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Role of herbal bioenhancers (Piperine and Curcumin) on the oral bioavailability of Tamoxifen using experimental rats

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Abstract
Tamoxifen is an anticancer drug which has very low oral bioavailability. It has been reported in the literature that the various P-gp and CYP3A4 enzyme inhibitors were co administered with drug to enhance their oral bioavailability. In the present report, the effect on the bioavailability of tamoxifen when co-administerd with herbal bioenhancers such as curcumin and piperine has been studied in experimental animals. The various pharmacokinetic parameters from different blood samples were determined using HPLC. It has been found that the co administration of selected bioenhancers significantly enhanced the oral bioavailability of tamoxifen.

Keywords: Tamoxifen, herbal bioenhancers, oral bioavailability

1. Introduction
Most of the world population used the plants and plant based medicines for the prevention and treatment of the various diseases. The modern pharmacopoeias also included the monographs of herbal medicines to ensure the efficacy and safety of herbal medicines. The main hurdle in the popularization of the herbal medicines is their low bioavailability on oral administration. The permeability of drug molecules is low mainly due to less water solubility and efflux by P-glycoprotein [1]. The improvement in the bioavailability of phytoconstituents has been the main issue for the formulation scientists. It has been achieved by the various approaches such as development of prodrugs, inclusion of absorption enhancers and development of novel delivery system like liposomes and self emulsifying system etc. [2]. The application of P-gp inhibitors in improving peroral drug delivery has been of special interest to the researchers [3]. Co-administration of the herbal bioenhancers can improve the bioavailability of drug and is generally regarded as safe approach. It is also evident from the concept of ayurveda in which black pepper has been included in the multi drug formulations as bioenhancer [4].
Tamoxifen is a nonsteroidal antiestrogen and is the agent of choice for treating and preventing breast cancer. The orally administered tamoxifen is metabolized by CYP3A4 both in the human liver and intestine and the absorption of tamoxifen in intestinal mucosa is inhibited by the P-gp efflux pump. Both CYP3A4, the major phase 1 drug metabolizing enzyme in human, and the multidrug efflux pump, P-gp are present at high levels in the small intestine, the primary site of absorption of orally administered drugs. Moreover these proteins have a broad substrate overlap, suggesting that they act synergistically during the first pass metabolism [5-6]. Hence dual inhibitors against both CYP3A4 and P-gp should have a great impact on the bioavailability of tamoxifen.
Since piperine and curcumin are the major inhibitors of CYP enzyme family and P-glycoproteins and has been used in past as the potent herbal bioenhancers against CYP and P-gp inhibited drugs [7-10]. We investigated the influence of piperine and curcumin, the naturally occurring phytoconstituents, on the bioavailability of tamoxifen in experimental animals (rats).

2. Material and Methods
Tamoxifen was procured as gift sample from Ar-Ex Laboratories Pvt. Ltd., Mumbai. Methanol was purchased from Loba Chemie. Heptane sulphonate was purchased from S D Fine Chem Ltd. Sodium dihydrogen orthophosphate was purchased from Loba Chemie Pvt. Ltd., India. All other reagents were of HPLC grade.

2.1 Collection and authentication of crude drug
The fruits of *Piper nigrum* (Piperaceae) and rhizomes of *Curcuma longa* (Zingiberaceae) were collected from local market of Rohtak, Haryana and were authentified by Dr. H.B. Singh, Chief scientist and Head, Raw Materials Herbarium and Museum (RHMD), NISCAIR, New Delhi. A voucher specimen of both the samples was deposited in department for further reference.

2.2 Extraction and isolation of phytoconstituents
The extraction and isolation of phytoconstituents was carried out from the crude drugs (fruits of *Piper nigrum* and rhizomes of *Curcuma longa*). The black pepper (25 g) was powdered using mixer and grinder and extracted with ethanol (95%) in a soxhlet apparatus until the drug was completely exhausted. The extract was filtered and concentrated using rotary evaporator. Then alcoholic potassium hydroxide (10%) was added with constant stirring and solution was filtered. The alcoholic solution was allowed to stand overnight to separate the needle shaped crystals of piperine [11].
The fresh rhizomes of turmeric (25 g) were cleaned washed with deionised water, sliced and dried in the sun for one week and again dried at 50°C in a hot air oven for 6 hours. The dried rhizomes were cut in small pieces and powdered by mixer and grinder. The sample was extracted by continuous hot percolation method in a soxhlet apparatus. The acetone was used as solvent for extraction process. The process was carried out continuously till the drug was completely exhausted. After complete extraction the dark brown extract was obtained which was cooled, filtered, concentrated using rotary evaporator and finally precipitated with petroleum ether and dried to obtain curcumin [12].

2.3 Experimental protocol
The protocol of animal studies was approved by Institutional Animal Ethical Committee (IAEC), Maharshi Dayanand University, Rohtak. Healthy Wistar albino rats weighing above 200 g, were used as *in-vivo* animal model, for the pharmacokinetic studies. The animals were procured from the Central Animal House, Haryana Agriculture University, Hisar. Wistar albino rats (200-300 g) were given a normal standard chow diet and tap water *ad libitum*. The animals were housed in laminar flow cages, three per cage, which was maintained at 22±2 ºC, 50–60% relative humidity, under a 12 h light-dark cycle throughout the experiment. The animals were allowed to acclimatize for at least one week prior to the experiment.
The rats were fasted for at least 24 hrs prior to the experiment but given free access to water. Each rat was anaesthetized with diethyl ether. Tamoxifen dose (10 mg/kg) was chosen in order to keep the plasma concentrations above the detection limit [13]. The formulation used for administration of was prepared by adding tamoxifen (10 mg/kg) in 0.9% NaCl solution containing 10% of Tween 80 (3.0ml/kg) [14]. The formulations used for the administration of curcumin and piperine were prepared by dissolving their respective doses in 1% Tween 80 as vehicle (about 1 ml) [9]. In curcumin treated test groups, the rats were kept for curcumin pretreatment for four consecutive days and were given free access to food and water while the dosing was done on experimental day 5 [10]. In control group, the rats were administered a dosage of 10mg/kg tamoxifen formulation only. In test group A, formulation of curcumin (5mg/kg) was given to rats and then formulation having tamoxifen (10 mg/kg) was administered after half an hour. In test group B, the rats were given a formulation of curcumin (10mg/kg) and then formulation of tamoxifen (10mg/kg) was administered after half an hour. In test group C, formulation of piperine (5mg/kg) was given to rats and then formulation having tamoxifen (10 mg/kg) was administered after half an hour. In test group D, the rats were given a formulation of piperine (10mg/kg) and then formulation of tamoxifen (10mg/kg) was administered after half an hour. The blood samples (2 ml) were withdrawn from the retro-ocular area at 1, 2, 3, and 4 hrs time interval after administration of drug and centrifuged at 2500 rpm for 15 min at 4 ºC. The plasma samples (1 ml) were collected and stored at -40 ºC in deep freezer and further analyzed by HPLC for determination of drug concentration in rat plasma [15].

2.4 HPLC assay of plasma samples
The plasma concentrations of tamoxifen were determined by HPLC assay. Before HPLC study, the plasma samples were processed by adding 1 ml of ammonium hydroxide (1 M) and then extracted with 10 ml of tetrabutyl ether. The solution was evaporated to dryness and the sample was redissolved in 100µl of eluant and then 20 µl of this solution was injected onto the HPLC. The mobile phase consisted of methanol and 0.01 M phosphate buffer (pH 3.0) in the ratio 80:20 (v/v) with addition of an ion pair i.e. heptane sulphonate in 50 mM concentration. The buffer was prepared by dissolving 780 mg of dibasic sodium phosphate in water, and the volume was made up to 500 ml with water and adjusting the pH (3) with Orthophosphoric acid. The flow rate was 1.5 ml/min. The detector was operated at 240 nm and the operating temperature kept at 25ºC. The chromatographic system consisted of an Agilent 1200 Series G1311A solvent delivery quaternary pump equipped with a 20 µl loop and rheodyne sample injector. The analytical column used was Zorbax SB-C8 (5 µm; 4.6×150mm). The UV detector used was of variable wavelength [15]. The mobile phase was freshly prepared before each experiment. The data were recorded using Empower software.

2.5 Pharmacokinetic analysis
The AUCs of plasma concentration (*Cp*) versus time (*t*) were determined by using non-compartmental pharmacokinetic analysis using the LAGRAN computer program which uses the LAGRAN method. Maximum plasma concentrations (C_{max}) and times to reach maximum plasma concentrations (T_{max}) were determined by visually inspecting the experimental data [13]. The relative bioavailability of tamoxifen citrate after the oral administration (10 mg/kg) with the herbal bioenhancers compared to the control group was calculated by using the formula:

$$\text{Relative bioavailability (RB\%)} = \frac{\text{AUC(coadmin)}}{\text{AUC(control)}} \times 100$$

$AUC_{(coadmin)}$ = Area under the test sample curve
$AUC_{(control)}$ = Area under the control sample curve

2.6 Statistical analysis
The data were presented as means and standard deviations (mean±S.D.). The Pharmacokinetic parameters were compared by one-way analysis of variance, followed by a posterior testing using the Dunnett's correction. The $P< 0.05$ was considered to be statistically significant.

3. Results and Discussion
On extensive literature survey, the piperine and curcumin were selected as herbal bioenhancers as they are major inhibitors of

CYP enzyme family and P-glycoproteins. The extraction and isolation of phytoconstituents was done from fruits of *Piper nigrum* and rhizomes of *Curcuma longa* as per the standard procedures. The influence of the isolated phytoconstituents (Piperine and curcumin) on the bioavailability was studied as per the described experimental protocol in wistar rats. The blank plasma samples taken from rats were spiked with known concentration of tamoxifen (25-500ng/ml) and then analysed by HPLC. The retention time (R_T) of tamoxifen was about 3.1 minutes. The calibration curve was linear over the range of 25 to 500ng/ml. The plasma samples from various groups were isolated and analysed by HPLC. The data obtained in HPLC are shown in figure 1.

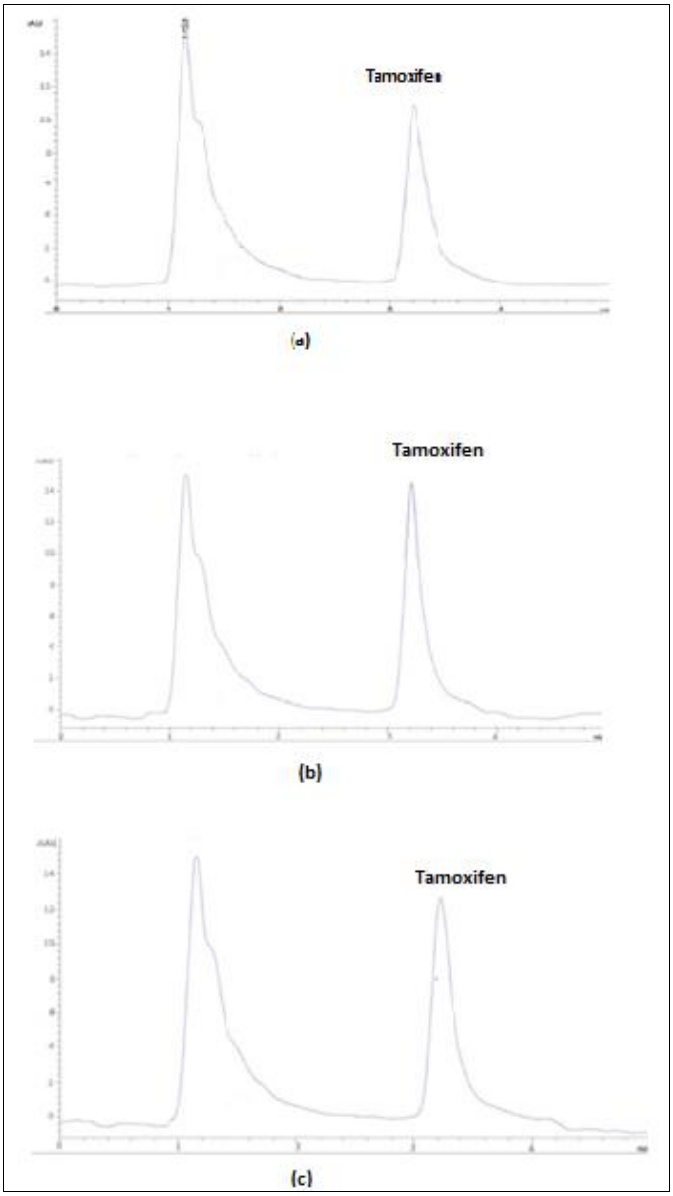

Fig 1: HPLC graph indicating the peak of Tamoxifen citrate (R_T-3.1 min) in plasma samples of different groups at 10mg/kg of dose after 2hr (a) Control (b) Curcumin (c) Piperine

The mean plasma concentration of tamoxifen in rat plasma after oral administration of drug with or without herbal Bioenhancers were calculated and expressed as the mean±SD. The mean plasma concentration of drug in different groups is shown in table 1.

Table 1: Mean plasma concentration (ng/ml) of drug in different groups

Time (hrs)	Control (Tamoxifen 10mg/kg)	Test group A (with Curcumin 5mg/kg)	Test group B (with Curcumin 10mg/kg)	Test group C (with Piperine 5mg/kg)	Test group D (with Piperine 10mg/kg)
1	67±6.2	82±6.1	89±4.5*	86±5.2*	92±6.3*
2	73±3.4	93±5.7*	113±7.3**	97±5.7*	119±8.1**
3	65±4.5	71±4.6	87±4.5*	78±4.1	88±4.4*
4	55±2.1	66±3.3	76±3.7*	68±3.2	80±3.9*

* $P < 0.05$, $P < 0.01$ as compared to control

The various pharmacokinetic parameters of drug like AUC, C_{max}, T_{max} and relative bioavailability in test groups as compared to control were determined and are shown in table 2.

Table 2: Pharmacokinetic parameters of orally administered tamoxifen (10 mg/kg) in different groups

S. No	Parameters	Control (Tamoxifen 10mg/kg)	Test group A (with Curcumin 5mg/kg)	Test group B (with Curcumin 10mg/kg)	Test group C (with Piperine 5mg/kg)	Test group D (with Piperine 10mg/kg)
1	AUC(ng/ml)	257.0±20	335.23±35*	411.66±30**	346.91±28*	424.55±31**
2	C_{max}(ng/ml)	73±3.4	93±5.7*	113±7.3**	97±5.7*	119±8.1**
3	T_{max}(h)	2	2	2	2	2
4	RB%	100	130	160	135	165

*P< 0.05, **P< 0.01 as compared to control

In evaluation studies, the drug was administered in rats with or without herbal bioenhancers in five groups (1 of control, 2 test groups with piperine and 2 test groups with curcumin). The selected herbal bioenhancers (piperine and curcumin) were given in 5 and 10mg/kg dose. The mean plasma concentration of tamoxifen citrate was found to be higher in all test groups as compared to control. The mean plasma concentration values were significant in test group A and B at 1 and 2 hr time interval. The mean plasma concentration values were significant in test group C and D at 1, 2, 3 and 4hr time interval. The significant value of AUC at 2 hr time interval with curcumin (5mg/kg), curcumin (10mg/kg), piperine (5mg/kg), and piperine (10mg/kg) were335.23±35 (P< 0.05), 411.66±30 (P< 0.01), 346.91±28 (P< 0.05) and 424.55±31 (P<0.01) respectively as compared to control which was 257.0±20. The significant values of C_{max} with Curcumin (5mg/kg), Curcumin (10mg/kg), Piperine (5mg/kg) and Piperine (10mg/kg) were 93±5.7 (P< 0.05), 113±7.3 (P< 0.05), 97±5.7 (P< 0.05) and 119±8.1 (P< 0.01) respectively as compared to control which was 73±3.4. The time required to reach the peak concentration (T_{max}) was not significantly altered by the administration of herbal bioenhancers. The relative bioavailability (RB%) of tamoxifen was also increased by 1.3-1.6 and 1.35-1.65 fold by curcumin and piperine pretreatment respectively.

4. Conclusion

Tamoxifen is a nonsteroidal antiestrogen and is the agent of choice for treating and preventing breast cancer. The orally administered tamoxifen is extensively metabolized by CYP3A4 the human liver and intestine. Moreover the absorption of tamoxifen in intestinal mucosa is also inhibited by the P-gp efflux pump. The piperine and curcumin has been reported to be inhibitor of CYP enzymes and P-glycoproteins, hence the effect of these herbal bioenhancers on bioavailability of tamoxifen has been studied in the present investigationl. The co-administration of piperine and curcumin (10mg/kg) with significantly enhanced the mean plasma concentration of tamoxifen after two hours. Further The relative bioavailability (RB%) of tamoxifen was also increased by 1.3-1.6 and 1.35-1.65 fold by curcumin and piperine pretreatment respectively. In a nutshell we can say that herbal bioenhancers likecurcumin and piperine significantly enhance the oral bioavailability of the drug tamoxifen citrate presumably via dual inhibition of CYP3A4 and P-glycoproteins.

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