Effect of Co-administration of Single and Multiple Doses of Piperine on the Pharmacokinetics of Tamoxifen in Rats

Suresh[a] P.S., Dilip[b] S., Balamuralidhara[b] V. G., Umesh[a] T, Rajnish[a] K., Purushottam[a] D, Zainuddin[a] M, Bhamidipati[a] R. K., Sriram[a] R., Mullangi R[a]*

[a] *Jubilant Biosys, Industrial Suburb, Yeshwanthpur, Bangalore, India*
[b] *J.S.S College of Pharmacy, Mysore, India*

Received: November 15th, 2016; Accepted: April 04th, 2017

The aim of this study was to investigate the effect of single and multiple oral administration of piperine on the pharmacokinetics of tamoxifen in rats. Tamoxifen was administered orally alone at 10 mg/kg or with singles (10, 25 and 50 mg/kg) or multiple (10 mg/kg) oral doses of piperine. In the present study, compared with the control group (tamoxifen alone) and piperine single and multiple dose administration showed significant increase in $AUC_{0-\infty}$ and $t_{1/2}$. The relative bioavailability of tamoxifen in the piperine treated groups was 2.15-2.39 times higher compared to control group. The present study revealed that piperine significantly improved bioavailability of tamoxifen and the influence of piperine on the pharmacokinetics of tamoxifen may be attributed to the inhibition of metabolism of tamoxifen.

Keywords: Tamoxifen, piperine, pharmacokinetics, drug interaction, rats

1. Introduction:

Tamoxifen, chemically (*Z*)-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]-*N*-*N*-dimethylethanamine is a selective estrogen receptor modulator (Furr and Jordan, 1984) and it is used in the treatment of metastatic breast cancer. Tamoxifen inhibits estrogen activity by binding to the estrogen receptors (Wang and Yin 2015). It is an approved drug for the treatment of metastatic breast cancer. The main adverse effects of tamoxifen in humans are increased risk of endometrial cancer and thromboembolic diseases (Fornander *et al.* 1993; Meier and Jick 1998). It is mainly metabolized by CYP3A4 into its major metabolite i.e. *N*-desmethyltamoxifen, through N-demethylation. Other minor metabolite, 4-hydroxytamoxifen is catalysed mainly by CYP3A4, 2D6, 2E1 and 2C9 (Jacolot *et al.* 1991; Mani *et al.* 1993; Crewe *et al.* 1997). The piperine (*Piper longum* and *Piper nigrum*) is an alkaloid, a major constituent of black pepper (Wu *et al.* 2004; Srinivasan 2007). Piperine has been demonstrated to antihypertensive, hepatoprotective, antioxidant, anti-thyroid, antitumor and anti-platelet activities (Koul and Kapil 1993; Khajuria *et al.* 1997; Lee *et al.* 1997).

*Corresponding author. E-mail: mullangi_ramesh@jubilantinnovation.com

Piperine is used as a bioenhancer and in several studies it ~~was~~ has been demonstrated to increase the bioavailability of several drugs (rifampicin, phenytoin, sulfadiazine, resveratrol, fexofenadine, tetracycline, propranolol, theophylline, linarin, emodin, ciprofloxacin and β-lactam antibiotics etc) (Feng *et al.* 2014; Balkrishna and Yogesh, 2002; Wadhwa *et al.* 2014) and other pharmacological active substances (curcumin, resveratrol, epigallocatechin-3-gallate etc) (Shoba *et al.* 1995; Di *et al.* 2015). We have also reported that piperine co-administration increases the plasma concentrations of ibuprofen and nateglinide, which translated into increased pharmacological activity of these two drugs (Venkatesh *et al.* 2011; Sama *et al.* 2012). Several mechanisms of piperine's bioavailability enhancement action have been postulated, including the increase in blood supply to the gastrointestinal tract, decreasing gastrointestinal emptying, inhibiting gut and hepatic metabolizing enzymes and inhibition of P-gp (Atal *et al.* 1985; Reen *et al.* 1993; Bajad *et al.* 2001; Han *et al.* 2008).

Tamoxifen known as a substrate for CYPs has an oral bioavailability, which seems to be affected by first-pass metabolism in intestine and liver. As a dual inhibitor of CYPs and P-gp, piperine might alter the pharmacokinetics of tamoxifen when co-administered. The aim of the present study was to investigate the effect of co-administration of single (at different doses) or multiple dose administration of piperine on the pharmacokinetics of tamoxifen in rats. To date many researchers have reported the bioenhancing effect of piperine when it was co-administered with several drugs known as substrates of CYPs and/or P-gp but to the best of our knowledge this is the first report evaluating the bioenhancing effect on multiple dose administration of piperine.

2. Materials and methods:

2.1. Chemicals and Reagents

Tamoxifen (purity >99%) was a gift sample from Dr. Reddy's Laboratories, Hyderabad, India. The piperine (purity >99%) was isolated from dried unripe fruits of *P. nigrum* (Sama *et al.* 2012). Ethylenediaminetetracetic acid disodium salt (Na_2.EDTA) and phenacetin (internal standard, IS) were purchased from S.D. Fine Chemicals, Mumbai, India. Dimethyl sulfoxide was purchased from Sigma Chemicals, Mumbai, India. Carboxy methyl cellulose was purchased from S.D. Fine Chemicals, Mumbai, India. HPLC grades acetonitrile and methanol were purchased from J.T Bakers, Avantor Performance materials, PA, USA. Analytical grade isoflurane was purchased from Abbott Laboratories, IL, US. All other chemicals/reagents were of research grade and used without further purification.

2.2. HPLC operating conditions

A Shimadzu VP (Shimadzu, Japan) LC system equipped with degasser (G1379A), quaternary pumps (10ADvp), column oven (CTO-10ASvp), auto-sampler (SIL-HTC) along with system controller (SCL-10Avp) was used to inject 2 µL aliquots of the processed samples on a Atlantis dC18 column (50 x 4.6 mm, 3 µm; Waters, NY, the USA), which was kept at ambient temperature. A gradient mobile

phase (0.2% formic acid:acetonitrile) was filtered through a 0.45 µm membrane filter (XI5522050) (Millipore, USA or equivalent) and then degassed ultrasonically for 5 min was delivered at a flow rate of 0.90 mL/min into the mass spectrometer electro spray ionization chamber.

2.3. Mass spectrometry operating conditions

Quantitation was achieved by MS/MS detection in positive ion mode for tamoxifen, piperine and IS using an MDS Sciex (Foster City, CA, USA) API-4000 Q-Trap mass spectrometer, equipped with a Turboionspray™ interface at 400°C. The common parameters viz., curtain gas, GS1 gas and GS2 gas were set at 30, 45 and 35 L/min, respectively, whereas the CAD gas was set at medium. The compound parameters viz., declustering potential (DP), collision energy (CE), enhance potential (EP) and collision exit potential (CXP) for tamoxifen, piperine and IS were 66, 47, 10, 12 V; 51, 39, 10, 12 V and 36, 29, 10, 6 V, respectively. Detection of the ions was performed in the multiple reaction monitoring (MRM) mode, monitoring the transition of the *m/z* 372 precursor ion to the *m/z* 72.0 product ion for tamoxifen, *m/z* 286 precursor ions to the *m/z* 115 product ion for piperine and *m/z* 180 precursor ions to the *m/z* 110 product ion for IS. Quadrupole Q1 and Q3 were set on unit resolution. The retention times of tamoxifen, piperine and IS were ~2.0, 1.6 and 1.9 min, respectively. The total run time is 3.5 min. The analytical data were processed by Analyst software (version 1.5.2).

2.4. Animal experiments and drug administration

All animal experiments were approved by the Jubilant Biosys Institutional Animal Ethics Committee, Bangalore, India (IAEC/JDC/2012/27) and were in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Environment, Government of India. Male Sprague Dawley rats (*n*=20, weighing 202-210 g) were procured from Vivo Biotech, Hyderabad, India. The animals were housed in Jubilant Biosys animal house facilities in temperature and humidity-controlled room with a 12:12 h light:dark cycles, had free access to rodent feed (Altromin Spezialfutter GmbH & Co. KG., Im Seelenkamp 20, D-32791, Lage, Germany) and water for one week before using for experimental purpose. For all the experimental work, animals were kept for ~12 h overnight fasting and during this time they were allowed to take water *ad libitum*. Feed was provided 2 h post-dose of tamoxifen and/or piperine administration and water were allowed *ad libitum*. Overnight fasted animals were divided into five groups (*n*=4/group). Group I animals received tamoxifen alone at10 mg/kg. Animals of Group II to Group IV received piperine orally at 10, 25 and 50 mg/kg along with 10 mg/kg of tamoxifen. Group V animals received piperine at 10 mg/kg once daily by oral route for 7 consecutive days and on day 7 along with piperine animals received tamoxifen 10 mg/kg. Both tamoxifen and piperine were administered to animals as a fine suspension (prepared using 20 µL of Tween-80 + 0.5% methyl cellulose). Serial blood samples (~100 µL) were collected under isoflurane anesthesia from the retro-orbital plexus into polypropylene tubes containing Na_2.EDTA solution (100 µL per mL blood) as an anti-coagulant at pre-dose, 0.25,

0.5, 1, 2, 4, 8, 10 and 24 h. Plasma was harvested by centrifuging the blood using Biofuge (Hereaus, Germany) at 1760 *g* for 5 min and stored frozen at -80 ± 10°C until analyzed by LC-MS/MS.

2.5. Plasma samples analysis

A simple protein precipitation process was followed for extraction of tamoxifen and piperine from rat plasma. To an aliquot of plasma (50 µL), IS solution (10 µL of 50 ng/mL) was added and mixed for 15 sec on a cyclomixer (Remi Instruments, Mumbai, India). After the addition of 200 µL of acetonitrile, the mixture was vortexed for 2 min; followed by centrifugation for 10 min at 3200 rpm on Multifuge 3SR (Heraus, Germany). The supernatant was separated and 2 µL was injected onto LC-MS/MS systems. The linearity range was 1.62-2030 and 5.33-3045 ng/mL for tamoxifen and piperine, respectively. In-study quality control (QC) samples, supplemented with concentrations of 4.87, 974 and 1624 ng/mL of tamoxifen and 16.0, 1446 and 2436 ng/mL of piperine, were analysed with the unknown test samples.

For plasma sample analysis of both tamoxifen and piperine, the criteria for acceptance of the analytical runs encompassed the following: (i) 67% of the QC samples accuracy must be within 85-115% of the nominal concentration (ii) 50% of each QC concentration level must meet the acceptance criteria. Following completion of the analysis both the linearity and quality control samples values were found to be within the accepted variable limits.

2.6. Pharmacokinetic analysis

Plasma concentration-time data of tamoxifen and piperine was analyzed by non-compartmental method using Phoenix Version 1.3 (Pharsight Corporation, Mountain View, CA).

The relative bioavailability of tamoxifen was calculated as follows:

$$\text{Relative bioavailability (\%RB)} = \frac{AUC_{tamoxifen+piperine}}{AUC_{tamoxifen}} \times 100$$

Data was expressed as the mean ± S.D (standard deviation). The p-value <0.05 was considered significant.

3. Results:

3.1. Effects of a single dose of 10 or 25 or 50 mg/kg piperine on tamoxifen pharmacokinetics

The mean plasma concentration-time profiles of tamoxifen in rats, which received tamoxifen alone (10 mg/kg) or up on co-administration of single oral administration (10, 25 and 50 mg/kg) of piperine are shown in Fig. 1. The corresponding pharmacokinetic parameters are shown in Table 1. The presence of piperine significantly altered the pharmacokinetic parameters of tamoxifen. Compared with the control group (tamoxifen alone), the co-administration of piperine at different doses viz., 10, 25 and 50 mg/kg significantly ($p < 0.05$) increased the area under the plasma concentration-time curve from zero to infinity ($AUC_{0-\infty}$ - 3648, 3925 and 4048 ng*h/mL versus 1697 ng*h/mL; Group II, Group III

and Group IV versus Group I) and terminal half-life ($t_{1/2}$) of tamoxifen. However, the, time to reach maximum plasma concentration (T_{max}) and mean residence time (MRT) were not different between the treatment groups. The relative bioavailability (%RB) of tamoxifen was increased by 2.15, 2.31 and 2.39-fold up on co-administration of piperine orally at 10, 25 and 50 mg/kg, respectively.

3.2. Effects of multiple dose of 10 mg/kg (for 7 consecutive days) piperine on tamoxifen pharmacokinetics

The mean plasma concentration-time profiles of tamoxifen in rats, which received tamoxifen alone (10 mg/kg) or up on multiple oral administration of 10 mg/kg of piperine for 7 consecutive days are shown in Fig. 2. The corresponding pharmacokinetic parameters are shown in Table 1. The repeated administration of piperine significantly altered the pharmacokinetic parameters of tamoxifen. Compared with tamoxifen alone group, the repeated administration of piperine at 10 mg/kg for 7 days significantly ($p < 0.05$) increased $AUC_{0-\infty}$ (3256 ng*h/mL versus 1697 ng*h/mL; Group V versus Group I) and $t_{1/2}$ of tamoxifen. However T_{max} and MRT did not change significantly. Relative bioavailability of tamoxifen was increased by 1.9-fold on repeated oral administration of piperine for 7 days.

Discussion:

Tamoxifen majorly undergoes metabolism by CYP3A4 and 2D6 (Jacolot *et al.* 1991; Mani *et al.* 1993; Crewe *et al.* 1997). Inhibition of this CYP mediated metabolism of tamoxifen might improve the oral bioavailability of this compound. It is well documented that piperine is a bioenhancer and in several studies piperine has increased bioavailability of several drugs by inhibiting CYPs and/or P-gp mediated efflux (Feng *et al.* 2014; Di *et al.* 2015). Previously, we have also reported increase in oral bioavailability of nateglinide and ibuprofen up on co-administration of piperine in rats (Venkatesh *et al.* 2011; Sama *et al.* 2012). In the current investigation, we have evaluated the effect of single or multiple administration of piperine on the oral pharmacokinetics of tamoxifen in rats to assess the potential for drug-drug interaction between these compounds.

The presence of piperine single (10, 25 and 50 mg/kg) or multiple (10 mg/kg for 7 days) demonstrated significant increase the $AUC_{o-\infty}$ tamoxifen without much influence on C_{max} values (Table 1). This finding is probably indicating that co-administration of piperine had resulted in the inhibition of CYP mediated metabolism of tamoxifen, which is evident from significant increase in plasma $AUC_{o-\infty}$ and in the extension of its plasma half-life. The change in the slope of the elimination profile (Fig. 1 and 2) on co-administration of single and multiple doses of piperine clearly indicate saturation in the clearance mechanism(s) of tamoxifen. Flavonoids like quercetin, morin, naringin, silybinin and natural phenol like curcumin were reported increasing the $AUC_{o-\infty}$ and C_{max} of tamoxifen. The fold increase in C_{max} and $AUC_{o-\infty}$ with these flavonoids and curcumin ranged between 1.15 to 1.95 and 1.11

to 2.88, respectively (Shin *et al.* 2006; Shin *et al.* 2008; Choi *et al.* 2008; Kim *et al.* 2010; Cho *et al.* 2012). On the other hand, kaempferol, a known inhibitor of both CYP3A and P-gp did not alter the pharmacokinetics of tamoxifen (Piao *et al.* 2008). The findings of our study are in consistent with these reported studies (Shin *et al.* 2006; Shin *et al.* 2008; Choi *et al.* 2008; Kim *et al.* 2010; Cho *et al.* 2012).

Conclusions:

In summary, piperine co-administration (single or multiple dose) significantly enhance the bioavailability of tamoxifen probably via inhibition of CYP mediated metabolism. If the results obtained from the rat model are confirmed in clinical trials, tamoxifen dose should be adjusted for potential drug interactions when tamoxifen is used with piperine or piperine-containing dietary supplements.

Table 1. Mean ± S.D pharmacokinetic parameters of tamoxifen up on co-administration of single and multiple doses of piperine

Group	Treatment	Dose (mg/kg)	$AUC_{0-\infty}$ (ng*h/mL)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	RB (%)
I	Tamoxifen	---	1697 ± 495	181 ± 56.4	3.00 ± 1.15	4.40 ± 1.11	100
II	Tamoxifen + piperine*	10 + 10	3648 ± 470	217 ± 43.8	4.00 ± 0.00	12.6 ± 1.68	215
III	Tamoxifen + piperine*	10 + 25	3925 ± 432	224 ± 36.7	4.00 ± 0.00	6.82 ± 0.88	231
IV	Tamoxifen + piperine*	10 + 50	4048 ± 713	231 ± 57.5	3.00 ± 1.15	12.1 ± 2.12	239
V	Tamoxifen + piperine**	10 + 10	3256 ± 504	256 ± 65.8	3.00 ± 0.00	10.2 ± 1.12	191

*piperine single dose; **piperine multiple dose; $AUC_{0-\infty}$: area under the plasma concentration-time curve from 0 h to infinity; C_{max}: peak plasma concentration; T_{max}: time to reach C_{max}; $t_{1/2}$: terminal half-life; RB: relative bioavailability.

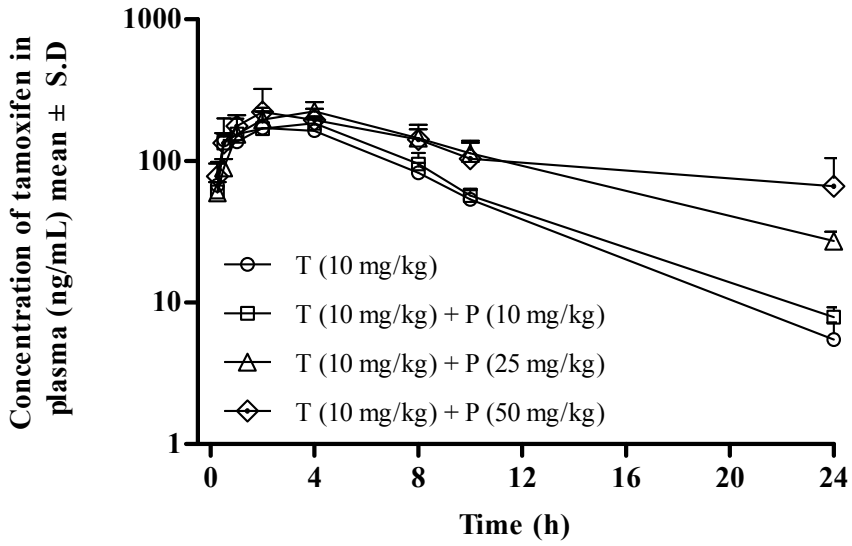

Figure 1. Mean plasma concentration-time profiles of tamoxifen after the oral administration of tamoxifen (10 mg/kg) with or without piperine to rats. Bars represent the standard deviation (n = 4). (O) Tamoxifen 10 mg/kg; (□) Tamoxifen co-administered with piperine 10 mg/kg; (Δ) Tamoxifen co-administered with piperine 25 mg/kg; (◊) Tamoxifen co-administered with piperine 50 mg/kg. T: tamoxifen; P: piperine.

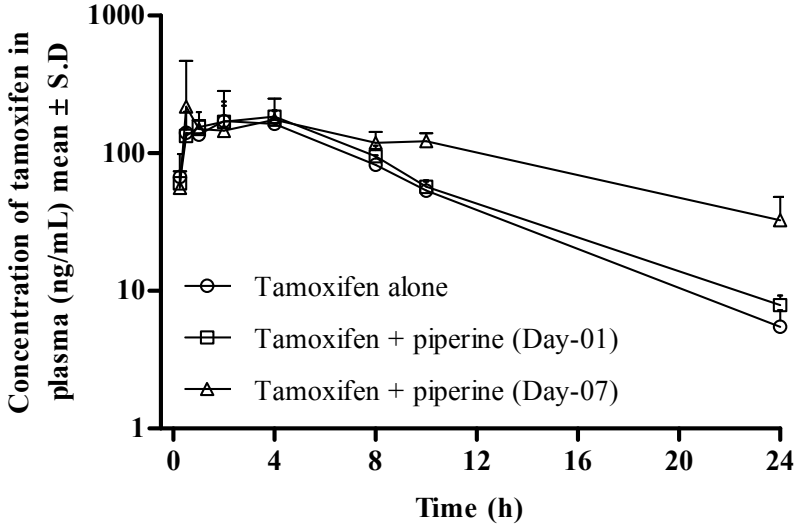

Figure 2. Mean plasma concentration-time profiles of tamoxifen after the oral administration of tamoxifen (10 mg/kg) with piperine single- and multiple-dose administration to rats. Bars represent the standard deviation (n = 4). (□)Tamoxifen 10 mg/kg; (□) Tamoxifen co-administered with piperine single dose; (Δ) Tamoxifen co-administered with piperine multiple dose.

Competing interests:

The authors have no conflicts of interest relevant to the ideas and/or contents of the manuscript.

References

Atal C. K., Dubey R. K., Singh J. (1985). Biochemical basis of enhanced drug bioavailability by piperine: Evidence that piperine is a potent inhibitor of drug metabolism. *J. Pharmacol. Exp. Ther.,* 232(1), pp258-262.

Bajad S., Bedi K. L., Singla A. K., Johri R. K. (2001). Piperine inhibits gastric emptying and gastrointestinal transit in rats and mice. *Planta Med.,* 67(3), pp176-179.

Balakrishna B. S., Yogesh P. V. (2002). Influence of co-administration of piperine on pharmacokinetic profile of ciprofloxacin. *Indian Drugs,* 39(3): pp166-168.

Cho Y. A., Lee W., Choi J. S. (2012). Effects of curcumin on the pharmacokinetics of tamoxifen and its active metabolite, 4-hydroxytamoxifen, in rats: possible role of CYP3A4 and P-glycoprotein inhibition by curcumin. *Pharmazie,* 67(2), pp124-130.

Choi J. S., Kang K. W. (2008). Enhanced tamoxifen bioavailability after oral administration of tamoxifen in rats pretreated with naringin. *Arch. Pharm. Res.,* 31(12): pp1631-1636.

Crewe H. K., Ellis S. W., Lennard M. S., Tucker G. T. (1997). Variable contribution of cytochromes P450 2D6, 2C9 and 3A4 to the 4-hydroxylation of tamoxifen by human liver microsomes. *Biochem. Pharmacol.,* 53(2): pp171-178.

Di X., Wang X., Di X., Liu Y. (2015). Effect of piperine on the bioavailability and pharmacokinetics of emodin in rats. *J. Pharm. Biomed. Anal.,* 115, pp144-149.

Feng X., Liu Y., Wang X., Di X. (2014). Effects of piperine on the intestinal permeability and pharmacokinetics of linarin in rats. *Molecules,* 19(5), pp5624-5633.

Fornander T., Hellström A. C., Moberger B. (1993). Descriptive clinicopathologic study of 17 patients with endometrial cancer during or after adjuvant tamoxifen in early breast cancer. *J. Natl. Cancer Inst.,* 85(22): pp1850-1855.

Furr B. J., Jordan V. C. (1984). The pharmacology and clinical uses of tamoxifen. *Pharmacol. Ther.,* 25(2): pp127-205.

Han Y., Tan T. M. C., Lim L. Y. (2008). In vitro and in vivo evaluation of the effects of piperine on P-gp function and expression. *Toxicol. Appl. Pharmacol.,* 230(3), pp283-289.

Jacolot F., Simon I., Dreano Y., Beaune P, Riche C., Berthou F. (1991). Identification of the cytochrome P450 IIIA family as the enzymes involved in the N-demethylation of tamoxifen in human liver microsomes. *Biochem. Pharmacol.,* 41(12), pp1911-1919.

Khajuria A., Thusu N., Zutshi U., Bedi K.L. (1997). Antioxidant potential of piperine on oxidant induced alterations in rat intestinal lumen. *Ind. Drugs,* 34(10), pp557-563.

Kim C. S., Choi S. J., Park C. Y., Li C., Choi J. S. (2010). Effects of silybinin on the pharmacokinetics of tamoxifen and its active metabolite, 4-hydroxytamoxifen in rats. Anticancer Res., 30(1), pp79-85.

Koul I. B., Kapil A. (1993). Evaluation of the liver protective potential of piperine, an active principle of black and long peppers. *Planta Med.,* 59(5), pp413-417.

Lee E. B., Shin K. H., Woo W.S. (1984). Pharmacological study on piperine. *Arch. Pharmacol. Research,* 7(2), pp127-132.

Mani C., Gelboin H. V., Park S. S., Pearce R., Parkinson A., Kupfer D. (1993). Metabolism of the antimammary cancer antiestrogenic agent tamoxifen. I. Cytochrome P-450-catalyzed N-demethylation and 4-hydroxylation. *Drug Metab. Dispos.,* 21(4), pp645-656.

Meier C. R. Jick H. (1998). Tamoxifen and risk of idiopathic venous thromboembolism. *Br. J. Clin. Pharmacol.,* 45(6), pp608-612.

Piao Y., Shin S. C., Choi J. S. (2008). Effects of oral kaempferol on the pharmacokinetics of tamoxifen and one of its metabolites, 4-hydroxytamoxifen, after oral administration of tamoxifen to rats. *Biopharm. Drug Dispos.,* 29(4), pp245-249.

Reen R. K., Jamwal D. S., Taneja S. C., Koul J. L., Dubey R. K., Wiebel F. J., Singh J. (1993). Impairment of UDP-glucose dehydrogenase and glucuronidation activities in liver and small intestine of rat and guinea pig in vitro by piperine. *Biochem. Pharmacol.,* 46(2), pp229-238.

Sama V., Nadipelli M., Yenumula P., Bommineni M. R., Mullangi R. (2012). Effect of piperine on antihyperglycemic activity and pharmacokinetic profile of nateglinide. *Arzneimittelforschung,* 62(8), pp384-388.

Shin S. C, Choi J. S, Li X. (2006). Enhanced bioavailability of tamoxifen after oral administration of tamoxifen with quercetin in rats. *Int. J. Pharm.,* 313(1-2), pp144-149.

Shin S. C., Piao Y. J., Choi J. S. (2008). Effects of morin on the bioavailability of tamoxifen and its main metabolite, 4-hydroxytamoxifen, in rats. *In Vivo,* 22(3): pp391-395.

Shoba G., Joy D., Joseph T., Majeed M., Rajendran R., Srinivas P. S. (1998). Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Med.,* 64(4), pp353-356.

Srinivasan K. (2007). Black pepper and its pungent principle-piperine: a review of diverse physiological effects. Crit. Rev. Food. Sci. Nutr., 47(8), pp735-748.

Venkatesh S., Durga K. D., Padmavathi Y., Reddy B. M., Mullangi R. (2011). Influence of piperine on ibuprofen induced antinociception and its pharmacokinetics. *Arzneimittelforschung,* 61(9), pp506-509.

Wadhwa S., Singhal S., Rawat S. (2014). Bioavailability enhancement by piperine: a review. *Asian J. Biomed. Pharmaceutical Sci.,* 4(36), pp1-8.

Wang Z. Y., Yin L. (2015). Estrogen receptor alpha-36 (ER-α36): a new player in human breast cancer. *Mol. Cell Endocrinol.,* 418(3), pp193-206.

Wu S., Sun C., Pei S., Lu Y., Pan Y. (2004). Preparative isolation and purification of amides from the fruits of Piper longum L. By upright counter-current chromatography and reversed phase liquid chromatography. *J. Chromatogr. A,* 1040(2), pp193-204.

Arabian Journal of Medicinal and Aromatic Plants

www.ajmap.org ISSN 2458-5920