration. After each withdrawal, animals were replenished with same amount of dextrose normal saline. Blood samples were collected into micro-centrifuge tubes containing EDTA. Blood samples were centrifuged at 5000 rpm for 15 min and plasma was separated within 30 min after blood sampling and stored at -20°C till the time of analysis.

**Extraction procedure:**

Liquid phase extraction procedure was used for extraction of tizanidine from plasma samples for HPTLC analysis. To a fix quantity of plasma (0.5 mL), 100 µL acetonitrile was added; the mixture was shaken and allowed to stand for 5 min. Free drug was extracted with methanol by vortexing followed by centrifugation at 1000 rpm for 10 min. Organic layer was separated and evaporated carefully on water bath. After cooling residues were reconstituted with 1 mL methanol and these were used as sample solutions for further analysis [19].

**Determination of plasma drug concentration:**

Plasma tizanidine concentrations were quantified by HPTLC system. The samples were spotted in the form of bands of width 6 mm with a Camag microlitre syringe on pre-coated silica gel aluminium plate 60F254 (20 cm×10 cm with 250 µm thickness) using a Camag Linomat IV. A constant application rate was 0.1 µL/s with bandwidth of 5 mm. Optimized mobile phase (12mL) consisted of toluene: acetone: ammonia (6:6:0.4 v/v/v) was used. Linear ascending development was carried out in twin trough glass chamber saturated with the mobile phase for 10 min at room temperature. The length of chromatogram run was 7 cm. Subsequent to the development; TLC plates were dried in a current of air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner 3 with Deuterium lamp and Wincats 1.4.2.81 software in the absorbance mode at 254 nm. The linearity of Tizanidine ($r2=0.991\pm0.001$) in plasma is found in range of 300–1100 ng/spot. Standard curve for plasma tizanidine concentrations range from 30-110 µg/ml with a mean correlation coefficient of $0.991\pm0.001$ shows acceptable linearity, precision and accuracy. Intraday and interday precision is less than 15 % RSD while average % recovery is found to be 99.72 with average % RSD 0.4865. The specificity of the HPTLC method for tizanidine was determined by spiking the tizanidine plasma sample with internal

*Section: Research Paper*
*ISSN 2063-5346*

standard. No interferences with the measurement of tizanidine by plasma constituents or internal standard were observed [19].

**Pharmacokinetic analysis:**

The pharmacokinetics of tizanidine was characterized by plasma Cmax, Tmax, AUC0-t, AUC0-∞, T1/2(z), λz, Vd/F, CL/F, AUMC0-t, AUMC0-∞, MRT0-t, MRT0-∞ and Fr values for reference as well as both the test groups. The pharmacokinetic calculations were performed with the "R software using bear version 2.5.3" developed by Hsin-ya Lee & Yung-jin Lee (Kaohsiung Medical University, Taiwan). Noncompartmental analysis (NCA) approach is used to compute AUCs and the terminal elimination rate constants λz for drug plasma concentration. The linear trapezoidal method is applied to calculate AUC (time 0 to the last measurable Cp). The extrapolated AUC (from time of the last measurable Cp to time infinity) is equal to the last measurable Cp divided by λz. λz is calculated using the Adjusted R Square method that excludes data point of Tmax and Cmax[20].

**Statistical analysis:**

Values were expressed as mean ± SD. Average bioequivalance data was analyzed from NCA using ANOVA for parallel study. The pharmacokinetic variables between the groups like AUCs and Cmax were compared by the ANOVA followed by Welch Two Sample t-test (% T/R); TOST and Anderson-Hauck Test to analyze statistical significance. The differences were considered statistically significant when $P < 0.05$. In both studies 90% CI were calculated for the mean differences of selected variables of the test and reference products. Bioequivalence acceptance criterion was set within the range of 80.000 - 125.000 % [20].

**RESULTS**

**Effect of Black grape juice on Tizanidine Pharmacokinetics in Rats**

The time-course of plasma tizanidine is shown in Figure 1. As per the figure, the bioavailability of tizanidine was remarkably improved when it was co-administered with black grape juice. Effect of black grape juice on the pharmacokinetic parameters of tizanidine is given in Table 1. There was significant increase in peak plasma concentration Cmax, AUC0-t, and AUC0-∞ by

*Section: Research Paper*
*ISSN 2063-5346*

31.51% (P<0.001), 104.65% (P<0.001) and 78.28% (P<0.001) respectively. When Tizanidine was coadministered orally with black grape juice marked decrease was observed in Tizanidine Tmax value from 1.5 hr to 1 hr and T1/2(z) value by 68.09% as compared to reference. At the same time terminal elimination rate constant (λz) of tizanidine was increased by 217.95% as compared to reference. In black grape juice co-administered group CL/F value and Vd/F value of Tizanidine were decreased as compared to reference by 43.95% and 82.17% respectively. AUMC0-t and AUMC0-∞ values were increased by 113.64% and 37.76% respectively as compared to reference. MRT0-t of tizanidine was increased by 4.47% and MRT0-∞ was decreased by 22.76% as compared to reference. Fr value of tizanidine was found to be 204.65%. Table 2 shows statistical analysis summary of pivotal parameters of bioequivalence Study.

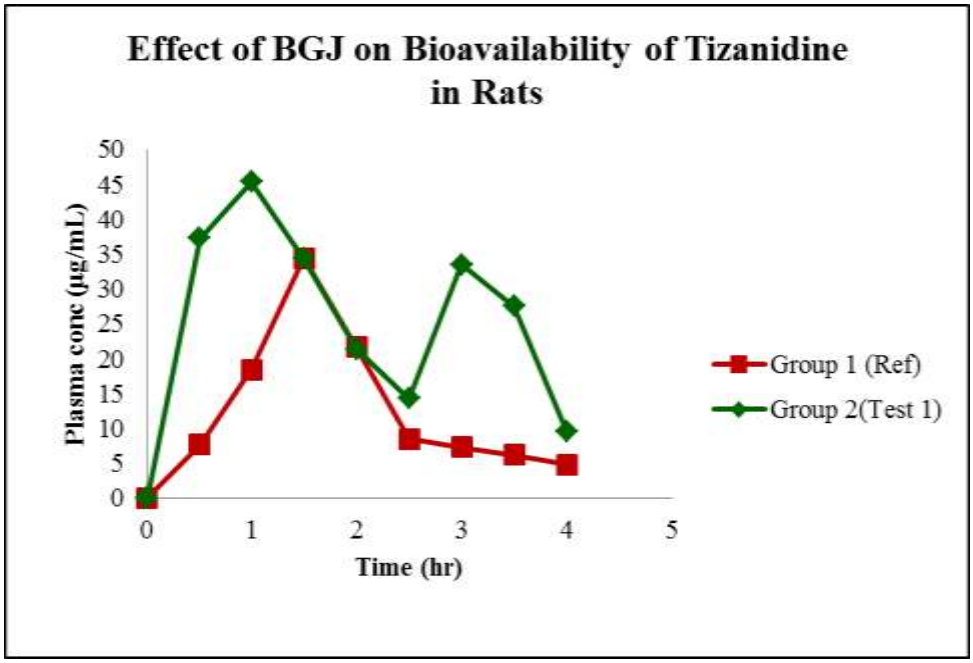


**Figure 1: Effect of BGJ on Bioavailability of Tizanidine in Rats**

| Pharmacokinetic parameters | Treatment groups | |
|---|---|---|
| | **Reference (Control)$^n$** | **Test 1(BGJ co-administrated)$^n$** |
| $C_{max}$ (µg/mL) | 34.46 ± 0.07 | 45.32 ± 0.12 |
| $T_{max}$ (hr) | 1.5 | 1 |
| $AUC_{0-t}$ (µg.hr/mL) | 53.33 ± 0.07 | 109.14 ± 0.13 |

| | | |
|---|---|---|
| $AUC_{0-\infty}$ (μg.hr/mL) | 65.56 ± 1.12 | 116.88 ± 0.30 |
| $T_{1/2}$(z) (hr) | 1.63 ± 0.18 | 0.52 ± 0.03 |
| $\lambda_z$ | 0.39 ± 0.03 | 1.24 ± 0.02 |
| $V_d$/F (mL/kg) | 269.85 ± 17.79 | 48.10 ± 0.67 |
| CL/F (mL/min) | 106.86 ± 1.9 | 59.89 ± 0.15 |
| $AUMC_{0-t}$ (μg.$hr^2$/mL) | 95.87 ± 0.20 | 204.82 ± 0.33 |
| $AUMC_{0-\infty}$ (μg.$hr^2$/mL) | 175.66 ± 9.90 | 242.00 ± 1.49 |
| $MRT_{0-t}$ (hr) | 1.79 ± 0.002 | 1.87 ± 0.002 |
| $MRT_{0-\infty}$ (hr) | 2.68 ± 0.10 | 2.07 ± 0.007 |
| Fr (%) | | 204.65 |

**Table 1: Effect of BGJ co-administration on pharmacokinetic parameters of Tizanidine in rats**

Values are mean ± SD; n= 18; Cmax: Maximum plasma concentration ; Tmax: Time to reach the peak concentration; AUC0-t: Area under the plasma concentration time curve (Time= 0 to time of the last measureable plasma concentration); AUC0-∞: Area under the plasma concentration time curve (time = 0 to infinity); T1/2(z): Terminal elimination half life; λz: Terminal elimination rate constant; Vd/F: Volume of distribution; CL/F: Total plasma clearance; AUMC0-t: Area under the first moment curve (Time= 0 to time of the last measureable plasma concentration); AUMC0-∞ : Area under the first moment curve (time = 0 to infinity); MRT0-t: Mean residence time (Time= 0 to time of the last measureable plasma concentration); MRT0-∞: Mean residence time (time = 0 to infinity); Fr: Relative bioavailability determined by comparing AUC0-t of test group I and AUC0-t of reference group.

| **Property** | **Classical 90% CIs** | **T/R (%)** | **TOST** | **Anderson-Hauck Test** |
|---|---|---|---|---|
| $lnC_{max}$ | 131.53 (131.35-131.71) | 107.74 | Upper P<0.001 Lower P<0.001 | P= 1.0000 |
| $lnAUC_{0-t}$ | 204.62 | 118.00 | Upper P<0.001 | P= 1.0000 |

| | | | | |
|---|---|---|---|---|
| | (204.47-204.78) | | Lower P<0.001 | |
| $lnAUC_{0-\infty}$ | 178.40 (177.13-179.68) | 113.84 | Upper P<0.001 Lower P<0.001 | P= 1.0000 |

**Table 2: Statistical analysis and 90% Confidence Intervals (CI) for Different Pharmacokinetic Parameters from Log-Transformed Data for Assessment of Bioequivalence.**

## DISCUSSION

Black grape juice co-administration improves oral bioavailability of tizanidine to a greater extent. This was probably by inhibiting the CYP1A2-mediated presystemic metabolism of tizanidine in liver as reported by Granfors [5]. The Cmax and AUC values of tizanidine were increased significantly, suggesting improved exposure of drug as explained by Chen et al. for improved exposure of raloxifene co administered with apigenin [21]. Revel et al. reported that resveratrol present in grapes inhibits a receptor AhR to which polycyclic aromatic hydrocarbons

bind [18]. So the increase in plasma concentration of tizanidine might be due to inhibition of this AhR by black grape polyphenolic constituents. The CL/F was found to be decreased due to black grape polyphenols. The reason behind this may be the inhibition of CYP1A2 mediated metabolism of drug in liver as suggested by Yamsani et al. [22]. Vd/F value was also found to be decreased but the reason behind this was not clear. Systemic absorption of tizanidine was found to be erratic when animals were co-administrated with black grape juice. The cause behind such irregular absorption and distribution was not exactly known. Tmax and T1/2 of tizanidine were decreased due to black grape juice co-administration. This suggested the faster onset of action as reported by Ching et al. for theophylline co-administered with curcumin [23]. The AUMC and MRT values were found to be increased indicating that tizanidine resides in its active form for longer time in body showing its therapeutic action for longer duration. In contrast to other pharmacokinetic determination the terminal elimination rate constant λz was found to be increased. The increase in elimination rate constant was may be due to increased rate of drug metabolism [24]. The percent relative bioavailability of tizanidine was found to be 204.65%

*Section: Research Paper*
*ISSN 2063-5346*

indicating greater improvement in Tizanidine oral bioavailability due to co-administration of black grape juice. This is a 2-treatment parallel single-dosed study. Log-transformed bioavailability measures were analyzed. The pivotal parameters of parallel bioequivalence studies were established using linear model (ANOVA) - statistical analysis. Here the bioequivalence acceptance criterion was set within the range of 80.000 - 125.000 % as per FDA guidelines [20]. These parameters include log transformed Cmax, AUC0-t and AUC0-∞ values of tizanidine. The classical (shortest) 90% CIs for log transformed Cmax (lnCmax), log transformed AUC0-t (lnAUC0-t) and log transformed AUC0-∞ (lnAUC0-∞) were estimated. T/R % values for each of the above parameters were calculated. Then the statistical significance of differences in pharmacokinetic parameters was determined by analyzing the data of log transformed values by TOST and Anderson-Hauck Test. The nullhypothesis was rejected at an upper significance level of 0.05 if the two one-sided tests for testing the ratio was less than 80% and greater than 125%, respectively, both were rejected at the significance level 0.05 (two one-sided test situation). It could be shown that this was equivalent to a CI for the true ratio; with confidence level 90% was entirely within the interval 80% to 125% [20]. Here classical 90% CI for lnCmax, lnAUC0-t and lnAUC0-∞ were 131.53% (131.35% - 131.71%), 204.62% (204.47%-204.78%) and 178.40% (177.13%-179.68%) respectively, which were not found to be within the interval 80% to 125%. In both of these tests at least the P values were greater than 0.05, thus we could not reject the null hypothesis. Rejection of null hypothesis concludes bioequivalence. Therefore from statistical analysis the test (Tizanidine + black grape juice) and reference (Tizanidine) were found to be bio-in-equivalent, suggesting the improved bioavailability of drug.

## CONCLUSION

Concomitant oral administration of black grape juice resulted in a significant increase in systemic tizanidine exposure after oral administration. The increased oral bioavailability of tizanidine may probably attribute to the inhibition of CYP1A2 mediated pre-systemic metabolism by oral black grape juice. These results indicated that some flavonoids present in black grape juice could enhance the absorption of co-administered drugs with low bioavailabilities, and thus enhance their therapeutic efficacy. No serious adverse events were observed during the study period and tizanidine was well tolerated in all animals. But the plasma

concentration of tizanidine if increasing drastically, it would lead to hazardous pharmacodynamic adverse effects, so we should aware the potential interaction of a drug with a narrow therapeutic index and the dose monitoring of both the drug and co-administered substance is necessary.

**REFERENCES**

1. Kawtikwar PS, Zade PS, Sakarkar DM. Formulation, Evaluation and Optimization of Fast dissolving tablet containing Tizanidine Hydrochloride. International Journal of PharmTech Research. 2009; 1(1): 34-42.

2. Wagstaff AJ, Bryson HM. Tizanidine: A review of its pharmacology, clinical efficacy and tolerability in the management of spasticity associated with cerebral and spinal disorders. Drugs 1997; 53: 435-452.

3. Dhaneshwar SR, Kaul N, Agrawal H, Kakad A, Patil B. Application of HPLC and HPTLC for the simultaneous determination of tizanidine and rofecoxib in pharmaceutical dosage form. Journal of Pharmaceutical and Biomedical Analysis 2005; 37: 27-38.

4. Lataste X, Emre M, Davis C, Groves L. Comparative profile of tizanidine in the

management of spasticity. Neurology 1994; 44(9): 553-559.

5. Granfors MT, Backman JT, LaltilaJ, Neuvonen PJ. Tizanidine is mainly metabolized by cytochrome p450 1A2 in vitro. British Journal of Clinical Pharmacology 2004; 57: 349-353.

6. Quintieri L, Bortolozzo S, Stefanostragliotto, Stefanomoro, Pavanetto M and

Albertonassi et al. Flavonoids Diosmetin and Hesperetin are Potent Inhibitors of Cytochrome P450 2C9-mediated Drug Metabolism in vitro. Drug Metabolism and Pharmacokinetics 2010; 25(5): 466-476.

7. Ahn D, Putt D, Kresty L, Stoner GD, Fromm D and Hollenberg PF. The effect of dietary ellagic acid on rat hepatic and esophageal mucosal cytochromes P450 and phase II enzymes. Carcinogenesis 1996; 17: 821-828.

8. Johanna W Lampe. Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. American Journal of Clinical Nutrition 1999; 70(3): 475S-490S.

9. Ishizuka M, Ohno M, Sakamoto KQ and Fujita S. Crude Cacao Theobroma cacao Extract Reduces Mutagenicity Induced by Benzo[a]pyrene Through Inhibition of CYP1A Activity in Vitro. Phytotherapy Research 2009; 23: 1134-1139.

10. Krishna DR, Narayana KR, Reddy MS, Chaluvadi MR. Bioflavonoids Classification, Pharmacological, Biochemical Effects and Therapeutic Potential. Indian Journal of Pharmacology 2001; 33: 2-16.

11. Ramchandani AG, Chettiyar RS and Pakhale SS. Evaluation of antioxidant and antiinitiating activities of crude polyphenolic extracts from seedless and seeded Indian grapes. Food Chemistry 2010; 119(1): 298-305.

12. Han X, Shen T and Lou H. Dietary Polyphenols and Their Biological Significance. International Journal of Molecular Science 2007; 8: 950-988.

13. Andrade ER, Piccoli JCE, Cruz IBM , Rocha JBT, Andrade VVR et al. Effect of Black Grape Juice Intake on Liver Lipoperoxidation and Body Weight Loss in Whole Body XIrradiated Rats. Journal of Medical, Chemical, Biological and Radiological Defence 2009; 7.

14. Li X, Wu B, Wang L, Li S. "Extractable amounts of trans-resveratrol in seed and berry skin in Vitis evaluated at the germplasm level". Journal of Agriculture and Food Chemistry 2006; 54(23): 8804-11.

15. Zendulka O, Zahradníková L, Juřica J and Totušek J. The Influence of Trans-resveratrol and Quercetin on the Activity of CYP1A2 in Rat. Czech Journal of Food Science 2004; 26: S60-S64.

16. Foti RS and Wahlstrom JL. The role of dietary suppliments in cytochrome P450- mediated drug interactions. Boletin Latinoamericano y del Caribe de Plantas Medicinales y Aromaticas 2008; 7(002): 66-84.

17. Mikstacka R, Gnojkowski J and Dubowska WB. Effect of natural phenols on the catalytic activity of cytochrome P450 2E1. Acta Biochimica Polonica 2002; 49(4): 917-925.

18. Revel A, Raanani H, Younglai E, Xu J, Rogers I, Han R, Savouret JF and Casper RF. Resveratrol, a natural aryl hydrocarbon receptor antagonist, protects lung from DNA damage and apoptosis caused by benzo[a]pyrene. Journal of Applied Toxicology 2003; 23(4): 255-61.

19. Sherkar DS, Bhamre VG, Narkhede MR and Nehete JY. Development and Validation of High-Performance Thin Layer Chromatographic Method for Quantitative Estimation of Tizanidine Hydrochloride in Plasma. Inventi Rapid: Pharm Ana & Qual Assur 2011; 2(1).

20. Sultana R, Ullah A, Akbor MM, Azad MAK, Latif AHMM and Hasnat A. Effect of Orange Juice on Bioavailability of Levofloxacin. The Journal of Applied Research 2008; 8(1): 34-42.

21. Chen Y, Jia X, Chen J, Wang J and Hu M. The Pharmacokinetics of Raloxifene and Its Interaction with Apigenin in Rat. Molecules 2010; 15: 8478-8487.

22. Yamsani MR, Yamsani SK, Gannu R, Devandla A, Yamsani VV, Palem CR et al. Effect of ketoconazole and java plum pretreatment on intestinal transport of buspirone across rat intestine . Acta Pharmaceutica Sciencia 2010; 52: 461-468.

23. Ching H, Tsai SY, Hsiu SL, Wu PP and Chao PDL. Effect of Curcumin on Theophylline Pharmacokinetics in Rabbits. Journal of Chinese Medicine 2001; 12(1): 51-59.

24. Helleberg L, Rubin A, Wolen RL, Rodda BE, Ridolfo AS and Gruber CM. A Pharmacokinetic Interaction in Man between Phenobarbitone and Fenoprofen, A New Anti-Inflammatory Agent. British Journal of clinical Pharmacology 1974; 1: 371-374.